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"To the solid ground
Of nature trusts the Mind that builds for aye"—WORDSWORTH

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Saturday, June 15, 1963.

PROVISIONS FOR UNIVERSITY EXPANSION IN BRITAIN

THE major pre-occupation of the general survey with which the University Grants Committee prepares its comparative statistics for 1960–61[*] is, as might have been forecast, finance. In the main, the Committee is content to present the facts rather than comment on the implications of the Government's refusal to accept the Committee's recommendations for the next quinquennium designed to provide places for some 150,000 students in four years time. Returns for existing universities indicated that they could increase their student numbers to 144,500 by 1966–67, dependent on the recruitment of staff and provision of adequate recurrent grants, and if the new universities, for the development of which the Committee had earmarked capital sums up to, and including, 1965, can provide 7,000 places by 1966–67 a total of about 151,000 should then be available, an increase of 36 per cent over 1961.

The Committee is satisfied that there is no shortage of applicants of the requisite ability, nor is it particularly disturbed at the rate of failures revealed by further inquiries on first-degree students. Of the 82·8 per cent admitted in October 1957 who obtained their first degree, 76·9 per cent did so in the normal period, and of the 14·2 per cent of 'failures', 11·8 per cent left for academic reasons and 0·1 per cent for disciplinary reasons. In medicine, dentistry and veterinary science, however, 29·9 per cent of the students required more than the normal time to achieve success.

Leaving for the moment the question of student numbers and quality with the rider that this evidence underlines all that the University Grants Committee emphasizes about the importance of academic staff of the highest quality, it should be noted that the steady rise in net expenditure on existing commitments itself limits the provision which the universities can make for expansion of the undergraduate population. Furthermore, the University Grants Committee has already earmarked sums totalling in the region of £2,000,000 by the end of the quinquennium to meet special needs in research and teaching, in the main at the Government's own request. The Committee recognizes that there is little money available in the new quinquennium for any other developments than those concerned with student expansion, and in the early years of that quinquennium the Treasury grants announced scarcely cover the increasing costs of existing commitments.

It is obvious that the University Grants Committee is relying on the indication in the debate in the House of Commons on April 5, 1962, that a fresh review of the universities' financial situation in two years time may lead to a further offer from the Government; however, the critical factor in expansion is not finance, at least not directly, but staff. It is this that gives such pertinence to the Committee's discussion of academic salaries, for obviously the ability to recruit a sufficiency of academic staff of high quality depends a good deal on the salaries offered. At the time of writing the report, the University Grants Committee was frankly concerned with the effects of the then suggested 3 per cent salary increase in the universities on the retention as well as on the recruitment of university staff. The Committee of Vice-Chancellors and Principals described this increase as entirely unrealistic and inadequate to enable universities to retain and recruit the academic staff required for the proposed expansion. However, in this context it remains to be seen how effective will prove the Government's present recommendations to the National Incomes Commission.

The University Grants Committee devoted considerable time during the year to the question of the ratio of academic staff to students, and it notes too that the increasing importance of research makes higher demands of staff time per student through the supervision involved. The present overall ratio, excluding Oxford and Cambridge, is estimated at 10·4, compared with 9·2 in 1954–55 and 11·7 in 1938–39; but it is pointed out that over the whole period the ratio in science and applied science has become worse: if staffing was in balance in 1938–39, these faculties were about 30–40 per cent understaffed in 1961–62, and a sample survey of academic posts vacant in June 1961 showed that 5·6 per cent of the posts were vacant. In the pure and applied sciences the figure was 6·5 per cent.

These figures, in fact, represent little more than the normal turnover in such posts, but at least they indicate the extent to which the universities have to compete with salary changes elsewhere in maintaining as well as recruiting staff. Moreover, although the Government's willingness to meet by supplementary grant the cost of academic salaries (about 44 per cent of the total) has made it possible to maintain the quinquennium system, this does nothing to cover the creation of new and higher posts or the reorganization that is often needed in anticipation as well as part of expansion. Furthermore, there have been increases of 98 per cent in national insurance, 37 per cent in pensions, 29·9 per cent in non-academic salaries and wages, 28 per cent in rates and taxes, and 24 per cent in the cost of books, periodicals and bindings between June 1956 and June 1961, the dates on which the universities framed their estimates for the 1957–62 and 1962–67 quinquenniums.

This represents an increase of some £4·7 million towards which the Government in 1959 made a supplementary grant of rather less than £1 million. The universities' expenditure on rates (about 2·2 per cent of the total in 1961–62) is indeed to be made the subject of earmarked grants during the coming quinquennium, in view of the changes made by the Rating and Valuation Act, 1961; this means that about 46 per cent of university income can now be augmented by supplementary grants to meet circumstances which are beyond university control. Nevertheless, the pressure remains particularly acute at precisely the point which is most critical for the expansion of the universities. Whatever may be possible in the way of providing new and less-expensive types of accommodation for students or making more effective use of existing accommodation and equipment, whether for teaching or research, the question of staff remains. Discount as we may the complaints that communication between staff and students is at present inadequate or non-existent, to raise it to the level of the past practice at present calls for some improvement in the ratio of staff to students. The expansion of postgraduate teaching as well as of research in itself calls for higher ratios, and the present evidence is that in many subjects the universities are already failing to retain their staff.

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[*] University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grants, Academic Year 1960–61. (Cmnd. 1855.) Pp. xxiv+48. (London: H.M. Stationery Office, 1962.) 5s. 6d. net [See also p. 24 of this issue of *Nature*.]

This question of student–staff relations is one of the most important points brought out by Prof. H. Butterfield in his Lindsay Memorial Lectures†. Universities, he maintains, ought primarily to be regarded as the arena in which occurs the electric contact between teacher and pupil. This should be the supreme object of the educational arrangements. It is the more important as the proportion increases of students who come from homes with no tradition of books or learning and who tend to regard the university as a mere production line. If arrangements so discourage this vital contact between teacher and student that the student never really experiences the university atmosphere at all, the university fails in its purpose, and this can result equally from the admission of too many undergraduates as from failure to provide the conditions of work or salary which attract and retain teachers of the requisite quality.

We are in danger, continues Prof. Butterfield, if we allow any pressure to train students for examination to lead us to overlook the urgent need to elicit the maximum intellectual power at every level. It is of the utmost importance for Britain as a whole—for her economic life, her intellectual development, the work of Government—that the universities should foster in their students imagination and flexibility of mind. The universities, moreover, have a creative work to perform both in handling young people and in the development of science, scholarship and thought, and it is at this point that Prof. Butterfield stresses the danger of allowing teaching and research to be divorced from one another. The place of research in the life of the university has become much more important in the past two decades, and he recognizes, too, that young men even in Oxford and Cambridge no longer have the same informal contacts with the dons, but he believes that it is far better to accept the existing tensions and heavy double programme of work than to admit the division between research and teaching which some have urged.

It is from this point of view that, in his second lecture, Prof. Butterfield discusses the academic profession. He emphasizes first the creative possibilities of teaching, the importance of the teachers in the progress of society and culture and the advantage that accrues when teachers perform their task in an atmosphere and mood of intellectual exhilaration. He is not uncritical of the present position and of some of the problems which arise in the administration or government of universities, but in this lecture he directs attention to what is commonly overlooked in discussions on academic salaries and university finance. It should be a first consideration of Government policy to promote just the conditions which he desiderates. Measures which are directed primarily at the expansion of student members to the detriment of those conditions are as unsound as they are wasteful.

Behind this, however, as he fully recognizes, lies the question of the relations between the university and society, and here again he has some wise words. With universities as with professional organizations generally, rights are correlative to responsibilities: academic autonomy is merely the requisite condition for fulfilling a university's responsibilities to society. There probably should always be a tension between universities and either society or Government, and it may be the duty of the university to maintain its side of the tension, if it is not to abdicate its responsibility for leadership. Prof. Butterfield believes that universities exist to serve society, but that it is a part of their service to conduct a search for truth which, if necessary, shall be independent of any immediate utilitarian end. He sees them as existing to produce good citizens, wise statesmen and able industrialists rather than first and foremost seeking to provide more dons and teachers.

Unless a university is able to perform these functions it will not render its most vital and characteristic service to the community, and Prof. Butterfield is well aware that some of the pressures for university expansion—not least the Government's own policy—could seriously impair that capacity. While, however, it will need a strong tradition and powerful professional organization to prevent universities from becoming mere factories for the production of examination candidates, Prof. Butterfield rests his main hope for the preservation of the academic ideal on the teachers themselves in contact with the young. The prime pre-occupation must be with that ideal, with maintenance of standards and the vindication of liberty, not with the development of particular branches of scholarship.

In his last lecture Prof. Butterfield refers again to the opportunities of the new universities, stressing in this context the extent to which the problems of the world are essentially humanist problems requiring insight and experience in respect of human personality, human issues and human relations. The notion of liberty of men and women in Britain, their democratic systems, their urbanities and tolerances and love of liberal-mindedness represent the late flowering of a humanist civilization which century after century has directed so much attention to men and problems of human relations. The transmission of these imponderables of tolerance and self-discipline is far more difficult than the transmission of science and technology, and in this transmission of our cultural heritage extra-curriculum activities play an important part. The preparation for responsibility in society, in fact, devolves more and more on such activities, and that once again indicates the vital importance of effective communication.

Until December 27, there had been no real indication that the Government appreciated the importance of this central issue which once again turns on the quality and the numbers of the academic staff. By putting first the expansion of the student population and failing to provide the resources which the universities themselves consider they need to deal effectively with that expansion, it is in danger of defeating its own purpose. Even if the universities are able to retain sufficient staff and increase the load on them, the most brilliant improvisation will scarcely ensure that more than a proportion of the students really receive a university education. If the Government is convinced that the nation can, in fact, not afford a public expenditure of more than £116 million in 1961–62 and £140 million in 1966–67, it would be sounder policy to consider diverting, for the time being, a larger proportion of the school-leavers to the colleges of technology and other institutions of higher education. Desirable as it may be to increase Britain's output of graduates, it is even more important that their quality should be maintained. It is obvious from the lively discussions already proceeding and from evidence tendered to the Robbins Committee that the efficiency of the universities could be improved in some respects and that there may be ways of reducing the cost per student. What is equally clear is that the financial pressure on the universities is already such as to raise serious concern as to their ability to deal adequately with their present responsibilities for teaching and research if the conditions that they are to offer their academic staff are not quickly improved.

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† *The Universities and Education Today*. The Lindsay Memorial Lectures given at the University College of North Staffordshire, No. 2. By Prof. Herbert Butterfield. Pp. viii+110. (London: Routledge and Kegan Paul, 1961.) 12*s*. 6*d*.

That the Government has now decided to refer the question of university salaries to the National Incomes Committee shows that it has at last accepted the advice of the University Grants Committee as to the need for immediate increases in university salaries. The announcement on December 27 of an actual salary increase of £3·5 million a year from April 1, 1963, sufficient to provide an overall increase of 10 per cent, provides the universities with an important salary basis for recruitment. However, in the present circumstances, it is unlikely by itself to remove the existing dissatisfaction in view of the claims previously advanced by the Association of University Teachers, which has already protested to the chairman of the University Grants Committee.

INFORMATION U.S.S.R.

Information U.S.S.R.

An Authoritative Encyclopædia about the Union of Soviet Socialist Republics. Edited and Compiled by Robert Maxwell. Pp. xii + 982 (illustrated). (Oxford, London, New York and Paris: Pergamon Press, 1962.) £10 net.

THIS is far more than a book—and one that ought to be in every library worth the name throughout the length and breadth of English-speaking lands. The *Great Soviet Encyclopædia* is a gigantic work of reference which can bear comparison with comparable encyclopædias in any country, and what Mr. Robert Maxwell and the Pergamon Press have done is to translate into English Volume 50 of the *Soviet Encyclopædia*—the volume which deals with the Soviet Union itself. What therefore is presented to the English-speaking world is the Russian world as seen and described by its own leading experts and as presented to its own people. It occupies 762 large quarto pages in double column, or much more than a million words, and, as if that were not enough, there follow 150 pages of statistics—the "U.S.S.R. in Figures, 1960", which is translated directly from the Russian handbook with the same title. Two exhaustive indexes of names and topics occupy nearly a further hundred pages, and so complete a monumental tome of approximately a thousand pages.

Robert Maxwell is a remarkable young man—still under forty—of Czech origin, who speaks and reads fluently at least nine languages, including Russian. After a distinguished war career, underground and later commissioned and decorated in the field, he was with the British Foreign Office until in 1947 he set up the scientific publishing house, Pergamon Press, now one of the largest organizations of its kind in the world. Holding that knowledge is the key to better world understanding, he regards *Information U.S.S.R.* as the first of a series of reference books, each to be written by the leading experts of their own country, which will eventually cover the whole world.

Information U.S.S.R. has been produced with the cordial collaboration of the Government Scientific Publishing House which produces the *Soviet Encyclopædia*, and already it has been discussed in principle that comparable works, *Information Great Britain* and *Information U.S.A.*, might be published simultaneously in Russian and English. Mr. Maxwell is already alive to the major problem of encyclopædias—that they quickly get out of date, and he plans annual or periodic supplements to keep them up to date.

Such a project staggers the imagination by its immensity, but what the Pergamon Press has already achieved in a dozen years of life leads one to believe that if anyone can see it through to fruition, that person is Mr. Maxwell. So much is at stake in a world rent by 'cold wars' that surely the effort deserves the fullest support: let every reader of this notice for a start make certain that his institution secures its copy of *Information U.S.S.R.*

To review the contents is an impossible task. If one dips into those sections covering one's own fields of study one is struck by the excellence and smoothness of the translation. Russian names which are so frequently mangled in English are given their correct transliteration and diacritical marks; for example, what normally appears in English as the Tian Shan are given as Tyan'-Shan' and Lwow as L'vov. One may object to anticlinoriums, but where rises and falls in the surface of the Palæozoic platform are designated anticlines and synclines one presumes this is the usage of the original. There are full references to the original literature, the works being given in Roman lettering, so that as with place-names one has a standard transliteration. Good tests of the adequacy of translation are afforded by the sections on soils and vegetation. Both are well done and it would be a severe critic who found serious fault. It is recognized that 'takyr', 'solonets' and 'solonchak' are not to be translated, and one is introduced to 'takyrization' quite naturally. Throughout 's' is used in preference to 'z' (podsol, not podzol). We are also given permanently frozen subsoil (*merzlota*). It should be noted that throughout the numerous maps and diagrams in the text and folding maps have been translated with equal care.

Section 15, "Science and Scientific Institutions", occupies no less than 140 pages. Undoubtedly the impression one gets is that science is a monopoly of the Soviet Union and in the main of the Soviet régime; it is scarcely possible to find other than a Russian name mentioned. It is thus a record of Soviet scientific achievement as seen by themselves: the Western reader must be left to see where the whole fits into the world pattern. But this is what is given to the students in Russian universities and to the intelligent reading public: it is right that readers in English should see for themselves exactly what is being disseminated entirely without editorial comment or alteration. L. Dudley Stamp

A DRIFT BETWEEN CONTINENTS

Animal Geography

By Wilma George. Pp. vi + 142. (London, Melbourne and Toronto: Heinemann Educational Books, Ltd., 1962.) 21*s*.

BOOKS on zoogeography are too few, too specialized or too inaccessible for a welcome to be withheld for one that "provides an introduction to the study of the distribution of animals . . . for those who are starting zoology at school and for those whose knowledge has progressed further but who require a brief survey of the subject". This latest book, excellent in so many ways, could meet the needs of the second category of reader but might fail for the beginner since it demands almost specialist knowledge for easy reading or ready understanding.

Only the vertebrates are dealt with, and among these the mammals are used predominantly to illustrate the principles. The text is divided into four parts: "Present", "Past", "Past to Present", and "Islands". In the first, we are introduced to the use of maps and to the classification of mammals, down to families, and to the 'thumbnail' pictures of mammals used as symbols throughout the book. The six zoogeographical regions are then explained, the methods of dispersal as well as the barriers to it, the outlines of evolution in mammals, and the three theories on the probable distribution of the land-masses in the past. The fourth part is devoted to the interpretation of island faunas.

Its author has the unenviable task of trying to simplify a tangled field of knowledge in which explanations must be attempted although so many unknowns and variables need to be reconciled. There is a pleasing reluctance to be dogmatic, and a commendable readiness to keep the reader reminded continually that so much that is said on this subject is based on theory. The style is straightforward and the diagrams clear, indicating the author's pioneering in the use of economy of words linked with visual aids (obviously with "those who are starting zoology at school" in mind). It is with reluctance, therefore, that one ventures to direct attention to what may be shortcomings, but this may be of help in a future edition.

It was with some slight surprise that, on finishing the book, one turned back to the title-page to be reminded that the author is "Fellow and Tutor in Zoology . . . [at] Oxford", instead of at Harvard. There is something non-British (and highly commendably so) in the remark (p. 33) that ". . . Darwin and Wallace announced the theory of natural selection . . ." (Wallace is usually dismissed in a single line.) There is an American flavour in other respects also. Instead of the familiar Palæozoic and palæontology we have paleozoic and paleontology, and Cænozoic becomes cenozoic. The loss of the diphthong may be a matter of taste, but the dropping of the capitals impedes one's reading whenever it occurs, perhaps only because the eye is not used to it. It may be that with the increasing interchange of American and English editions the time is already here when uniformity should be sought in such matters or imposed by pioneers like Wilma George.

Even so, the use of conies for hyraxes, with no explanation at all, seems unjustified. The word originally referred to *Oryctolagus cuniculus*. It is true that we have corruptedly standardized the name for a young cony (that is, the rabbit) by applying it to adults as well, and admittedly the conies of the Bible refer to hyraxes. Yet even in the United States, where some authors tend to use it alone when they mean hyraxes, the best-informed mammalogists render it as hyraxes or 'conies'.

The otherwise commendable brevity in style of this book can at times go too far. It is, for example, doubtful whether many Fellows of the Royal Society even, much less beginners in schools, would know offhand what a degu is, or a blesmol, even if they knew that Rhyzomyidae (p. 28) should be Rhizomyidae ; and a "Dalliid blackfish" is, to say the least, unfamiliar and should be given a brief amplification, unless the aim is to compel the beginner zoologist to go rooting around among specialist books or the best encyclopædias. Maurice Burton

ORGANIC MICROANALYSIS

Methods of Organic Elemental Microanalysis

By G. Ingram. Pp. xvi + 511. (London: Chapman and Hall, Ltd., 1962.) 75s. net.

The periodical literature on organic microanalysis has now reached vast proportions, and the newcomer in this field—and, indeed, the moderately experienced technical analyst—needs some sort of 'filter' which will offer to him the critically assessed best of what have been proposed in the way of methods. To act as such a 'filter' has been the aim of Mr. Ingram, and he has achieved this very well indeed. Previous text-books on organic elementary analysis have tended to be the products of 'schools'—where sometimes one might suspect a certain amount of special pleading. Mr. Ingram is not a member of any school or, in a sense, perhaps he is his own school. Out of a severely critical approach and a vast experience he has presented the best of the published methods, and has argued his choice competently.

He has elected to take a quite unusually wide interpretation of 'organic elements'. Instead of restricting himself to the usual ten, or at most about sixteen elements usually dealt with under this heading, he has recognized that more than fifty elements may be required to be determined in organic materials; he has widened his net accordingly.

He has also taken note of the remarkable advances in the ultra-micro range in the past few years, and has included a section dealing with the elementary analysis of samples of the order of 50 μg.

Mr. Ingram comments on one interesting anomaly: that although the techniques of organic elementary analysis have altered vastly in the fifty years that have passed since they were first devised by Fritz Pregl, the fundamental basis for the determination of some of the elements, for example, carbon and hydrogen, is still that evolved more than a hundred years ago. He suggests, with some justification, that more thought could be given to this by organic microanalysts than has been done in the past.

This book is a valuable contribution to the literature. It makes one hope that the author, in due time, may turn his attention to the field which here he has deliberately excluded—organic functional analysis.

C. L. Wilson

BIOLOGY IN AMERICAN HIGH SCHOOLS

High School Biology

Revised edition. American Institute of Biological Sciences. Biological Sciences Curriculum Study Committee on Innovation in Laboratory Instruction for Experimental Use During the School Year 1961/62.

A. (1) Blue Version. (2) Yellow Version. (3) Green Version (each version consisting of Text, in three parts, The Laboratory, in three parts, and The Laboratory (Teachers') Guide, in three parts).

B. Laboratory Blocks of : (1) Microbes : Their Growth, Nutrition and Interaction, by Alfred S. Sussman ; (2) Animal Growth and Development, by Florence Moog ; (3) The Ecology of Land Plants and Animals, by Edwin A. Philips; (4) Interdependence of Structure and Function, by A. Glenn Richards ; (5) Animal Behavior, by Harper Follansbee ; (6) Plant Growth and Development, by Addison E. Lee ; (7) Regulation in Plants by Hormones, by William P. Jacobs and Clifford E. LaMotte.

C. The Teacher's Handbook (all-Text Version, parts 1, 2 and 3).

D. Biological Investigations for Secondary School Students, in two parts.

(The series are not in fact classified A, B, C . . . 1. 2, 3; this is done here purely for simplicity.)

(Washington, D.C. : American Institute of Biological Sciences, 1961 and 1962.)

Curriculum reform in American education has been carried out on a truly large scale in recent years at a cost of four million dollars furnished by the National Science Foundation. Starting in 1959, seventy teachers in schools and universities, together with other scientists, set about a comprehensive re-orientation of school biology. A first writing conference produced three different types of high school courses which were used in 1960–61 by 118 teachers and their 14,000 pupils in seventeen States. A second writing conference produced revised versions of the three courses which have been used in 1961–62 by 541 teachers and their 56,000 pupils in thirty-five States, while a final version is to be made available on the American market in September, 1963, following a third definitive writing conference. It is the second revision which is the subject of this review.

To appreciate the significance of this remarkable *tour de force* both for British and American education it is essential to understand routes by which British school biology has reached its present scope and acquired its present character. At least 80,000 boys and girls enter for biology each year at the Ordinary Level of the General Certificate of Education in England and Wales, and thus exceed the entries for any other single science by some 18,000; and three-quarters of these entrants are girls. The ratio of degrees awarded each year in biological subjects to passes each year at Advanced Level and Ordinary Level is approximately 1 to 7·5 and 1 to 94 respectively. Thus the bulk of school biology is not concerned with either entry into university or university type of work, a fact which has to be balanced against the currently fashionable idea that mathematics, physics and chemistry is the only proper intellectual diet for science sixth formers. Another striking fact is the extent to which Advanced Level examination questions are predominanty descriptive and out of phase with contemporary biological thought and emphasis.

In this situation the moves afoot to re-align school biology are nationally important, vigorous and extensive as a study of the publications of the past three or four years shows well. Britain's 'gadgetry' is excellent, several stimulating schemes for reform are in existence, and nationally important bodies provide several types of facilities to teachers. The needs are to raise the standard of intellectual rigour of biology by modernization, shift of emphasis and reduction of study of too much dead material. In the long run new forms of training for teachers will be necessary, a far closer co-operation between school and university called for and a big increase in the supply of laboratory technicians in the schools at a far greater cost than now. Yet no real change is possible until the examination boards alter their policies and syllabuses and type of examination.

The American problem is essentially like Britain's, and has been stressed in urgency by reaction in the 1950's to Soviet performances. This is why the publication under review, running as it does into thirty-eight volumes, is bound to have a world-wide impact.

The American panels first set out nine basic biological concepts all of current research interest:

Evolution, diversity of animal and plant life in relation to unity of phylogenetical patterns, genetic continuity, relation of organism to environment, relation of structure to function, biological roots of behaviour, homoeostasis, and science as inquiry. Intellectual history of biological concepts is woven into the treatment.

From this three courses were prepared, each comprising three text-books to cover a yearly course and containing a greater volume of material than the pupil is expected to remember. Each course also has three laboratory manuals, one for each text-book and forming the main course to which the reading in the texts is a background. There are also three teacher's handbooks with each course.

The 'Green' course starts with ecology and relationships and works up to biochemistry; the 'Blue' course emphasizes biochemistry and works by stages towards ecology, while the 'Yellow' course begins with cytology and works in two directions from it, one towards biochemistry and the other towards ecology via various levels like physiology and systematic biology. 70 per cent of the matter is common to all three courses. The level of presentation is directed at the 16–18-year-old pupil.

In assessing the extent to which these works achieve their aims it is necessary to realize the completeness and the revivifying effects of the break with traditional methods and thoughts. The practical work impels the course on its way and is not didactically presented but is introduced as a form of research. The ingenuity of the practical methods is excellent. In addition, supporting volumes set up two new themes. One is the laboratory block by which single or small teams of pupils are given a stretch of six weeks full-time laboratory work. "During the next six weeks we want you to work like a scientist" is the opening sentence of the block. The second new theme is the provision of volumes written by research biologists stating 150 research problems within the capacity of the pupils and to which the answers are as yet unknown. Throughout, the style of writing is exciting and I myself have tested the effects of it on my own pupils and am convinced of its adequacy for its avowed purpose. The quality of the illustrations is not high; but it has to be remembered that one is seeing an interim version, not a final commercial production.

The success of this splendid experiment will be measured in proportion to its effectiveness in stimulating and shifting opinion outside the United States. [See also *Nature*, 194, 242; 1962.] L. M. J. Kramer

PHYSICO-CHEMICAL TECHNIQUES IN PHYSIOLOGY

Physical Techniques in Biological Research

Edited by William L. Nastuk. Vol. 4: Special Methods. Pp. xiii+410. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 13 dollars.

The previous volumes of this series were concerned with optical techniques (1955), physico-chemical techniques (1956), physical methods of observation of cells and tissues (1956).

Now, after an interval of six years, a new volume with a new editor, dealing with "Special Methods", has appeared. This is concerned with various specialized physico-chemical techniques with applications in physiology. S. Chien and M. I. Gregersen have written a comprehensive monograph on the determination of the volumes of body fluids which will be invaluable to all who are concerned with this. E. M. Renkin writes on the techniques of vascular perfusion—a method which has extensive uses in studying the functions of different tissues or organs. A more physico-chemical parameter, which is of considerable interest in connexion with tissue metabolism and has importance in radiotherapy, is the oxygen tension. P. W. Davies discusses the use of oxygen cathodes of various types in these determinations. It appears that successful determinations can be made by using the ability of oxygen to depolarize cathodes, the extent of depolarization depending on the diffusion of oxygen in the medium. A. B. Otis describes physical techniques used in the examination of respiration—mainly involving the analysis of the gases involved. M. Margoshes describes recent studies of flame photometry—a method of determining certain metals which has been greatly refined by the more exact control of temperature. Finally, W. T. Fry and F. Dunn contribute a lengthy monograph on ultrasound, which deals very comprehensively with the methods of producing ultrasound and its physical properties. This section contains much more information than most biological users of ultrasounds could be expected to require. Certain biological applications are rather briefly described.

The articles differ considerably in their comprehensiveness, and in several cases go much beyond the declared aim of the series—to provide critical and useful information for graduate students and also investigators needing to use a particular type of technique.

Two more volumes of this useful series are promised, to deal mainly with certain electrophysiological techniques and types of apparatus. It is to be hoped that these will not be delayed too long. J. A. V. Butler

INTERNATIONAL GEOPHYSICAL CALENDAR 1963

THE International Geophysical Calendar designates selected days and intervals for special attention for geophysical experiments and analysis, and is thus a framework for world-wide co-ordination. The Calendar serves mainly the branches of geophysics dealing with the Earth's atmosphere in which many phenomena vary significantly during the course of a year. In some experiments, such as the routine recording of variations of the Earth's magnetic field, the observing and analysis programmes at observatories are normally carried out at a uniform level throughout the year; in these cases the Calendar is not needed. However, in many other experiments (for example, rocket experiments), it is not practical or meaningful to carry out the same programme on each and every day. Here the Calendar can provide a useful mechanism for co-ordination; experimenters will know that their colleagues in other countries, in other laboratories, and in other disciplines will tend also to carry out experiments on the days or intervals marked on the Calendar. In this way, results of experiments may later be more easily and usefully compared.

In some scientific fields, international scientific organizations have made specific recommendations for programmes to be done on days or intervals marked on the Calendar. In others, the arrangements are informal or self-evident. Some examples are given here.

Regular World Days (*RWD*)

These are intended for observations or analyses or special experiments which as a practical matter can be performed for only about 10 per cent of days and should be in groups of three consecutive days and spaced throughout the year. One day of each group of three is designated as the *RWD* with highest priority and may be used for similar work which can be undertaken for only one day each month. Examples of suitable programmes in ionospheric physics are: oblique incidence pulse transmission and reception; absorption measurement by pulse reflexion technique; extended observing schedule for whistlers and very-low-frequency emissions; analysis of vertical sounding ionograms by f-plot, h'-plot, etc.; hourly reduction from ionograms of F-region true height parameters hc and qc. It is recommended that exchange of copies of original ionograms in ionospheric vertical sounding work be made for the *RWD* with highest priority.

World Geophysical Intervals (*WGI*)

These are intended for experiments which for practical reasons cannot be carried on continuously, but for which statistics of seasonal variations are especially needed. The choice of intervals has been heavily influenced by the needs of the meteorology discipline. The intervals in 1963 are placed about a month after the equinoxes and solstices which are times of marked seasonal change in certain upper-air meteorological phenomena. The co-ordinated international programmes for meteorology call for extra effort in synoptic rocket sounding and balloon sounding during the *WGI*. Other programmes such as ionospheric drift and high-atmosphere wind measurements are other examples of suitable programmes for such intervals.

Other Special Days

Other special days marked on the Calendars include rocket-winds days, the days of solar eclipses, and days of unusual meteor shower activity. The *rocket-winds* days (February 24, 25, 26) have been selected by the Committee on Space Research (here referred to as COSPAR) for a series of simultaneous rocket measurements of winds in the lower ionosphere (up to about 200 km) by techniques such as grenades, sodium clouds and visible grenade glows. COSPAR further recommends that simultaneous ground-based measurements by radio methods also be made on these dates. The solar eclipses (January 25, July 20, 1963; January 14, 1964) are occasions when special programmes may be expected to be carried out in appropriate parts of the world to study the Sun and any eclipse effects on the Earth-atmosphere. The geomagnetism discipline has directed special attention to these eclipses. Ionospheric stations customarily increase their observing programmes even if the magnitude of eclipse at their location is small. Many solar activity observatories take extra observations and issue specially detailed reports to assist the interpretation of the geophysical effects. The days with unusual meteor shower activity include some of the important visual meteor showers and also unusual showers observable mainly by radio and radar techniques. Attention is directed to these days in case ionization produced by meteors may account for unusual effects in other geophysical experiments. The annual World Meteorological Day, selected by the World Meteorological Organization (WMO) as March 23 (not marked on the Calendar), was first celebrated in 1961. Its purpose is to make the services which national meteorological services can render to the various branches of economic development, as well as the activities of WMO better known and appreciated by the public of all countries.

World Days for the International Years of the Quiet Sun, 1964–65 (IQSY)

This present Calendar also shows January 1964, which is the first month of IQSY. This month therefore shows a pattern of world days which is somewhat different from 1963, but which will be followed in the International Geophysical Calendars for the IQSY period. The Regular Meteorological Days are each Wednesday (U.T.), on which it is recommended that particular efforts be made to obtain the maximum quantity of data, including rocket ascents and ozone radiation sonde ascents, as well as maximum altitude balloon ascents at 0000 and 1200 U.T. The World Geophysical Intervals for IQSY have been extended to cover 14 days.

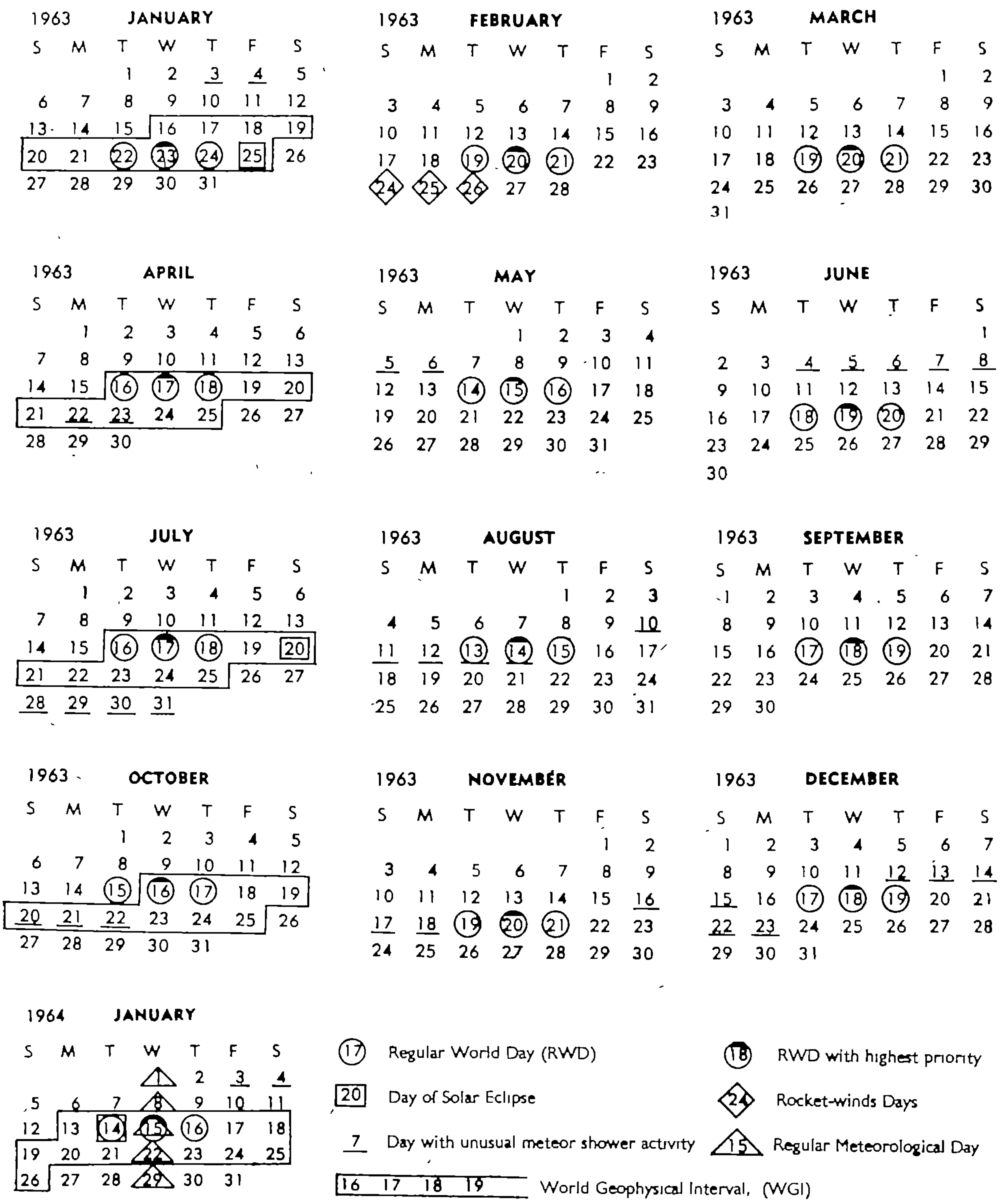
Special Intervals not appearing on Calendar

Periods of great magnetic, auroral and ionospheric disturbance are also of considerable geophysical interest. World-wide co-ordination of observation is especially useful for stations not near the auroral zones, that is, places where the beginning of a major disturbance may not be immediately apparent from local observations. Notices of the Geophysical Alerts and Special World Intervals (*SWI*) are distributed by telegram or radio broadcast on a current basis by the solar-geophysical Regional Warning Centres of the *IUWDS*, the telegraphic addresses of which are as follows:

AGIWARN WASHINGTON (U.S.A.); DENPA KOKUBUNJI (Japan); NIZMIR MOSCOW (U.S.S.R.); IONOSPHARE DARMSTADT (G.F.R.) or GENTELABO PARIS (France) or AGI NEDERHORSTDENBERG (Netherlands). The meteorological telecommunications network co-ordinated by WMO carries such information once daily soon after 1600 U.T. Many geophysical stations increase their programmes or carry on special experiments during disturbed periods. Prompt notification of immediately significant geophysi-

International Geophysical Calendar 1963

Issued October 1962 by the International Ursigram and World Days Service,
under the auspices of U.R.S.I.



1963 **JANUARY**

S	M	T	W	T	F	S
		1	2	$\underline{3}$	$\underline{4}$	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	㉒	㉓	㉔	$\boxed{25}$	26
27	28	29	30	31		

1963 **FEBRUARY**

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	⑲	⑳	㉑	22	23
◇24	◇25	◇26	27	28		

1963 **MARCH**

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	⑲	⑳	㉑	22	23
24	25	26	27	28	29	30
31						

1963 **APRIL**

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	⑯	⑰	⑱	19	20
21	$\underline{22}$	$\underline{23}$	24	25	26	27
28	29	30				

1963 **MAY**

S	M	T	W	T	F	S
			1	2	3	4
$\underline{5}$	$\underline{6}$	7	8	9	10	11
12	13	⑭	⑮	⑯	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

1963 **JUNE**

S	M	T	W	T	F	S
						1
2	3	$\underline{4}$	$\underline{5}$	$\underline{6}$	$\underline{7}$	$\underline{8}$
9	10	11	12	13	14	15
16	17	⑱	⑲	⑳	21	22
23	24	25	26	27	28	29
30						

1963 **JULY**

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	⑯	⑰	⑱	19	$\boxed{20}$
21	22	23	24	25	26	27
$\underline{28}$	$\underline{29}$	$\underline{30}$	$\underline{31}$			

1963 **AUGUST**

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	$\underline{10}$
$\underline{11}$	$\underline{12}$	⑬	⑭	⑮	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

1963 **SEPTEMBER**

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	⑰	⑱	⑲	20	21
22	23	24	25	26	27	28
29	30					

1963 **OCTOBER**

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	⑮	⑯	⑰	18	19
$\underline{20}$	$\underline{21}$	$\underline{22}$	23	24	25	26
27	28	29	30	31		

1963 **NOVEMBER**

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	$\underline{16}$
$\underline{17}$	$\underline{18}$	⑲	⑳	㉑	22	23
24	25	26	27	28	29	30

1963 **DECEMBER**

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	$\underline{12}$	$\underline{13}$	$\underline{14}$
$\underline{15}$	16	⑰	⑱	⑲	20	21
$\underline{22}$	$\underline{23}$	24	25	26	27	28
29	30	31				

1964 **JANUARY**

S	M	T	W	T	F	S
			△1	2	$\underline{3}$	$\underline{4}$
5	6	7	△8	9	10	11
12	13	$\boxed{⑭}$	△⑮	⑯	17	18
19	20	21	△22	23	24	25
26	27	28	△29	30	31	

⑰	Regular World Day (RWD)	⑱	RWD with highest priority
$\boxed{20}$	Day of Solar Eclipse	◇24	Rocket-winds Days
$\underline{7}$	Day with unusual meteor shower activity	△15	Regular Meteorological Day
16 17 18 19	World Geophysical Interval, (WGI)		

cal observations and of major solar flare events which have important and sometimes long lasting geophysical effects, are also undertaken through the Regional Warning Centres.

The International Ursigram and World Days Service (*IUWDS*)

This is a permanent service of the International Scientific Radio Union (URSI) adhering to the Federation of Astronomical and Geophysical Services of the International Council of Scientific Unions (ICSU). This Calendar has been drawn up by A. H. Shapley and J. V. Lincoln in consultation with interested ICSU unions and committees, and representatives of the WMO. A fuller description of such Calendars has appeared in the *URSI Information Bulletin* and various widely available scientific publications. Further copies of the Calendar can be obtained from the Secretary-General, URSI, 7 Place Danco, Brussels, 18.

EDUCATION AND THE HUMANIST REVOLUTION*

By SIR JULIAN HUXLEY, F.R.S.

MANY people have made pronouncements about education: T. H. Huxley drew a memorable picture of a liberally educated man, and Dorothy Parker has maliciously described it as "casting sham pearls before real swine". But I want to define education as a social process. I see education as an organ of man in society, whose basic function is to ensure the continuity and further advance of the evolutionary process on Earth by the transmission and transformation of tradition.

To look at education in the perspective of evolution shakes us out of over-preoccupation with our immediate difficulties. It provides a corrective to the tendency of our neotechnic industrial civilization to think in terms of quantity rather than quality, and it gives new dignity and importance to the whole educational process.

Education helps to transmit experience and its results across the gap between generations. There are three stages of its evolution. In the first, transmission bridges only one generation-gap, as in carnivores like fox or lion, where the young animal, after a period of self-education by play, learns to hunt by accompanying its parents.

The second is marked by the beginnings of tradition, a set of social habits which endures through a number of generations. The most striking example is the Japanese monkey. Here each social group has its own traditional food-habits, but these are occasionally modified by adventurous innovations, which take two or three years to spread through the little community. This is a big advance, but it involves no real educational system.

The third, found only in man, is that of cumulative tradition, based on conceptual thought and communicated mainly by artificial symbols that must be learnt. As the tradition grows more complex and the system of communication more elaborate, organized education becomes a necessary part of the machinery for their transmission.

Cumulative tradition provides man with a second mechanism of inheritance and evolution superimposed on the primary genetic-evolutionary mechanism of his chromosomes, and often overriding it. This has pushed man across a critical threshold, from the biological to the psychosocial phase of the universal evolutionary process. In this new phase, evolution is manifested primarily and most strikingly in cultural change, only secondarily in change in genetic constitution.

Not only is cultural evolution much faster than biological: it also shows acceleration. In the lower palaeolithic a major cultural change took perhaps 100,000 years; in early civilization a few hundred; and now barely a decade—with all sorts of implications for education.

Yet, in spite of all his achievements, we must remember that man is an extremely recent phenomenon. He cannot be much more than a million years old, and that, in the perspective of life's evolution, is a bare half-minute out of a twenty-four-hour day; while the whole of civilization is compressed within one tick of the cosmic clock. What is more, he is sadly incomplete, an unfinished type, needing a great deal of improvement before he can do his evolutionary job properly. Human individuals, too, are obviously unfinished when first thrust into the world, and need a great deal of improvement before they can qualify as satisfactory social beings: and their improvement as individuals, as well as the eventual improvement of man as a type, depends largely on the improvement of education.

Improvement during biological evolution is manifested in the improved construction, working, and behaviour of animals and plants. As Darwin pointed out, it is the inevitable result of the blind and non-purposeful agency of natural selection, operating via the genetic mechanism of transmission and variation—the gene-complex.

Improvement during human evolution, on the other hand, is manifested in the organization, working and achievements of human societies and cultures. It is primarily the result of the collective action of psychological and social forces, operating via the psychosocial mechanisms of transmission and transformation, of course, including cumulative tradition.

We are only beginning the detailed exploration of the agencies of psychosocial evolution, but some important points are emerging. First, it is the general idea-system, the pattern of knowledge and belief, which essentially characterizes human cultures, and indeed is the prime determiner of their social organization, their material sub-structure, and their achievements.

Secondly, everything in human history which deserves the title of advance or progress, every improvement or hope of improvement of the human lot and man's hard destiny, has sprung from the discovery and dissemination of new knowledge and new ideas. But new ideas, and the knowledge of which they are the vehicles, must operate within the contemporary idea-system, and will also modify it. Education should help in ensuring that they do so effectively.

Major advance in evolution takes place in a series of steps, by a succession of dominant types, each new one with an improved organization, while the older types persist in an inferior position and in reduced numbers. During biological evolution, what is improved is bodily organization. But in human evolution, what is improved is psychosocial organization—the organization of knowledge, ideas and beliefs and the cultures and social systems related to them. The industrial national type of organization is higher than the literate barbaric, that in turn higher than the illiterate tribal type. Magic, myth, monotheism, scholasticism, science succeed and replace each other as dominant patterns of human thought; though here, too, the older organizations persist in a subordinate position.

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* Substance of the Ninth Fawley Foundation Lecture delivered at the University of Southampton on November 27.

The knowledge explosion of the past hundred years has given us a new vision of human destiny—of the world, of man, and of man's place and role in the world. It is evolutionary and monistic, showing us all reality as a self-transforming continuous process, with no dualistic cleavage between soul and body, matter and mind, life and not-life, natural and supernatural. All phenomena, from worms to women, from radiation to religion, are natural.

It leads inevitably to a new dominant organization of thought and belief, and, after centuries of ideological fragmentation, to a new and comprehensive idea-system, which I call 'evolutionary humanism'.

Its upshot is clear. Man is not merely the latest dominant type produced by evolution, but its sole active agent on Earth. He is responsible for the future of the evolutionary process on this planet. Whatever he does, he will affect it. He must try to understand it and its mechanisms, and to steer it in the right direction.

In carrying out this operation, he must not sit back and expect supernatural aid. Its overriding aim must be defined in positive terms, like fulfilment and achievement —greater fulfilment (and less frustration and misery) for more human individuals, interlocking with fuller achievement (and less muddle and failure) by more human societies. This must be secured by a fuller realization of human possibilities.

To realize this aim man must learn to live in harmonious symbiosis with his planetary environment—a responsible partner instead of an irresponsible exploiter. He must abandon the heuristic idea of conquering Nature; he must co-operate and conserve.

If this view of our destiny is correct, clearly our educational system must come to terms with it.

Most educational systems are highly change-resistant because they are controlled by dogmatic religious or State organizations, because they are linked with the established social order, or just because of inherent conservatism. But our new system must itself be evolutionary, not change-resistant but change-promoting; it must transform as well as transmit. In part, this can be achieved by making girls and boys understand the moral duty of helping and guiding the evolutionary process in a desirable direction. But something more practical is also needed. If our aim be greater fulfilment, the next step in psychosocial evolution must be from the Welfare State towards a 'Fulfilment Society'. A humanist educational system will put the idea of a fulfilment society before children, and will provide them with opportunities for actual personal fulfilment—through knowledge (I remember Bertrand Russell once exclaiming: "How nice it is to *know* things !"); through disciplined adventure; through expeditions and travel; through painting and acting and making music; through beauty; through study projects and discussions; through responsible participation in group activities. It will have a curriculum of experience as well as a curriculum of subjects.

In adapting our old educational system to our new vision, much cargo will have to be jettisoned—once-noble but now mouldering myths, shiny but useless aphorisms, Utopian but unfounded speculations, nasty projections of our prejudices and repressions.

Thus, man was not created in his present form a few thousand years ago. Mankind is not descended from a single couple. Children are not born with a load of original sin derived from a "Fall", nor with a *tabula rasa* of a mind. There never was a Golden Age, nor a Noble Savage. There are no pure races, nor any Superior or Master Races. There are no Absolutes of Truth or Virtue, only possibilities of greater knowledge and fuller perfection.

On the contrary, mankind originated from a population of ancestral anthropoids about a million years ago. His subsequent evolution has been marked by the increasing complexity and improvement of his material, social and psychological organization, but also by the increasing magnitude of his crimes and follies. Human children are not a set of uniform learning machines, but highly complex and varied psychosocial organisms engaged on the difficult attempt of developing into satisfied and satisfactory human beings.

Education has to cope simultaneously with several distinct problems—the imparting of knowledge; the learning of skills and social habits; the transmission of traditions and beliefs; the formation of character and personality; and the crucial passage from childhood to adult life.

In tribal societies, children gain the knowledge they need by imitating the adults and participating in their activities. Punishment is rare. Their theoretical instruction, in tribal myth, morality and forms of adult behaviour is imparted by selected elders, usually with the aid of esoteric ceremonies and painful ordeals. There is no examination to test their knowledge, only an ordeal to prove their adulthood; no certificate to frame and cherish, only the fact of their acceptance as full tribal members.

We still inflict painful ordeals on our children, in the shape of a whole series of examinations; we still celebrate their successful survival of these ordeals by ceremonial activities, most elaborately by the solemn pomp of university commencements and degree days. Most psychiatrists will agree that one reason for the stiffness of examinations is psychological—the subconscious desire of the adult to revenge himself for past ordeals by subjecting the young to similar trials; and assuredly the gorgeous robes of the academic procession are worn not only to impress the community gathered for the occasion but also as a permitted outlet for donnish egos. Yet it is still true that a tribal boy's education may be a better preparation for life in a tribal society than is ours for life in our vaunted technological society.

Education must come to terms with humanism over its curriculum, over its relations with society, and over its methods of fostering the development of personality.

As regards curriculum, the solution is, in principle, simple. The advance of knowledge has at last given us a unitary picture of phenomenal reality: the curriculum must reflect this unity and itself become unified instead of consisting of a number of unrelated fragments called 'subjects'.

Simple in principle, but not so simple in practice ! The first difficulty is quantitative—the mere bulk of knowledge is too great. However, the prime function of education is not to impart the maximum amount of factual information, but to provide comprehension: and for this we need what we can broadly call 'ideas', together with the necessary modicum of facts.

Some ideas are better than others—they can handle more apparently disparate phenomena, bridge larger gaps, simplify greater diversity. Thus the single idea that the planets move around the Sun in ellipses, at one stroke simplified for us the whole working of the solar system.

Evolution is the most powerful and the most comprehensive idea that man has ever had. It helps us to understand our own nature, and our relations with the rest of Nature. It shows us the major trends of evolution in the past and indicates a direction for the future. It gives us a new sense of excitement and wonder, a new view of our relations with our planetary habitat and its resources, a new perspective in time, and a new sense of universal interrelatedness. We are made of the same matter, we operate by the same energy as the rest of the cosmos; we maintain and reproduce ourselves by the same mechanisms as the rest of life, unique only in having reached the psychosocial stage.

Thus the evolutionary idea must provide the main unifying approach for a humanist educational system, and evolutionary biology should become a central or key subject in its curriculum. Another unifying idea of great importance is the ecological. Ecology is scientific natural history: the science of relations, between organisms and their environment, and of organisms with each other.

It deals with the dynamic balance of Nature. Its central concept is the ecological community—an assemblage of different types of organism, inter-adjusted to give optimum utilization of the resources of the habitat. If the balanced pattern of relations is interfered with, the whole habitat may be damaged, together with the whole community inhabiting it.

From earliest times, man has been interfering ignorantly with more and more natural habitats and has exploited their resources more and more pitilessly. He has converted vast areas of once-fertile land into arid semi-deserts. Now that his exploding population is subjecting more areas to more technologically efficient exploitation, he is in danger of becoming the cancer of the planet instead of its guide.

In the educational systems of under-developed territories, children should be introduced to science by the biological way of ecology and physiology and their applications in conservation and health, not by way of physics and chemistry and their applications in technology and industry; and in all countries, ecology is essential as a basis for good land-use and productive development.

Man lives in a triple tier of environments—material, social and psychological. Ecology in the customary sense deals with man's relations with the forces and resources of external nature. Social ecology deals with man's social problems, within and between human societies. Psychological ecology deals with man's relations with the forces and resources of his inner nature, and the environment of ideas and beliefs with which he has surrounded himself.

How should our evolutionary-humanist approach take effect in education? We must end the fragmentation of the present system. Education must be comprehensive, reflecting all aspects of life; it must have a unitary pattern, reflecting the unity of knowledge and the wholeness of experience; and it must give growing minds a coherent picture of Nature and themselves.

For this, we need an integrated curriculum, in which separate subjects can link up with each other—physiology with chemistry and heredity, ecology with geography, soil science with agriculture and meteorology: reproduction leads on to biological evolution, evolution links back with geology and cosmology, and on to archaeology and human history: history links up with economics and social studies. Art and architecture, law and morality, science and technology are best treated as functions of evolving social man: they too evolve, and accordingly profit by being treated historically, not merely as separate packages of facts and principles.

Just how to plan and introduce such a curriculum is a matter for the educational profession. It will not be easy, in face of the competitive claims of traditional subjects and established departments, and of the portentous growth of factual knowledge and theoretical scaffolding. It will mean scrapping a great deal of dead wood and dead weight, and some sacrifice of specialists' *amour propre*. But I am sure that it can be done.

There are already sporadic beginnings. Medicine is throwing overboard a mass of unnecessary anatomical and other detail: universities are introducing courses of general studies: sixth forms are taking steps to correct earlier over-specialization. The American Institute of Biological Sciences has worked out a remarkable integrated curriculum for high-school biology, complete with new text-books, new prospects, new laboratory and field methods [see p. 4 of this issue]; and the Gulbenkian Foundation is following suit in Great Britain. But these are isolated sporadic measures. The task will only reach a satisfactory conclusion when we realize that, here as elsewhere, unity brings strength: in a unified curriculum, separate subjects need not compete for prestige and place but can reinforce each other.

This could give a new dynamic to the educational process. Children have a natural interest and curiosity; they want to know about this strange world, about human life and how to live it, to find out what it all means. If their education helps them in this instead of setting up a number of hurdles in their way, they will enjoy it, and will become willing co-operators instead of reluctant or obstructive victims. From being merely a preparation for a job or a career, education could become an integral part of life, an instrument of social evolution.

A humanist education should also help children to realize their capacities and to develop into well-integrated personalities. For this, besides the integration of subjects, we need an integration of activities. The educational system must set itself, not merely to by-pass frustration and overcome apathy, but also to provide opportunities—for active living, for satisfying achievement, for feeling significant.

This again will not be easy. Apart from difficulties of staff and facilities, and of linking school life, home life and social life, there is the difficulty of time. Once more, however, it not only can but must be done.

Here again, unification can lead to mutual reinforcement. So-called extra-curricular activities can be made to serve curricular ends, and vice versa. Children have a natural desire to achieve, to exercise their capacities, to count for something in life. If they find that their education helps them in this instead of treating them as temporary inmates to be dealt with by a combination of bribes and penalties, they will become willing participants.

Then the educator must be reminded that the mind-bodies he has to deal with are extremely diverse—they exhibit a high degree of variance, both genetic and non-genetic.

Unfortunately, ideological warfare between the environmentalists and the hereditarians is still rampant in the social sciences. The biologist must point out that their dispute is as silly as that between Bigendians and Little-endians in *Gulliver's Travels*. Neither environment nor heredity is the more important. Both are necessary: all characters of all organisms are the result of their joint effect—though the share of either in the combined operation may differ greatly. Thus the share of genetic determination is high in mammalian hair- and eye-colour, in human colour-blindness and various forms of mental defect, while that of environmental modification is high in size of flowering plants, or health and physique in human beings.

If a diversified population is made genetically uniform, as when man makes pure strains of dogs or wheat, more and more of its visible variation will be modificational, due to differences in environment. Conversely, if its environment is made uniform, as when an unselected strain of plants is grown in controlled conditions, more and more of its visible variation will be genetic in origin.

A surprising recent modificational change in man is the trend towards earlier maturity, which has been going on in Western countries for more than a century. The onset of puberty has been advanced by some four months every decade, from above seventeen in 1850 to about thirteen to-day. This is correlated with an acceleration of growth: during every decade of the past seventy years, Western adolescents have become bigger by about an inch in height and four pounds in weight. Whatever its cause, this trend has had various interesting consequences. Boys' schools are faced with a shortage of trebles for their choirs; and an average boy of fifteen is quite likely to be bigger than his woman teacher, and is fairly certain to have stronger sexual urges: while the average girl of fifteen is now psycho-physiologically a woman. The adolescent crisis has been shifted to an earlier age: our educational systems must adjust themselves to this fact.

Then there are various kinds of genetic difference for education to cope with. There are large quantitative differences in general intelligence, and also in the rate of mental development, notably between rapid developers and slow maturers: clearly the educational system must take account of such facts.

There are also qualitative differences in what I may call the psychometabolism of children—the way their minds handle the raw materials of experience, notably between visualizers, auditory types, concrete manipulators, and abstract verbalizers. The traditional verbal type of education is not very good for visualizers or manipulators.

There are genetic differences in creativity and in the capacity for absorbing and regurgitating academic knowledge: the two are not by any means always strongly correlated—indeed, a recent American study has come out with the suggestion that the U.S. educational system may actually be eliminating some of the best creative talent.

Moreover, there are psychosomatic differences, of body-build and temperament. We cannot expect that a skinny ectomorph will react to the educational process in the same way as a comfortable endomorph with double the ratio of guts to muscle and a radically different pattern of hormones in his bloodstream.

In this field, as elsewhere, the abnormal may shed light on the normal. Let me give two examples. Myopia—severe short-sightedness—is a genetic abnormality found in more than one per cent of children. It was probably of selective value during those stages of man's history when fine craftsmanship was needed and craftsmen were kept out of the fighting; but in the modern world it can be a severe handicap. However, the modern world has found a way to circumvent the genetic handicap, by correcting the defect with spectacles.

My second example concerns schizophrenia. This disordered condition of mind, which all too often leads to certifiable insanity, affects nearly one in every ten of all human beings. It almost certainly has a genetic basis and is due to a metabolic error which produces an abnormal chemical compound related to adrenaline. This interferes with the working of the brain's mechanisms for building up the chaos of our sensations into an orderly system of perceptions.

The schizoprone child begins to manifest overt schizophrenia when the disorderliness of his perceptual world throws him thoroughly out of gear with the ordered 'normal' world of his fellows. This usually happens during the adolescent crisis. Psychiatrists are busy devising chemical tests for the schizophrenia-provoking compound in the blood and urine and psychological tests for its effects on perception. These could be carried out in school: and then the schizoprones could be given treatment to correct their defect. This possibility, of helping to lift some of the heavy burden of schizophrenia from the shoulders of humanity, is a notable challenge to the educational profession.

The general challenge of human diversity remains. Man is the most variable of all organisms—anatomically and physiologically, intellectually and temperamentally. Furthermore, a large degree of diversity is a source of strength to human societies, especially to high civilizations.

It would be a good thing if the numbers of the too abnormal and the defective could be reduced, those of the more intelligent and more gifted increased; and perhaps one day eugenics will get busy on this. Meanwhile, the problem is how to utilize the existing diversity to the best advantage.

Prof. Roger Williams has proposed "Free but Unequal" as the motto of a modern society. Freedom in inequality is a good basis for an educational system to work on: but as a goal for it to work towards, I would suggest "Varied Excellence".

Educationists must struggle against conformism and resist the imposition of all dogmatisms. They will remember that cultural and individual diversity is precious in itself, and will strive against monotonous mediocrity. They will try to ensure that the more gifted children are not bored and frustrated by being kept back, the less-gifted not made to suffer by being pushed beyond their capacities. They will hold fast to the humanist vision of variety in unity.

The increasing complexity of modern societies demands an increasing number of men and women of great ability to run them. To meet this demand, applied genetics and education must join hands. We need a comprehensive selection system to catch as many potential geniuses and top people as possible, and an educational system designed to help them realize their capacities fully.

In doing justice to human variety, educationists will be accused of encouraging an *élite*, and of promoting new class differences. That must be faced. Nature is not egalitarian; societies must always be stratified in some way; and, whether you call them an *élite* or anything else, outstanding people are needed at the top.

A proper understanding of the developmental process is important both for educators and for their political masters. Development must be studied as a natural process, not in the abstract or scholastic terms of some intrinsic vital principle or ideal purpose, nor in the reductionist terms of elements and origins. In regard to human (and animal) behaviour, the reductionists are still very vocal; orthodox psychoanalysts spend most of their time searching for origins, over-zealous behaviourists proclaim that mind is merely an epiphenomenal resultant of matter, the Skinnerian school asserts that nothing but learning is of much importance in behaviour.

We must beware of all such 'nothing-buttery'. Whenever anybody states that something is 'nothing but' something else, or is explicable 'merely' in terms of its origins, we can be sure he is wrong. In opposition to 'reductionism' I suggest 'eductionism' as a rallying cry to constructive thought in this field. It means that in considering any developmental process, we must begin with its operative function—how its end-result works. Then we can analyse it into its elementary components and search for its origins. Finally, we must examine the process itself to find out how it educes its results out of its original components.

Human development is not a mere unfolding of a miniature model; the developing human being passes through a series of radically different stages, each of them demanding different educational treatment. Yet many educational systems have insisted on treating the child as a miniature man, and others as so much blank or plastic material, to be written on or moulded at the educator's will.

The development of all organisms is a cybernetic process full of feed-back mechanisms, and produces both complexity and emergent novelty.

We are beginning to explore the mechanisms by which the genetic code inscribed in the chromosomes of the fertilized egg is translated into bodily and mental organization. An important one is what Waddington calls the canalization of development into a number of channels, each leading towards a specific kind of end-result, while any departure from their 'right' or normal course is automatically corrected.

Some more plastic canalization systems may permit the organism to wander off in various directions over the developmental landscape. This applies especially to the behaviour-systems of higher vertebrates, whose learning capacities permit them to adjust their activities to their experience.

The canalization system concerned with human behaviour is so plastic that it not only provides for an extensive wandering in later stages of development but also can be markedly influenced at its roots and in the main trunk of its growth.

It has to integrate competing and even conflicting drives and desires into some sort of wholeness. Integration can be prevented by depriving the child of some basic ingredient for proper development, as Bowlby has shown with maternal deprivation. It can be traumatically distorted by internal conflict culminating in repression, which then leads to the projection of one's own repressed aggression outwards on to others, or inwards on to oneself. An excess load of infantile guilt may produce anything from a cruel super-ego and a harsh morality to an inferiority complex and an over-meticulous conscience. A load of sexual guilt at adolescence coupled with failure to achieve social adjustment may lead to amoral and asocial indifference, to retreat into neurotic depression, or to assertion of the ego's significance by violent and antisocial actions: meaninglessness is the parent of delinquency.

Clearly educational systems cannot supply psychoanalytic treatment, nor take over from parents the job of looking after babies. But they could do much to correct infantile distortions, to by-pass adolescent frustrations, and to replace meaninglessness with significance. They could do so by providing varied opportunities for fulfilment and satisfaction, especially through activities which make boys and girls feel that they mean something, to themselves and other people.

In general, educators should try to provide children with effective systems of canalizing their own development: but how to achieve this is a matter for much research and experiment. Thus, group-projects can obviously help in giving a sense of significance; but we want to know what sort of project and what type and size of group gives what degree of significance to what kinds of boys and girls.

Meanwhile, ethology is giving a useful lead, by investigating the formation of behavioural bonds in animals. In higher vertebrates such bonds involve emotions: so they do in ourselves, but in ourselves they can be reinforced by empathy. They include the parent–offspring bond, the pair-bond tying mated birds together, the gregarious bond in bird flocks or caribou herds, the social bond in wolf-packs or rookeries.

Some important points are emerging. First, all behavioural bonds have a genetic built-in basis, but only develop to full effectiveness through some sort of learning by experience. Thus, the basis of the offspring–parent bond in monkeys is merely the experience of something soft and furry: but to make it into an operationally effective bond between a mother and her offspring, a long further chain of reciprocal experiences is needed.

Again, close and intimate relations between individuals often involve elements of hostility and fear as well as of attraction. Thus, in bird courtship, the attitudes of the mates as they approach each other are the resultant of a mixture of drives—attraction, attack, escape. These attitudes have then been seized on by natural selection and turned into formal displays serving as moves in the reproductive game. Or to take an example from a mammal which (like man) is both social and fierce, if a younger and weaker wolf is in danger during a fight, he crouches and presents the back of his head to his more powerful enemy; and this automatically inhibits the enemy's aggressive urges. The basis for this valuable bit of behaviour is built in to the genetical equipment of wolves.

All such behaviour has been largely ritualized—turned into formal activities of functional value; in the process, the element of aggression is made to serve some useful purpose, whether by re-directing it against other objects or by utilizing its energy in the performance of the bonding ceremony. Rituals are thus methods for transforming frustrating conflict situations into biologically significant activities. Clearly, something similar could be achieved in education.

All primitive societies cope with the adolescent crisis by elaborate *rites de passage*, laden with significance. Can our over-intellectual and over-technological societies not devise something similar? Could not one age-group undertake a series of challenging individual exploits, another embark on group adventures and projects, and yet another to engage in some sort of community service? Could not the end of school life be celebrated in a more significant way than now? Could not the passage to adult life be linked with the larger community of the nation and the world in something which would really deserve the title of national, or even international, service? The beginnings are there, in organizations like the Peace Corps and the Youth Conservation Corps, in exploration societies and social study-projects. We should try to link them all up into some flexible and imaginative scheme which would give outlets for many types, would provide some sense of direction to many chaotic lives, and would become an important part of the educational system.

In this, as in other ways, education could be made a more integral process, and the split between its two functions of intellectual instruction and professional training on one hand, and moral education and character-formation on the other, which began two thousand years ago with the rise of the Sophists in Greece, could be repaired and bridged.

Education still has an unfortunately low status in our competitive technological world. Yet it has a crucial role in helping the technological world through its present crisis. To-day, the overall evolutionary process, in the person of post-Darwinian man, is at last becoming conscious of itself; it is time that the educational process, in the person of the educational profession, should become conscious of itself as the essential psychosocial organ for transmitting and transforming human culture.

A first step might be the establishment of an Education Council, on a par with the Arts Council, the Medical and Agricultural Research Councils and the Department of Scientific and Industrial Research. The overriding question for such a body to consider would be this: Howe an education help in bringing coherence, significance, and direction into our fragmented, bewildered and frustrated world? It could do so in two interdependent ways: one primarily concerned with knowledge and the handling of outer experience, the other with personality and the handling of inner experience.

An obvious task is to hammer out the details of a general curriculum which would reflect the unitary vision provided by modern science and learning, and would by-pass the split between the so-called 'two cultures'; its very unity would give it a new dynamism, with sciences and humanities reinforcing each other instead of being driven into mutual hostility.

I believe that it is perfectly possible to give everybody a general understanding of what science and history and literature and industry are about, by the age of fifteen or sixteen, though it would be much easier if secondary education were extended to seventeen or eighteen: and the first year of college or university life should supplement necessary specialization with some reasonably advanced study of complementary fields.

One sub-group might study examination systems, with particular reference to methods of testing understanding as well as knowledge, and assessing creativity as well as academic performance; while another might look into ways of preventing the growth of anti-rational ideas and superstitions.

The second major study would be concerned with inner development—how to promote the growth of integrated personalities and so by-pass the split between intellectual and moral education. For this, the educational process must be designed to help children resolve their conflicts, and must provide them with opportunities not only for enjoyment but for fulfilment of many kinds.

Such a study will be difficult. It will need help from a whole range of 'ologies'—anthropology, ethology, psychology, physiology, to mention only a few. It will involve facing the problem of evil and what theologians call original sin, and reconciling some violently opposed points of view. But it could lead to new conceptions of the functions of education and a revolutionary overhaul of its methods. It could also enable education to affirm its importance as a transformer of society as well as a transmitter of culture; for the products of such a personally fulfilling education would undoubtedly press for the establishment of a Fulfilment Society.

Then there is the exciting territory of non-verbal education to be explored. There is the problem of education's link with the arts: the educational system is being called on to supply large numbers of scientists and technicians; should it be required to furnish a similar quota of artists, architects, decorators and designers? There is the question of giving schoolmasters (and, of course, schoolmistresses) opportunities for research and creative work. There is the tricky problem of tests—intelligence tests, open-ended tests, imagination tests, personality tests. There is the problem of how far meditation could replace or supplement formal religious services.

Then there is the world problem. The world has become one *de facto*: it must achieve some unification of thought if it is to avoid disaster, and this can only come about through more and better education. Yet more than half the world's total population is still illiterate, and its provision for education at all levels is grossly inadequate. The under-developed countries are all demanding more and better education. Britain, as an ex-colonialist country and the senior partner in the Commonwealth, has a special responsibility for meeting these demands.

In the background there looms the great question-mark of so-called adult education. With the combination of more automation, more compulsory leisure and greater spending power, we shall soon have to extend our educational system to meet the continuing needs of the entire adult population: perhaps we should envisage a National Education Service.

Obviously, our university departments of education will be required to enlarge their functions, and will become the academic agencies of the educational system. By fully establishing the claims of education to equal rank with other subjects in the university curriculum, both as a field of research and as a course of study, as well as training for a job or a career, they could do much to improve its public image.

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It is a strange and rather disturbing fact that in the one-volume abridgement of Toynbee's monumental *Study of History* there is only one short section on education. If the educational profession is successful in meeting the challenge of the new knowledge and the new vision which it reveals, later histories of mankind will not only devote much more attention to education as a major social function but will also single out our age as the historical moment when education was reorganized as an integral part of the psychosocial process, and became pre-eminent among all the agencies concerned with human destiny.

BIOSYNTHESIS OF PROTEIN-VITAMIN CONCENTRATES FROM PETROLEUM

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IN 1957, a microbiological research organization was founded at Lavera with the aim of studying the possibility of using micro-organisms for the treatment of petroleum fractions. These investigations soon showed that a large variety of microbes were able to live and reproduce themselves actively at the expense of hydrocarbon mixtures, not only in laboratory cultures but also at the bottom of storage tanks, in refinery settling beds, in soils saturated with oil and even under bituminous road surfacings. These micro-organisms have a high protein content.

On May 22, 1959, at a meeting of l'Association Nationale de la Recherche Technique, which was held for the purpose of suggesting broad themes of fundamental research to the Comité Consultatif de la Recherche Scientifique, one of the present authors (A. C.) proposed "Microbiology with Respect to the Conversion of Petroleum into Proteins"[1].

The microbiological research of the Société Française BP was directed to this problem and for two years the laboratory at Lavera has been producing small quantities of protein concentrates analogous to animal proteins and rich in growth vitamins. It has been found that the petroleum fractions used as a substrate for the cultivation of the chosen micro-organisms are extensively dewaxed by these micro-organisms. There are thus new possibilities from the point of view of the petroleum refiner, in addition to the production of protein-vitamin concentrates in place of waxes since wax is sometimes undesirable because of its tendency to crystallize out in cold weather. The transformation of the paraffinic hydrocarbons metabolized into protein-vitamin concentrates may reach a 100 per cent yield as a result of the fixation of atmospheric oxygen by the hydrocarbons/from biosyntheses which elaborate cellular material. Nitrogen, phosphorus, potassium, and trace elements, all of which are indispensable, are supplied by fertilizers and by low-priced chemical products in an aqueous nutritive medium.

Proteins are present in appreciable amounts in cereals such as wheat, maize, rice, etc., which are the basic and often the only foodstuffs for the majority of the world's populations, either because of poverty or through ignorance of nutritional requirements. But the amount of amino-acids essential for life (that is, those which the human organism is incapable of synthesizing) which these proteins contain is very low. Only in animal proteins are these amino-acids found in sufficient quantity, and in the requisite proportions. However, it has been found that the majority of micro-organisms are capable of performing this same synthesis, with a product distribution approaching that found in animal proteins.

Table 1 gives the composition of a typical protein-vitamin concentrate prepared at Lavera and examined at length. Table 2 gives details of the distribution of the essential amino-acids present in this concentrate and, for comparative purposes, in various basic foodstuffs. Table 3 gives the vitamins contained in the concentrate, in comparison with those of various foodstuffs.

Although the carbohydrates and the fats present are also of interest nutritionally, it is the essential proteins and the water-soluble vitamins which give the new foodstuff its value.

Table 2 shows first of all that the wheat proteins are gravely deficient in lysine. This amino-acid is found in requisite proportion only in animal proteins: meat, fish,

Table 1. BP Protein-vitamin Concentrate

	Analysis (per cent by wt.)
Moisture	7·03
Dry matter	92·97
Total nitrogen	6·92
Proteins	43·6
Lipids	18·5
Carbohydrates (starch)	21·9
Ash	4·43
Calcium	0·211
Phosphorus	1·250
Potassium	0·500
Sodium	0·060

Table 2. COMPOSITION OF PROTEINS FROM DIFFERENT SOURCES (DRY BASIS)

	Wheat flour[1]	Beef[1]	Cow's milk[1]	Dry *Torula* yeast[8]	BP protein-vitamin concentrate
Protein (dry basis) (per cent)	13·2	50·4	33·1	44·4	43·6
Essential amino-acids (per cent wt.)					
Leucine	7·0	8·0	11·0	7·6	7·0
Isoleucine	4·2	6·0	7·8	5·5	3·05
Valine	4·1	5·5	7·05	6·0	8·40
Threonine	2·7	5 0	4·7	5·4	9 10
Methionine	1·5	3·2	3·2	0·8	1·20
Cystine	1·9	1·2	1·0	1 0	0·10
Lysine	1·9	10 0	8·7	6·8	11·6
Arginine	4·2	7·7	4·2	4·1	8 0
Histidine	2·2	3·3	2·6	1·7	8·10
Phenyl-alanine	5 5	5·0	5·5	3·9	7·90
Tryptophan	0 8	1·4	1·5	1·6	1·17

Table 3. WATER-SOLUBLE VITAMINS

	Thiamine B1	Riboflavine B2	Nicotinic acid PP	Pantothenic acid	Pyridoxine B6	Cobalamine B12
Function	Carbohydrate metabolism	Oxidative dehydrogenation	Hydrogen transfer	Acetylation synthesis of fatty acids	Transamination	Purine synthesis
Daily requirement for adults (mg)	2	3	15	3	2	0·01
Effect of deficiency	Beriberi neuritis	Arrested growth	Pellagra	Arrested growth	Dermatitis	Pernicious anæmia
Foods						
Beef	1–3	2	40–100	7–21	1–4	
Beef liver	5–10	16	75–275	30–60	5	8
Milk	0 3–0·7	1–8	1–5	1–4	1–3	
Cereals	0·5–7	1·0–1·5	10–30	5–20	3–6	
Oil cake	7–14	3–10	10–250	12–50		
Dry yeast	2–20*	30–60	200–500	80–200	40–50	
BP protein vitamin concentrate	8–16*	75	180–200	150–192	23	0·11

Quantities of vitamins are expressed in mg/kg: they were taken from refs. 1, 4, 5.

* Dependent on conditions of drying the final product.

milk, and also in yeasts. "Lysine in particular is the limiting factor for the growth of animals. Cereal proteins are lamentably poor in lysine"[1] with the exception of soya. Thus a cereal without any supplementary material is not a good foodstuff for cattle[1], or for human beings. Addition of lysine to cereal foodstuffs is now normal practice[5]. The proteins of the new concentrate are related to animal proteins and more closely to those of yeast, the nutritive quality of which is already well established. The concentrate is, in fact, produced by a variety of yeast which has adapted itself to petroleum.

The composition of the protein-vitamin concentrate shows that it can supply the essential proteins and the growth vitamins at one and the same time to mixed foodstuffs. Its richness in riboflavine (vitamin B2) and in pantothenic acid gives it an exceptional value from this point of view.

Nutritional tests are in progress on populations of rats and chickens. The growth of these animals is being compared with that of control animals which are receiving conventional protein rations.

The daily human requirements for animal protein are 30 g for adults, 70–100 g for pregnant women, nursing mothers, and children[6]. In order to satisfy these requirements in full, it would be necessary to make up an annual world deficit in animal protein of the order of 3 million tons[7]. This would correspond to 15 million tons of lean meat. It is not very likely that the cultivation of new land followed by the raising of selected strains of cattle could realize this production within a period of (say) 40 years. In 40 years time, however, the present world population of 3 thousand million may well have doubled.

From a ton of normal paraffins, which do not need to be separated from the petroleum fraction which contains them, it should be possible to obtain a ton of the protein-vitamin concentrate containing 45–50 per cent protein. At the same time, the corresponding petroleum fractions are dewaxed and in certain cases their value is increased.

For an annual production of 1,000 million tons of crude petroleum, it may be assumed that 700 million tons are paraffinic. The production of 7 million tons of protein-vitamin concentrate (equivalent to 3 million tons of proteins) would consume only 1 per cent of these 700 million tons of crude. An ample supply of raw material is therefore available. The time required for the construction of petroleum fermentation units would be about 1–2 years.

The rate of production of proteins by the new process may be compared with that for proteins produced naturally in the conventional manner: one 500-kg cow suitably fed by grazing synthesized 0·5 kg protein in 24 h[8]. 500 kg of living micro-organisms in a continuous fermentation unit, suitably fed with hydrocarbons, nitrogen, phosphorus, potassium and air, should produce, according to our experience, 2,500 kg micro-organisms in 24 h, which may be 1,250 kg protein. Industrial production may thus be 2,500 times as fast as the natural method.

We thank Dr. Jacques Senez of the Centre National de la Recherche Scientifique for his contribution to the fundamentals of this work.

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NOTIONS OF NICHE AND COMPETITION AMONG ANIMALS, WITH SPECIAL REFERENCE TO FRESHWATER FISH

By DR. A. H. WEATHERLEY
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CONCRETE definitions will be offered here of the terms 'niche' and 'competition'. Both notions have been fruitful 'operational concepts' in ecology for many years; but for lasting usefulness their scope should be delineated more clearly, even though this will inevitably be a somewhat arbitrary undertaking. The terms may then continue to serve, since their more felicitous application will become possible, even if their use is more limited, because more strictly prescribed.

Elton[1], probably the first to popularize the use of the term 'niche' in animal ecology, certainly appears preeminent in applying it mainly to an animal's nutritional status in its ecosystem. He also included, in the meaning of the niche, the animal's relations to other animals, and its general space occupancy. It has long been common to use the word in some very broad sense, and in my view it is this comprehensiveness or inclusiveness of meaning that threatens the usefulness of the term because it reduces its precision of application. Thus, other authors[2–4] besides Elton have considered the niche mainly as the complex of environmental conditions under which the organism lives—conditions among which it may, indeed, be obliged to live through having become adapted to them—and the manner in which it lives among them. It is thought that by thus living under these 'niche' conditions the organism may continue to exploit the environment in various ways that permit the survival of it and its progeny.

Dice[5] departed from a formal point of view so far that he failed to restrict, or specify with any adequacy, the meaning he attached to the niche. However, he did claim that "The term does not include, except indirectly, any consideration of the function the species serves in the community", whereas, as Odum[3] points out, Elton pioneered the use of the "term 'niche' in the sense of the functional status of an organism in its community".

Sometimes the meaning of niche has been cast in such broad terms as to make it almost useless to the ecologist. As Bodenheimer[4] wrote: "It is erroneous to expand it to a meaning synonymous with ecoworld. . .". After all, this is virtually to make niche substitute for ecology itself, or anyway a large part of ecology.

It is proposed that if the niche be retained as a valid concept in ecology its value as a tag or label be borne in mind. Elton[1] used the term to label an animal's characteristic role in the ecosystem, especially with respect to the foods it ate and the importance of this for other organisms of the ecosystem. Elton pointed out that in ecosystems widely separated from each other—a continent, even a hemisphere, apart—remarkable parallels in niche occupancy could occur among animal forms that were widely different in the systematic sense. He wrote that there is a "rabbit niche . . . filled by rabbits and hares in the palaearctic region and in North America, by the agouti and viscacha in South America, by wallabies in Australia and by . . . the hyrax, the springbok, and the mouse deer in Africa". A conception of the niche as powerful as this provides excellent means for deriving profitable abstractions about ecosystems. For example, in comparative surveys ecologists are often faced with a difficulty of finding common underlying facts or principles that will indicate presence or absence of essential similarities between different ecosystems. The amount of parallelism or diversity between the niches occupied in the different ecosystems would go far towards revealing basic similarity or divergence of ecological constitution. If restricted to an organism's feeding relations the niche concept will be of most value, as it is on a common fabric of available food that all patterns of animal activities are worked out. Modern studies on the ecology of production can benefit if the niches of the various organisms of the ecosystem are well known. Furthermore, the completeness with which two or more species occupy the same niche is of great importance in evaluating competition between them.

The following definition of the niche is proposed: The niche is the nutritional role of the animal in its ecosystem, that is, its relations to all the foods available to it.

Since we are concerned especially with the relevance of the niche for freshwater fish, it is important to show that at least some of them actually occupy niches.

Das and Moitra[6,7] described alimentary systems of various tropical species from the Uttar Pradesh, India. There were many omnivores, and also strongly carnivorous and herbivorous species. The findings are briefly set out as follows:

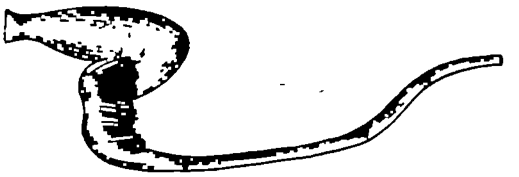
(*a*) Herbivorous fish lack teeth, but possess fine gill-rakers that can sieve microscopic plants from the water; they lack a true stomach, but have a long, thin-walled intestine.

(*b*) Carnivorous fish have teeth well developed to seize, hold and tear; gill-rakers are modified to grasp, retain, rasp and crush prey; there is a true, flask-like stomach for trituration and storage of food, and the intestine is short and elastic with thick walls.

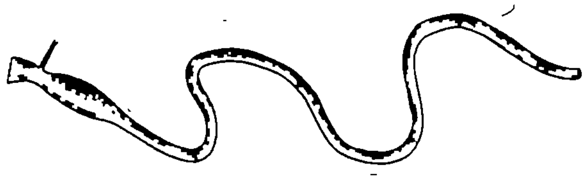
(*c*) Omnivorous fish have alimentary systems more or less intermediate in form between the herbivorous and carnivorous extremes.

These authors also found that, relative to body-length, the alimentary canal was much longer among herbivorous than carnivorous fish, with the omnivores again occupying an intermediate position.

Many fish species of Lake Nyasa have remarkable adaptations of mouth structure to feeding habits[15]. Perhaps most striking of these, *Genyochromis mento*, lives on scales and fin-rays of other fish, thus occupying an extremely narrow niche.



Salmo trutta



Carassius carassius

Fig. 1. Morphology of the alimentary canals of two temperate species of freshwater fish, showing adaptation to dietary habits

It is easy to find similar adaptations of the alimentary system among freshwater fish of temperate regions. Thus the brown trout is strongly carnivorous, while the crucian carp feeds heavily on algae and detritus of largely vegetable origin (Fig. 1). In these forms, as with the tropical Indian fish, morphological differences clearly reflect dietary differences.

Another sort of evidence in support of the niche can be found by examining data on biomasses of fish in many reservoirs in the mid-western United States[8]. It was found that total biomass tended to increase with number of species present in a community. This suggests that the tendency for different species to occupy different niches permits several or many species to utilize more completely the total food resources of the environment, than can one or a few species, resulting in higher total biomasses.

Notwithstanding the evidence that many freshwater fish occupy fairly definite niches, there are many others the wide, variable food habits and unspecialized alimentary systems of which practically preclude their having clear-cut niches. Several investigations have indicated[9–11] that multispecific fish communities can exist stably. Fish species in communities of this sort, though most of them are drawing on one general supply of food, may engage in sporadic and unpredictable changes of diet and can scarcely be thought of as occupying definite and discrete niches. Thus while many fish species may be firmly assigned to a niche, others cannot be so treated.

This article is concerned with competition for food among fish, and with the relations between competition and the niche. Before considering this latter topic, a definition of competition must be decided on, and evidence advanced of its existence among freshwater fish.

Recently, the conceptual confusion that surrounds competition was clearly shown[12]. Furthermore, there has long been a tendency to infuse definitions of competition with a flavour of 'conflict'. Thus Odum[3] has suggested that competition is an interaction between species "in which each population adversely affects the other in the struggle for food, nutrients, living space, or other common need". Key words here are 'adversely' and 'struggle'. Harper has been quoted[13] as proposing that "Competition is the endeavour of two (or more) animals to gain the same particular thing, or to gain the measure each wants from the supply of a thing when that supply is not sufficient for both (or all)". Apart from the highly questionable use of such words as 'endeavour' and 'wants' in the second definition, there resides in both an implication that competition will result in advantage or sufficiency for one species, with the opposite results for others. This objection can be avoided[13]: "Competition is the demand, typically at the same time, of more than one organism, for the same resources of the environment *in excess of immediate supply*". This is not a perfect definition, being somewhat marred by the ambiguousness of the final clause (italicized); but, if this is deleted, it is satisfactory in telling what competition is about, without the objectionable feature of going on to predict what its results may be. This sort of thing is a weakness in any definition, since the more inclusive a definition is made the greater will be the likelihood that exceptions to its application will arise.

The idea of deleterious effects arising from competition for food among fish must, in general, be set aside. This is mainly because fish have such flexible growth rates. The growth of fish in Nature is largely dependent on density of population. This is well shown for pond fish[14] and Beckman's work[15-17] elegantly shows the stimulus to growth of lake fish communities, of several species, that followed massive field mortalities. Recently[18] there has been clear evidence of greatly increased growth of perch in Windermere resulting from drastic lowering of population density through many years of trapping. These are just selected examples to illustrate the general relationship. They invite the inference that fish compete for food, so that intensity of competition is also dependent on density. The further inference follows that growth rate may be increased, without lowering population density, by artificially stimulating the production of organisms on which fish feed. This has, in fact, been accomplished for both pond and wild populations of fish on a number of occasions[19-21].

Andrewartha and Birch[22] emphasized the scarcity of 'direct' evidence of competition in Nature; but as with many ecological realities, the kind of interaction which can be called competition is usually recognizable mainly through its effects. It is not the less real for this.

The actual nature of competition will vary. Sometimes competitive interaction may be of that very direct kind where two animals actually struggle together over a single food item. Probably it more frequently takes the form of a behavioural inhibition that limits feeding territory. However, its precise character in a given instance is not of general significance for ecology. Fig. 2 is an attempt to show what happens to assimilation (growth, weight increment) in a fish population as numbers increase. There will be some critical low population density below which effects of competition for food will not be present, that is, the fish will be assimilating at their maximal rate for that particular set of environmental conditions. Now given a certain level of available food, the principal limitations to a fish's feeding capacity reside in the efficiency of its feeding organs (mouth, teeth, gill-rakers, alimentary canal) and in its feeding habits. The principal limitations to its assimilative capacity reside in the balance between its catabolic and anabolic physiology, which also involves the matter and energy it must consume in its search for food. At a critical level of population density varying with the species involved, fish will begin to interact significantly with each other, so that their feeding habits are interfered with, and the distance they must travel to find food will, on the average, be increased. This will result in a change in the balance between catabolism and anabolism, and assimilation will decrease, and will continue to decrease with increasing population density (that is, intensity of competition). These principles will operate whenever fish are competing for the same food, and will therefore operate at both the intra- and inter-specific levels. The most complete form of competition for food will obviously take place between species occupying much the same niche, that is, eating much the same food in the one environment.

Many modern ideas about the niche and competition stem from highly artificial laboratory experiments, such as the famous studies of Gause[23] on protozoans. Thus Odum[2] provides several examples of natural populations of organisms where the 'Gaussian' principle, that more than one species cannot continue to occupy the one niche, can be used to explain or interpret differences in distribution between species. Now the fact that in this article the niche has been defined somewhat strictly, and has a more restricted scope for application than is usual, does not permit us to beg the question implied by the investigations mentioned above. This question is, essentially: Where two (or more) species are eating exactly the same kind of food in the same ecosystem, occupying the same general space at the same time, and exploiting the same habitat conditions, will one species eventually extinguish the other(s)? Fryer[24] has, indeed, asserted that among herbivorous fish of Lake Nyasa: "Several closely allied species co-exist in the same micro-habitat and take the same food". His explanation is that: "Such co-existence is at variance with the so-called Gaussian hypothesis and seems possible because of a superabundance of the algal food on which the species feed". This is a possible interpretation; but perhaps Fryer rather overlooks the fact that the essential consequences of competition for food among fish, whether at the intra- or inter-specific level, are lowered growth rate rather than density-dependent mortality effects. Even if two fish species occupy the same general space and habitat (as Fryer believes his fish to do) eventual extinction of one of the species need not follow. If some females were impaired in their spawning through overcrowding, this might lower recruitment; there is little reason to think that the lowered population densities that would eventually result would be serious for either competing species.

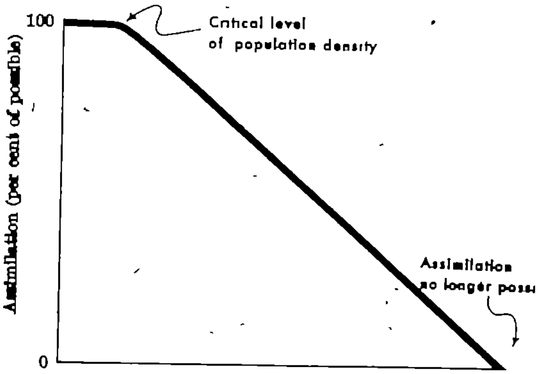


Fig. 2. Dependence of assimilation of food by freshwater fish on population density

Of course, for the multi-specific fish communities of no fixed feeding habits, which have already been mentioned, competition would probably never be more than a fleeting problem. These fish change their diets so readily and can grow satisfactorily on such a wide range of items that they are scarcely likely to suffer prolonged disadvantage from competition-induced food shortage. In addition, in these communities, through differences in behaviour, general habitat preference, activity patterns (which may or may not include seasonal migrations), oviposition sites, etc., few species indeed will be likely to occupy persistently the same space as others.

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OBITUARIES

Dr. G. H. Cunningham, C.B.E., F.R.S.

GORDON HERRIOT CUNNINGHAM, pioneer in plant pathology and taxonomic mycology in New Zealand and formerly director of the Plant Diseases Division, Department of Scientific and Industrial Research, died at Auckland on July 18, 1962.

Born at Moa Flat, a high country sheep station in Central Otago, New Zealand, he spent his childhood in the wide spaces of the tussock lands, which induced in him a lasting love of outdoor life. He received his early education at the local Dunrobin school and at Tapanui High School.

As a young man he gained wide practical experience in sheep farming, fruit growing and forestry work in both New Zealand and Australia. During the First World War he served with the New Zealand Expeditionary Forces, but, after being wounded during the Gallipoli campaign, he was invalided home.

Debarred by his injuries from rejoining the Armed Forces and being keenly interested in fruit growing, he joined the New Zealand Department of Agriculture as an orchard instructor in 1917, the year in which he met Madge Leslie McGregor, whom he married the following February. Cunningham's knowledge of, and interest in, the diseases of fruit trees so much impressed the director of his Division that he was transferred (in 1918) to the Department's Biological Laboratory at Weraroa, near Levin, to specialize in plant pathology. Realizing the need for academic training, he requested permission to attend university and moved to Wellington early in 1920.

This was the beginning of an extraordinarily active career in plant pathology and systematic mycology. During the eight years he was stationed at Wellington, while fulfilling his normal duties as plant pathologist, he passed his university entrance examinations and gained the degrees of B.Sc. (1924), M.Sc. (1925) and Ph.D. (1927). He published his first book, *Fungous Diseases of Fruit Trees in New Zealand* (1925), wrote thirty papers on plant pathology and thirty-two on taxonomic mycology, made disease surveys throughout the country and many excursions into the field to collect specimens for the establishment of a herbarium of New Zealand fungi. His taxonomic research, which included systematics and developmental studies of the Ustilaginales, Uredinales, Gasteromycetes and Polyporaceae, set a pattern for his later work within the Basidiomycetes. During the winters he visited the main fruit-growing areas to lecture to orchardists on the diseases of fruit trees. In 1924, on the recommendation of Dr. E. J. Butler, director of the Imperial Mycological Institute, London, who had visited New Zealand the previous year, he was sent to England to represent the Dominion at the first Imperial Mycological Conference.

When agricultural research was re-organized in 1928 and the Plant Research Station was formed with headquarters at Palmerston North, Cunningham was placed in charge of the Mycological Laboratory of the new institution. In the following year he attended the second Imperial Mycological Conference in London, visiting research institutions in Britain, on the Continent and in North America. This enabled him to meet most of the world's leading plant pathologists and fungous taxonomists, contacts which had a lasting influence on his work. During the eight years that he was in charge of the Mycological Laboratory he organized a team of plant pathologists to work in the fields of economic mycology, bacteriology, virology, plant physiology and plant protection. He continued his systematic mycology, and in addition to writing many taxonomic papers he published his book, *The Rust Fungi of New Zealand* (1931), for which he was awarded the degree of D.Sc. At the same time, he maintained a keen interest in plant-pathology and published articles dealing with chemicals used for the control of plant diseases and pests. This work culminated in 1935 in the publication of a book, *Plant Protection by the Aid of Therapeutants*. In the same year he was awarded the Hutton Memorial Medal for research in mycological botany.

A further re-organization was made in 1936 when plant research was transferred from the Department of Agriculture to the Department of Scientific and Industrial Research. A Plant Diseases Division, responsible for plant pathology, entomology and pomological aspects of fruit growing, was formed, Cunningham becoming its director. His first task, apart from choosing a suitable site in Auckland, was to plan buildings for the new Division. A modern laboratory block on the slopes of Mt. Albert was ready for occupation in January 1938. Although much of his time was taken up in planning and organizing during this period, he still continued his research, producing a steady flow of scientific papers and reports.

With the formation of the Plant Diseases Division, the acquisition of land and erection of laboratories, Cunningham, for the first time in his career, had adequate facilities to pursue his ideal of creating a plant protection service adequate to the Dominion's needs. He introduced, in 1938, a voluntary therapeutant certification scheme which revolutionized chemical control of diseases and pests in New Zealand. It was also through his encouragement and assistance that the New Zealand Fruitgrowers' Federation, Ltd., established a Fruit Tree Nursery at Levin, from which, for the past twenty years, orchardists have been able to secure pedigree fruit trees growing on improved rootstocks.

In spite of the difficulties in organizing a plant protection service, he still found time for his taxonomic studies and began to prepare the manuscript of a book on the Gasteromycetes of Australia and New Zealand. The development of the Division, however, suffered a severe setback with the outbreak of the Second World War. As a veteran of the First World War, he viewed sympathetically his staff's desire to join the Armed Services. As soon as permission could be secured, therefore, more than half his trained professional staff volunteered for active service. His own particular contribution was to the linen flax industry, which operated at that time in the Dominion.

With the War over and his officers returning from duty overseas, he set about rebuilding his plant protection organization. He also again took up his taxonomic studies, his book, *The Gasteromycetes of Australia and New*

Zealand, appearing in 1944. In 1948 he was awarded the Hector Medal, and in 1949 the C.B.E. In 1950 he was elected a Fellow of the Royal Society.

During the ten years before his retirement in 1957 at the age of sixty-five he devoted much time to the study of systematics. Although he died when he was sixty-nine, in those four years he prepared the manuscripts of one book on the Thelephoraceae and another on the Polyporaceae of Australia and New Zealand. The former is due to appear early in 1963, while arrangements have been made to publish the latter.

Dr. Cunningham was a man of great physical and mental energy. In his youth he earned a reputation both as a boxer and as a racing motor-cyclist, and in later life became a keen mountaineer. An indefatigable collector, until his last illness he spent much time in the field gathering material for the Plant Diseases Division Mycological Herbarium which, begun by him more than forty years ago, is now one of the finest in the southern hemisphere. His work was characterized by a remarkable perception and soundness of judgment. This is shown by the fact that his early papers in both plant pathology and systematics were, even on present-day standards, of exceptional merit. In addition to 220 scientific papers, including six books, he wrote a biography on the life of Squadron Leader McGregor—*Mac's Memoirs*. He had an absorbing interest in farming with a keen desire to assist the man on the land. A delightful companion, he will be greatly missed by his many colleagues and friends throughout the world. To science his death is a very serious loss, but his huge output of work is a monument in itself. He is survived by a wife and married daughter.

E. E. Chamberlain

Prof. E. J. King

Earl Judson King, professor of chemical pathology in the University of London at the Postgraduate Medical School, died suddenly at his home in London on October 31, at the age of sixty-one years.

Prof. King was born in Toronto in 1901, the son of the Rev. C. W. King. He was a student at Brandon College and then attended McMaster University, where he graduated with honours in chemistry and biology. He then proceeded to the master's degree in chemistry and, having been awarded a National Research Council bursary, went to work with Prof. F. B. Allan in the University of Toronto, where he obtained his Ph.D. in 1926. During the following year he was research associate in the Department of Medical Research at Toronto and then entered the Banting Institute in 1927. While here he spent two periods of leave of absence—one at the Lister Institute in London and the other at the Kaiser Wilhelm Institut in Munich. He returned to Toronto as assistant professor of medical research and director of the Biochemical Section of the Banting Institute. In 1934 he was invited to become reader in pathological chemistry and head of the Chemical Pathology Department at the newly founded Postgraduate Medical School of London. He took up these duties in 1935 and was appointed professor of chemical pathology in 1944. He was admitted to the fellowship of the Royal Institute of Chemistry in 1948 and appointed an examiner in 1952. He eventually obtained the degree of D.Sc. of the University of London.

Prof. King's two major scientific interests were concerned with research on silicosis and the phosphatase enzymes. In each of these fields he gained world-wide recognition. The work on phosphatase was commenced at the Lister Institute with Robison in 1929 and later in Toronto with A. R. Armstrong he devised the well-known method for estimating alkaline phosphatase in blood. The King–Armstrong unit has become a household name in all laboratories concerned with clinical chemistry. In this respect, it is interesting that Prof. King was the main originator of proposals to define enzyme units on a more rational basis, which would mean that the unit with which his name was associated would be abolished. The work on phosphatase could justly be regarded as the forerunner of the general use of enzymology in clinical practice as well as in clinical research.

During his period at the Postgraduate Medical School, Prof. King developed many of the micro-chemical methods now in use in clinical chemistry and did much to introduce photo-electric equipment concerned with colorimetry, flame photometry and automatic analysis. He was a constant inspiration to those who worked with him; a notable number of people who worked in his Department have achieved senior status. One of the most admirable aspects of Prof. King's character was the great trouble to which he went to help promote the interests of the junior members of his staff as well as the encouragement he gave them.

Prof. King served with distinction on a remarkably large number of committees and held many administrative appointments. He was one of the founders and the first secretary of the Canadian Biochemical Society. He was appointed sub-dean of the Postgraduate Medical School in 1944. He was secretary of the Board of Studies in Biochemistry in the University of London during 1943–46 and served as chairman from 1951 until 1955. He also served as chairman of the Committee on Clinical Chemistry of the International Union of Pure and Applied Chemistry and was president of the Biological Section. He was chairman of the International Federation of Clinical Chemistry during 1952–60 and chairman of the Central Academic Council of the British Postgraduate Medical Federation from 1952 until 1955. He was president of the British Occupational Hygiene Society (1954–55) and chairman of the Editorial Board of the *Biochemical Journal* from 1946 until 1952. In 1957 he became chairman and director of the Postgraduate Medical School's combined Departments of Bacteriology, Biophysics, Chemical Pathology, Hæmatology and Morbid Anatomy and Histology.

He played a great part in the formation of the Association of Clinical Biochemists; he was chairman during 1953–55 and president in 1956.

In addition to numerous scientific papers, Prof. King published *Chronic Pulmonary Disease in South Wales Coalminers: III—Experimental Studies* (M.R.C. special report) and was the original author of *Micro-Analysis in Medical Biochemistry* and co-editor (with R. H. S. Thompson) of *Biochemical Disorders in Human Disease*.

During the Second World War he was sector chemical pathologist in the Emergency Medical Service and consultant in pathology to the R.A.M.C. In 1945 he went to India with the rank of brigadier to act as adviser on chemical pathology.

Among numerous honours that he received were life membership of the American Association of Clinical Chemists, the Queen's Coronation Medal, honorary membership of the Canadian Physiological Society, and the Canadian Society for Clinical Chemistry. He was the recipient of a number of honorary degrees, including an honorary M.D. by both the University of Oslo and the University of Iceland. These latter were more than appropriate, since Prof. King had contributed so much to medicine.

Earl Judson King was typically colonial in his manner. He was always very forthright and direct, but, nevertheless, the kindliest of men, who made friends throughout the world. He was an extraordinarily good host and it was in this role that many obtained their first glimpse of the remarkable breadth of his culture.

Although during the last years of his life he suffered from serious illness, this was only allowed to interfere with his activities as little as possible.

In 1927 he married Hazel Keith, a former class-mate at Brandon College. He is survived by his wife and two daughters.

A. L. Latner

Dr. F. U. Klaehn

Dr. Friedrich U. Klaehn, associate professor of sylviculture at the State University College of Forestry at Syracuse University, died suddenly of a heart attack in Crouse-Irving Hospital on October 21, aged forty-six.

An international authority on forest tree genetics and efforts to improve forest tree species, Dr. Klaehn was born and educated in Germany. He was a native of Essen-Steele, Ruhr, where he was born on October 27, 1915. He was a holder of forestry and Ph.D. degrees from the University of Göttingen, Göttingen, West Germany. His two degrees were earned in 1949 and 1950, respectively. He went to Syracuse on his appointment to the College of Forestry faculty on October 1, 1955. He was a member of the faculty and research staff of the Department of Silviculture.

He is survived by his widow, the former Miss Ingrid H. Schrader, two children and his parents.

Dr. Carl R. Eklund

Dr. Carl R. Eklund, noted Antarctic explorer and chief of Polar and Arctic research for the United States Army, died suddenly on November 4, 1962, in Philadelphia, where he had been lecturing at the Museum of Natural Science. He was fifty-three years old.

A native of Tomahawk, Wisconsin, he was given the key to the city last year as an "honoured son", during its Diamond Jubilee celebration. Dr. Eklund graduated from Carleton College in Minnesota, in 1932, and began his twenty-nine years of Government service as a forestry foreman in Virginia's Shenandoah National Park. In 1936, he transferred to the Fish and Wildlife Service as a research biologist and ornithologist, serving in various locations, including Michigan, Oregon, Illinois, and Washington. He received his master's degree from Oregon State College in 1936. During the Second World War, he served as a major in the Arctic section of the Arctic Desert Tropic Information Center of the Army Air Forces and was assigned to missions in Greenland and Canada. His last post with the Fish and Wildlife Service before his resignation in 1957 was as assistant regional director in Atlanta, Georgia. During the International Geophysical year, 1957–58, Eklund served for eighteen months as scientific leader at Wilkes Station, Antarctica. His thesis for his Ph.D. degree at the University of Maryland in 1959 was based on his work at Wilkes, where he solved the mystery concerning the penguin egg's incubation temperature by 'borrowing' an egg from the Adelie penguin, implanting a tiny temperature sensor and radio transmitter, and replacing the egg in the nest. He then monitored its average temperature, which he found to be 92·7 degrees, only 11·1 degrees lower than the body temperature of the bird which was incubating them, despite the surrounding sub-zero temperatures. In 1958, on his return from the Antarctic, he was appointed chief of the Polar and Arctic Branch of the Army Research Office.

Dr. Eklund made his first trip to the South Polar continent during 1939–41 as a member of the late Rear-Admiral Richard E. Byrd's expedition, during which he and Finn Ronne made one of history's major Antarctic treks. They travelled 1,264 miles by dog-sled in 84 days, charting and mapping more than 350 miles of the Antarctic coast, and discovered a group of islands in King George VI Sound, which has since been named the Eklund Islands. He and Ronne also determined that Alexander I Land is an island, linked to the mainland only by floating ice in a trough more than three times the length of Long Island Sound.

Dr. Eklund received international recognition for his work as an explorer and scientist. Recently he had returned from Paris, where he was an official representative of the Committee on Polar Research of the United States National Academy of Sciences. He was a member of the Explorers Club and was founder and first president of the Antarctican Society in Washington, D.C.

Dr. Eklund will be missed by his many friends and colleagues. He was one of the rare men who had no enemies; he had a great sense of humour and a kind attitude to his fellow man which made him a valued companion.

He is survived by his widow, Harriet, and two daughters, Linda and Signe.

Kaare Rodahl

Mr. N. M. H. Lightfoot

Nicholas Morpeth Hutchinson Lightfoot, principal of the Chelsea College of Science and Technology, died on November 20 at the age of sixty. Lightfoot was educated at Jarrow Grammar School, Co. Durham, proceeding from there by scholarship to Jesus College, Cambridge, to read mathematics. During 1929–43 he served on the mathematics staff of the Heriot-Watt College, Edinburgh, first as lecturer and later as head of the Department. In 1943 he was appointed principal of the South-East Essex Technical College, Dagenham. He moved to Chelsea in 1950, where he was principal until his death.

At the beginning of his career he published papers on the mathematical theory of cooling steel, but later became primarily a teacher and then administrator.

During his long service as principal, first at Dagenham and later at Chelsea, he played an active part in matters concerning technical education and was elected president of the Association of Principals of Technical Institutions in 1955–56. He also served on the National Council for Technological Awards, the Parliamentary and Scientific Committee and the Regional Advisory Council.

His period as principal at Chelsea was marked by rapid developments in the College as the result of the change of status to a College of Advanced Technology, by which the old Chelsea Polytechnic became the Chelsea College of Science and Technology. This change required important decisions as to the proper direction for the future of the College. Up to the time of designation as a college of advanced technology, the College had been providing courses mainly for internal degrees of the University of London in science and pharmacy. Unlike all the other colleges of advanced technology, there was no school of engineering. It was chiefly as a result of Lightfoot's deliberations that the College continued to provide courses such as were traditional, but to develop research and postgraduate courses of an applied nature. Because of this decision, Chelsea College has a higher percentage of postgraduate work than any other college of advanced technology despite great limitations of space. In this way, Lightfoot felt that the College would play a proper part in its new capacity.

As a man, Lightfoot was quiet and retiring in his behaviour. In all that he did he was extremely conscientious with a sharp eye for detail. He came to a decision only after mature thought, but once he had made up his mind he went ahead with determination. He was a man of the highest integrity and because of this his staff knew that he could always be trusted. Likewise, he never interfered with his heads of Departments, leaving it to them, as experts in their own particular fields, to run their departments in their own way. In his unobtrusive way he has left his mark on the College and he will be sadly missed.

Lightfoot's death came tragically soon after that of his wife in December 1961, and our sympathies go out to his only daughter in losing both her parents in so short a time.

C. C. Hentschel

Mr. E. C. Stubbens

Ernest Charles Stubbens, who died on November 16 at his home in Shelford, was born in Cambridge on May 3, 1899. He began a lifetime devoted to the scientific instrument industry with an apprenticeship to the Cambridge Instrument Co., which he entered in 1913. After two years in the armed forces in the First World War, Mr. Stubbens spent four years with British Thomson-Houston Ltd. in Rugby but took an early opportunity of returning to his native city when he was appointed chief inspector with Pye Radio Ltd. in 1925.

In August 1934 Mr. Stubbens joined Unicam Instruments, the new enterprise started a few months earlier by his brothers, the late Mr. Sidney Stubbens and Mr. John Stubbens, who is the present head of the model shop at Unicam. Pioneer conditions made it possible—essential even—for "E. C. S." to show his many talents, and he served his new company in several capacities before finding his true métier when, during the Second World War, he took charge of production at the London Instrument Co., seconded to Unicam for the duration. At the end of hostilities he returned to the main Unicam factory as works manager and began the happy association with old colleagues and new friends that has now come to an end.

He held strongly to the belief that efficiency is closely related to congenial working conditions and set himself to create both, as much by his personal example as by his executive activities. His success contributed largely to the major expansion of Unicam in recent years. A lively personal interest in the welfare of his staff found expression in his work as chairman of the Company's Sports and Social Club, to which he devoted his time and energies in full measure.

Mr. Stubbens leaves a widow to whom his many friends extend their profound sympathy.

NEWS and VIEWS

Soil Mechanics at Manchester: Prof. P. W. Rowe

Dr. Peter Walter Rowe, at present reader in soil mechanics in the University of Manchester, has been granted the status of professor of soil mechanics at that University. Dr. Rowe went with a Merchant Venturer Scholarship from Bristol Grammar School to the University of Bristol, where he graduated with first class honours in civil engineering in 1942. After three years as a junior scientific officer at the Royal Aircraft Establishment, Farnborough, he joined Sir Robert McAlpine and Son, Ltd. In 1947 he was appointed lecturer in civil engineering at University College, Dundee, where he conducted research on anchored sheet pile bulkheads, for which he was awarded the degree of Ph.D. Dr. Rowe went to Manchester in 1952 as a lecturer in engineering and was promoted to a senior lectureship in 1954. He was awarded the degree of D.Sc. in 1956 and was appointed reader in soil mechanics in 1958. He has organized in Manchester short postgraduate courses in soil mechanics which have attracted senior workers from many parts of the United Kingdom and abroad. He has lectured widely in the United States and at international conferences.

Genetics at Liverpool: Prof. P. M. Sheppard

Dr. P. M. Sheppard, reader in genetics at the University of Liverpool, has been appointed to the newly established chair of genetics at that University. Dr. Sheppard, who is forty-one years of age, was educated at Marlborough College and at Worcester College, Oxford, where he graduated with honours in zoology in 1948, and was awarded the degree of Ph.D. in 1951. From 1951 until 1956 he served as a research officer in the Department of Geology and Comparative Anatomy at the University of Oxford. During 1954–55 he held a Rockefeller fellowship and worked at Columbia University, New York. He was appointed a senior lecturer in genetics at the University of Liverpool in 1956 and a reader in 1959. Dr. Sheppard has served on the Committee of the Genetical Society of Great Britain and has worked extensively in the field of natural selection and heredity. He is especially interested in the genetics of butterflies and moths.

Signals Research and Development Establishment, Christchurch, Hants: Mr. R. J. Lees

Mr. R. J. Lees has been appointed director of the Signals Research and Development Establishment at Christchurch, Hants, in succession to Mr. R. V. Whelpton. Mr. Lees, who is at present head of the Instruments and Air Photography Division of the Royal Aircraft Establishment, was born in 1917. He joined the Telecommunications Research Establishment, Malvern (now the Royal Radar Establishment), in 1939, where he remained until moving to his present post at the Royal Aircraft Establishment in 1959. While at Malvern he was largely concerned with work on guided missiles, being superintendent of the guided weapons section in 1951. In 1953 he was appointed superintendent, experimental physics, in 1955 director of scientific research (guided weapons), and in 1956 head of airborne radar at the Royal Radar Establishment.

Ministry of Aviation Science Officers in Industry

In a written answer in the House of Commons on December 10, the Minister of Aviation, Mr. J. Amery, stated that in the past ten years applications to take up posts with industrial firms had been approved from one senior principal scientific officer, one principal scientific officer, four senior scientific officers, three senior experimental officers, two chemists and nine engineers. Mr. Amery emphasized that the Department employed several thousands of scientists, engineers and technical staff of potential value to industry, and that it had been the object of successive Ministers to ensure that, while industry was not denied the services of some very able people, nor they themselves the chance to make a new career, this fruitful exchange should not be allowed to influence the placing of contracts or to lead to favours being sought or given.

Grants for Education Research in Great Britain

In a written answer in the House of Commons on December 11, the Minister of Education, Sir Edward Boyle, listed grants totalling £183,000 for the financial year 1962–63 made or approved under the Educational Services and Research Regulations, 1946. Besides £58,000 over five years to the National Foundation for Educational Research for a study of the effects of streaming in primary schools and £21,000 over three years to the same Foundation, as well as £20,000 for a four-year study of the merits of block- and day-release, these include: £20,000 to the Brunel College of Technology for a five-year study of the learning of intellectually complex material; £13,000 to the Department of Education, Sheffield, for a three-year project on programmed learning; £15,000 to the Educational Foundation for Visual Aids for the audio–visual aids experimental development unit; £10,000 to the Department of Sociology, Manchester, for a four-year study of the effects of home life on school performance; £6,000 to the Department of Education, Birmingham (in association with the Department of English, Leeds), for a three-year project of oral English tests in the Certificate of Secondary Education; £5,000 to the Department of Education, Swansea, for a three-year study of teaching educationally sub-normal children. Grants totalling £46,800 for special education services include £14,500 to the Central Bureau for Educational Visits and Exchange,

£14,000 to the National Council for Diplomas in Art and Design, £6,500 to the National Council for Technological Awards, and £4,000 to the Field Studies Council.

British Space Research in 1961–62

British activities in space research in the year ending in May, 1962, are described in the *United Kingdom Report for 1961 to the I.C.S.U. Committee on Space Research* submitted by the British National Committee on Space Research to the fifth meeting of the Committee on Space Research in Washington during April 30–May 9 (Pp. 23. London: The Royal Society, 1962). The report gives a good summary of the varied investigations in progress, which are classified under the following headings: tracking and data recovery; atmospheric structure and composition; radio propagation and the ionosphere; geomagnetism; orbital analysis; micrometeorite studies; cosmic radiation; electromagnetic radiation and astronomy. There is also a selected list of more than seventy British papers published in late 1960 or 1961 on these and related subjects. The most striking event of the year under review, however, came at the very end, with the launching of the first Anglo-American satellite, *Ariel 1*, on April 26, and the booklet includes a photograph and cutaway drawings of *Ariel*. An appendix gives details of the experiments to be flown in the second Anglo-American satellite, which is to measure galactic noise, atmospheric ozone and the flux of micrometeorites.

Uniform Time and Constant Frequency for the Year 1963

Following the decision of the International Committee of Time at Berkeley, in August 1961, and the general assembly of the Union Radio Scientifique Internationale at London, in September 1960, and the Study Group VII of the Comité Consultatif International Télégraphique in Geneva, during April–May 1962, the International Bureau of Time consulted the observatories which have caesium atomic resonators with the view of comparing their astronomical observations. It has now calculated the nominal value of the frequency to transmit during the year 1963 as -130×10^{-10} with regard to the scale of time such that the frequency of caesium has the value: $f(\mathrm{Cs}) =$ 9,192,631,770 c/s. The value indicated will not change during the course of 1963. It is the same as the 1962 value. The International Committee of Time, the Union Radio Scientifique Internationale and Study Group VII of the Comité Consultatif International Télégraphique recommend that the value of the frequency indicated by the International Bureau of Time be utilized by the organizations desiring to transmit uniform time and constant frequency.

Personnel Management

An important development in the activities of the Institute of Personnel Management as shown in its annual report for 1961–62 is the increasing attention it is now giving to overseas contacts (Pp. 28. London: Institute of Personnel Management, 1962). Among the projects the Institute will undertake during 1963 to celebrate its jubilee is the foundation of a senior lectureship in industrial relations at University College, Ibadan, in Nigeria. The Institute already has about 600 overseas members, and during the past year the Institute's officers have visited several countries including India, Cyprus and Israel. Links with European personnel managers have been strengthened during the year, these having been stimulated by Britain's possible entry into the European Economic Community. European salary structures featured at the Institute's Conference in May, and labour relations in the European Economic Community were discussed at the South-West Regional Conference in April. At the end of June, a two-day meeting of chief personnel managers from West Germany and Britain was held in London and it is proposed to hold a similar meeting in Germany in 1963. In June a meeting was called by the Organization for European Co-operation and Development to discuss the formation of a European Personnel Management Group for liaison between different institutes and associations and for the exchange of information about personnel practices in the different countries.

Careers in Biochemistry

The Biochemical Society has published a revised version of the booklet *Careers in Biochemistry*. It starts by describing what biochemists do and the various types of laboratories in which they work. There are sections on educational qualifications and professional training, on opportunities and prospects, and on salaries. A short reading list is provided. This booklet should be very helpful to boys and girls considering taking up biochemistry and to those responsible for advising them. It is obtainable, price 2*s*. post free, from the Administrative Secretary, the Biochemical Society, 20 Park Crescent, London, W.1.

National Museum of Wales

For the first time in thirty years, extensions are being built for the National Museum of Wales, and a public appeal for at least £150,000 towards the cost has been launched. The Museum at Cardiff is less than half complete, and the present collections have outgrown the space available in which to exhibit them. Congestion is especially severe at the Welsh Folk Museum at St. Fagan's, and in the Welsh Museum's School Service. The building of the new wing has been authorized by the Government and the Treasury will meet 90 per cent of the cost. It is hoped that the appeal to the public will be successful—£91,697 was received before launching it.

Museum of Applied Arts and Sciences, Sydney

With a striking original design on the cover, the annual report of the Museum of Applied Arts and Sciences, Sydney, for the year ended December 31, 1961, is a record of much activity (Pp. 24. Sydney: Museum of Applied Arts and Sciences, 1962). The increased attendances are attributed to two main factors: first, a planned advertising programme; and secondly, that during the year under review special efforts were made to attract more organized parties from secondary schools. It was also in 1961 that Royal Assent was given to an Act which altered the name of the institution from Museum of Technology and Applied Science to Museum of Applied Arts and Sciences. Re-organization of the exhibition staff has permitted a greater degree of specialization according to special qualifications and talents. This has resulted in the formation of the nucleus of a design section, charged with the task of design in general as it affects museum activities of any kind and of co-ordinating the various contributory skills, for example, artist, ticket-writer, carpenter or painter. The chief event of the year in the exhibition galleries has been the re-display of the arms and armour collection.

Associated Electrical Industries' *Electron Microscopy*

Associated Electrical Industries' first scientific film, *Electron Microscopy*, which received four awards at the second International Industrial Film Festival in Turin in 1961, has now received the Nikola Tesler gold medal. This was the premier award at the third International Festival of Scientific and Technological Films in Belgrade, Yugoslavia. *Electron Microscopy* shows the principles, construction and operation of the electron microscope and the techniques of preparing specimens. A number of uses of the machine in research and industry is demonstrated, including the application of the image intensifier. The latest scientific film to be made by Associated Electrical Industries, *Analysis by Mass*, has been selected for showing at the annual meeting of the American Association for the Advancement of Science in Philadelphia at the end of December.

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U.S. Television 'Journal' for Science and Engineering

Scientific Information Notes for October–November 1962 (4, No. 5), issued by the National Science Foundation, notes that the Foundation is supporting an experiment in the use of television as a medium for communication from scientists to scientists. The experiment is being administered by the American Association for the Advancement of Science, and, with the co-operation of several scientific societies, is being developed as a television series to function as a 'journal' of science and engineering at professional level. The Foundation is also supporting a two-year study at Lehigh University, Pennsylvania, of methods of analysing the information needs and patterns of use of scientists.

Twentieth Anniversary of the First Nuclear Reactor

THE International Atomic Energy Agency has issued a special number of its *Bulletin* to mark the twentieth anniversary of the first nuclear reactor becoming critical on December 2, 1942. An introduction by the director-general of the Agency, Dr. A. S. Eklund, is followed by articles by L. Meitner on roads to the discovery of nuclear energy; Dr. O. Hahn, on E. Fermi and uranium fission; S. K. Allison, on initiation of the chain reaction; G. T. Seaborg, on the first nuclear reactor, the production and chemical extraction of plutonium ; Sir John Cockcroft, on the early days of the Canadian and British atomic energy projects; B. Goldschmidt, on France's contribution to the discovery of the chain reaction; V. S. Emelyanov, on the first atomic reactor in the U.S.S.R.; H. DeWolf Smyth, on the publication of the Smyth Report; E. P. Wigner, on the anniversary of the first reactor; J. A. Wheeler, on "Fission Then and Now"; G. de Hevesy, on the reactor and the production of isotopes; L. Fermi, on some personal reminiscences; C. Allerdic and E. R. Tragenell, on the first atomic pile.

William Dampier

A SHORT account ot the life of Dampier as explorer, seaman and scientist has been prepared by J. C. Shipman, of the University of Kansas (University of Kansas Publications, Library Series, No. 15: *William Dampier: Seaman-Scientist*. Pp. 63. Lawrence, Kansas: University of Kansas Libraries, 1962. 1.50 dollars). Shipman describes Dampier's voyages around the world, the many plants and animals attributed to him, as well as his many observations in oceanography. The book provides a valuable supplement to the larger existing works.

Anti-knock Efficiency of Methyl*cyclo*pentadiethyl Manganese Tricarbonyl

A. Girelli and C. Orlandi have studied the knock rating of methyl*cyclo*pentadiethyl manganese tricarbonyl (1), using that compound alone or in conjunction with tetraethyl lead (2), in eight normal production oil fuels from Italian refineries, each being of different chemical composition. The results of their investigations were given at the ninth National Congress of Chemistry, held in conjunction with the Swiss Chemical Society, at Naples, during May 27–June 2, 1962, and summarized in *La Rivista dei Combustibili* (16, 9; September 1962). Their findings are significant. With straight-run, paraffin-base petrol the octane number increases more with addition of (1) than of (2), giving the same concentration of grammes of metal per litre. Much the same is found in petrols produced by 'cracking' processes: for the same metal content the octane number rises more with (1) than (2). With increase of aromatic content in the distillates, however, the effect of additive (1) becomes less progressively compared with the more commonly used tetraethyl lead. Carrying the research a stage further, the authors tested the anti-knock behaviour of both (1) and (2) in combination added to the same series of petrols. They found that the synergic effect of this procedure was greatest in the paraffin-base petrols and decreased in proportion to rise in aromatic content. The chain of chemical reactions involved in these experiments is complex, but it is believed that the behaviour of (1) in the presence of (2) in the varying types of petrol used is due to the retarded action of (2) compared with (1). This can be explained by assuming that the reactive compound (1) in the form of manganese oxide precedes the influence of the lead oxide in (2) in the course of the combustion process. It is argued that, in consequence of this preference, the role of compound (1) is such that it breaks the chain of reactions ". . . which, in the presence of (2) alone, would continue undisturbed until knocking occurs before the appearance of the active form". These results apparently are in agreement with those of other workers on this problem and would seem to establish the fact that the anti-detonating efficiency of the manganese compound in light petroleum distillates, compared with tetraethyl lead, must now be reckoned with in modern refinery projects.

Gerontology in the United States

THREE brochures reviewing different aspects of research programmes in ageing conducted and supported by the National Institutes of Health during 1961 have been issued by the U.S. Public Health Service. The publications are prepared annually by the Centre for Ageing Research. *Research Highlights in Ageing* presents a review of a selected number of scientific papers on research in ageing, ranging from fundamental studies in the biology of ageing to studies concerned with the physical, psychological and social problems of ageing people (Publication No. 921. Pp. v+29. Washington, D.C.: Government Printing Office, 1962. 20 cents). *Activities of the National Institutes of Health in the Field of Gerontology* lists research and training grants either primarily or secondarily related to ageing (Publication No. 935. Pp. viii+47. Washington, D.C.: Government Printing Office, 1962. 35 cents). In each category the grants are classified under general headings and under such special groupings as physiological and biochemical aspects in ageing, psychological aspects, social aspects and disease processes. The third brochure, *Research Programmes in Ageing*, briefly summarizes the research programmes at the National Institutes of Health in this field as well as programmes at other institutions where the National Institutes of Health grants are sponsoring research in the increasing medical, social, economic, physiological and psychological problems associated with the ageing process (Publication No. 939. Pp. v+17. Washington. D.C.: Government Printing Office, 1962. 15 cents). Single copies of the pamphlets are available from the Centre for Ageing Research, National Institutes of Health. Bethesda 14, Maryland.

Animal Health Yearbook

THE *Animal Health Yearbook* is issued jointly by the Food and Agriculture Organization, United Nations, the World Health Organization and the International Epizootics Office, Paris. The 1961 issue provides tabulated statistical information concerning farm animals, including birds, in the countries which fall within the activities of these international bodies, this being the major portion of the text (Pp. 315. Rome: Food and Agriculture Organization, 1962. 15*s*.). There are brief accounts of five important laboratories which deal with matters that concern the disease problems of these countries in so far as they have, to that extent, application to their own countries (the Republic of South Africa, the United States and Britain). In addition, there is a brief account of the operations of the Inter-African Bureau of Animal Health in Kenya. This is a publication for the senior administrative office of a veterinary service, for veterinary libraries and other organizations interested in animal health, as well as for general workers in the interests covered by its contents. There is a mass of information about present-day affairs not available elsewhere as a whole within one book. In such a compendium the accuracy of figures may vary from country to country. The figures given could not be obtained elsewhere, except by laborious inquiry or search from a number, a very large number, of sources. To provide such a mass of information within a reasonable space, a very elaborate system of notation is used, this in itself requiring to be used when gleaning the data provided. There is an elaborate and useful index. The whole of the book is printed in three languages—English, French and Spanish.

Fertilization in Farm Animals

J. L. Hancock, of the Animal Breeding Organization, Edinburgh, has reviewed fertilization in farm animals (*Animal Breeding Abstracts*, 30, 285; 1962), and dealt with some 200 references of recent years. The text covers "Physiological Aspects of Fertilization" (duration of oestrus, time of ovulation, site of fertilization, time taken for spermatozoa to reach site of fertilization, numbers of spermatozoa at site of fertilization, functional life-span of spermatozoa in the female tract, functional life-span of the ovum, penetration of ova, morphological changes preceding and following fertilization, changes in unfertilized ova, criteria of fertilization, anomalies of fertilization), "Failure of Fertilization" (failure of sperm transport, defective gametes) and "Fertilization in Hormone-treated Females" (fertilization *in vitro* after ovum transfer, selective fertilization). Hancock emphasizes that basic information on the physiology of fertilization in animals is derived largely from studies of small laboratory animals.

Courses in Research and Development

The Comparative Administration Trust is organizing practical courses on "Administration and Management of Research and Development" in collaboration with Brooklands Technical College, Weybridge (January 14–February 8), Gonville and Caius College, Cambridge (July 14–August 9), Queen's College, Oxford (September 1–28), and Sundridge Park Management Centre, Bromley (November 10–December 7). The courses will be open to those with science or engineering degrees or equivalent qualifications, and are aimed at improving the practical effectiveness of those engaged in administration and management of technical work in industry, in industrial research associations or publicly sponsored research establishments. Forms of application and further information can be obtained from the Comparative Administration Trust, 30 St. George Street, Hanover Square, London, W.1.

Nato Advanced Study Institute on Palaeoclimates

An Advanced Study Institute on Palaeoclimates will be held under the auspices of the Northern Atlantic Treaty Organization's Science Office at King's College, Newcastle upon Tyne, during January 7–12. The programme has been divided into eleven sessions dealing with: the palaeoclimatic significance of coal; Cretaceous and Tertiary palaeobotany; palaeontology and palaeoecology; palaeontology; glaciation; Eo-Cambrian glaciation and Devonian climate; deserts and palaeowind aspects; evaporites; Permian climate; soils; meteorological aspects. The director of the Institute is Prof. S. K. Runcorn, head of the Physics Department, King's College. Further information can be obtained from the organizing secretary, Dr. A. E. M. Nairn, Nato Advanced Study Institute on Palaeoclimates, King's College, Newcastle upon Tyne.

The British Society of Acoustic Technology

Under the directions of committee of representatives from universities, local government, industry and the Royal College of Advanced Technology at Salford, and headed by Dr. L. H. A. Pilkington, of Messrs. Pilkingtno Bros., St. Helens, a Society of Acoustic Technology is to be formed. A programme has been prepared which includes both lectures and visits; topics which the Society will consider in the first half-session (beginning in January) include airport noise, psychological problems caused by excessive or particularly penetrating noise, measurement of noise-levels and the design of lightweight partitions intended to reduce noise. The inaugural meeting of the Society will be held on January 17 at the Royal College of Advanced Technology, Salford, when Prof. E. J. Richards, of the University of Southampton, will give an address on "Future Problems in Noise Abatement". The joint honorary secretaries of the Society are Dr. P. Lord, of the Department of Pure and Applied Physics, Royal College of Advanced Technology, Salford, and Dr. H. D. Parbrook, of the Sub-department of Acoustics, University of Liverpool. Further information concerning the inaugural meeting can be obtained from Dr. Lord.

Announcements

Sir John Wolfenden, vice-chancellor of the University of Reading, has been appointed to succeed Sir Keith Murray as chairman of the University Grants Committee. Sir Keith Murray will relinquish the post on September 30, by which time he will have served for ten years.

Dr. R. Franklyn Morris, of the Federal Department of Forestry, Fredericton, New Brunswick, has been awarded the first Gold Medal of the Entomological Society of Canada for his work in the field of insect population dynamics. Dr. Morris's work has provided a scientific basis for attempting the control of budworm.

M. S. Moss has been appointed to take charge of the Animal Health Trust's forensic laboratory at the Equine Research Station, Newmarket. Mr. Moss has been a member of the Metropolitan Police Laboratory, New Scotland Yard, where he has investigated the techniques of extraction and identification of drugs. The forensic laboratory at the Equine Research Station has been established to implement some of the recommendations contained in the report of the committee, under the chairmanship of the Duke of Norfolk, which inquired into the question of 'doping' of racehorses.

The British Association for Commercial and Industrial Education is organizing a special conference to consider the White Paper (Cmnd. 1892) *Industrial Training: Government Proposals*, which will be held at the Grand Council Chamber, Federation of British Industries, 21 Tothill Street, London, S.W.1, on January 15. Further information can be obtained from the Conference Secretary, BACIE, 26*a* Buckingham Palace Road, London. S.W.1.

A meeting of the Systematics Association on "The Place of Taxonomy in the Teaching of Biology" will be held in the Department of Botany, University of Birmingham, on January 26. Further information can be obtained from the Honorary Botanical Secretary, the Systematics Association, c/o British Museum (Natural History), Cromwell Road, London, S.W.7.

"The Role of Chemical Engineering in the Space Age" is the title of a lecture to be given by Prof. Ju Chin Chu, professor of chemical engineering at the Polytechnic Institute of Brooklyn, at the Geological Society, Burlington House, London, W.1, on January 28. The lecture is being organized by the Institute of Chemical Engineers, and further information can be obtained from the General Secretary of the Institute, 16 Belgrave Square, London, S.W.1.

GOVERNMENT SUPPORT FOR UNIVERSITIES AND COLLEGES IN GREAT BRITAIN

FOLLOWING last year's precedent, the University Grants Committee again prefaces its annual returns from universities and colleges in Great Britain in receipt of Treasury grants for the academic year 1960–61[*], now covering 22 universities and 3 colleges, with an introduction which in effect amounts to an interim report. This note refers to the establishment of an *ad hoc* advisory panel to deal with proposals for the development in the universities of studies in teaching English as a second or foreign language. Four universities have already initiated such studies and other universities have put forward proposals, but the rate of expansion is severely limited by the shortage of trained teachers and for the first years of the next quinquennium resources will be concentrated in the four universities where the studies are already established. Financial provision of £10,000 in 1962–63 rising to £70,000 in 1966–67 has been earmarked for these developments.

The University Grants Committee has also earmarked a sum of £560,000 in 1962–63, rising to £640,000 in 1966–67, for support of research projects from which the Research Councils are withdrawing support at the end of the current quinquennium but which the universities consider important enough to be given priority with, or immediately after, existing commitments ahead of any new developments. Following acceptance of the recommendations of the Hayter Committee on the further development of Oriental, Slavonic, East European and African Studies, involving expenditure rising from £120,000 in 1962–63 to £290,000 in 1966–67, an interim scheme for the award of special postgraduate scholarships to commence in October 1962 has already been adopted. Discussions are already proceeding with the universities on the further allocation of these funds and nine centres for area studies have already been selected. Fourthly, the Committee has earmarked special funds, rising from £185,000 in 1962–63 to £750,000 in 1966–67, for the development of dental schools, both for teaching and research. This will permit the intake of dental students being raised, as requested by the Government, from 620 to 940 a year.

The University Grants Committee is also discussing with the universities the meeting of two other national needs brought to its notice. First, the Government requested the Committee to consider the possibility of increasing the intake of the medical schools by 10 per cent above the level recommended by the Willink Committee and in the initial discussions it was assumed that the recurrent grants for 1962–67 would allow for a reasonable development of the medical schools. Following the Government's rejection in March of the Committee's recommendations for recurrent grants during that quinquennium, further advice was submitted to the Government in July. The new recommendations were accepted by the Government, and, in addition to capital grants of about £100,000, recurrent grants for this purpose, rising from £135,000 in 1962–63 to £245,000 in 1966–67, will be provided.

The second matter was raised by the Foreign Office late in the year and relates to the further development of Latin-American studies, and a committee, with Dr. J. H. Parry as chairman, has been set up to review developments in the universities in the field of Latin-American studies and to consider and advise on proposals for future developments. The University Grants Committee also notes that, while the proposals originally put forward by the universities provided for the 500 additional places in University Departments of Education requested by the Ministry of Education for the next quinquennium, the funds made available under the Government's announcement last March fall short of the universities' and the Committee's estimates of their needs. While the Committee makes no direct comment on this decision, it does not conceal its concern at the probable consequences of the decision on standards of teaching and research in the universities or on the ability of the universities to provide the number of student places desired by the Government. There will clearly be little money available for other developments during the quinquennium and, small as are the earmarked grants already mentioned, against this background the risk of distortion is greater.

Assessment of the universities' recurrent financial needs during the coming quinquennium was indeed the Committee's main concern during the year. In this connexion it points out that net expenditure on existing commitments is bound to increase, particularly in respect of salaries and building programmes, and it concludes that to meet existing commitments the universities as a whole would need a 3·5 per cent increase in grants over 1961–62 in 1962–63 and 15·9 per cent in 1966–67. The Committee's proposals contemplate provision of places for 151,000 students by the end of the quinquennium, an increase of 36 per cent over 1961. The calculations on which the Committee's report to the Chancellor of the Exchequer on the 1962–65 building programme was based showed that if standards of admission were not raised, by 1966–67 at least, 180,000 if not 200,000 places might be required for those academically qualified. Of the 30 per cent increase to 144,500 students (compared with 111,100 in 1961–62) about two-thirds will take place in the pure and applied sciences. The respective percentages are: pure science, 41·7; applied science, 60·4; medicine, 8·4; dentistry, 35·2; agriculture and forestry, 20·9; veterinary science, 13·7.

For the rest, this general survey notes the establishment of a small *ad hoc* group to consider in principle and advise on the advantages which can be secured from a proposed grouping of postgraduate institutes and hospitals and their joint use of facilities. Furthermore, in view of the National Coal Board's intention to reduce considerably its demand for graduates in coal-mining and to concentrate its assistance to university mining departments on five instead of nine, the University Grants Committee is participating in relevant discussions to effect this concentration without hardship to academic staff or students concerned. The Board wishes to concentrate its help in the mining departments of King's College, Newcastle upon Tyne, the University College of South Wales and Monmouthshire, the University of Nottingham, either the University of Leeds or the University of Sheffield, and in one centre in Scotland. The question of academic salaries is discussed at some length (this and other financial matters are considered on p. 1 of this issue of *Nature*).

The number of full-time students in British universities increased to 107,699, compared with 104,009 in 1959–60, and of these 17,925 were in the Universities of Oxford and Cambridge, 21,995 in the University of London, 42,819 in other English universities, 6,431 in the University of Wales, and 18,529 in Scottish universities. There were 11,608 students from overseas, 6,932 of whom were from within the Commonwealth, and 5,242 of whom were postgraduate. Of the full-time students 27·4 per cent were in colleges or halls of residence, 50·7 per cent in lodgings and 21·9 per cent at home; these figures compare with 28·1, 40·9 and 31·0 per cent, respectively, in 1953–54; these proportions varied widely in different universities and for men and women. In Scotland only 9·6 per cent of men and 20·0 per cent of women were in colleges or halls of residence, while 50·7 per cent of men and 45·4 per cent of women were at home.

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[*] University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grants, Academic Year 1960–61. (Cmnd. 1855.) Pp. xxiv+48. (London: H.M. Stationery Office, 1962.) 5s. 6d. net.

Of full-time men students 25·4 per cent were in arts and 11·5 per cent in social studies; 25·5 per cent in science, 19·5 per cent in technology and 11·5 per cent in medicine; for women the corresponding figures are: 52·7; 9·4; 21·7; 1·3; and 11·2. Of those that were 'full-time', and apart from students engaged in research or other advanced work, 27,702 were in arts, 10,115 in social studies, 21,395 in pure science, 13,597 in technology and 11,199 in medicine. Among the 17,836 advanced students, 3,551 (2,193 men) were taking postgraduate courses in teacher training; 3,326 of the others were in arts, 1,692 in social studies, 5,058 in pure science, 2,628 in technology, and 1,077 in medicine. Of full-time students 84,999 were reading for a first degree, 3,958 for a first diploma and 908 attending courses not leading to a degree or diploma; the proportion of men (75·5 per cent) and women (24·5 per cent) was again little changed from 1938–39 (76·7 and 23·3 per cent, respectively). Of the 29,510 full-time students admitted for the first time in 1960–61, 32·7 per cent were in arts, 12·0 per cent in social studies, 26·4 per cent in pure science, 15·1 per cent in technology and 8·8 per cent in medicine, and over the past four years increases in pure science and technology have been about twice as great as in arts.

The proportion of assisted students was 82·4 per cent, as in 1959–60, which compared with 71·9 per cent in 1953–54, ranging from 92·6 per cent in Wales, 89·2 in English universities excluding those at Oxford, Cambridge and London, to 72·8 per cent for Scotland. There were 17,831 part-time students, 4,860 from overseas, and of these 13,478 were in English and 4,132 in Scottish institutions; 6,234 were engaged in research or other advanced work. Full-time teaching and research staff, excluding Oxford and Cambridge, increased to 12,105 (11,474 in 1959–60) and 25·7 per cent are now in science, 14·8 per cent in technology and 17·4 per cent in medicine. The percentage of professors was 12·0, readers and independent lecturers 6·2, senior lecturers 12·3, and lecturers 47·7. Including Oxford and Cambridge the grand total was 12,417.

Of the recurrent increase of £68,706,388 (£59,800,092 in 1959–60), £48,399,076 (70·4 per cent) was from Treasury grants, and £1,224,802 from grants from Government departments; £6,317,603 from fees (9·2 per cent), £1,963,347 (2·9 per cent) from endowments, £1,577,564 (2·3 per cent) from local authority grants, £506,806 from donations and subscriptions, and £6,215,400 (9·1 per cent) from payments for research. It is expected that the increase in students' fees will provide a further £1·3 million or 1·7 per cent of total income in 1962–63. For English, Welsh and Scottish Institutions the totals are £55,506,730, £3,437,275 and £9,762,383, respectively. Non-recurrent Treasury grants amounted to £22,199,128, an increase of £2,346,381 over the previous year, and of this £14,576,504 was spent on building work, £2,212,152 on professional fees, £4,123,907 on furniture and equipment and £1,286,565 on site and property purchases. Of the recurrent expenditure of £67,822,988—an increase of £9,317,916 on 1959–60—6·7 per cent was spent on administration, 44·1 per cent on salaries and superannuation of teaching staff, 11·2 per cent on departmental wages, 14·1 per cent on departmental and laboratory maintenance, 3·3 per cent on repairs and maintenance of buildings and 9·8 per cent on rates, insurance, heat, light, etc. Expenditure on libraries, at £2,624,625, fell slightly to 3·9 per cent of the total.

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1962, includes the Minister's Statement, the report of the Council and the Secretary's report, as well as reports from the various Divisions of the Department, the incorporated Research Associations and on grant-aided research in the Cawthron Institute, Lincoln College, Massey College and the Industrial Development Department of the University of Canterbury*. Lists of publications during the year are appended to these reports and to the whole is appended the fourth annual report of the Ross Dependency Research Committee. The latter records reconnaissance geological and mapping surveys of 27,000 square miles in the Beardmore Glacier region, a unique study of temperature gradients in Lake Vanda in the Wright valley, and a new study of penguins, seals and marine biological problems in the McMurdo Sound area.

The Council's report records the adoption by the Government of the Council's proposals for changes in the basis of support by State Departments of research in the universities. Under these, State Departments will sponsor and provide funds for research units in the universities in fields of study deemed essential in the national interest and in which an outstanding man is available in the university, enter into research contracts with the universities for specific research projects, and provide studentships in fields of science of special interest to the department concerned. Meanwhile, the Department of Scientific and Industrial Research will provide funds for the establishment in 1962 of research units in agricultural economics at Lincoln College and in molecular biology at the University of Auckland. The Council has also considered strengthening the Auckland Industrial Development Laboratories and re-equipping the workshops of the Dominion Physical Laboratory and is negotiating with the University of Canterbury on the future work of its Industrial Development Department.

Of the total vote of £2,192,000 in 1961–62, £225,000 was spent on grants to other organizations. The total compares with an average of £1,895,000 over the three years 1958–61, which represented 0·149 per cent of the gross national product, compared with 0·134 per cent in 1955–58 and 0·154 per cent in 1949–52. The total staff on March 31, 1961, of 1,148, including 422 scientific officers, increased to 1,170 by the end of the year, including 442 scientific officers. The Council comments on the inadequacy of the amount of capital available for renewal and purchase of scientific equipment and on the demand for research into an ever-increasing number of fields. Grants to research associations in 1961–62 totalled £147,850, and to Canterbury and Massey Agricultural Colleges and the Industrial Development Department, University of Canterbury, £24,149, while the Commonwealth Agricultural Bureaux received £18,680 and the Cawthron Institute £12,400.

Commenting on the economic value of the Department's research work, the Secretary in his report refers to the importance of the work done on the control of bloat in dairy cattle for which a cheap and efficient method has been found by spraying peanut oil and tallow on the pasture. The discovery by the Entomology Division that small amounts of DDT give effective control of the grass grub has resulted in it being kept well under control in most parts of the country; as a pest, the grass grub has previously cost New Zealand some £6 million a year. Damage done by the white butterfly and the diamond black moth has been reduced to a low level by the introduction of their natural predators, and a safe and effective hormone–fungicide spray has been developed for control of grey mould or botrytis on tomatoes, while acceptable methods of spray application of diphenylamine have been developed which are expected to reduce scald of apples in storage to negligible proportions. Savings in thinning costs of Statesman apples, estimated at £20,000 a year, have been obtained by the introduction of hormone sprays, and the short-rotation rye-grass developed by the Grasslands Division is now valued at £150,000 a year in seed alone, while Savoy cabbage strains bred by the Crop Research Division are yielding about 2·5 tons per acre more than the best commercial strains and are superior in uniformity and time of maturity. Between 1948 and 1960 the yield of tobacco leaf per acre was raised from 789 to 1,856 lb. with a simultaneous improvement in quality, and the study of curing conditions in the kiln has led to the use of a down-draught kiln which gives more uniform curing, reduces fuel consumption by one-third and drying time by 24 h.

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* New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1962. Pp. 87. (Wellington: Government Printer, 1962.) (H.34.)

The Secretary's report also mentions the Soil Bureau's discovery of the importance of molybdenum as well as fluorine in connexion with dental caries, which could have a profound effect on the condition of children's teeth. Of the present research work, the report notes that the study of foraminifera is well advanced in New Zealand and that the Geological Survey's palynologists were called on to examine fossil pollen samples from coal-bearing strata penetrated by the drills. Field trials have shown that the high-temperature Geiger tubes developed within the Institute of Nuclear Sciences can operate for long periods in the geothermal bores at Wairakei and that the counters can detect small differences in the radioactivity of the rocks and aid the correlation of the geological strata from hole to hole. A design procedure that permits an estimate of the safety of any proposed building and its occupants if subjected to earthquake has now been developed to an intermediate stage and results of the study of a number of standard buildings have been issued in a handbook. A method has been devised to assist the diagnosis of heart defects consisting of an abnormal connexion between the chambers of the right and left sides of the heart in which a sample of dye is rapidly injected into the blood stream at a selected site and afterwards detected downstream from the injection. A detailed study of clay fractions from different horizons of 54 soils has been made with the electron microscope and by chemical and mineralogical analysis. Reported sulphur deficiencies in New Zealand soils appear to be confined to soils with a very low absorbed sulphate content and a low absorption capacity, so that added sulphate is not retained in competition with phosphate, and when the two anions are added together, as in superphosphate, the sulphate is rapidly leached from the soil. Work on oestrogenic substances in red and subterranean clover has confirmed that the activity of red clover can affect the reproductivity of grazing cows and has provided a basis for investigating the heritability of oestrogens in clover progenies. Bitter pit in Cox's Orange and Golden Delicious apples has been reduced by calcium nitrate sprays.

NOMENCLATURE OF ISOLATES OF VIRUS FROM TRACHOMA AND INCLUSION BLENNORRHEA

At an informal meeting following the New York Academy of Sciences symposium on "Biology of the Trachoma Agent" in May 1961, an *ad hoc* committee was formed to try to establish a uniform system of nomenclature for trachoma virus isolations. The various institutions represented by the committee members are: Prof. J. H. S. Gear, the South African Institute for Medical Research, Johannesburg; Dr. F. B. Gordon, Naval Medical Research Institute, Bethesda 14, Maryland; B. R. Jones, Institute of Ophthalmology, London, W.C.1; Dr. S. D. Bell, jun., Harvard University School of Public Health, Boston 15, Massachusetts (chairman of the committee).

At the eighth International Congress of Microbiology, held during August 1962, the committee presented the views of the majority of persons interested enough to communicate their thoughts on the subject. The final recommendations which were voted by those present at Montreal followed the system proposed by Dr. Leslie Collier in almost all respects The elements are as follows:

(*a*) (*b*) (*c*) (*d*) (*e*)
TRIC/antigenic group/country or State/number/origin

(*a*) The term *TRIC* refers to the trachoma-inclusion conjunctivitis group of viruses.

(*b*) This space would be left blank or can be filled in when this information is available.

(*c*) The country or state from which this specimen was obtained is to be designated by the internationally recognized automobile plate letters, with such further geographic designation as is necessary for clarity.

(*d*) If this system can be administered by a central agency such as the World Health Organization, sequential numbering following the country of origin is favoured. If no central agency is available, it was thought best to insert a laboratory designation before the number.

(*e*) The origin of the clinical material would be inserted here as follows with the *O* or *G* optionally followed by a *T*, *C* or *N*: *O* = from the eye, *G* = from the genital tract, *T* = diagnosis of trachoma, *C* = diagnosis of conjunctivitis, *N* = diagnosis of ophthalmia neonatorum.

The full designation of the *G*–1 strain[1] therefore would be: *TRIC*/ /*WAG*/*MRC*–1/*OT*. *WAG* = West Africa Gambia, *MRC* = Medical Research Council.

Similarly the nomenclature for the *SA*–2 strain[2] assuming acceptance of the immunological groups proposed at present would be: *TRIC*/2/*SAU*/*HAR*–2/*OT*, *SAU* = Saudi Arabia, *HAR* = Harvard, and the strain of inclusion conjunctivitis[3] now called *IC*–*Cal*–1 would be: *TRIC*/ /*USA*–*Cal*/*Cal*–1/*ON*.

Whether or not strains already isolated should be incorporated into this system is left to the individual laboratories concerned, but the *ad hoc* committee feels that in the interests of uniformity and ease of communication, it is desirable that all new isolates should be officially designated by the above system as from January 1, 1963, onward.

J. H. S. GEAR
F. B. GORDON
B. R. JONES
S. D. BELL, JUN.

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[1] Collier, L. H., and Sowa, J., *Lancet*, 1, 993 (1958).

[2] Bell, jun., S. D., Snyder, J. C., and Murray, E. S., *Science*, 130, 626 (1959).

[3] Hanna, L., Zichosch, J., Jawetz, E., Vaughn, jun., D. G., and Thygeson, P., *Science*, [illegible], 1660 (1960).

INFORMATION IN BIOLOGY

An international colloquium on information in biology was recently held at Royaumont, near Paris. The aim of the organizing committee was to bring together workers interested in various fields of biology in which problems of information are faced—genetics, biochemistry, immunology, embryology, neurophysiology. Certain sessions were devoted to reports covering a broad field, followed by a general discussion; others were seminars with brief reports and original contributions on a limited subject. The discussions used to continue around the luncheon table, in the cloister or in the gardens of the Abbey. The value of such a meeting resides as much in these conversations, in improvised discussions and remarks, as in the prepared reports and contributions themselves.

M. Delbrück was chairman of the seminar on genetic information which occupied the first half of the colloquium. It is only one year since Nirenberg reported the specific stimulation by synthetic polynucleotides of amino-acid incorporation into protein material and an almost complete amino-acid code has already been proposed. Nirenberg and Lengyel both reported experiments which led to assigning one or more nucleotide triplets to each amino-acid; there is good agreement between most results of the two laboratories. The genetic code, however, is not yet completely solved; many questions and some difficulties remain. M. Bretscher directed the attention to two sources of error: in many of the experiments from which the present tentative code was derived the composition of the synthetic polynucleotide was assumed to be the same as that of the mixture of nucleoside diphosphates used to make them. According to M. Bretscher and M. Grunberg-Manago, this assumption is not warranted, and in many cases therefore the calculated frequencies of triplets on which the code rests might be wrong. The assumption that phenylalanine is coded by *UUU* exclusively is also uncertain and the triplet assignments rest on this assumption. Nirenberg reported that most of the polynucleotide added to the *E. coli* preparation is destroyed within a few minutes; a small part only, which is bound to the ribosomes, remains and acts as the template. It is not certain whether all the polynucleotides have equal chances of being bound. The presence of *U* in all the proposed coding units is not what one would expect, for no known nucleic acid is as rich in *U* or *T* as would be a template corresponding to the proposed code. According to the Cambridge–Paris group, poly-*A*–*C*, free of *U*, does stimulate the incorporation of three amino-acids, indicating the existence of codons (a word proposed by Crick for coding sequences) which do not contain any uracil. The proper interaction of polynucleotides with the ribosomes may depend on the solubility or the secondary structure, and *U*-containing polynucleotides might have an advantage over the others in this respect when assayed in the Nirenberg system.

The present code is but a first approximation: after these discussions, one would even doubt its significance, if it were not for the striking agreement between the code as it stands and many of the replacements of amino-acids observed in mutants. That Nirenberg's system can indeed make defined proteins when a natural RNA is provided to it, is shown by experiments from Fraenkel-Conrat's laboratory. Isolated tobacco mosaic virus RNA added to Nirenberg's system stimulates the incorporation of amino-acids into protein material which can be precipitated with an antivirus serum. A fraction of this material closely resembles the coat protein of tobacco mosaic virus; by hydrolysis, it gives apparently the same peptides. This fraction, however, is not perfect coat protein, for it does not aggregate into rods as does the real tobacco mosaic virus protein, possibly because the acetylation of the NH_2 end of the polypeptide fails to occur in *E. coli* extracts.

Incidentally, tobacco mosaic virus RNA causes the synthesis of other polypeptides besides that which resembles the coat protein, for the RNA stimulates the incorporation into protein material of two amino-acids which are not found in the coat protein. It is indeed most probable that tobacco mosaic virus RNA, like phage DNA, also codes for enzymes required for virus multiplication, and not only for the protein sheath of the virus. The previously established fact that many tobacco mosaic virus mutants have no amino-acid replacements in their coat protein already indicates that only part of the RNA molecule codes for the virus protein and that the rest of the RNA nevertheless is important for the process of virus infection.

Wittmann reported on further investigations concerning the amino-acid replacements obtained in nitrite mutants of tobacco mosaic virus. A given amino-acid is replaced by a very restricted choice of other amino-acids only, indicating that the number of meaningful substitutions within a codon is small. Since the replacement of an amino-acid does not lead to the replacement of its neighbours, the coding units are largely independent—the code is probably non-overlapping: but it is degenerate, for part of the amino-acids are coded by more than one triplet. If one assumes that de-amination results in the replacement of *C* by *U* and of *A* by *G* (*H* pairs like *G* and is ultimately replaced by *G*), the 64 possible triplets can be ranged in 8 groups of filiation; within each group, each triplet is related to three others by a one-step mutation obtainable by de-amination.

It is striking that all the transitions so far observed fall into three of the eight possible filiation groups. Unless some restriction to the observable transitions is imposed by factors irrelevant to the code, Wittmann's results suggest that the degeneracy of the code might obey certain rules. An interesting proposal for a logically degenerate code was presented by Woese on theoretical grounds. It is assumed that the three bases in each codon do not carry the same amount of information. In one of the positions, the four bases are recognized as different, but in the other two positions, only the nature of the group (NH_2 or OH), either in position 2 or in position 6 of the heterocyclic ring, is read. This is a degenerate code since in the member which codes according to its position 6, *U* is equivalent to *G* and *C* to *A*, and in the member which codes for ring positions 2, *C* is equivalent to *U*. Such a code would account for the wide differences in DNA composition of different micro-organisms without much change in protein composition, and also for the high *U* content of the codons derived from Nirenberg's system, since in one of the three members of the triplet *U* would have the same meaning as *G* and in another member *U* would be equivalent to *C*. Woese's code fits rather well with the coding assignments of the *E. coli* system, and with existing replacement data. In an excellent discussion, F. Crick analysed the present state of the coding problem, concluding that the genetic code is probably universal, partly degenerate, that there is little, if any, overlapping of the codons, and that the codons may indeed be made of three nucleotides.

The information coded in the template is read by transfer RNA. This is clearly proved by works done by F. Chapeville in F. Lipmann's group: cysteine bound to its specific transfer RNA was chemically changed into either alanine or cysteic acid and the resulting product assayed for its transfer properties in a *E. coli* system programmed by poly-*UG* which normally stimulates cysteine incorporation. The modified amino-acids when bound to cystein transfer RNA behaved like cysteine, showing that the positioning of an amino-acid on the template does not depend on the amino-acid itself, and that it is determined exclusively by the properties of the transfer RNA to which it is bound. By fractionating transfer RNA's from *E. coli*, Sueoka showed the existence of more than one transfer RNA for certain amino-acids, for example, two each for Leu, Ser, Try, Asp, Glu, three for Ile. Moreover, results from S. Benzer's laboratory show that the two separable transfer RNA's specific for Leu must carry different reading sequences since one of them transfers Leu to *UC* polymers only, the other one to *UG*, not to *UC*; therefore, the two transfer RNA's which read leucine are not interchangeable. The concept of degeneracy will have to be reconsidered in the light of such experiments: the code is degenerate in this sense that one amino-acid can be coded by more than one codon, but if these different codons are nevertheless recognized as different by the reading system, the code is not degenerate in the same sense as Woese's code. Plurality, rather than degeneracy, is indicated by these results. It will be interesting to know whether the different leucine residues of the same protein are coded by the same word or whether certain positions are coded in one language and others in the other.

Certain mutants can be restored to an almost wild phenotype by mutations occurring far from the structural gene in which the original mutation occurred. Genetic studies on these 'suppressor' mutations provide extremely interesting data on the reading mechanism. An example was presented by A. Garen, who studied mutants of the alkaline phosphatase cistron, which do not produce any cross-reacting material, and therefore possibly do not produce any protein in place of the phosphatase in the locus of which mutations occurred. It is assumed that the mutation in the phosphatase locus changes a codon into a nonsense triplet, with the result of blocking the synthesis of the phosphatase polypeptide. The synthesis of active enzyme with normal immunological properties can be restored by a 'suppressor' mutation. This second mutation probably changes the structure of a transfer RNA in such a way that this RNA now reads the nonsense triplet as if it was a good codon. The function of the normal transfer RNA which was changed by the mutation cannot be lost, otherwise the mutation would be lethal; on the other hand, the change in reading specificity should not cause too much damage to the other proteins; there are several escapes to these difficulties. A misreading, anyway, is better than no reading at all, and this might justify the existence of several codons for the same amino-acid: the more there are of meaningful codons, the smaller are the chances for a mutation to produce a nonsense triplet (cf. Benzer).

As an introduction to the next section of the discussion, F. Jacob briefly outlined the problem of transfer of information from the gene to the machine which makes proteins; he summarized his theory of information transfer and regulation and emphasized the support it receives from current works on short-lived RNA's and on the Nirenberg system. Jacob then raised a few questions which introduced the next papers: Do rapidly labelled RNA's carry genetic information? What are the biochemical and physical properties of messenger RNA? At what level does regulation operate?

Rapidly labelled RNA now appears very heterogeneous in size. It is quite reasonable to assume that large messengers carry the message for long polypeptides. By combining chromatography and density gradient centrifugation, R. Monier was able to resolve rapidly labelled RNA into several components. Certain fractions have a sedimentation constant as high as $30S$; this corresponds to rather big RNA molecules, larger than those of ribosomal RNA, and large enough to code for several proteins. One wonders whether messages for several proteins can be delivered as a unit to the ribosomal machine, whether some messenger RNA's contain the information of a complete operon. F. Gros showed that the activity of various RNA fractions when assayed in Nirenberg's system depends essentially on their content in rapidly labelled RNA. Free ribosomal RNA is inert; it is not even attached to ribosomes. Another question is whether each molecule of messenger RNA is able to direct the synthesis of only one copy of a polypeptide or whether it is used over and over again. The differential effects of inhibitors on the synthesis of proteins and of messenger RNA in bacteria indicate that each molecule of messenger RNA can function several times, perhaps 10 or 20 times before losing its activity.

Evidence that the different genes of T_2 bacteriophage send their messages at different times after infection was presented by two independent groups (R. Hessin and S. Spiegelman). Short pulses of tracer were given at various times after infection, the labelled complementary RNA was isolated by hybridization with phage DNA; the RNA's which are produced at various times after infection do not compete for the DNA, indicating that they correspond to different regions of the DNA molecule.

Another investigation by S. Spiegelman concerns the fate of the infecting RNA of a small RNA bacteriophage. Phage RNA binds to the $80S$ particles and stays there for a long time without being degraded. The association between this RNA and the ribosomes is very stable even at low magnesium concentration. This is in sharp contrast with what happens when the same virus RNA and ribosomes are confronted *in vitro*; association depends on high magnesium concentration, a large part of the RNA is destroyed during incubation and lowering the magnesium concentration results both in the separation of virus RNA from ribosomes and in the dissociation of the ribosomes themselves. Spiegelman also reported that this phage RNA causes the labelling of two proteins in the *E. coli* system. One of them is the coat protein; the function of the other one is not known.

The series of lectures on information in protein synthesis was closed by a report by M. Beljanski. In a soluble preparation from *A. faecalis* amino-acids bind to RNA's isolated from ribosomes; the binding depends on the presence of nucleoside triphosphates; analogues of purines or pyrimidines interfere with this fixation. Under certain conditions, rather large free peptides are formed.

M. Cohn very clearly summarized basic facts of immunological response, the intricate problems they raise and the current theories, of which he considered only the genetic models. The specificity is assumed to reside in the genetic material, the antigen regulating the production of a specific antibody or selecting the antibody producing system among the many existing potentialities. M. Cohn then summarized present knowledge on the structure of γ-globulin as deduced from immunological, chemical and genetic studies. The structural basis of antibody activity was discussed by R. Porter; his approach may answer the fundamental question as to whether the specificity of antibodies rests on differences in amino-acid sequence. Rabbit γ-globulins appear to be made of two identical *A* chains, flanked by two smaller *B* chains. Papain splits the antibody into three fragments: one of them, which was crystallized, is made of a rather large piece of the double *A* chain; it does not carry specific combining sites. The other two are probably identical to each other, each containing one of the two combining sites; they are formed of a *B* chain associated with the remnant of an *A* chain. The *B* chain was isolated; by itself it does not possess binding specificity. The fragment of *A* associated with *B* could not be studied easily because of a tendency to aggregate at neutral *p*H but such activity as survived the separation was always in the *A* chain. Comparable studies by G. Edelman and B. Benacerraf on guinea pig antibodies indicate differences in electrophoretic mobility between the *B* chains of different antibodies; it would seem that *B* may take part in the specificity although it does not bind antigen when separated from *A*. The combining site appears to be in the *A* chain remnant, but it may be formed by the association of *B* with part of *A*.

The amount of work presented and discussed at Royaumont was so great that when the programme was half way through, it was realized that there was not enough time left for the second half. It was agreed that the discussion on information in embryology would be replaced by two brief talks meant to state the problems and present perspectives. Thus C. H. Waddington considered essential problems of embryonic differentiation in terms of the mechanisms which control the synthesis of specific proteins. Experiments on nuclear transplantation and other less-direct evidence indicate that differentiation does not necessarily depend on permanent genetic changes; differentiation must involve switching on or off whole batteries of genes. Obvious questions occur: how are genes co-ordinated? How rigid is this co-ordination? What is the nature of 'genotropic substances', that is, of cytoplasmic substances which must control the activity of the genes? For if the cytoplasm in bacterial systems appears ancillary in regard of the all-mighty genome, embryologists view the cells and embryos as a much more involved system in which the cytoplasm exerts a very strong control on the genome.

Reasons why embryologists give a great importance to the cytoplasm were also illustrated by J. Brachet, who summarized fundamental facts about the early determinism of morphogenesis during cleavage. During this period of development, DNA replicates whereas protein and RNA synthesis are non-existent. Ribosomes isolated from eggs at this stage nevertheless incorporate amino-acids *in vitro*; the inactivity of ribosomes *in vivo* at that stage thus raises a clear problem of regulation. Hybrids between different species offer a means of studying the specific interactions between a genome and a foreign cytoplasm. Thus hybrids between frog and toad show a normal cleavage but are blocked precisely at the stage when specific protein synthesis normally begins. RNA metabolism in many nuclei of such hybrids is disturbed.

After this glimpse over challenging problems facing the molecular biologist of to-morrow, the symposium turned to considerations on enzyme structure and function, which were introduced by a brief report by R. Wurmser. The information content of an enzyme can be looked at from different points of view. Considering only the specificity of enzymes toward substrates, one obtains a small amount of information; but if one considers the actual support of enzyme activity, the protein itself, and calculates the amount of information from the negentropy of such a structure as estimated from the possible permutations of the amino-acids, figures of the order of several thousand bits are obtained. The reports and discussions which followed showed indeed that many structural features of the protein are important for enzymatic activity.

B. Keil raised the question as to whether the arrangements of amino-acids in protein chains obey certain rules. Common themes in amino-acid vicinity are obviously connected with function: thus the presence of aspartic acid close to the reactive serine is a common feature of esterases. Analogies of sequences between enzymes of similar function can also be recognized in the rest of the molecule, although this requires much more faith or enthusiasm. The notion of an active centre comprised of a few chemical groups is too narrow and altogether inadequate; the catalytic properties of an enzyme indeed depend on the rest of the protein as well —they can be altered by subtle changes occurring in other parts of the protein. Activation of trypsinogen and of chymotrypsinogen are good examples. Here the active centre is created by a transition of the secondary and tertiary structures made possible by the rupture of one peptide bond. P. Desnuelle gave another example: lipases which are active on insoluble substrates only, as if adsorption at the interface would cause a structural change necessary for enzymatic activity to appear. J. Yon discussed factors from which the activity of trypsin may depend. A fragment of the trypsin molecule containing less than one-third of the polypeptide chain can be isolated by pepsin treatment. It is enzymatically active, but its specificity is not as restricted as that of intact trypsin; it can hydrolyse substrates of chymotrypsin, beside those of trypsin. On the other hand, the specificity of trypsin becomes narrower in the presence of copper ions: activity on lysyl linkages is increased, whereas hydrolysis of arginyl linkages is inhibited.

Another example was given by F. Labeyrie. Mild treatments may change the properties of a yeast lactic dehydrogenase: the enzyme which has been crystallized does not have the same affinity for a competitive inhibitor, D-lactate, as the native enzyme. A very striking type of enzyme modification was discussed by G. Tomkins. A glutamic dehydrogenase with a molecular weight in the range of one million can dissociate under various conditions into four molecules which have lost the ability of oxydizing glutamic acid, but which now possess all the properties of an alanine dehydrogenase. The type of chemical reaction catalysed is the same; but the substrate specificity of the enzyme depends on its state of aggregation. Extracts of HeLa cells contain alanine dehydrogenase, but no glutamic dehydrogenase. Addition of ADP causes the aggregation of alanine dehydrogenase into glutamic dehydrogenase. ADP in this case is not a metabolite, it acts physically, causing association; it is a kind of 'configurational co-factor'. Certain steroid hormones, on the contrary, split glutamic dehydrogenase into alanine dehydrogenase. These and similar processes suggest new mechanisms for the regulation of enzyme activity.

Delicate interactions between proteins or regions of proteins are also illustrated by complex enzymes. C. Gunsalus discussed the stereospecificity of dehydrogenases involved in the metabolism of camphor, the co-operation of two enzymes in a process of lactonization and the induction specificity of these enzymes. E. Racker described the case of glyceraldehyde dehydrogenase, a double-headed enzyme which catalyses two successive reactions; the oxidation of the aldehyde by DPN with the formation of the acylenzyme, and the phosphorolysis of the acylenzyme with formation of free acylphosphate. Chymotrypsin splits the dehydrogenase releasing a smaller protein which still catalyses the first reaction, but has lost the phosphorolysis activity. Erythrose diphosphate inhibits the first reaction in the native enzyme; but it has no effect on the split enzyme; binding of this inhibitor depends on a part of the protein which does not contain the active centre. Negative feed-back, that is, the process by which the end-product of a metabolic chain inhibits the enzyme which catalyses the first step in that particular chain probably rests on effects of a comparable type. G. Cohen gave a few examples of feed-back regulatory processes, with emphasis on branched metabolic chains. The kinetics of inhibition of the controlled enzymes is not quite of the competitive type; the structure of the inhibitor is indeed very different from that of the substrate of the enzyme, hence the suggestion to name this type of process 'allosteric inhibition'. The sensitivity of enzymes to allosteric inhibition is often a very labile feature of the enzyme protein; it can be abolished without impairment of the catalytic activity of the enzyme. Beside the catalytic site, there must be an allosteric site specific for the controlling metabolite.

On the basis of these facts, J. Monod proposed a generalization according to which induction and repression of enzyme synthesis as well as feed-back inhibition of enzyme activity operate by allosteric action. A regulated enzyme has two sites: the enzymatic site and the allosteric site. Fixation of the regulating metabolite on the allosteric site slightly changes the shape of the protein and thus abolishes the activity of the enzyme. In the case of repression and induction of protein synthesis, the repressor produced under the control of the regulator gene is now assumed to be a protein (the evidence in favour of its not being a protein was not very good, and in the case of a lysogenic system there is now evidence that the repressor is probably a protein). The repressor-protein is believed to possess two sites: one of them recognizes the operator and interacts with it, thus locking the operon; the other site can bind the inducer metabolite. When the inducer metabolite is bound, the repressor protein is slightly changed, it loses affinity for the operator and the operon unlocks; a repressor metabolite, on the contrary, increases the affinity of the repressor protein for the operator. This new hypothesis affords a simple model of action of the inducing or repressing metabolite and thus removes one of the greatest difficulties of the previous models of enzyme induction. It also introduces more unity since the regulator gene is now regarded as a structural gene which specifies the repressor protein. The last day of the colloquium was devoted to a completely different field, namely, information in neurophysiology. A. Fessard gave a fascinating course on the mechanisms of brain, the nature of the messages, how they are processed and stored. He first outlined the several levels of organization: wiring diagram of neurones, neurone populations, cellular organization, synapses, membranes, macromolecular structures. He described the activity of the nerve fibre as revealed by the action potentials, and discussed how modulation of the time sequence of successive signals carry information. Depolarization, hyperpolarization and other processes occurring in the dendrite, and the role of the trigger zone in transmitting the messages were described. Then came the spatial aspect of impulse reception in the brain, the interconnexions between neurones, the filtration of signals and the increase of contrast. Great emphasis was laid on the fact that the signals arriving to the brain are not restricted to a small primary area, that they go to many other parts where signals from other types of excitations also arrive. The code in which a certain excitation is translated is assumed to be in terms of patterns.

Finally, the problem of memory was outlined. Fessard described striking experiments on short-term and long-term memory, insisted on the absence of localization of the stored information, and indicated present interpretations. When hearing this lecture after those on enzyme structure and regulation one was led inescapably to think of memory as a regulation process, and to imagine a model in terms of allosteric effects on some synaptic structure. The conclusion of Fessard's lecture was that many of the elaborate properties of the nervous system can be explained by random connexion between neurones, regulated by external actions. A similar conclusion applies to the evolution of the highly organized structure of proteins and nucleic acids. The high information content of the genes, the 'antihasard' which is so obvious in the structure of a protein like hæmoglobin or a double-headed enzyme can have their origin in known random events corrected by the purely deterministic challenge of the external medium. Teleological features of living matter are thus already accounted for in terms of causality.

The participants in this symposium are grateful to their French colleagues, especially Prof. E. Aubel, secretary of the organizing committee, and to the Délégation générale à la Recherche scientifique et technique which provided financial support. The Abbey of Royaumont, the gardens, the musical evenings, and supper by candlelight are delightful memories for those who were privileged to take part in this most successful colloquium.

H. CHANTRENNE

THE INTERNATIONAL ATOMIC ENERGY AGENCY

AN outline of the programme for 1963 of the International Atomic Energy Agency is given in the October issue of the Agency's *Bulletin* (4, No. 4, 11; 1962). A total budget of 9,562,100 dollars has been proposed, representing an increase of approximately 7·5 per cent on the budget for 1962. Estimated expenditure under the heading of the "Regular Budget", financed out of assessed contributions by member states of the Agency, amounts to 7,337,500 dollars. The remainder of the total budget is assessed under the heading of the "Operational Budget" and is financed out of voluntary contributions by member States and other resources, such as funds made available under the United Nations Expanded Programme of Technical Assistance.

The Agency's technical assistance programme is to be extended. During 1962 requests for assistance involving the services of 40 experts, and equipment worth nearly 230,000 dollars were approved. This was in addition to some 36 experts already serving during 1962 under previously sanctioned projects. During 1963 the need will arise for the services of some 90 field experts, in addition to those whose assignments continue from previous years. Requests for equipment are expected to exceed the value of 250,000 dollars.

The Agency's training programme includes the award of fellowships and research grants. The number of applications for fellowships has increased steadily, but there has not been a corresponding increase in the funds available for the purpose. Hence during 1963 more extensive use is to be made of cost-free fellowships made available by member States, but even then it is anticipated that not all deserving requests will be able to be met. For use in training on the spot the Agency provides two mobile laboratories. Approximately 750 trainees in the Far East, Latin America and Europe have used these laboratories in the past years, and it is expected that one of the laboratories will move to Africa late in 1962 or early in 1963.

A number of conferences and symposia are planned for 1963. A panel of experts is to meet to discuss the Agency's report on the results of an investigation into the cost of nuclear power in an integrated system and its comparison with the cost of conventional power production, and another group of experts will discuss the physics of heavy-water lattices. Symposia are to be held on materials for reactor control, including their metallurgical and physical properties and their design and fabrication; on new nuclear materials technology, with special reference to the use of such materials in the fabrication of non-metallic fuel elements; on the use of radioisotopes and radiation sources for the control of plant pests; on the use of isotopes in hydrology; on the biological effects of neutron irradiation and on the effects of radiation on the endocrine system; and on radiological health and safety in the mining and milling of nuclear materials; and the Agency's Scientific Advisory Committee has recommended that a seminar be held on some aspect of nuclear, solid-state or plasma physics.

Research contracts in tropical medicine are to be awarded with the view of promoting the application of established radioisotope techniques to the study of diseases affecting large groups of people in the less-developed countries in tropical regions. A review is to be made of the results of the Agency sponsored research on calcium-47, and work on the calibration and standardization of measurements of uptake of radioiodine by the thyroid gland is to continue. The distribution of calibrated radionuclide samples will be extended during 1963 and new calibration methods will be developed. Participation in the international intercomparison measurements of radionuclides organized by the International Bureau of Weights and Measures will continue. The comparative study of methods used in different laboratories for the chemical analysis of nuclear materials; the determination of trace elements in the marine environment; and in mass spectroscopy, the programme of the application of stable isotopes to analyses by the isotope dilution method, and the direct analysis by the mass spectrograph of trace elements in sea-water and nuclear materials, are some of the investigations to continue or be commenced during the year.

There are indications that during 1963 a number of nuclear facilities will be subject to Agency safeguards, and plans are being made to buy the essential minimum of portable equipment needed for the inspections. The general problems of emergency conditions in the event of a serious accident in nuclear installations are to be reviewed and the Agency's plans for the provision of international emergency assistance to member states are to be extended. Increased attention is to be directed to the development of techniques to deal with the disposal and management of radioactive waste, atmospheric pollution, and the transport and distribution of radioactive material. In this connexion, any necessary revision to the Agency's regulations for the safe transport of radioactive materials will be considered.

S. WEINTROUB

FOREST RESEARCH

THE report on forest research for the year ended March 1961* shows the wide range of work being carried out by the Forestry Commission Research Staff and for the Forestry Commission, with assistance by workers at universities and other institutions.

Much more attention is now being paid to the forest soil. Drainage is often a great problem. Wet soils may have a severe effect on tree roots by inhibiting growth and also by making the trees vulnerable to windblow. With the machines now available, the initial drainage system for a bare area may not be a very difficult task but a great deal more information is required on the depth drains should be on a given site and how far apart they should be spaced in order to provide reasonable conditions for afforestation purposes. The subsequent problems include the maintenance of the drains and, unfortunately, there is quite a lot of evidence in Britain of neglected drains. However, ways and means for ensuring the provision of good drainage in forest areas are being investigated. Nutritional work is also being undertaken. The analysis of foliar samples from fertilizer trials is providing data on nutrient deficiencies. Another series of experiments is attempting to elucidate the role of mites in the breakdown of forest litter.

For the first time a report on work study is included. The activities of the section dealing with this cover investigations on tools, equipment, machinery, piecework and other matters affecting production operations at all levels.

A prototype direct-reading calliper has been developed and trials of this instrument have demonstrated that enumeration work can be speeded up and so costs are reduced. As the measurements are transferred directly on to a tape by a punch system, errors in recording can be avoided. Two plates show some details of this calliper but no details are given of its weight. At least two other types of self-recording callipers are in use in Scandinavia.

In the report of the New Zealand Forest Service's Forest Research Institute for 1961†, mention is made of the new requirement that although officers out with the Research Division are still encouraged to conduct their own research projects, these must be approved by the Director of Research. This is a wise ruling. The individual is not thwarted, co-ordination is possible and one of the great drawbacks to individual research in forestry will be avoided, namely, the discontinuance of an experiment through the transfer of an officer to another territorial charge.

It is good to read that one of the planned developments is in physiology and the reason given is "because it is basic to an understanding of many problems in the fields of silviculture, forest-tree improvement and forest pathology". Another development is the appointment of a "silvicultural economics" team with the object of constructing financial yield tables for exotic species. This shows a realistic approach to land-use evaluation and will form a proper basis for the assessment of the relative merits of agriculture and forestry whenever such is required and it is expected to be more needed in the future. In addition, it is felt in New Zealand that a new approach is required in forest economics which, for so long, has been conditioned by European practice.

There is nothing perfunctory about the forest research described in this report. Indeed, the reader is convinced that forest research in New Zealand is well planned and vigorous, and that, in addition to trying to solve present-day problems, it is also forward-looking in a very commendable way.

C. J. TAYLOR

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* Forestry Commission. Report on Forest Research for the Year ended March 1961. Pp. ix+200+12 plates. (London: H.M.S.O., 1962.) 14s. net.

† New Zealand Forest Service. Forest Research Institute, Rotorua. Report for the year 1961. Pp. 75. (Wellington, N.Z.: Government Printer, 1962.)

ASPERGILLUS FLAVUS AND GROUNDNUT TOXICITY

IN 1960, large numbers of young turkeys in Britain died (and, later, other domestic birds and farm animals were shown to be affected) for a reason that was at first unknown and was called 'turkey X disease'. This was later shown to be due to the presence of a toxic batch of groundnut meal in their 'feed'. As a result of this, a number of Government and other research laboratories studied this toxicity as a matter of urgency. Real progress became possible with the demonstration that the toxin was not intrinsic to the groundnuts themselves but was the product of a mould (a strain of *Aspergillus flavus*) growing on them. This toxin-producing mould is widespread, and it was not surprising, therefore, that the toxin was identifiable on some samples of groundnuts or meal from all the main producing areas.

Because the problems posed overlapped the responsibilities of a number of Government departments, an

Interdepartmental Working Party was set up to review and co-ordinate research relevant to groundnut toxicity. In August 1962 the report * of this Working Party was circulated to overseas Governments and to the Press, and was made available to those in Britain and overseas known to be concerned in the production and utilization of groundnuts.

The report provides an account in general terms of the nature of the problem and means of identifying affected batches of nuts or meal. Suggestions are made as to how mould growth can be minimized and toxin-levels be kept down.

The report should prove of value to those who produce, market, process or use groundnuts, and should also stimulate necessary further research on groundnut toxicity, especially in the producing areas themselves.

P. C. Spensley

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* *Toxicity Associated With Certain Batches of Groundnuts*. Report of the Interdepartmental Working Party on Groundnut Toxicity Research (1962). Pp. 16. (Cyclostyled.) Obtainable from the Department of Scientific and Industrial Research, State House, High Holborn, London, W.C.1.

POLARIZATION AND CLASSIFICATION OF RADIO SOURCES

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THE detection of polarized radio emission from the radio sources Cygnus *A* (ref. 1) and Centaurus *A* (refs. 2 and 3), and a group of weaker sources[4], opened a new phase on the investigation of these objects. As well as the angular structure and spectrum, the percentage and position angle of polarization of radio sources are now available. This article gives the results of an examination of ten further radio sources of small angular diameter and relates the present polarization data and that obtained by Gardner and Whiteoak[4] to other known properties of radio sources.

The receiver used was basically that utilized for the Zeeman experiment[5], but was modified to measure the difference of intensity between two orthogonal planes of linear polarization. The frequency was 1,410 Mc/s and the band-width 5 Mc/s. Using a 20-sec time-constant, it was possible to detect differences in polarization of 2×10^{-27} Wm^{-2} (c/s)$^{-1}$ in the weaker radio sources studied. Measurements were taken by allowing the beam of the 250-ft. radio telescope to drift across the source at one position angle of the horn feed system and then repeating at a position angle 45° different. Sources were observed at several different hour angles. The system gave a spurious response of several per cent of the source flux which was constant and was allowed for. The measurements were made in the first week of October 1962.

This programme was confined to a group of 3*C* sources which had a galactic latitude (b'') greater than 12° and included representatives of the classes of radio objects identified with stars, double sources and sources with a core and halo. They also showed a wide range of spectral types, brightness temperature and redshift. The new polarization data obtained at Jodrell Bank are shown in Table 1.

The optical results quoted in Table 1 are those given by Maltby, Matthews and Moffet[6] and by Burbidge[7], and the radio information about the structure of the sources comes from published measurements at 160 Mc/s (refs. 8 and 9), 960 Mc/s (ref. 10) and 1,420 Mc/s (ref. 11). The results agree with the Sydney data for the sources common to both lists, namely, 3*C*348 and 3*C*353. In addition, it was found that Cassiopeia *A* was less than 0·3 per cent polarized and Taurus *A* was 1·0 ± 0·2 per cent polarized at position angle 82° ± 8°.

This data can be combined with that obtained in Sydney, also taken at 1,400 Mc/s. However, in order to make the measurements on the extended objects Centaurus *A* and Fornax *A* uniform with the present measurements the integrated polarization of the entire object was calculated. In Centaurus *A* the central double core is 7 per cent polarized and the large double halo was estimated to be 11·5 per cent polarized at most. The integrated emission from Fornax *A* was 7·4 per cent polarized. The combined data included 11 sources which are double systems at radio frequencies, 3 which consist of a bright central core and an extended halo of lower luminosity and 3 which have been tentatively identified with stars[7].

Cygnus *A* will be included in the following discussion although it lies only 6° above the plane and its polarization is effected by Faraday rotation in the Galaxy which amounts to about 10 rotations at 1,400 Mc/s, corresponding to an emission measure of ~ 200 in the outlying areas of Cygnus *X* which envelop Cygnus *A*. A variation of 10 per cent in the Faraday rotation across the source will be sufficient to cause complete depolarization at this frequency. At 3-cm wave-length the total rotation would amount to ~ 100° and the depolarization would be expected to be negligible. Thus the observed[1] polarization of 8 per cent found at 3 cm will be adopted as the polarization of the signal leaving Cygnus *A*. This step is justified by the observations of Centaurus *A* at $b'' = +20°$, which show the same polarization of the bright central component from 1,400 Mc/s to 10,000 Mc/s and also by the manner Cygnus *A* fits into the picture of double radio sources in the following discussion.

The observed position angle of the received signal from the sources in Table 1 show no correlation with the axes of symmetry of the associated optical objects. This is in agreement with the Sydney[4] and N.R.L.[1] findings and is the result of different amounts of Faraday rotation between the source and the receiver for different sources.

Table 1. PERCENTAGE POLARIZATION AND POSITION ANGLE OF THE ELECTRIC VECTOR FOR TEN SOURCES MEASURED AT 1,410 Mc/s AT JODRELL BANK

Source	Polarization (per cent)	Position angle	Identification	Distance (Mpc)	Comments
3*C*48	1 ± 1	70° ± 10°	Star	—	Diameter less than 1″ at 160 Mc/s.
3*C*84 Perseus *A*	5 ± 1	36° ± 10°	*NGC* 1275 Two spiral galaxies	53	Appears to be a core and halo at radio frequencies.
3*C*123	4·5 ± 1·5	8° ± 10°	—	—	Two components 12·5″ apart E.W. Component size 5·3″.
3*C*196	10 0 ± 0·5	49° ± 2°	Star	—	Diameter ~ 5″.
3*C*274 Virgo *A*	< 0·3	—	*NGC* 4486	12	Core and halo at radiofrequencies associated with elliptical galaxy and jet.
3*C*286	12 ± 1·5	31° ± 2°	Star	—	Diameter ~ 1″.
3*C*295	6·0 ± 1·0	162° ± 4°	20·9 m galaxy	1,380	Two components 4″ apart. Component size ~ 1·7″.
3*C*348 Hercules *A*	2 ± 0·5	6° ± 5°	19 m galaxy	460	Two components 1·95″ apart. Component size ~ 0·75″.
3*C*353	6 ± 0·5	7° ± 10°	16·8 m galaxy	(250)	Two unequal components. 2·5′ apart. Component size ~ 1·4′.
3*C*405 Cygnus *A*	< 0·3	—	15·3 m galaxy	170	Two components 1·58′ apart. Component size ~ 0·7′.

Polarization and spectral characteristics. The relationship between the percentage polarization P and the detailed spectral information given by Conway, Kellerman and Long[12] was investigated. No close correlation between polarization and the mean spectral index in the range 38–3,200 Mc/s was found although there is a tendency for the spectral index (α) to increase from 0·5 for the strongly polarized sources to 0·9 for the weakly polarized sources. However, a much stronger correlation (Fig. 1) exists between the polarization amplitude and ρ, the ratio of expected flux at 3,200 Mc/s extrapolated from the low frequencies to the observed flux at 3,200 Mc/s; ρ is a measure of the curvature of the spectrum at the high frequencies. The 7 double sources show a close correlation between P and ρ; the limits of the range are represented by 3C273 for which $P < 1$ per cent and $\rho = 0·95$, and by 3C270 for which $P = 8$ per cent and $\rho = 1·82$. The stars and single sources with halos show a scatter on Fig. 1.

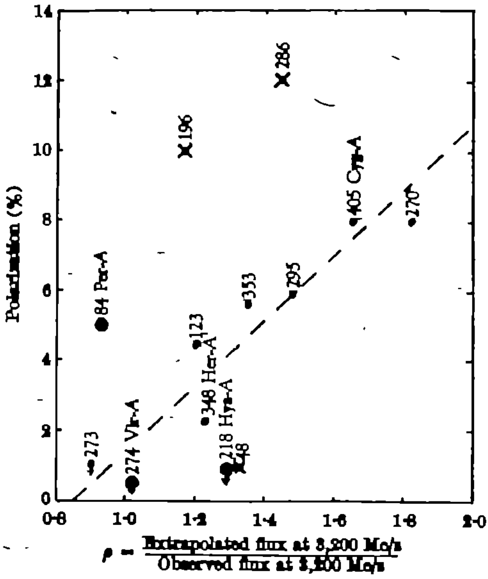

Fig. 1. Percentage polarization (P) plotted against ρ the ratio of the flux at 3,200 Mc/s extrapolated from low frequency measurements to that actually observed at 3,200 Mc/s

Stars are shown as ×, sources with a halo and bright core as ⊙, and double sources as ●

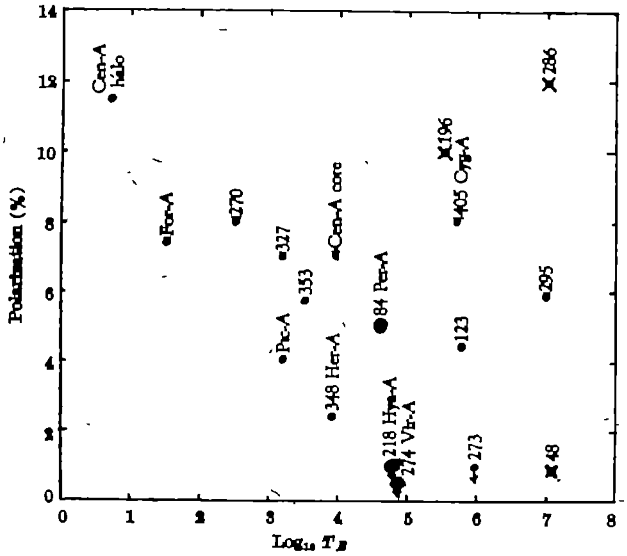

Fig. 2. Percentage polarization (P) plotted against the brightness temperature of the source. In the case of sources with a core and halo the brightness temperature of the core is taken. For Centaurus *A* the double core and the double extensions (halo) are plotted separately. The symbols are as for Fig. 1

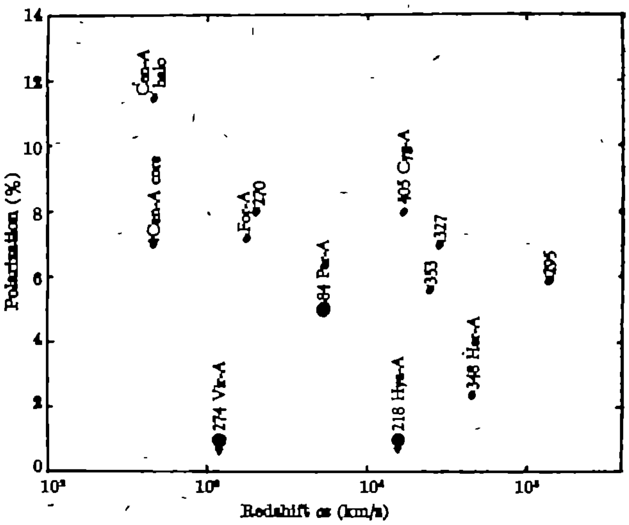

Fig. 3. Percentage polarization P plotted against redshift cz in km/s. The symbols are as for Fig. 1

Polarization and brightness temperature. A plot of P against T_B, the brightness temperature, of the sources studied is shown in Fig. 2. The correlation between P and T_B is small. If the sources identified with stars are excluded there is a weak tendency for the polarization to fall with increasing brightness temperature. However, the scatter is so great that this tendency is unlikely to be significant.

Polarization and redshift. The redshift of extragalactic objects in the combined list of sources which have been investigated for polarization are shown in Fig. 3, as a function of polarization. Again, there is no correlation with redshift even out to the distance of 3C295 for which $d\lambda/\lambda = 0·46$ (ref. 13). This result is significant as it indicates that there is no depolarization in the intergalactic medium. Complete depolarization would occur during an increase of a factor of two in distance where differential rotation increases from 45° to 90°, say, and this would appear as a rapid fall in P with redshift in Fig. 3.

Properties of different classes of object. The radio properties of the 17 observed sources in three classes are summarized in Table 2. It is tentatively suggested from these results that the three types of object may be distinguished by their radio properties alone. Two of the three so-called stars have a polarization of 10 per cent or more and it seems that this class of object is characterized by high polarization and high brightness temperature. The three objects consisting of a bright core and a halo show low polarization, nearly straight spectra ($\rho \simeq 1·0$) and intermediate brightness temperatures. The one object in this class which shows any significant polarization is Perseus *A* (3C84); it may possibly be part of a double system consisting of 3C84 and 3C83.1; measurements were not made on 3C83.1, so it is not at present possible to tell whether this double system fits the other data for doubles presented here or whether it is a halo object like Virgo *A* (3C274). The double sources show a strong correlation between the polarization P and ρ which is a measure of the curvature of the high-frequency part of the spectrum.

The classification of radio objects using the properties outlined above may be important in assessing the nature

Table 2. RADIO PROPERTIES, 'STARS', HALO SOURCES AND DOUBLE SOURCES

	1,400 Mc/s polarization (%)	Spectral characteristics α	ρ	Brightness temperature (T_B (°K))	Structure
Stars	~ 10	~ 0·5	~ 1·8	> 10⁶	Single
Halo sources	$\lesssim 2$	~ 0·8	~ 1 0	~ 5 × 10⁴	Core and halo
Double sources	0–8 (correlation with ρ)	0·4–1·0	1·0–1·8	10¹–10³	Double

of the unresolved radio objects at large distances and those for which optical identifications cannot easily be made or are in question. Further observations are needed to verify this classification.

Double radio sources. The close correlation between P and ρ for the double sources is of particular interest as it throws light on the nature of these objects. One picture of these objects which will give rise to this correlation will be presented. It is proposed that these are expanding objects of various ages in which synchrotron radiation is emitted by relativistic electrons generated in the system which spiral around the lines of a magnetic field also generated in the system. Thus at an early stage the proportion of high-energy electrons will be greater and the spectrum will be enhanced at medium and high frequencies. However, these high-energy electrons radiate more quickly than low-energy electrons and there will be a sharp drop in the spectrum at the high frequencies. As the object ages the contribution from the high-energy electrons disappears and the spectrum reduces to a constant slope over most of the frequency-range. In the early stages the proportional rate of increase of volume is greatest and the field lines are being rapidly expanded to give a more uniform field through the object than at a later epoch when the rate of expansion is slower. The percentage polarization observed in synchrotron emission increases with the uniformity of the field and will thus be greatest for a young object. Accordingly, both P and ρ will be indicators of the age of double systems on this model. The duplicity of the systems may be the result of enhanced radiation at the two mirror points in the enclosed magnetic field of the expanding object.

If this picture is correct the spectrum of the young objects will be flatter than that of older objects and accordingly there should be a negative correlation between P and α, the mean slope of the spectra. Such a tendency has already been noted in a previous paragraph.

At first sight it might be expected that the brightness temperature should be high in young sources with a high energy relativistic electron component. However, it must be remembered that T_B is also a function of the number density of relativistic electrons and of the magnetic field and variations from source to source in the latter quantities may remove any correlation between P and T_B. Indeed, this lack of correlation is evident in Fig. 2, and it was also found that for the sources studied there was no close correlation between T_B and ρ.

Cosmological implications. Since $3C295$ shows the expected amount of polarization for its value of ρ then the amount of depolarization it has undergone in the Milky Way halo, in the intracluster medium of our own system of galaxies, in the intracluster medium of the system of galaxies to which $3C295$ belongs and in the intergalactic medium must be less than ~ 30 per cent. This would be caused by differential rotation between the two components of the source of less than 30° or 0·08 rotation. Such a difference of rotation would occur between two paths which leave $3C295$ 27 kpc apart, the separation of the components of $3C295$, then pass through our local cluster at a mean distance of 0·5 Mpc at 10 pc apart and enter the halo of the Milky Way 0·2 pc apart.

In the case of the interglactic medium the differential rotation will occur owing to variations of magnetic field or electron density or both between the two paths. If the intergalactic medium has sub-units 2 Mpc in diameter 30 Mpc apart which eventually form clusters of galaxies with density variations of 10 : 1 about the mean over this distance then the density variation is 10 per cent of the mean density for 10 kpc, the mean separation of the two paths. A variation in the direction and magnitude of the intergalactic magnetic field alone would give a Faraday rotation variation of the same order for each sub-unit. If there are about 20 sub-units ~ 50 Mpc apart lying between our Galaxy and $3C295$ this would lead to an increase of about 5 and a total differential Faraday rotation of ~ 100 per cent on the paths to the two components of $3C295$. Even if only 3 : 1 variations in density and field were assumed the differential rotation would still be 30 per cent of the whole and thus the total rotation to avoid depolarization would need to be much less than 0·25 rotations. At 1,410 Mc/s the number of rotations $= 6 \times 10^3\ Ne\ H\ L$ where Ne is the number of electrons per cm^3, H is the field in gauss and the path length L is in parsecs. Thus the mean value of $Ne\ H$ is $< 3 \times 10^{-14}$ for the intergalactic medium between the Milky Way and $3C295$.

A similar calculation for the intracluster case assuming density and field variations of 3 : 1 over 300 kpc (the distance apart of galaxies) in a cluster with a diameter of 2 Mpc shows that the mean rotation need only be 0·08 rotation. The corresponding value of $Ne\ H$ for the $3C295$ cluster is $< 1{\cdot}3 \times 10^{-11}$.

Both these results appear to be in conflict with the hot version of the steady-state universe proposed by Gold and Hoyle[14] in which the intergalactic and intracluster gas is ionized hydrogen having densities of 2×10^{-5} and 10^{-3} cm^{-3} respectively. With an intergalactic magnetic field of 10^{-7} gauss[14] the density is $< 3 \times 10^{-7}$, which is more than an order of magnitude less than that proposed. With an intracluster field of 3×10^{-7} gauss the corresponding density is $< 4 \times 10^{-5}$, again an order of magnitude less than the predicted value. In a more recent discussion[15] the intergalactic magnetic field strength is suggested to be 10^{-6} gauss, which would result in an upper limit of the intergalactic density 600 times less than required in the steady-state theory and an intracluster density more than a factor of 100 times less than required.

This result for the cluster medium also has a bearing on the discussion[2,15,16] of the origin of the Faraday rotation between Centaurus A and the Earth. If the density derived here for the cluster with which $3C295$ is associated is typical of the local cluster then for a field of 10^{-6} gauss $Ne\ H\ L < 10^{-5}$ for a depth of 1 Mpc, whereas the value required is $\sim 10^{-4}$. Thus the cluster medium cannot give the rotation required and it is likely that the nearer parts of the Milky Way provide the rotation as argued by Cooper and Price[16]. A region which can provide the required rotation is the galactic spur if it is considered as a typical old supernova remnant[17]. It extends into the vicinity of Centaurus A. The expanding shell will have a hydromagnetic shock region which compresses both the magnetic field and the gas which will be ionized. A model of the spur object[18] gives densities in the brighter regions of ~ 10 cm^{-1} and a magnetic field of $\sim 10^{-5}$ and a shell thickness of ~ 5 pc when observed at the edge. The corresponding value of $Ne\ H\ L$ is 5×10^{-4}, which is sufficient to give the rotation.

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SIMULTANEOUS RADAR AND REFRACTOMETER SOUNDINGS OF THE TROPOSPHERE

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IN research on tropospheric wave propagation, the explanation of the transmission of very-high-frequency signals to points well beyond the horizon has been a subject of considerable interest for many years. Several workers[1,2] have suggested that, in the absence of super-refraction, the propagation mechanism is one of 'forward scatter' from random irregularities in refractive index which are always present, to some extent, throughout the troposphere. However, the validity of these theories, which are based on the statistical analysis of atmospheric turbulence, is still uncertain. Recently, other models of the troposphere have been proposed which, while retaining the concept of scattering as the operative mechanism in certain conditions, emphasize the importance of extended 'layer-type' irregularities[3]. These features are thought to exist in their most pronounced form in the stable air of temperature inversions.

Experimental investigations of refractive index irregularities in the troposphere are of fundamental importance in clarifying these problems, and it is the purpose of this article to summarize some recent work in this field at this Radio Research Station. The objective in the experiments was to compare the data provided by soundings of the troposphere by radar, as suggested in the early work of Friend[4], and by airborne microwave refractometers. The latter instruments were flown in aircraft of the Meteorological Research Flight, Farnborough, and spiral ascents and descents near the vertical beam of a radar transmitter were made whenever possible during the period July–November 1961 over a site at Bramshill Park in Hampshire. Special efforts were made to carry out joint soundings in clear weather conditions to avoid any confusion due to echoes from cloud and precipitation.

The airborne refractometers used for measuring the atmospheric refractive index were of the type developed by Birnbaum[5], and some details of their construction and of the procedure followed in airborne measurements have been given elsewhere[6]. With this type of refractometer the air passes continuously through a substantially open-ended microwave cavity resonating (in this case) near 10 Gc/s. The exact resonant frequency is inversely proportional to the instantaneous value of refractive index to be determined, and is measured automatically by a sweep-frequency technique. The refractive index fluctuations so determined were recorded on photographic paper, and height markers were inserted manually on the record as required. The soundings were afterwards re-plotted in profile form showing refractive index as a function of height. The rate of ascent or descent was generally 5 m/sec.

The vertically pointing radar equipment used in the tests operated with a single paraboloid aerial 8 m in diameter having a gain of 34 db at 3 Gc/s. The peak power of the transmitter was 500 kW and the pulses were 0·1 μsec wide repeated 500 times per sec. With a receiver band-width of 30 Mc/s and a noise-factor of 8 db a signal of strength 121 db below 1 W produced a root mean square signal/noise ratio of unity. The echoes were displayed on a vertical time-base by brilliance-modulation adjusted to produce limiting brightness at a signal-level just above that of the majority of the thermal noise peaks. This vertical line was moved horizontally at a steady slow

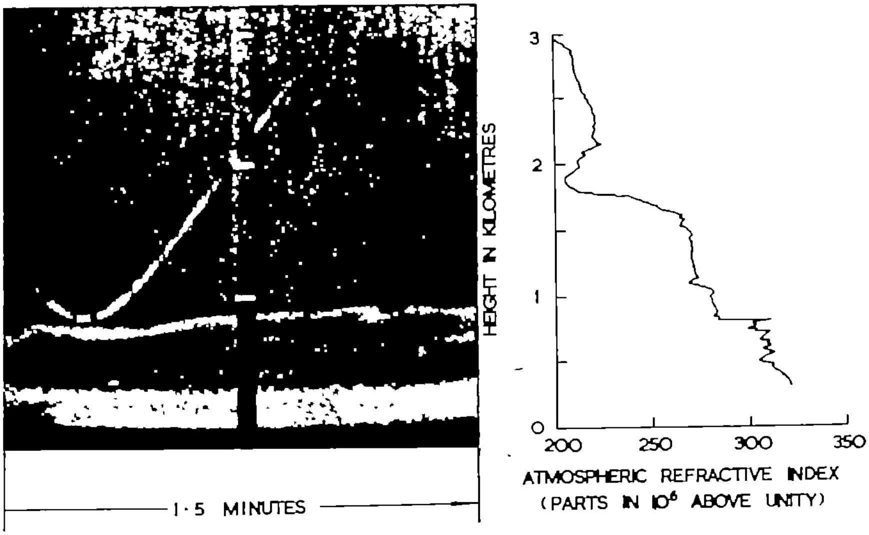

Fig. 1. Simultaneous radar and refractometer soundings at 15 10 h G.M.T. on August 29, 1961

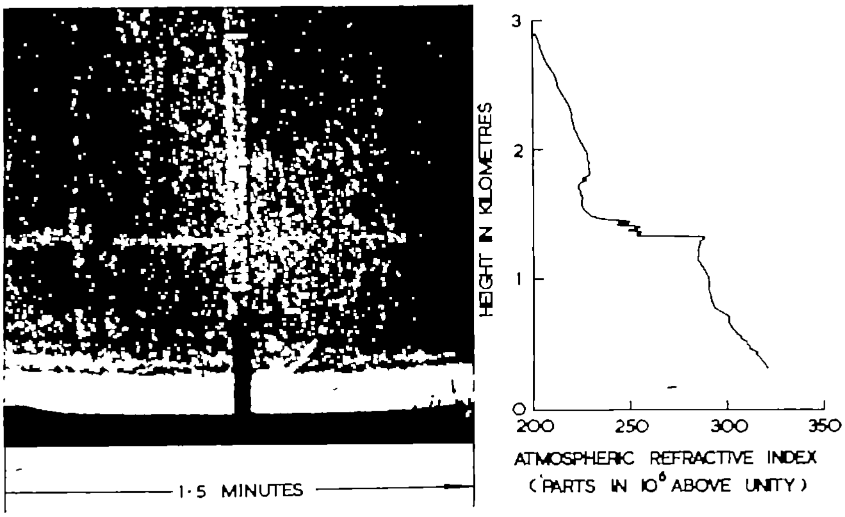

Fig. 2. Simultaneous radar and refractometer soundings at 17.55 h G.M.T. on August 29, 1961

rate to cover a photographic plate in about 1·5 min, and produced the pictures illustrated in Figs. 1 and 2, where height-markers 1 km apart are also shown. A useful amount of 'integration' was introduced by the photographic process, and enabled signals to be detected at a level of 134 db below 1 W corresponding to an amplitude reflexion coefficient of about 3×10^{-8} at a layer at a height of 1 km.

The results of the experiments may be summarized as follows. The characteristic, layer-type echo sometimes observed by radar[7] was generally found to correlate with a sharp gradient in the refractive index profile, at the corresponding height. On the other hand, the refractometer sometimes recorded a marked irregularity which was not observed by radar. These different characteristics reflected not only the effect of the overall sensitivity of the equipment, but also the degree of coherence in the scattered radiation together with the detailed structure and orientation of the layers. When the refractometer sounding showed a well-mixed atmosphere with no pronounced irregularities in the profile, no layer-type radar echoes were detected. Figs. 1 and 2 illustrate some of the results obtained in joint soundings made during anticyclonic conditions, at 15.10 h and 17.55 h G.M.T. on August 29, 1961. In Fig. 1, both techniques reveal a major irregularity in the refractive index profile at a height of about 0·8 km, the radar echo indicating the presence of an extended, slightly undulating layer. Ground-clutter extending to a range of about 0·3 km is also visible in Fig. 1, together with a curved trace showing the variation of aircraft range as it flew through the aerial side-lobes. At 15.10 h, no definite echo could be detected from the refractive index irregularity at 1·8 km, the height of the main subsidence inversion. By 17.55 h, however, the lower layer had disappeared from both records and the joint soundings in Fig. 2 show a single major irregularity at a height of 1·4 km. Detailed inspection of the original refractometer data shows that in the lowest portion of the irregularity illustrated in Fig. 2 the refractive index apparently changes by 30 parts in 10^6, in a vertical interval of a few metres. These soundings, and others made on the same afternoon, indicate that the main irregularity or layer shown in Fig. 2 was descending at a rate of about 100 m/h.

The results confirm the presence of extended layers in the refractive index structure of the troposphere and emphasize the value of combined radar and refractometer soundings in the investigation of the distribution of refractive-index irregularities and their scattering properties. A full account of this work is in the course of preparation.

This work has involved the participation of many of our colleagues and members of other organizations, in particular, the Meteorological Office and the Royal Aircraft Establishment. It is part of the programme of the Radio Research Board, and is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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HELIUM ION BELT IN THE UPPER ATMOSPHERE

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EXPERIMENTAL evidence for the presence of helium ions in the upper atmosphere in accordance with Nicolet's[1] suggestion is now available from ion- and electron-density profiles[2,3], ion probe retarding potential experiments[4,5], and direct measurements with radiofrequency ion spectrometer[6]. Hanson[2], who first inferred the presence of He+ from an experimental ion-density profile obtained by Hale[7], concluded that the thickness of the belt of helium ions is of the order of 2,000 km, extending from 1,200 km to about 3,400 km.

Because of the presence of He+, the upper ionosphere has to be considered a ternary ion mixture (O+, He+ and H+). Although the diffusive equilibrium distribution of a minor ion species in an ion mixture is influenced by the others (unlike neutral constituents) because of the electric field resulting from the slight charge separation between electrons and positive ions[8,9], the relative concentrations of ionic species behave like neutral constituents. The boundaries of a region where an ionic species predominates can be defined by the transition or equal-concentration levels, that is, the levels where the concentration of this species is equal to that of the neighbouring ionic species.

The equal-concentration level (h_{ij}) can be expressed in terms of the geopotential height parameter[10]:

$$z'_{ij} = H_{ij} \ln \eta_{ij} \qquad (1)$$

where $z'_{ij} = h'_{ij} - h'_0$ is the geopotential distance between the equal-concentration level and the reference-level h'_0 at which $[X_i^+]/[X_j^+] = \eta_{ij}$, and $H_{ij} = kT/(m_i - m_j)\, g_0$ with k the Boltzmann constant, T the absolute temperature, m_i and m_j the masses of the ionic species X_i^+ and X_j^+ and g_0 the acceleration of gravity at the Earth's surface. The geopotential altitude h' is related to the geometric altitude h by $h' = \int_0^h (g(h)/g_0)\mathrm{d}h$. In this discussion the subscripts 1, 2 and 3 refer to O+, He+ and H+, respectively.

The actual situation is complicated by the fact that the relative ion concentrations at the reference-level (η_{ij}) are also a function of temperature. This is particularly true for the ratio η_{31} since the neutral hydrogen concentration at a constant altitude depends on the rate at which it escapes from the upper atmosphere. Since this escape rate increases with temperature, the concentration of hydrogen at a constant level increases with decreasing temperature. The ratio [H]/[O] at an altitude of 500 km, which we shall adopt as reference-level h_0, increases by a factor of 100 for a temperature decrease from 2,000° K to 1,000° K[11,12]. If the proton concentration is governed by the charge exchange equilibrium condition[11] the ratio η_{31} varies with temperature in the same proportion as the ratio of the neutral constituents. The same argument applies if photo-ionization rather than charge exchange is responsible for the protons. Similarly, a proportional variation of [He]/[O] and η_{21} with temperature can be assumed. The helium concentration at 500 km varies only slightly with temperature[12] so that the ratio η_{21} will not vary as strongly with temperature as η_{31}.

The following values have been adopted for the present model: The concentration ratio [H+]/[O+] at 500 km varies from $\eta_{31} = 10^{-4}$ at 1,600° K to $\eta_{31} = 10^{-8}$ at 600° K, while the ratio [He+]/[O+] at 500 km varies from $\eta_{21} = 5 \times 10^{-2}$ at 1,600° K to $\eta_{21} = 5 \times 10^{-3}$ at 600° K. The temperature-range corresponds to the extremes for the present level of solar activity. The chosen variation with temperature of η_{31} is in good agreement with Bates and Patterson[11], and that for η_{21} is consistent with the variation of the helium concentration of Nicolet and Kockarts[12].

Fig. 1 shows the transition-levels h_{12} ([He+] = [O+]) and h_{23} ([H+] = [He+]) as a function of atmospheric temperature for the assumed variation of η_{ij} at the reference level h_0 = 500 km. The altitude difference $(h_{23} - h_{12})$ between the two equal-concentration levels may be called the thickness τ of the helium ion belt. It is obvious that this thickness is drastically reduced as the temperature decreases.

If the thickness τ is less than the local scale height for helium ions ($\tau < H(He^+)$), that is, if the two equal-concentration levels h_{12} and h_{23} occur within one scale height, no distinct slope corresponding to He+ will be distinguishable in an ion or electron density profile. This is the case for temperatures of the order of 1,000° K or less. Fig. 2 shows normalized charged particle density profiles based on a ternary ion mixture[10] for 800° K, 1,000° K and 1,300° K and corresponding η_{ij} versus geopotential distance z' above the reference-level, illustrating the fact that identification of helium ions from profiles is virtually impossible for low temperatures. A recent night-time electron density profile[13] failed to show evidence for the presence of He+. In the light of the foregoing discussion, failure to detect He+ from a profile does not prove the absence of He+ but can be explained by the fact that the thickness of the helium ion belt at that time was comparable in magnitude to the scale height of He+.

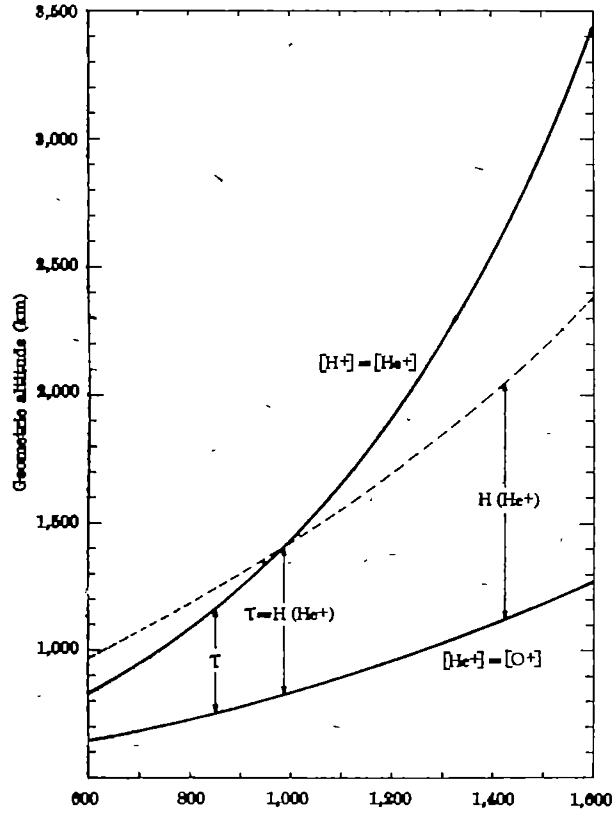

Fig. 1. Equal concentration-levels [He+] = [O+] and [H+] = [He+] on a geometric altitude scale versus atmospheric temperature. τ represents the thickness of the helium ion belt, while the local scale height for He+ is indicated by the distance between the dashed curve and the [He+] = [O+] curve. The equal-concentration-levels are based on the temperature-dependent ion concentration ratios (η_{ij}) at 500 km given in the text

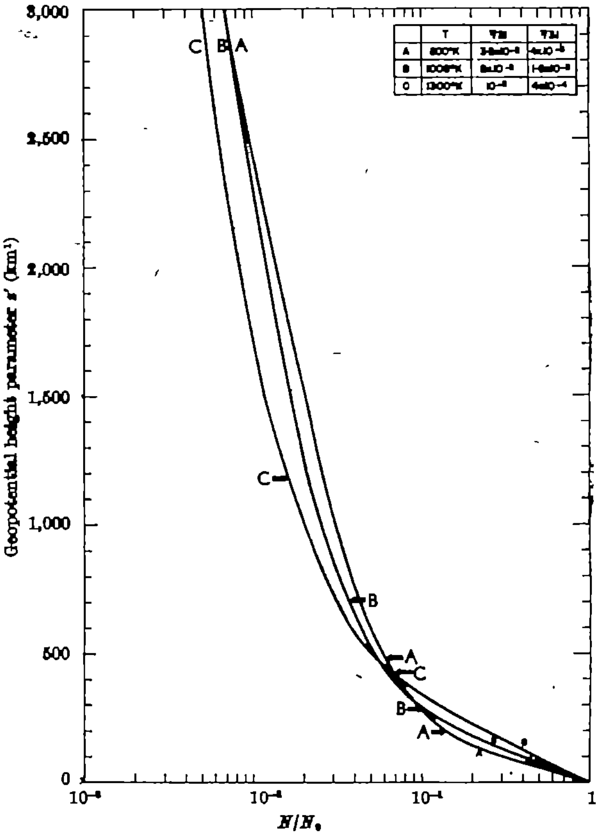

Fig. 2. Normalized charged particle density profiles for a ternary ion-mixture as a function of geopotential distance z' above the reference-level, corresponding to the listed ion concentration ratios (η_{ij}) and atmospheric temperatures. The solid arrows indicate the equal concentration level [He+] = [O+] and the open arrows [H+] = [He+]

In fact, from another recent night-time ion-density profile measured by a National Aeronautics and Space Administration rocket, the presence of helium ions is also not obvious[6]. However, a preliminary retarding potential analysis of the same data indicates the presence of all three ionic constituents (O+, He+ and H+). The combined ion density and composition data are thus consistent with the concept of a thin helium ion belt at low atmospheric temperatures.

Further refinements of the model presented here can be expected as the result of the ion-composition measurements on the *Ariel* satellite[14].

Note added in proof. From the final analysis of a recent ion density profile[6], it appears that the equal-concentration levels at the corresponding temperature of 800° K are about 200 km lower than indicated in Fig. 1, while the thickness of the helium ion belt is in good agreement with the present model. This fact seems to imply an even stronger temperature-dependence of the ion concentration ratios η_{ij} than was assumed here.

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METEORITE IMPACTS, LUNAR MARIA, LOPOLITHS AND OCEAN BASINS

On the assumption that one giant meteorite has fallen about every 10,000 years on our planet, R. Dietz estimates that some 50,000 of such missiles may have struck during the past 500 million years, or since the beginning of Cambrian times[1].

It is not very clear what is meant by "giant meteorites". If we refer to the data given by astronomy, the masses of the cosmic bodies indicated by Dietz may roughly be evaluated at 10,000 metric tons each[2]. Following the calculations made by M. Innes[3], such meteorites would produce craters of about 750 metres in diameter at an impacting speed of 20 km/sec.

As the question of the mass of striking bodies is still subject to controversial discussion, it would appear better to define a "giant meteorite" by the dimension of the crater it produces and to admit conventionally that those words refer to a missile able to produce a crater of at least 1 km in diameter at the impacting speed of 20 km/sec. In this case, the Barringer Crater (Arizona) represents the scar of a giant meteorite of about 5×10^4 metric tons, as calculated by Innes[3].

Rudaux and de Vaucouleurs mention that a meteorite of 5×10^4 metric tons—or more—strikes the Earth about every 100,000 years. They emphasize that this estimate is very conservative as most of these impacts must have escaped direct observation[2]. There is thus a good agreement between the evaluations given by geophysicists and those accepted by astronomers.

As there is no doubt that cosmic bodies have fallen at all times on our planet[4] we may conclude that some 30,000 giant meteorites can have reached it since the Earth seems to be 4·5 thousand million years old, although 'geological time', that is, the record of the rocks, began only about 3 thousand million years since the oldest rocks known were implaced in the continental nuclei, or in the past 3×10^9 years or so. This supposes that the overall rate of fall was practically the same during this period as that assumed for 500 million years. If we admit a uniform distribution for the impacts of giant meteorites on the Earth's surface (about 5×10^8 km²), there may have been one impact about every 17,000 km², or, lineally, a crater-centre roughly every 130 km. Of course, uniform distribution is illusory, and one must expect that the craters would group in clusters with many consequent overlappings.

Geologists argue strongly against such a wealth of meteoritic features on the ground that well-established cosmic scars are rare. The answer to this is that at least 70 per cent of the missiles may have fallen in the oceans and that the majority of the remaining 30 per cent may lie buried under younger geological strata, or have been eradicated by erosion and tectonic movements, or distorted by overlappings and foldings. In addition, the criteria used to identify meteoritic structures have not the absolute character that is attributed to them, with the result that many features, actually ascribed to volcanic activity, may well be of meteoritic origin.

Meteoritics is a young science. Only since around 1945 has extensive research been undertaken. The results of field research have been spectacular. More and more geological features of possible or probable meteoritic origin have been found with diameters ranging from 10 to 20, 200 and even up to 450 km (ref. 5). It is true that many conclusions arrived at are still controversial, but we are only at the 'beginning'.

The discovery of possible or probable meteoritic structures of huge diameter raises the question of possible cosmic catastrophes. Can the Earth's movements have been disturbed by impacts of giant meteorites? If so, in which conditions and to what extent? Several geophysicists have lately directed attention to this problem, but, to my knowledge, no satisfactory mathematical study on this subject seems ever to have been published.

Indisputably, there exists a cosmic body of given mass which, on impact at a known speed, is able to shift the polar axis. This is explicitly recognized by Laplace in his *Exposition du Système du Monde*, in which he describes vividly the devastating results of such an encounter.

If m is the mass of a cosmic body, V its speed on impact, M the mass of the Earth ($= 6 \times 10^{27}$ g), R the equatorial radius ($=$ 6,370 km) and ω the Earth's angular velocity ($R.\omega = \frac{40,000}{86,400}$ km/sec), the angle α of shifting of the polar axis is given by the equation:

$$\frac{x}{100} \times \frac{1}{2} m V^2 = 1{\cdot}6 \times M \times (R\omega)^2 \times \sin^2 \frac{\alpha}{2} \text{ (ergs)} \quad (1)$$

$\frac{x}{100}$ represents the percentage of the total kinetic energy (produced by the meteorite on impact) used for the shifting. Only the balance of energy is considered here and it is supposed that, after impact, the angular velocity of the Earth has not been altered ($\overline{OM} = \overline{OM'}$) (Fig. 1).

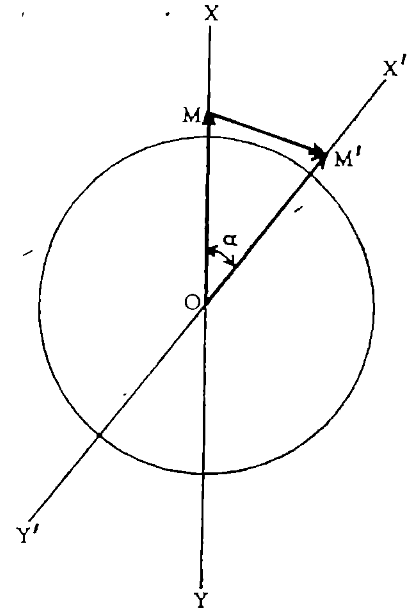

Fig. 1

XY and $X'Y'$ give the positions of the polar axis before and after impact.

The vector $\overline{MM'}$ represents the difference between $\overline{OM}$ and $\overline{OM'}$ and its value is:

$$2 \times \overline{OM} \times \sin\frac{\alpha}{2} \text{ or } 2.\omega.\sin\frac{\alpha}{2}$$

The quantity of energy used for the shift therefore is:

$$\frac{2}{5}.M.R^2 \times 4.\omega^2.\sin^2\frac{\alpha}{2} \text{ or } 1{\cdot}6 \times M \times (R\omega)^2 \times \sin^2\frac{\alpha}{2}$$

Innes has calculated the energy necessary for crater formation[3]:

$$E_c = 8{\cdot}44 \times 10^{12} \times D^2 \times d \text{ (ergs)}$$

in which D = diameter of crater in metres and d = density of impacted rock. If,

$$E_s = \frac{y}{100} \times \frac{1}{2} m V^2 \qquad (2)$$

and if V = 20 km/sec and d = 3, the value of α is easily obtained from (1) and (2):

$$\sin\frac{\alpha}{2} = 11 \times 10^{-13} \times \sqrt{\frac{x}{y} \times D^3}$$

The great difficulty is to assess acceptable values for x and y.

Innes gives y = 47 (ref. 3), adopting the results obtained in underground nuclear explosions[6]. For Öpik, y = 3 (ref. 7), but this value is too low in comparison with the results obtained by Johnson, Higgins and Violet[6]. It may be objected that Innes's figure is too high, for in meteoritic impacts the explosions do not occur in enclosed areas and the craters are hemispherical. In huge impacts able to shift the polar axis, one must expect that the energy required for such a shift is obtained mainly at the expense of that for crater forming and elastic effects. It may be assumed that the amount of energy used for shifting may be of the same magnitude as that spent in crater formation, if we do not take into consideration other possible phenomena, such as crust-slipping. Therefore, I propose as a working hypothesis the figure 30 for y and, in view of simplifying calculations in this work, I adopt:

$$x = y = 30$$

Then the following simple expressions may be used:

$$D = 0{\cdot}28 \times \sqrt[3]{m} \qquad m = 42{\cdot}2 \times D^3$$

$$\sin\frac{\alpha}{2} = 11 \times 10^{-13} \times \sqrt{D^3}$$

Further investigations will show to what extent the values of x and y must be modified.

Among the geological features accepted as being of probable meteoritic origin, the 'Vredefort Ring Structure' of South Africa, is outstanding. The question of its real diameter has not yet been settled. Daly[8] and Dietz[1,9] rate it at about 50 km. But Dietz stresses the point that the entire deformation reaches a diameter of 210 km (130 miles). Gilvarry[10] gives a diameter of 130 km. The central granitic core of the structure may well be the eroded root of an ancient dome, in which case, the original Vredefort feature could have been similar to the peculiar lunar craters with central uplift.

If we adopt 130 km, the structure may be the scar of an impacting body of 9·2 × 10[10] metric tons (V = 20 km/sec) shifting the polar axis by about 22″. If the diameter is 210 km, the mass of the missile was 3·9 × 10[11] metric tons and the shifting was about 38″.

An asteroid of the size of Juno (diam. about 190 km) would shift the polar axis by 45′ (V = 20 km/sec) and produce a crater of about 3,300 km in diameter. This is consistent with Gilvarry's hypothesis on the meteoritic origin of the oceanic basins[10].

It should be borne in mind that the above considerations do not take into account the possible 'slipping' of the lithosphere and the upper mantle on the underlying 'plastic layer', on meteoritic impact. This phenomenon would combine with the shifting of the polar axis and the energy required for it would certainly have to be deducted from that used for the shifting. There are good reasons for believing that this 'slipping' may well reach important values.

To conclude, I realize that my working hypothesis can only be the starting point of further investigations and that it involves many important geological and geophysical problems with far-reaching results. In the light of the actual known facts and the field research in progress, I feel confident that features of probable meteoritic origin, having disturbed our planet's movements, have already been found and that others are in progress of identification.

I thank Dr. D. Belorisky, of the Observatory of Marseilles, who helped me with astronomical aspects of this work.

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May I compliment Colonel Gallant on his interesting communication. His central thesis that giant meteorite impacts are capable of disturbing the Earth's polar axis of rotation may well be correct. However, I would like to take this opportunity to comment on some other subjects raised by his paper.

I differ from most other geologists in assigning an important role to meteorite impacts. Based on the shock criterion of shatter-coning alone, I[1] have recognized about a dozen ancient geological structures which appear to be bona fide 'astroblemes' (star-wounds). But as a marine geologist I cannot believe that ocean basin features bear any close resemblance to those on the Moon as Chenoweth[2] has written. Neither can I accept the view that ocean basins are simply giant meteorite craters as Gilvarry[3], Harrison[4] and now Gallant suggest.

I have indulged in this speculation myself[5], but with the more conservative approach that large impacts might have fractured the Earth's primeval sialic crust in 'pre-geological' time (that is, pre-rock record but post-Earth, or between 3·0 and 4·5 thousand million years ago). I suggested that the shattered fragments then may have completely rearranged themselves in what we now observe as the continents and the ocean basins. Under this scheme, the early impacts would only account for the discontinuity of the sialic crust and not the present shape or morphology of ocean basins. (Nearly all geologists, including myself, will dismiss out of hand the suggestion that continental slopes are crater walls, that the marginal mountains are explosion debris, etc. Geological evidence points quite clearly to the development of ocean basins by slow evolution rather than catastrophic birth.) This idea still seems to me to have as much merit as, for example, another better-known catastrophic hypothesis—the breaking up of an initial crust by the Fisher–Darwin hypothesis of pulling the Moon out of the Pacific. If the Moon was cosmically bombarded, certainly it is reasonable to suspect that the Earth was similarly bombarded at some early time. But now I am inclined to accept a cold accretion model for the Earth under which proto-Earth had no sialic crust at all as we now know it. The water, the air and the crust probably are all secondary, squeezed out of the mantle by processes related to thermal convection circulation as evidenced by sea-floor spreading[1]. Accordingly, early Earth impacts would have encountered no crust to fragment.

The diameter of the hypothetical Vredefort Ring[6] crater is more definable than Colonel Gallant suggests. The 40 km across central plug of Old Granite must define the crater's true diameter (that is, diameter below original ground-level). Using Baldwin's (in the press) scaling factor for the original apparent diameter (from the top of one rubble rim to the opposite rubble rim), the crater would have been 48 km across. My figure of 210 km for the limit of deformation refers to folding far outside

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of the crater *per se*. This figure was taken from Bishopp[7], but he recently[8] indicated that he now considers this figure as extravagant.

Gallant suggests that, in the absence of erosion, the Earth's surface would look like the Moon's crater-pocked face. I believe that geologists can state almost categorically that our geological maps do not permit this interpretation even in the shield regions where the basement complex is not obscured by sedimentary strata. Structures that can be interpreted by any stretch of the imagination as eroded impact craters are rather uncommon. Doubtless, we will find many more impact sites than are now recognized; but nothing comparable to the Moon.

Let us turn now to the question of the lunar maria or 'seas'. These do bear some resemblance to ocean basins in that they are depressed regions lined with dark rock; and although even Mare Imbrium is only the size of Texas, maria are, like ocean basins, dominating structures. Among those of us who consider maria as simply giant lunar impact craters, it might seem that we would extend this impact rationale to ocean basins; but I do not think it can be.

My reasoning concerning why the Earth's surface structures are fundamentally different from those of the Moon, without going into detail, is as follows. The primary diastrophic force, including the origin of ocean basins, is the thermal convection circulation in the Earth's mantle. Similar convection seems to be absent in the Moon, probably because of its comparatively small size. This I conclude from the simple observation that the lunar craters and maria, although mostly extremely old, do not reveal any significant tectonic distortion. On the Earth, they would have been compressed and thrust beyond all recognition.

The Moon must be a virtually crustless and coreless body. This agrees with the common assumption that the Moon is a primeval body resembling the early Earth and with a composition like that of a stony meteorite. The composition of tektites have a bearing on this subject since they may come from the Moon. Those who deny this origin rely as a main argument on the high silica and alkali content of tektites which is unlike that of stony meteorites. Here I would like to insert a *caveat*: magmatic differentiation well may have occurred in connexion with the evident outpourings of magma on the lunar surface. A welded tuff surface differentiate of this magmatism would likely be of rhyolitic composition which would closely resemble that of tektites.

The lunar maria appear to be filled with large saucer-shaped bodies of solidified magma rather than a series of thin lava flows. The rills and serpentine ridges suggest this, as they can be most easily explained by the contractional and compressional movements of a thick cooling magma. If so, they may be like extrusive lopoliths. Most geologists consider lopoliths as intrusive bodies, but some of us believe that many of them are extrusive (for example, Hamilton[9]).

It seems to me that we might look to lopoliths as possible terrestrial analogues of lunar maria. I have recently attempted this interpretation for the Sudbury lopolith and surrounding structure in Ontario[10]. I believe it to be an astrobleme involving an impact energy of about 3×10^{29} ergs, or twice the size of the Vredefort event. The Sudbury structure is my candidate for a maria-like feature on the Earth, rather than the ocean basins.

In conclusion, I believe that geologists are indeed guilty of looking down all of the time, when sometimes we should be looking up. On the other hand, the geological position of relying on conventional tectonic, plutonic and volcanic explanations is not arbitrary but well founded.

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RATE OF FLOTATION IN A HALLIMOND TUBE

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THE flotation behaviour of small quantities of a mineral can be determined in the laboratory by means of the Hallimond technique[1–4]. It is usual to design the experiment so that the results can be expressed as a percentage of the original mineral which is recovered by flotation after an arbitrary time interval. The time selected is generally between 1 and 5 min and is usually of sufficient duration to ensure that all the floatable material of the sample has been recovered.

However, if it is desired to investigate quantitatively the relation between flotation and variables such as collector concentration or parameters such as degree of adsorption of collector reagent, it seems preferable to arrange the experimental procedure so that the results can be expressed as a rate of flotation. Thus a flotation time shorter than that required for complete recovery of all the floatable material is necessary. To use the technique successfully in this quantitative fashion, it is then necessary to understand the importance, or otherwise, of operating variables such as gas flow rate, size of mineral bed and flotation time, and to know their effects on the observed flotation rate. No investigations of these aspects of the Hallimond technique are recorded in the literature, although Hallimond[4] noted that the gas flow rate should be enough to stir the bed, and such that the bubbles followed one another up the tube at intervals of 0·75 in. However, Fuerstenau[5] has shown that for a laboratory flotation tube with a stirred mineral bed, the amount of mineral recovered increases linearly with time, at constant gas flow rate, until recovery is almost complete, and that the recovery rate during this period of time is proportional to gas flow rate. The object of the experiments reported here was therefore to investigate these problems when the conventional Hallimond technique is used for the measurement of flotation rates.

The mineral used was beryl, crushed and dry sieved to a size range between 100 and 300 B.S.S. mesh size. It was pre-treated by standing in 1 per cent cold aqueous hydrofluoric acid (10 ml./mineral) for 0·5 h with occasional stirring, then washed with distilled water until the conductivity of the washing water was unchanged by contact with the mineral, and finally dried at 70° C overnight.

The desired weight of mineral (0·35–0·65 g) was then contacted with 65 ml. of a solution of the collector reagent for 30 min, with occasional shaking. The collector used

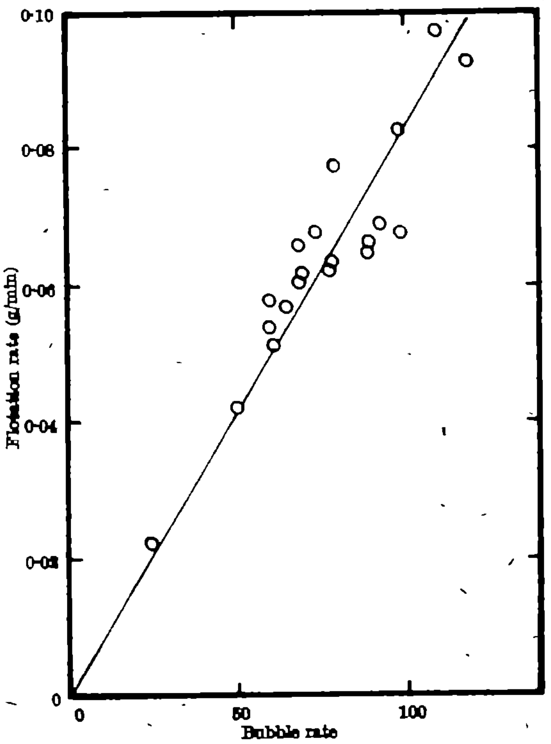

Fig. 1. Influence of gas flow rate on the rate of flotation

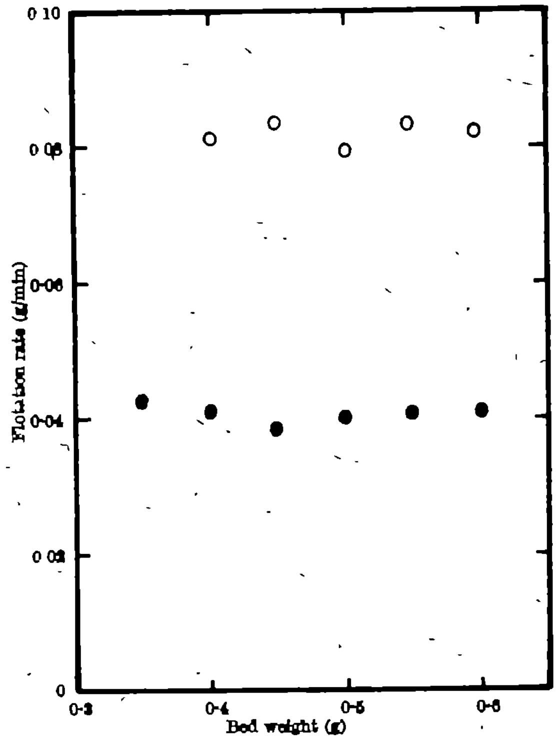

Fig. 2. Effect of mineral bed weight on rate of flotation

was 'Aero-promotor 824', at a concentration of 0·04 g/l., and hydrochloric acid was used to adjust the pH to the desired value. The slurry was then transferred to a Hallimond tube similar to that described by Sutherland and Wark[8], and air bubbled through at a steady rate. The same Hallimond tube was used for all the measurements.

In the first series of measurements, mineral beds of 0·50 g were used with gas flow rates between 25 and 120 bubbles per min. Flotation was continued until only about 0·1 g of the bed remained. Experiments involving flotation of the last 0·1 g of bed gave results which were difficult to reproduce because of the difficulty of regulating and measuring the bubble rate in these final stages. The results, reproduced in Fig. 1, show that within experimental error, the rate of flotation was directly proportional to the bubble rate.

In a second series of measurements, various bed weights (0·35–0·65 g) were used with bubble rates controlled within the range 80–100 bubbles/min. The observed rates of flotation were multiplied by (80/actual bubble rate) to give the flotation rate corresponding to a gas rate of 80 bubbles/min. Results for two series of measurements at different pH's are shown in Fig. 2 and indicate that the flotation rates are independent of bed size, over the range

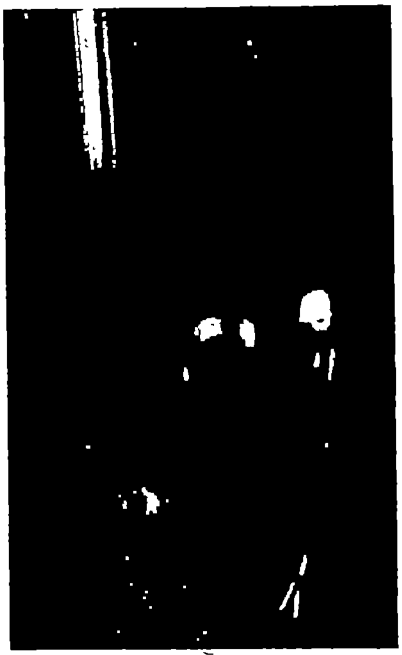

Fig. 3. Formation of a normal size bubble at the surface of the mineral bed in a Hallimond tube. The streaks on the underside of the bubble are formed by mineral particles being shed from the bubble

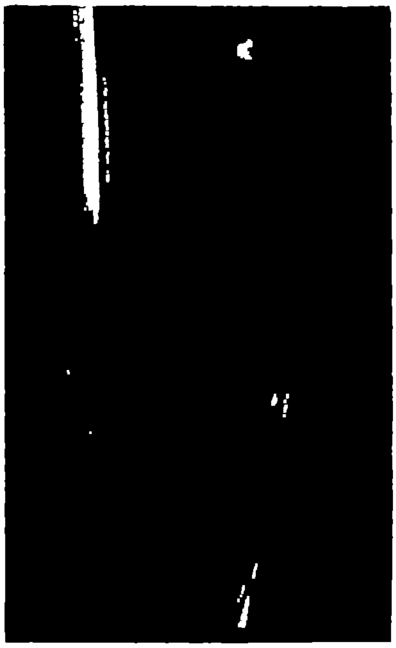

Fig. 4. Small heavily loaded bubble released shortly after a normal size bubble. Such small bubbles were always completely covered with mineral, so that they rose as 'solid' spheres without circulation of the gas within the bubble, and without formation of new interface at the front of the bubble. The normal bubbles were loaded only at the rear

used. Similar results were obtained in several other series of experiments using beryl pre-treated and conditioned in different ways.

In another series of measurements the influence of mineral size distribution was investigated. The beryl sample was separated by dry sieving to obtain the +300 −200, +200−170, +170−150, +150−100 fractions. Flotation rates, using the preceding technique, were the same for all samples, within experimental error.

Thus, for these systems, flotation rates can be compared by this modification of Hallimond procedure, providing the gas flow rate is accurately recorded; close control of the initial size of the mineral bed is not necessary. It is concluded that, for a given system, the mineral load carried by each bubble is the same, regardless of the rate of formation of the bubbles or depth of the bed. Measurement of the rate of flotation of a mineral by this procedure thus provides a quantitative measure of the floatability of the mineral, and the method has been successfully used in an extensive study of the flotation of beryl, which will be described elsewhere.

However, for the most readily floatable samples, some discrepancies were observed. Systems with flotation rates larger than 0·15 g/min at 80 bubbles/min gave results which were not accurately reproducible. Careful observation of these systems revealed the presence of two kinds of bubbles during flotation. In addition to the normal size, partly loaded bubbles (Fig. 3) were observed in all systems, and there were also a variable proportion of smaller bubbles (Fig. 4). These bubbles were always released from the bed immediately before or immediately after a normal bubble. Since their proportion was variable and could not be controlled, considerable variation resulted in the measured rate of flotation. Though the cause of the phenomena can only be a matter of speculation, it is suggested that the normal size bubble may be formed at the upper end of a continuous channel or void extending from the sintered disk to the upper boundary of the mineral bed. It is likely that such channels are less stable in beds of easily floatable mineral, and their collapse may lead to small pockets of air being trapped within the bed. The disturbance associated with the formation of a new channel would cause release of the pocket of air as a small bubble, at a point of time close to the moment of formation of the next normal bubble.

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INTERACTION OF AMINO-ACIDS, PROTEINS AND AMINES WITH CHLORANIL

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FOR several years there has been speculation about the possible role of charge-transfer complexing in different biological systems[1–4]. $\pi-\pi$ charge-transfer complexes, where the donated electron forming the complex is a π-electron, have been mainly considered. Less attention has been given to the possibility of $n-\pi$ charge-transfer complexes being formed, although many biological materials contain suitable lone-pair electrons.

One common acceptor which readily forms charge-transfer complexes is chloranil (tetrachloroquinone). $\pi-\pi$ complexes of the polycyclic aromatic hydrocarbons with chloranil have been studied by several authors[5–8]. $n-\pi$ complexes involving chloranil have also been observed[9–11]. Although chloranil does not occur in natural systems, its behaviour may be considered indicative of that of other quinones, such as ubiquinone, which are of biological interest.

In the present work the absorption spectra of mixed solutions of amino-acids and chloranil and of proteins and chloranil have been studied. It is observed that, on adding an amino-acid to a 10^{-3}–10^{-4} M solution of chloranil in 50 per cent aqueous ethanol buffered to a suitable *p*H, a change occurs in the solution spectrum. The absorption band *A* of chloranil at 295 nm is decreased and a new band, *B*, appears at longer wave-lengths. This behaviour is shown in Fig. 1. The position of band *B* is dependent on *p*H, varying between 380 nm and 330 nm as the *p*H of the solutions is varied between *p*H 4 and *p*H 12, but it appears to be independent of the nature of the amino-acid. Some absorption spectra of amino-acid–chloranil mixtures at various *p*H are shown in Fig. 2. Table 1 lists the amino-acids studied and the wave-lengths of maximum absorption of the new bands.

The effect is reversible. Increase in temperature causes the band *B* at 370 nm to decrease in intensity and the chloranil absorption band *A* at 295 nm to increase in intensity as shown in Fig. 3.

In alkali solution a small fraction of the chloranil is changed, probably to chloranilic acid[12]. Fig. 4 shows some spectra of new and old solutions of chloranil at different *p*H and some aqueous solutions of chloranilic acid at different *p*H. It is seen that the fraction of chloranil degraded is very small, the positions of the chloranilic acid absorption band at 330 nm are scarcely affected by the changes in *p*H. It is clear that chloranilic acid is not responsible for the effects observed in chloranil–amino-acid solutions.

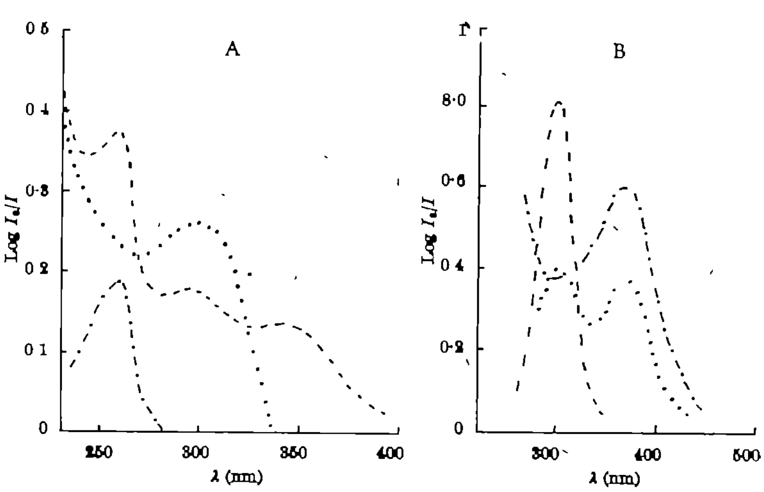

Fig. 1. Absorption spectra of: *A*, *a*, Phenylalanine ($1·06 \times 10^{-3}$ M) (— · —); *b*, chloranil ($2·27 \times 10^{-3}$ M) (· · ·); mixture of *a* and *b* (– – –); all in 50 per cent ethanol buffered to *p*H 7. *B*, chloranil (8×10^{-3} M) (– – –); plus glycine (10^{-3} M) (— · —); plus alanine (about 10^{-3} M) (· · ·); all in 50 per cent ethanol

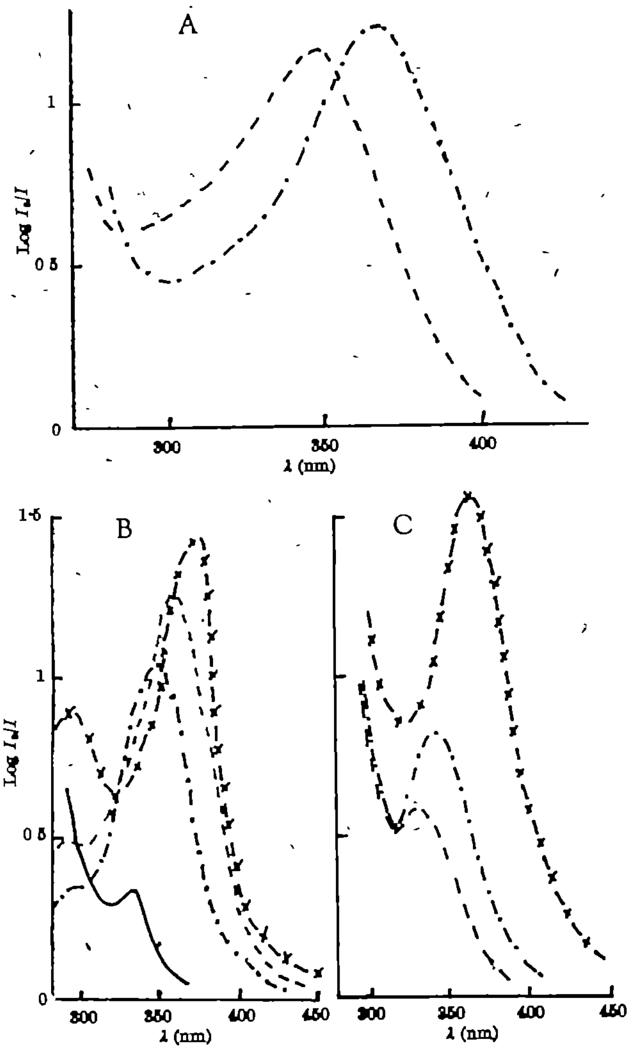

Fig. 2. Absorption spectra of: *A*, chloranil (about 2×10^{-4} M) and leucine (about 10^{-1} M) in 50 per cent ethanol buffered to *p*H 7 (—·—); chloranil (about 2×10^{-4} M) and glycine (about 10^{-1} M) in 50 per cent ethanol buffered to *p*H 9 (– – –). *B*, chloranil plus glycine in 50 per cent ethanol buffered to *p*H 7 (—×—); *p*H 9 (– – –); *p*H 11 (—·—); *p*H 13 (——). *C*, chloranil plus tryptophan in 50 per cent ethanol buffered to *p*H 7 (—×—); *p*H 9 (– – –); *p*H 11 (—·—)

The inverse concentration of amino-acid has been plotted against the inverse optical density of band *B*. An approximately linear relation is found corresponding to the Benesi–Hildebrand equation for 1 : 1 complexes[13], namely:

$$\frac{[b]\,l}{\log\frac{I_0}{I}} = \left(\frac{1}{K\varepsilon_c}\right)\left(\frac{1}{[a]}\right) + \left(\frac{1}{\varepsilon_c}\right), \text{ for } [a] \gg [b]$$

where $[a]$ and $[b]$ are the molar concentrations of the two components. K is the equilibrium constant of the complex given by:

$$K = \frac{[ab]}{[a-ab]\,[b-ab]}$$

where $[ab]$ is the molar concentration of the complex, l is the optical path length and ε_c is the molar extinction coefficient of the complex species. The values of the equilibrium constant K evaluated from the intercept and slope are listed in Table 2. K appears to decrease with increasing *p*H. Typical plots are shown in Fig. 5.

The absorption spectra of mixed solutions of proteins and chloranil in 50 per cent aqueous ethanol have also been studied. Typical results are shown in Fig. 6. Table 3 lists the positions of the new bands observed. The spectra of chloranil–protein mixtures are very similar to the spectra of chloranil–amino-acid mixtures except that, for a given *p*H, the position of the band *B* lies at shorter wave-length in the protein systems than in the amino-acid systems. Comparable weights of protein and amino-acid

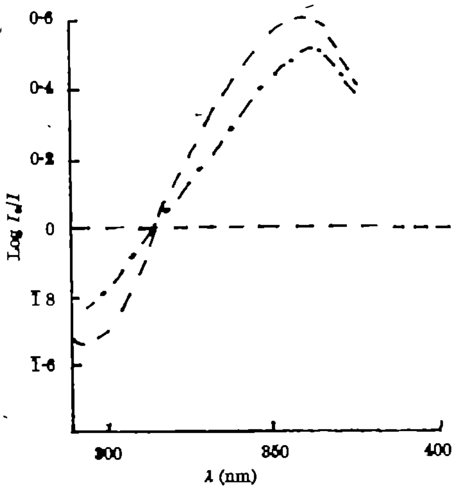

Fig. 3. Difference spectra between chloranil (about 2×10^{-4} M) and mixture of chloranil and glycine (about 10^{-1} M) in 50 per cent ethanol buffered to *p*H 7 at 20° C (– – –) and at 40° C (—·—)

Table 1. WAVE-LENGTH OF MAXIMUM ABSORPTION OF BAND OCCURRING IN AMINO-ACID–CHLORANIL MIXTURES IN 50 PER CENT ETHANOL

Amino-acid	Wave-length (nm)	*p*H
Tyrosine	360	7
	330	12·2
Leucine	370	5
	360	7
	355	7·6
Alanine	370	4·8
	360	7
	360	7·5
	355	8
Tryptophan	370	5
	360	7
	355	8
	340	10·4
	330	13
Glycine	370	5
	360	7
	350	9
	340	11
Phenylalanine	380	4
	370	4·6
	370	5·2
	360	7·4
Proline	370	5
	360	8
	335	12

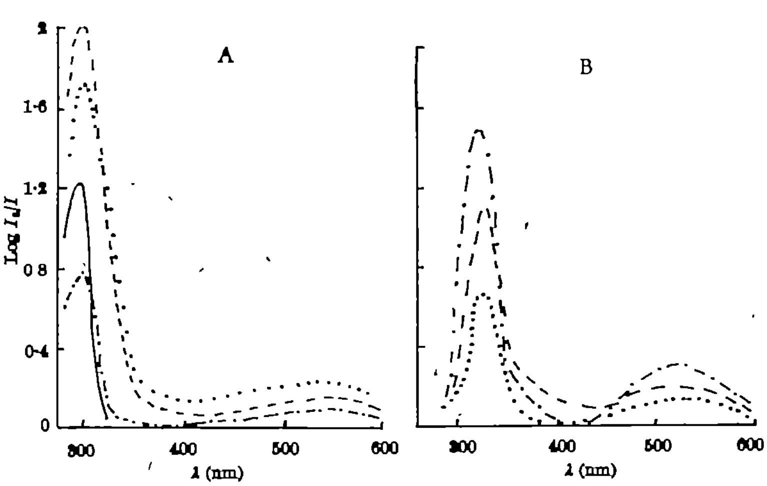

Fig. 4. Absorption spectra of: *A*, chloranil ($2\cdot3\times10^{-4}$ M) in 50 per cent ethanol buffered to *p*H 12 (· · ·); chloranil ($2\cdot1\times10^{-4}$ M) in 50 per cent ethanol buffered to *p*H 7 (– – –); chloranil ($1\cdot18\times10^{-4}$ M) in 50 per cent ethanol, freshly prepared (——); chloranil ($1\cdot18\times10^{-4}$ M) in 50 per cent ethanol four weeks after preparation (—·—). *B*, chloranilic acid in water buffered to *p*H 4 (—·—); *p*H 8 (– – –); *p*H 10 (· · ·)

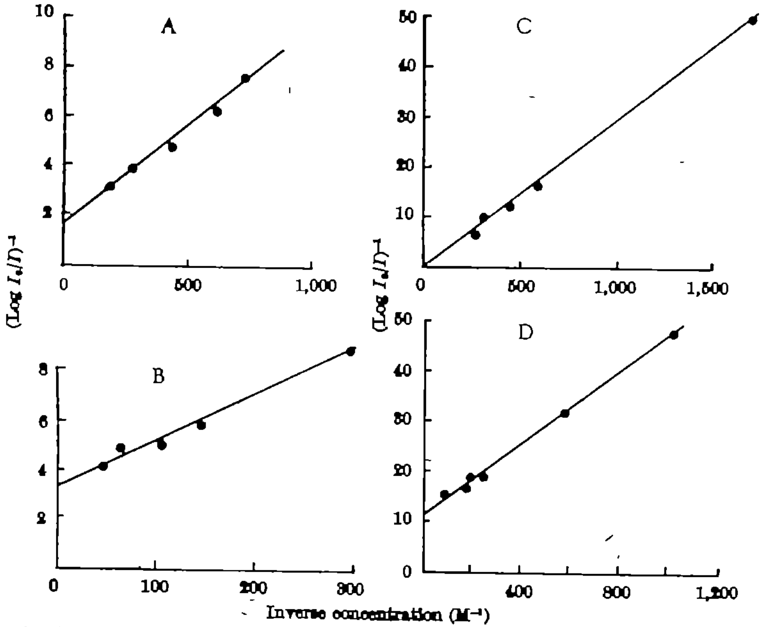

Fig. 5. Plots of inverse optical density against inverse concentration of amino-acid. *A*, glycine with chloranil ($3·6 \times 10^{-4}$ M) in 50 per cent ethanol buffered to *p*H 8 at 350 nm; *B*, glycine with chloranil (6×10^{-4} M) in 50 per cent ethanol buffered to *p*H 10 at 350 nm; *C*, tryptophan with chloranil (4×10^{-4} M) in 50 per cent ethanol buffered to *p*H 12 at 255 nm; *D*, alanine with chloranil (2×10^{-4} M) in 50 per cent ethanol buffered to *p*H 8 at 350 nm

Table 2. EQUILIBRIUM CONSTANTS OF AMINO-ACID–CHLORANIL COMPLEXES IN 50 PER CENT ETHANOL

Amino-acid	*K*	*A*	*D*	λ	*p*H
Glycine	298	1×10^{-4}	$4·8 \times 10^{-2}$–$1·14 \times 10^{-1}$	390	5-6
Glycine	215	$3·6 \times 10^{-4}$	$1·42 \times 10^{-3}$–$1·35 \times 10^{-2}$	350	8
Glycine	127	6×10^{-4}	3×10^{-3}–$1·8 \times 10^{-2}$	350	10
Tryptophan	176	$3·4 \times 10^{-4}$	$1·9 \times 10^{-4}$–$7·3 \times 10^{-3}$	360	8
Tryptophan	~0	4×10^{-4}	$1·57 \times 10^{-3}$–4×10^{-3}	255	12
Leucine	224	5×10^{-4}	$8·4 \times 10^{-4}$–$8·7 \times 10^{-3}$	360	7
Alanine	318	2×10^{-4}	9×10^{-4}–$1·8 \times 10^{-3}$	350	8

K, equilibrium constant l./mole at 20° C; *A*, concentration of chloranil moles/l.; *D*, range of concentrations of amino-acid in moles/l.; λ, wave-length (nm) at which *K* was determined.

Table 3. WAVE-LENGTH OF MAXIMUM ABSORPTION OF BAND OCCURRING IN PROTEIN–CHLORANIL MIXTURES IN 50 PER CENT ETHANOL

Protein	Wave-length (nm)	*p*H
Fibrinogen	360*	5
	350	7
	330	12·1
γ-Globulin	—	5
	340*	7
	350	8
Horse serum albumin	—	5
	350	8
Trypsin	340*	5
	345	8

* Poorly resolved.

in about 10^{-4} M solutions of chloranil give comparable optical changes. On adding alkali to solutions of protein and chloranil, a purplish precipitate is formed. The precipitates from a bovine-serum albumin–chloranil solution and a fibrinogen–chloranil solution are found to exhibit semi-conductor behaviour and a d.c. conductivity of 10^{13} Ω cm. This is in reasonable agreement with the data of Eley *et al.*[4], who also showed that a bovine-serum albumin complex behaved like a semi-conductor with a conductivity of about 10^{12} Ω cm.

Absorption spectra of mixed solutions of chloranil and amino-acid analogues have also been studied. Typical spectra are shown in Fig. 7. The spectrum of chloranil in acetic acid is similar to the spectrum of chloranil in different inert solvents[14]. On the other hand, the spectra of the various chloranil amino-compound mixtures are similar to those of the amino-acids and proteins. The new bands observed in these mixtures are also reversible, decreasing in intensity with increase in temperature. Solutions of these mixtures in 50 per cent aqueous ethanol exhibit only a new sharp band in the region of 350–420 nm (ref. 11), but in solutions in carbon tetrachloride an additional broad band, probably corresponding to the conventional charge-transfer band, is often observed at rather longer wave-lengths[18]. Anthranilic acid in carbon tetrachloride turns a bronze colour on adding chloranil, but the other amino-benzoic acids do not give any visible coloration in the presence of chloranil. The positions of these new absorption peaks and the *p*H of the solutions are tabulated in Table 4.

The absorption spectra of chloranil–amino-acid and chloranil–protein mixtures are very similar to those of chloranil–amine mixtures. It is concluded that the amino-groups in the amino-acids and proteins form *n*–π charge-transfer complexes with chloranil. The new band *B* has already been interpreted as being due to a red-shift of the chloranil band on complexing[11]. The studies reported here support this interpretation. The complex is probably formed by a lone-pair electron from the nitrogen in the amino group being donated to the chloranil. Although the normal charge-transfer band has not been observed in these amino-acid systems, it has been shown that some aliphatic amines show such a charge-transfer band in carbon tetrachloride but not in 50 per cent aqueous ethanol.

Booth and Boyland[15] found that various hydrocarbons form 1 : 1 complexes with purines in 50 per cent aqueous ethanol. It was later shown[16,17] that the infra-red spectra and crystal structure of these complexes are very similar to those exhibited by charge-transfer complexes. Booth and Boyland concluded, however, that these hydrocarbon–purine complexes could not be charge-transfer complexes because of the absence of the normal charge-transfer band. The present work suggests that charge-transfer complexes may be formed without the appearance of a typical charge-transfer absorption band.

Although the amino-acids exist as dipolar ions in neutral solution, in the presence of chloranil they behave as though they still possess an un-ionized amino group. This raises the question as to whether the equilibrium constants given in Table 2 are correct. If the amino-acids exist in both ionized and un-ionized form in the presence of chloranil, then the concentration of the amino-acid used to evaluate the equilibrium constant should be that of the un-ionized amino-acid.

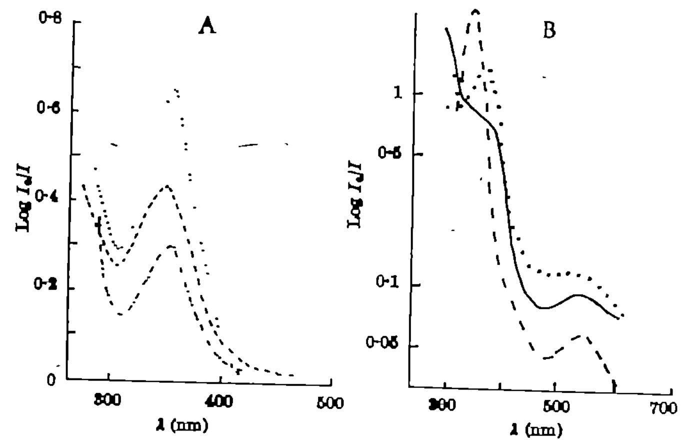

Fig. 6. Absorption spectra of: *A*, mixtures of chloranil and trypsin (···); horse serum albumin (– – –); γ-globulin (—·—); all in 50 per cent ethanol buffered to *p*H 8. *B*, mixture of chloranil and fibrinogen in 50 per cent ethanol buffered to *p*H 7 (——); *p*H 8 (···); *p*H 10 (– – –)

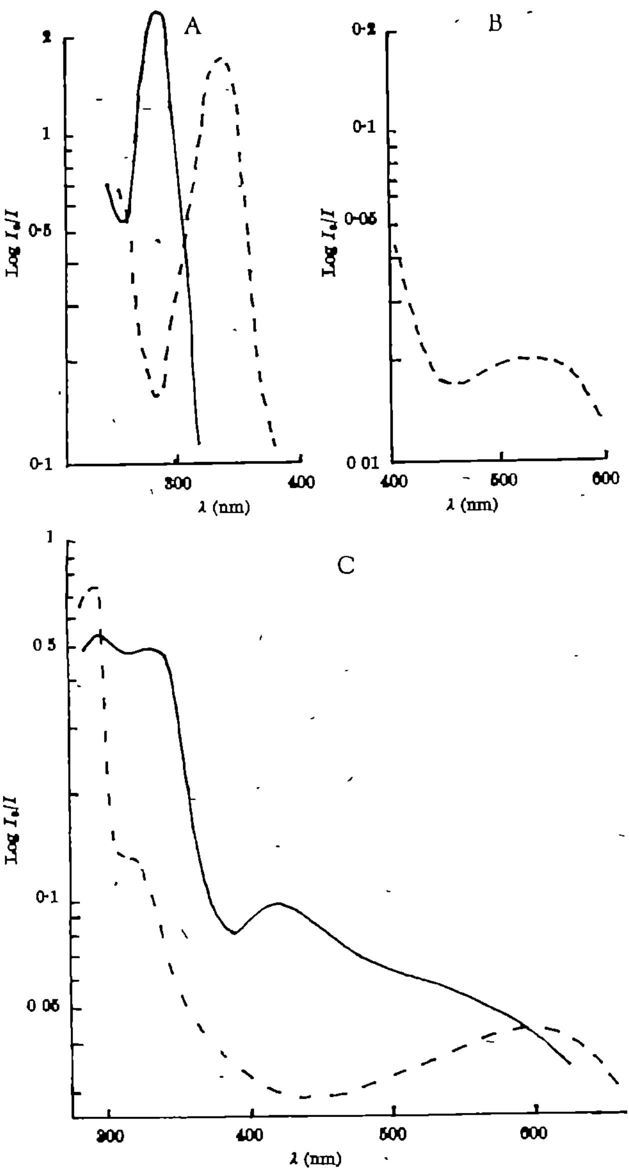

Fig. 7. Absorption spectra of: *A*, *B*, chloranil in: acetic acid (——), chloranil in ethylamine (1 M) in 50 per cent ethanol (– – –). *C*, diethylamine (1 M) and chloranil in carbon tetrachloride (– – –); anthranilic acid and chloranil in 50 per cent ethanol buffered to *p*H 4 (——)

Table 4. WAVE-LENGTH OF MAXIMUM ABSORPTION OF BANDS OCCURRING IN AMINE–CHLORANIL MIXTURES

Donor	Solvent	λ (nm)	*p*H
Ethylamine (ref. 11)	50 per cent ethanol	345	12
,,	carbon tetrachloride	350, 525	
Diethylamine	50 per cent ethanol	345	12
,,	carbon tetrachloride	320, 605	
,,		— 625 (ref. 10)	
Triethylamine (ref. 11)	triethylamine	315	12
,,	carbon tetrachloride	320, 640	
,,		— 655 (ref. 10)	
Anthranilic acid	50 per cent ethanol	425	3·8
,,	carbon tetrachloride	— 500	
m-Amino-benzoic acid	aqueous buffer	450	4
,, ,,	,, ,,	385	7
,, ,,	,, ,,	365	9
,, ,,	carbon tetrachloride	385	
p-Amino-benzoic acid	50 per cent ethanol	388	4·2
,, ,, ,,	,, ,, ,,	382	6
,, ,, ,,	,, ,, ,,	340	12
,, ,, ,,	carbon tetrachloride	368	
Dimethyl aniline (ref. 9)	butyl ether	345, 625	
Methyl dimethyl aniline (ref. 9)	,, ,,	380, 660	

Increasing the *p*H of the solutions causes the band *B* to shift to shorter wave-lengths. If this band is the original chloranil band shifted to longer wave-length on complexing, this would indicate that at higher *p*H the complexes are weaker. Amino-groups are expected to be better electron donors in acid solution than in alkali solution. Table 2 also shows a decrease in the equilibrium constants of the complexes on increasing *p*H. The observation that comparable weights of protein and amino-acids produce similar changes in the spectrum of chloranil suggests that many of the amino-groups in the protein complex with the chloranil.

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ABSORPTION SPECTRA IN IRRADIATED WATER AND SOME SOLUTIONS

Absorption Spectra of 'Hydrated' Electron

IN studying the absorption spectra of various aqueous solutions after exposure to a pulse of 1·8-MeV electrons, we have discovered a broad transient absorption band extending from wave-lengths below 5000 Å to beyond the limit of the emulsion sensitivity at 8800 Å, and having a peak near 7000 Å. We believe that this band is due to the hydrated electron, the existence and properties of which were predicted by Platzman[1]. It was first observed (Fig. 1) in a de-aerated 0·5 molar solution of sodium carbonate, but has since been found with somewhat lower intensity in pure de-aerated water (Fig. 2). It is strong in solutions containing sodium sulphate, hydroxide or chloride, but is absent in sodium bicarbonate or formate. Solutions of lithium and potassium carbonate and chlorides also give some degree of enhancement. In each of the solutions referred to, and in pure water, essentially the same band appears, with small differences of detail which are probably not significant. We have found the band in neutral and in alkaline solutions, but if it is present at all in strongly acid solutions it is very weak. A low concentration of dissolved oxygen reduces the absorption.

The experimental arrangement consisted of a 6-cm long all-quartz cell with a thin window on one side through which the electron beam entered. A dose of some 4–10 k.rads was given in a 2-μsec pulse. The absorption spectrum was taken on a Hilger intermediate quartz spectrograph (*E*.486) using Kodak III. scientific plates and a spark light source with a time duration of some 4 μsec (ref. 2). When the spark was triggered simultaneously with the electron pulse the absorption band illustrated in Fig. 1 was present. More than half this absorption had vanished in a delayed spectrogram taken 10 μsec after the pulse, but traces were still detectable for more than 20 μsec.

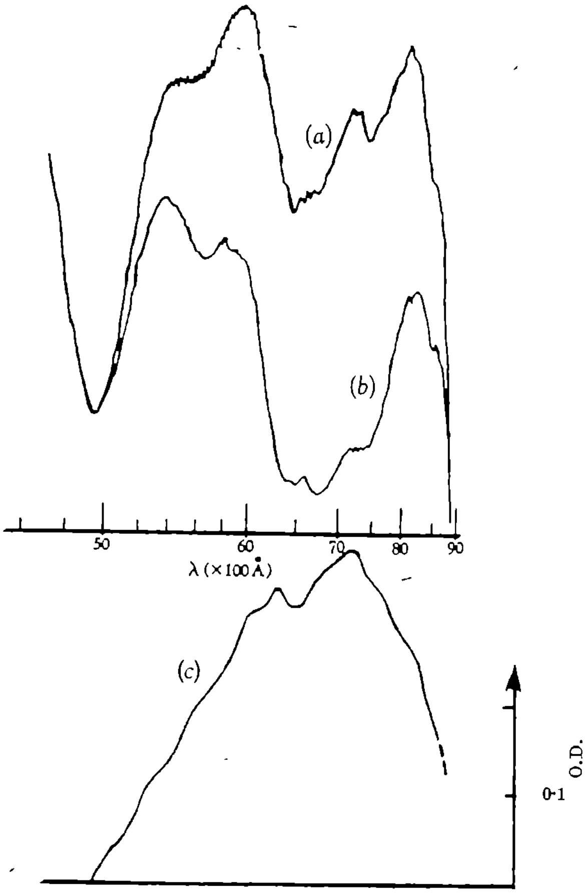

Fig. 1. Transient absorption in 0 5 M aqueous solution of sodium carbonate. *a*, Densitometer trace of part of a spectrogram taken through unirradiated solution (the peaks are due to the sensitizers in the emulsion); *b*, densitometer trace of same part of spectrum taken simultaneously with a 2 µsec electron pulse (*c*. 4 k.rads); *c*, difference curve

The presence of solvated electrons in solutions of the alkali metals in liquid ammonia, in methylamine or in ethylamine has been recognized for many years[2]. Evidence for the existence of trapped electrons in these liquids is provided by the form of their optical absorption spectra[4] as well as by their high conductance[5], paramagnetic susceptibility[6] and electron spin resonance spectrum[6,7]. The optical absorption spectrum of the solvated electron in liquid ammonia is a broad band with a peak at 14,500 Å (ref. 3) and in pure methylamine it is a broad band with a peak at 7000 Å (ref. 8). In mixtures of methylamine with ammonia the peak at 7000 Å appears when the percentage of methylamine in the mixture exceeds 70 per cent[9].

We prepared aqueous solutions of ammonia of various concentrations, and observed the absorption spectra when these were exposed to a pulse of some 10 k.rads of 1·8 MeV electrons. For ammonia concentrations of 1·2 molar or lower we found the absorption band characteristic of water, but in a 12 molar solution the absorption increased continuously to the limit of the emulsion sensitivity at 8800 Å, without showing a peak (Fig. 3). It resembled closely the tail of the absorption spectrum of solvated electrons in liquid ammonia, although the proportion of ammonia to water molecules in our solution was only about 1 : 4.

Since the transient spectrum we have observed is produced by the irradiation of pure de-aerated water, is affected in intensity but not in character by the presence of various alkali metal ions and various anions, is suppressed by oxygen, is of short life, and is a broad band resembling the spectrum of the solvated electron in liquid ammonia, methylamine and ethylamine, we believe that it is due to the electron trapped in a similar manner in water, as predicted by Platzman[1].

The difference curves in Figs. 1–3 were obtained after bringing the two densitometer traces arbitrarily into coincidence at 5000 Å, where the emulsion sensitivity has a minimum. This expedient was adopted in order to

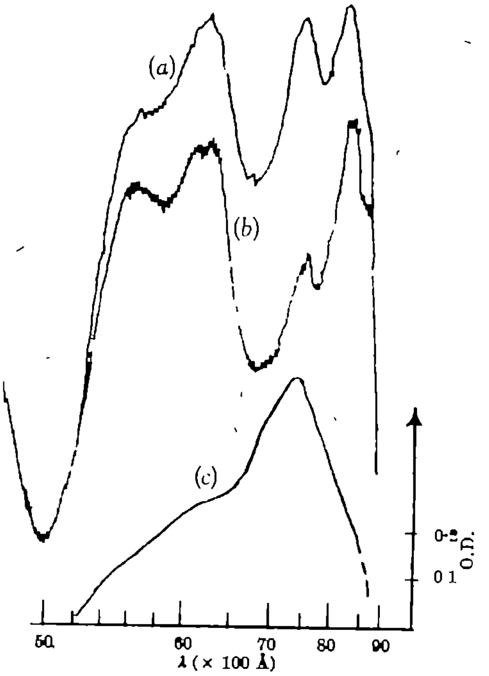

Fig. 2. Transient absorption in pure de-aerated water. *a*, unirradiated water; *b*, simultaneous with 2-µsec electron pulse (*c*. 10 k.rads); *c*, difference curve

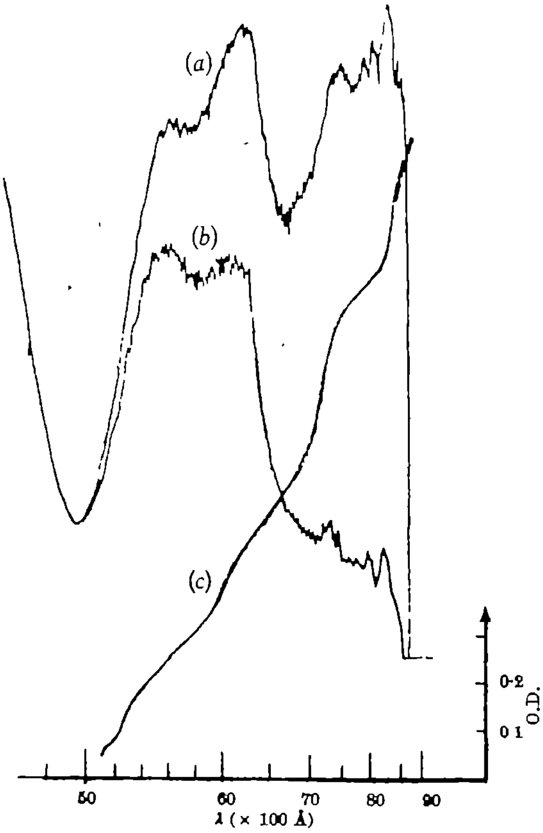

Fig. 3. Transient absorption in 12 M solution of ammonia in water. *a*, unirradiated solution; *b*, simultaneous with 2-µsec electron pulse (*c*. 10 k.rads); *c*, difference curve

eliminate from the comparisons small variations in total light output between successive sparks. The absorption band has, however, a long tail which extends throughout the visible region of the spectrum.

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Optical Absorptions in Irradiated Water

Boag and Hart (preceding communication) have observed a short-lived optical absorption band in water and various aqueous solutions irradiated with 2 μsec pulses of fast electron radiation. They attribute this band, which has a peak at ~ 7000 Å, to the presence of 'hydrated' electrons. In their experiments a light flash and spectrograph were used to produce a photograph of the absorption spectrum. I have used the complementary technique with a steady light source and photocell to obtain a cathode-ray oscilloscope trace showing the absorption as a function of time at any chosen wave-length. In this way more detailed information is obtained concerning the lifetimes of short-lived species but absorption spectra can be investigated only by repeated measurements at a series of wave-lengths. The apparatus has already been described and reference made to the existence of optical absorption in pure water[1,2].

The present communication describes some of my observations with water in more detail. The results obtained were the same in distilled water and in water redistilled from alkaline permanganate in silica. Also, the irradiation vessel was long enough to prevent irradiation of the optical end-plates by scattered electrons from the beam. It is believed, therefore, that the results can only be due to radiation effects in water and their modification by the presence of dissolved gases. De-aerated water was produced by bubbling argon for 24 h in the irradiation apparatus immediately before the exposure. The dose per pulse was ~ 5,000 rads and the optical path in the irradiated volume ~ 2 cm.

During the 2-μsec pulse Čerenkov light was produced in the water and under some conditions increased the apparent transmission above 100 per cent. This is shown in Fig. 1 for aerated water at 3100 Å. In general, the deflexion during the pulse is a combination of loss in light due to absorbing substances produced by the radiation and gain in light from the Čerenkov effect. The true drop in transmission can then be obtained by comparing traces obtained with the lamp shutter open and closed, the latter giving the amplitude of the Čerenkov light alone. In the case of Fig. 1 identical deflexions were obtained, thus showing the absence of detectable amounts of absorbing substances during the pulse itself. The blurring and brightness modulation on the trace are due to electrical interference from the linear accelerator, but its general shape shows that the beam intensity was reasonably constant during the pulse. Immediately after the pulse the transmission is 99 per cent and it then returns slowly towards 100 per cent, showing the presence of transient absorbing substances. This was clearer with increased amplifier gain[1] and has been found to consist of two components with half-times of ~ 10 μsec and ~ 1,500 μsec. These two components were only present in the ultra-violet beyond 3500 Å and measurements at a series of wave-lengths showed that they had similar (but not identical) absorption bands. No change in the faster component was produced by oxygenating or by de-aerating but the slower component was enhanced by oxygenating and was absent in de-aerated samples. At 5430 Å the Čerenkov radiation was much weaker and with aerated water a rapid drop in optical transmission to 95·8 per cent (after correction for Čerenkov light) occurred at the beginning of the pulse followed by recovery to 100 per cent immediately after the pulse. This is shown in Fig. 2. In de-aerated water at this wave-length the drop in transmission was five times greater and had a slower rise and fall (Fig. 3). In oxygenated water using increased amplifier gain it was found to be five times smaller and had the same flat base as in the aerated samples. The

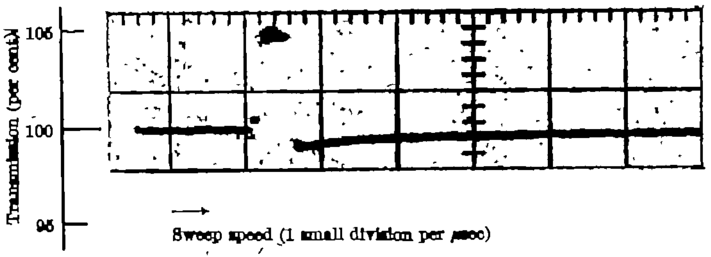

Fig. 1. Optical transmission of aerated water at 3100 Å before, during and after a 2 μsec electron pulse. Čerenkov emission occurs during the pulse and a small optical absorption immediately afterwards

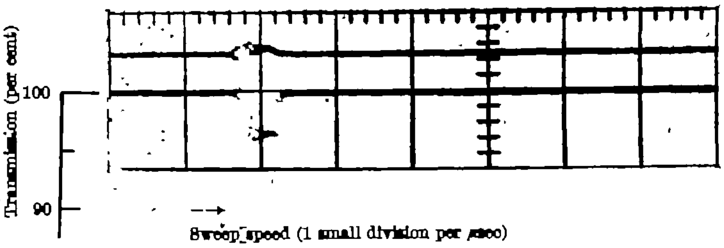

Fig. 2. Lower trace: optical transmission of aerated water at 5430 Å before, during and after a 2 μsec electron pulse. Upper trace: separate sweep with light shutter closed showing Čerenkov emission only

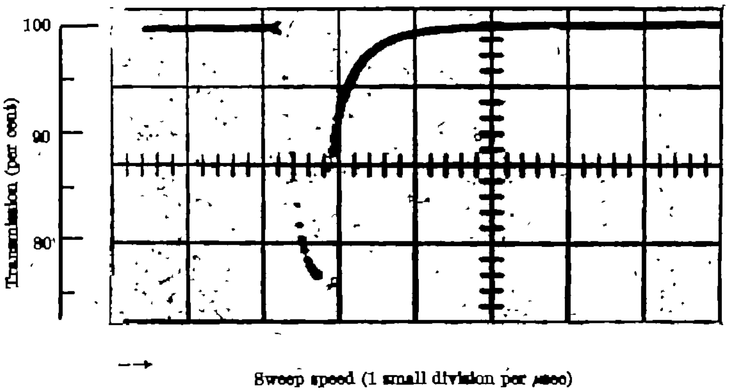

Fig. 3. Optical transmission of de-aerated water at 5430 Å before, during and after a 2 μsec electron pulse

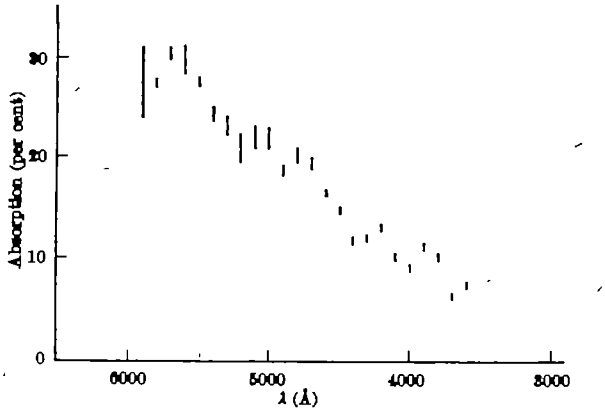

Fig. 4. Absorption spectrum of de-aerated water immediately before the end of a 2 μsec electron pulse

absorption spectrum was independent of dissolved oxygen (apart from amplitude) and is shown in Fig. 4 for de-aerated water. The vertical lines are equivalent to 1 mm on the oscilloscope screen. They do not include errors due to variation of dose from pulse to pulse but these were usually small as was shown by separate measurements on individual pulses with an integrating secondary emission chamber. The spectrum is not inconsistent with the band observed by Boag and Hart and may therefore be due to the same absorbing substance. Lack of photocell sensitivity made observations difficult beyond 6000 Å and the position of the absorption maximum could not therefore be established.

These results suggest that an absorbing substance with the spectrum of Fig. 4 is produced at a rate proportional to the initial slope of Fig. 3 (– 18 per cent per μsec) when water is irradiated with an ~5,000 rads pulse of fast electrons. If this substance does not react with dissolved carbon dioxide or nitrogen then it presumably reacts with oxygen to reach equilibrium concentrations corresponding to the 4·2 per cent drop in transmission shown in Fig. 2 in the case of air saturated water ($0·3 \times 10^{-3}$ molar oxygen) and the five times smaller drop observed in the case of oxygen saturated water ($1·4 \times 10^{-3}$ molar oxygen). From these figures the rate constant for the oxygen reaction can be calculated to be $1·5 \times 10^{10}$ l. mol^{-1} sec^{-1}. In de-aerated solution the recovery to 100 per cent after the pulse fits neither an inverse concentration plot nor an exponential. Thus removal of the substance in zero or very low oxygen concentration may occur by more than one reaction. Irradiation of 5×10^{-3} molar ferrous sulphate in 0·8 N sulphuric acid under the same conditions produced a drop in transmission at 3100 Å from 100 per cent to a steady value of 49 per cent after allowing 30 sec for the hydrogen peroxide reaction to go to completion. Comparison of this result with the initial slope of Fig. 3 gives a product of *G* value and extinction coefficient of 18,700 for the substance produced in water, taking *G* (Fe^{+++}) as 15·6, extinction coefficient (Fe^{+++}) as 2.100, and 2 μsec for the pulse-length.

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DISULPHIDE—SULPHYDRYL EXCHANGE IN DOUGH

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IT has long been recognized that the protein complex, gluten, is the principal component responsible for the visco-elastic properties of doughs made from wheat flour. Sullivan *et al.*[1] suggested that bromate or persulphate, the mild oxidizing agents used as dough 'improvers' by the baking industry, exert their action by causing the formation of additional protein disulphide bonds. More recently, Goldstein[2] examined the effect of oxidizing and sulphydryl blocking reagents on the rheological properties of dough and postulated an interchange of disulphide and sulphydryl groups which would account for the relaxation with time of induced strains in doughs and the failure of these strains to relax when dough had been treated with such reagents. Frater *et al.*[3,4] and Mecham[5] have elaborated this theory and have postulated that the strength of a dough is related to the number of intermolecular disulphide bonds in gluten and that relaxation of strains in dough is a function of the rate at which 'available' or 'accessible' sulphydryl groups exchange with disulphide bonds.

The experimental evidence for such sulphydryl–disulphide interchange in dough was lacking until recently, when McDermott and Pace[6] made use of the hydroxyproline-content of thiolated gelatin to demonstrate that, on mixing, thiolated gelatin becomes bound to gluten proteins.

In order to examine the chemical interactions which have taken place it is useful to separate the components of dough after mixing. It was felt undesirable, for several reasons, to use exhaustive solvent extractions for this purpose, and therefore a technique of dough fractionation which involved no extractants or diluents was developed. The dough, after mixing and resting, was subjected to preparative ultracentrifugation for 1 h at 30,000 r.p.m. (Spinco model *L*, head 30) and 30° C. This process was found to resolve dough into 5–7 distinct layers depending on the previous dough treatment. The main layers are, in ascending order of sedimentation rate, a lipid layer, a viscous, amber, dough liquor, a damaged-starch layer, a main gluten layer and a granular starch layer. The chemical composition of the principal layers is to be reported elsewhere.

It should be pointed out that only one of these layers, namely, dough liquor, which contains the components of dough soluble in dilute salt-solution, can be directly obtained uncontaminated by other layers. The remaining layers all contain dough liquor to a varying extent.

An examination of the incorporation of ^{35}S-cysteine and ^{14}C-leucine into the proteins of dough has been made by following the recovery of labelled compound in dough liquor, and the results are summarized in Table 1.

50 g of wheat flour were mixed with 31·7 ml. of salt solution containing the appropriate concentration of

Table 1. PERCENTAGE OF ADDED COUNTS IN FRACTIONS

Experiment	Dough treatment		Dough liquor	Dough liquor non-dialysable	Dough liquor, dialysable		Gluten
	Mixing time	Resting time			PTA sol.	PTA insol.	
I	3·5 min.*	60 min.	96	0	86	10	4
II	3·5†	60	76	11	35	30	24
III	3·5†	200	68	12	31	25	32
IV	60†	60	60	4	27	29	40
V	3·5 min. (under N_2)†	60	71	21	43	7	29

* 1 μmole ^{14}C-1-DL leucine. † 0·5 μmole ^{35}S-cysteine.

labelled compound. After resting, the dough liquor was separated by ultracentrifugation.

Preliminary experiments with ^{14}C-labelled leucine established that the isotope was present in dialysable form in the dough liquor in almost the expected concentration, indicating that only 4 per cent of ^{14}C-leucine had become bound to the non-dialysable components of dough (Table 1, Exp. I).

When 0·5 μmole of ^{35}S-cysteine (52 mc./m.mole) was added to dough during mixing, 24 per cent of the added isotope was bound to gluten and 11 per cent to the soluble proteins of dough liquor (Exp. II).

Dialysis of a suspension of the gluten or soluble proteins against 8 M urea containing 0·5 M sodium chloride, did not remove the isotope. It was removed, however, by dialysis against 8 M urea containing 0·25 M sodium sulphite, or by performic acid oxidation, suggesting that the ^{35}S-cysteine was bound to protein through disulphide–sulphydryl interchange according to reaction 1, or (after oxidation to cystine) by 2, or by oxidation via reaction 3:

$$\text{Protein S—S—protein} + \text{Cy}\overset{*}{\text{S}}\text{H} \rightleftharpoons \text{Protein S—}\overset{*}{\text{S}}\text{Cy} + \text{Protein SH} \qquad (1)$$

$$\text{Protein SH} + \text{Cy}\overset{*}{\text{S}}\overset{*}{\text{S}}\text{Cy} \rightleftharpoons \text{Protein }\overset{*}{\text{S}}\text{—}\overset{*}{\text{S}}\text{—Cy} + \text{Cy}\overset{*}{\text{S}}\text{H} \quad \text{(Hird and Frater}^{7}\text{)} \qquad (2)$$

$$\text{Protein SH} + \text{Cy}\overset{*}{\text{S}}\text{H} + \tfrac{1}{2}\text{O}_2 \rightarrow \text{Protein S—}\overset{*}{\text{S}}\text{Cy} + \text{H}_2\text{O} \qquad (3)$$

Half the dialysable radioactivity of dough liquor was present as phosphotungstic acid-precipitable material and appeared to be either cystine or other low molecular weight disulphides, suggesting that some oxidation of cysteine to cystine does occur.

The disulphide content of 50 g of flour was found by amperometric titration with methyl mercuric nitrate to be 480 μmoles, the total sulphydryl 63 μmoles, and the dialysable sulphydryl groups (both endogenous and that added) initially present was calculated to be 12 μmoles; thus, despite the high ratio (nearly 40 : 1) of protein disulphide to dialysable sulphydryl groups, the reaction does not go to completion.

The reaction of ^{35}S-cysteine with dough proteins appears to be far from equilibrium since the reaction constant (calculated for reaction (1)) was found to increase linearly with increasing cysteine-levels. Furthermore, increased incorporation of the isotope into gluten, but not the dough liquor proteins, occurred with increased times of both mixing and resting (Exps. III and IV).

As indicated by reaction 3, it is possible that cysteine may be bound to gluten by oxidative formation of disulphide bonds with protein sulphydryl groups. However, mixing under nitrogen resulted in a greater incorporation into the proteins of dough. If incorporation were oxidative in nature, less, not more, ^{35}S-cysteine would be bound to protein under these conditions (Exp. V). While oxidative incorporation remains a possibility, disulphide–sulphydryl interchange appears to play the major part. The relative importance of reactions 2 and 3 cannot easily be determined, but ^{35}S-cystine is incorporated to a lesser extent than cysteine.

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UNEQUAL BILATERAL SODIUM EXCRETION IN SHEEP WITH AN EXTERIORIZED URETER

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A METHOD of chronic unilateral urinary diversion in the sheep by means of a cutaneous ureterostomy is being developed in this laboratory. The nine animals so far studied all showed the odd characteristic of excreting more sodium through the operated than the intact ureter, the magnitude of the effect being illustrated in Table 1. The excretion values in Table 1 were calculated from 24-h urine samples with the animals on a low sodium intake (about 20 m.equiv./day). Urine from the fistulated ureter (*U*) consistently contained more sodium than urine from the intact ureter and bladder (*B*); the ratio of excretion on the two sides (*U*/*B*) varied widely both within and between animals.

With the exception of two sheep (1780 and 1784) in Table 1, further observations on renal excretion were cut short by the intervention of permanent ureteral stricture. The two animals referred to were examined for several months after exteriorization of the ureter and the *U*/*B* ratio for sodium excretion in the late post-operative periods is shown in Fig. 1. Urine specimens (24 h) from the ureter and bladder were compared for sodium content. The points on the graph represent the average *U*/*B* ratio for the number of collections made during 10-day intervals. Fig. 1 shows that there was a distinct change in the *U*/*B* ratio with the lapse of time after the operation. Fluctuations in daily values were not so marked in the later periods, and the kidneys began to show a more equal excretion of sodium 3–4 months after ureteral surgery. Increasing the sodium intake by 200 m.equiv./day did not, as Fig. 1 shows, affect the *U*/*B* ratio for sodium excretion.

The cause of the divergent handling of sodium by the two kidneys is unknown and is not to be found in the more obvious processes that might feasibly affect the appearance of sodium in urine. During the time the sheep were exhibiting the effect shown in Fig. 1, there was nothing to suggest from other observations that the kidneys were grossly different. Functional comparison of the separate kidneys showed that they were similar with respect to glomerular filtration (creatinine clearance), effective renal plasma flow, tubular secretion of *p*-aminohippuric acid, urea clearance, potassium and hydrogen ion excretion and the ability to elaborate hypertonic urine. The dis-

Table 1. BILATERAL SODIUM EXCRETION. URINE FROM THE EXTERIORIZED RIGHT URETER (U) COMPARED WITH SAMPLES FROM THE INTACT BLADDER (B)

Sheep No.	No. of daily bilateral urine collections	Sodium excretion calculated from 24-h samples (μequiv./min) *U* range	*B* range	Sodium excretion ratio (ureter/bladder) Range	Average
1784*	40	0·40–22·4	0·08–2·9	1·7–48·0	10·5
1780*	18	0·42–46·2	0·1–12·2	1·6–22·9	4·6
1782	17	8·1–58·6	0·7–22·6	1·5–12·8	6·2
1171	13	0·9–6·3	0·1–1·3	3·3–27·0	12·5
1776	12	2·3–12·5	0·2–2·0	3·3–22·4	10·2
1194	12	0·5–19·4	0·07–2·1	2·2–70·5	8·1†
1773	5	1·1–14·5	0·02–0·7	10·0–103	19·9†
1783	2	8·5–11·1	0·6–2·5	3·4–18·5	11·0
1777	1	14·8	3·3		4·5

* Further data in Fig. 1. † Highest value excluded.

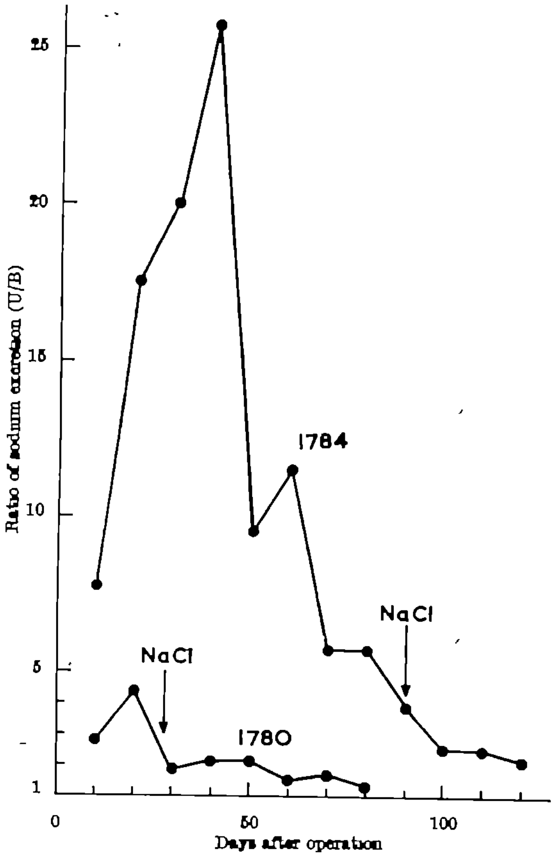

Fig. 1. Ratio of sodium excretion by the separate kidneys in two sheep (1780 and 1784) with explanted right ureters. Values were calculated from daily urine samples; sodium intake increased from 20 to 200 m.equiv./day at the points indicated

proportionate excretion of sodium occurred when the urine was sterile and free of protein.

In the first few days after the operation, all the animals except one experienced occasions when urine-flow from the fistulated ureter was temporarily obstructed, often by small blood clots, for an hour or more. The effect of such obstruction on electrolyte excretion in the sheep is unknown. Transient ureteral occlusion in the dog does not cause an increase in sodium on the obstructed side[1], although clinical studies in man[2] have revealed abnormal renal losses of sodium and water following the relief of complete obstruction of the urinary tract.

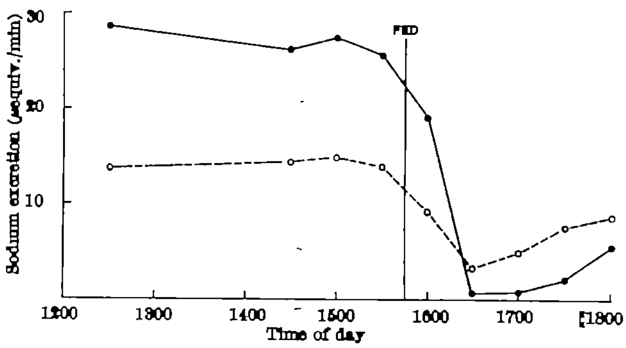

Fig. 2. Postprandial decrease in sodium excretion showing comparison of samples collected from the exteriorized ureter and the bladder; sodium intake 200 m.equiv./day (sheep 1780). ○, Bladder; ●, ureter

Despite the chronic nature of the effect, the kidney on the side of the exposed ureter did not show the renal abnormalities characteristic of salt-losing conditions such as adrenocortical insufficiency or salt-losing nephritis. Total renal excretion did not exceed dietary intake so that the development of sodium deficiency was unlikely. Furthermore, in some circumstances the suspect kidney was remarkably efficient at conserving sodium, clearly demonstrating that it did not continuously excrete salt entirely regardless of, physiological needs. The act of feeding was found to elicit numerous changes in renal behaviour, one of which was a sharp post-prandial fall in sodium excretion. Fig. 2 shows that the kidney with the exteriorized ureter was readily able to respond to this normal stimulus to conserve sodium.

From these observations on the sheep it is concluded that chronic unilateral ureteral fistulation, accompanied by early temporary obstruction, may disturb the ratio of sodium excretion in the two kidneys without obviously affecting other aspects of renal function.

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INTERACTION OF MECHANISMS CAUSING EYE AND EYELID MOVEMENT

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THE eyelids may be considered to be an auxiliary visual apparatus. Vision requires that the eyelids be kept open; conversely, it is interrupted when the eyelids are closed as in blinking, voluntary eye closure and closure at the onset of sleep. Also the eyelids tend to follow the vertical movements of the eyes. Thus, there are two modes of interaction between the eyes and the eyelids; one associated with closure, the other with eye 'following'.

Past work has shown that different mechanisms subserve these two functions. The mechanism of blinking described by Gordon[1] and Bjork and Kugelberg[2] consists of a brief activation of the palpebral orbicularis oculi with simultaneous inhibition of the levator palpebrae superioris. Visual following differs in that no active mechanism has been found to cause a lowering of the upper eyelid. The only muscular change detectable is a gradual decrease of levator palpebrae activity as the lid moves down.

The two functions, blinking and visual search, have been shown to be mutually inhibitory. Thus, during a demanding visual task, blinking is reduced in frequency[3,4], and, conversely, blinking occurs when the task is interrupted or ceases[5]. In the course of experiments on eyelid reaction during simple visual tasks (fixation of a spot of light), Glaser and Kennard[6] observed that blinking occurred at points of relaxation of attention.

We describe here an interaction between blinking and vertical following movements of the eyes and lids. Such an interaction has not been described previously. Movements of the visual apparatus in the vertical plane influence the frequency and form of blinking. Conversely, blinking is integrated into the normal movements of eyes and lids.

These two patterns of movement—lid closure (blinking) and vertical lid following—interact and provide interesting but complex examples of nervous integration.

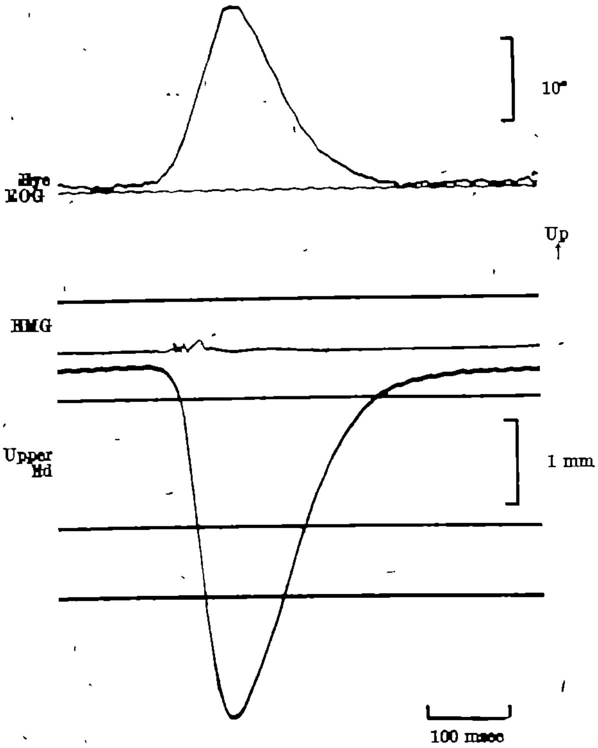

Fig. 1. A blink. The record shows simultaneous traces of the upper lid, the position of the eye determined electro-oculographically (EOG), and the electromyogram (EMG) of the upper palpebral orbicularis oculi muscle

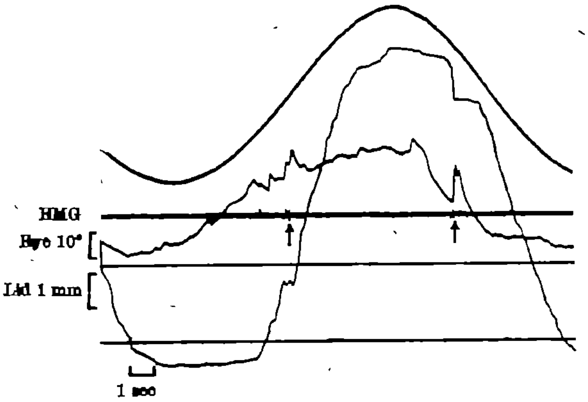

Fig. 2. Eye and upper lid movements during vertical visual tracking of a moving spot of light. Two miniature blinks occur (arrows). On the downward tracking movement the miniature blink is simultaneous with a downward lid saccade. On the upward movement a miniature blink is simultaneous with a hesitation in the lid record

The subject was required to fixate a spot of light moving in the vertical plane and following a variety of wave-forms —square step, sinusoidal and triangular. The eyelid movements were recorded with a photo-electric transducer[7] attached by means of a lever. Eye movements were recorded electro-oculographically. The electromyogram of the upper palpebral orbicularis oculi was recorded.

The simplest form of interaction observed was that between overt blinking and vertical tracking movements. Occasionally, during a demanding tracking task, blinks may not be completely inhibited, and fully formed but partial blinks occur during both the up and down movements. In common with other workers we have found the majority of blinks to be incomplete or partial, as the upper lid may stop considerably short of apposition with the lower lid. Such overt blinking may possibly be due to some source of irritation. In such circumstances evidence of facilitation is present in that the frequency of blinking is greater during downward movement than upward. We have measured such records and found the differences to be statistically significant. Sometimes simple inspection of tracking records alone suggests this fact.

However, as described here, when the visual task such as tracking is sufficiently demanding, overt blinks may be totally or very largely inhibited. Nevertheless, under such conditions evidence of blinking activity may still be present. These may be termed small or miniature blinks.

The eyelids perform the two types of movement that have been described for the eye, a smooth tracking motion, and a rapid step-like saccadic adjustment of position[6]. In executing these two types of movement the eyelids and the eye are found most often to act in unison. Thus saccades of the eye and lid occur simultaneously, though not always so. This is not the only pattern of interaction, as frequently lid saccades invoke a blink mechanism. It is understandable for this to occur during downward movement, since a downward saccade of the lid closely resembles in direction and velocity the active phase of blinking. Our records show some saccades to be associated with the typical blink movements of the eye. Thus, Fig. 1 shows that during a blink the eye moves up as the upper lid comes down. Fig. 2 shows that during downward tracking, downward lid saccades occur at the same time as upward eye rotation of 100–300 msec duration. The association with the blink is further supported by the electromyogram of the orbicularis oculi, which shows the characteristic activation of the initial phase of a blink. All grades of such blinking may be found during tracking, from the barely perceptible to the fully evolved blink.

In the course of upward tracking, such miniature blinks are also seen, but their effect on the lid is slight. They merely cause a hesitation or small notch in the record of lid movement (Fig. 3). Thus, there is mutual facilitation between the two similar functions in downward movement, although not in upward movement, and this may be considered a useful function.

Passing now to easier tasks, requiring lesser degrees of attention, a simpler pattern of interaction is found which probably represents the most elementary form of integration of these two mechanisms. Fig. 3 shows the record of the upper lid and eye obtained during upward and downward tracking of a spot of light moving slowly (3°/sec). The eye and lid follow the general motion of the target, but inspection of the record reveals superimposed minor fluctuations, which have a reciprocal relationship to each other. They can scarcely be termed blink-like or saccadic. During downward tracking, upward movements of the eye occur simultaneously with small downward lid movements. These may perhaps be considered the reverse of a blink. The record demonstrates a reciprocity between the movements of the two structures: a characteristic feature of the blink or closing action.

The central nervous mechanisms are worth discussing. The integration of the two movements probably takes

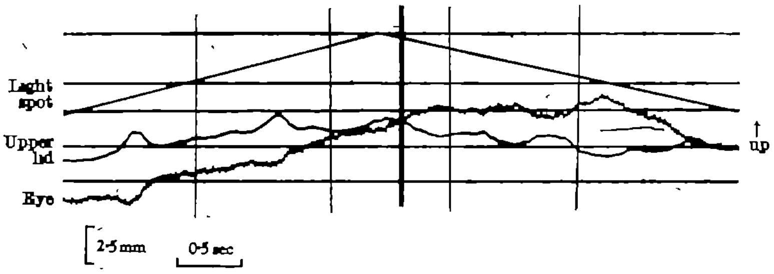

Fig. 3. Eye and upper lid movement during slow vertical visual tracking of a moving spot of light. The record shows a reciprocal relationship between the movements of the eye and lid, that is, small displacements of the lid are associated with eye displacements in the opposite direction

place at higher levels than the motor nuclei of the effector muscles, probably at the superior colliculus, which has been shown to be the nervous centre of integration of visual information and sensory data from the extra-ocular muscles. The eyelid muscles are probably to be included as extra-ocular.

We have shown that the graded mutual inhibition between the mechanisms of attention and blinking is modified by movements of the visual apparatus. Blinking is subject to phasic influence from the movements of the eyes and lids, downward movement being facilitatory and upward probably inhibitory. This interaction occurs at a centre such as the colliculus, where the neuronal fields overlap. The nervous excitatory state for blinking may be insufficient to bring about overt action, but activity of the same sign, such as downward movement, summates with it and then leads to the greater development of blinking both in amplitude and frequency. Future study of the nervous mechanisms in the responsible brain centres should provide interesting information about the performance of complex integration in the brain.

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THYMUS-LYMPHATIC NODES INTERRELATIONS FOLLOWING INJECTION OF FREUND'S ADJUVANT

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UNTIL recently it has been generally accepted[1] that the thymus lacks plasma cells and antibodies. However, Ross and Korenchevsky[2] described plasma cells in the thymus of rats treated with sex hormones. Marshall and White[3] have recently reported about the formation of antibodies, plasma cells and lymphoid follicles in the thymus of guinea pigs following direct administration of antigens, and Burnet *et al.*[4] have discovered lymphoid follicles in the thymus of mice of a strain with a hereditary and auto-immunological disease.

We have observed formation of numerous plasma cells and of lymphoid follicles along with epithelial glandular structures in the thymus of albino rats treated with Freund's adjuvant.

The adjuvant, prepared as described elsewhere[5], was administered to non-inbred rats at a rate of 0·1 ml. into each of four paws and 0·2 ml. twice beneath the skin of the abdomen. 5–15 rats were killed after 2–42 days and the lymphatic nodes, the thymus and spleen examined histologically (total, 100 rats). 48 h after administration of the adjuvant in half the cases conventional accidental involution was noted, varying in degree in the thymus and lymphatic nodes as characteristic of any stress[6]. Afterwards, hyperplasia and mass production of plasma cells progressed in the lymphatic nodes, at first in the regional and then in the distant ones, as previously described[7]. During the twentieth to thirtieth days epithelioid tubercules were also formed.

The thymus likewise increased in size, and during the fifteenth to twentieth days its average weight exceeded 1·5 times that of intact rats. The correlation between the cortical and medullary substance was normal or the thymocyte-rich cortical substance somewhat predominated. In most thymuses (particularly during the thirtieth to fortieth days) typical glandular structures were noted, such as epithelial tubes or cavities lined with epithelium (Fig. 1). These were localized mostly on the surface of the cortical layer or passed throughout the cortical layer to the medullary one. Less frequently they occupied 1–2 small lobes or were located in the interlobar connective tissue. They are usually connected with epithelial strands emerging from the surface of the lobe. In the lumen of these tubes homogeneous secretion can be noted. Drops of such secretion stained intensely with pyronine were noted in the cells of the epithelium of the tube walls themselves. Close to these structures groups of mature, transitional and immature pyroninophilic plasma cells are located (Fig. 2). The plasma cells form large groups sometimes in the cortical, particularly in the medullary substance. Moreover, in the medullary substance clusters of lymphocytes can be seen in the form of follicles, sometimes with light germinal centres and transitional cell varieties. In rats the germinal centres

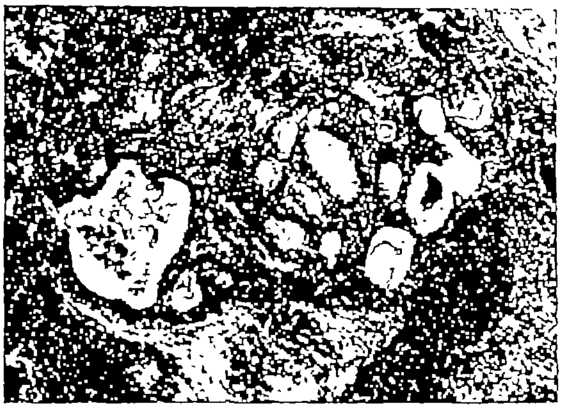

Fig. 1. Epithelial glandular structures (tubes) in the thymus of rat on the forty-second day following injection of Freund's adjuvant. (Stained with haematoxylin-eosin, × c. 52)

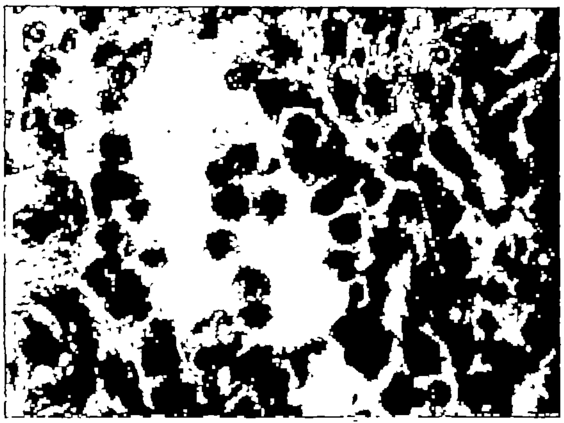

Fig. 2. Plasma cells in the rat thymus on the thirtieth day following injection of Freund's adjuvant. (Stained with pyronine-methyl green stain immersion)

are of very rare occasion, but we have observed typical germinal centres in the thymus of monkeys treated with a mixture of the brain with the adjuvant (Fig. 3). In the cortical substance of the rat thymus groups of eosinophils occur, and in the connective tissue, numerous mast cells.

In the thymuses of guinea pigs we have detected[5]; beginning with the seventh to tenth day after injection of the adjuvant, giant Hassal bodies infiltrated with polymorphonuclear leucocytes, and later on, increased development of the lymphoid cortical substance. This pattern is somewhat opposite to accidental involution.

Epithelial tubular structures have previously been found in the thymus of infantile rats[6], and in adults—during accidental involution[6], after administration of testosterone[8], fat[9], or DOCA along with accidental involution of the thymus but with hyperplasia of lymph nodes[10].

According to our results the increase of the thymus, of its lymphoid follicles, epithelial tubes and plasma cells invariably follows intensive plasma cell formation of many lymphatic nodes. A similar pattern was recorded in the thymus of pregnant non-inbred rats. In 'normal' rats with lymphatic nodes containing, if at all, but a few plasma cells no such changes were recorded in the thymus or there occurred single tubes without secretion as well as single pyroninophilic cells.

Large numbers of plasma cells in the thymus were always associated with secretion containing epithelial structures. There exists a definite functional interrelation between them. The secretion might possibly contain the hormone of cell maturation, of plasma cells in particular.

The extract of activated thymuses inhibited the development of epithelial structures in the rat thymus. Neither in intact nor in adrenalectomized rats did the extract significantly affect the production of Vi-typhoid antibodies.

In adrenalectomized rats administration of the adjuvant stimulated the appearance of plasma cells in the lymphatic nodes. Experiments on the role of adrenals in the activation of thymus are being carried out.

A quite opposite suggestion might also be made, namely, maturation of plasma cells might be attended by the formation of a substance which induces the development of epithelial structures and of the lymphoid elements of the cortex (according to the feed-back principle: lymphatic nodes–thymus). This suggestion has been tested experimentally.

It was found that the extract of lymphatic nodes abundant in plasma cells contains a substance (apparently of polynucleotide nature) which within 48–72 h stimulates in adrenalectomized rats increased reproduction of reticular cells in lymphatic nodes and the spleen and a drastic inhibition of reproduction and maturation of lymphoid and plasma cells in the lymphatic nodes and the thymus (there remain small lymphocytes-nuclei almost without cytoplasm). It will be noted that in the thymus not only do no glandular tubes appear but also its entire epithelial stroma is inhibited.

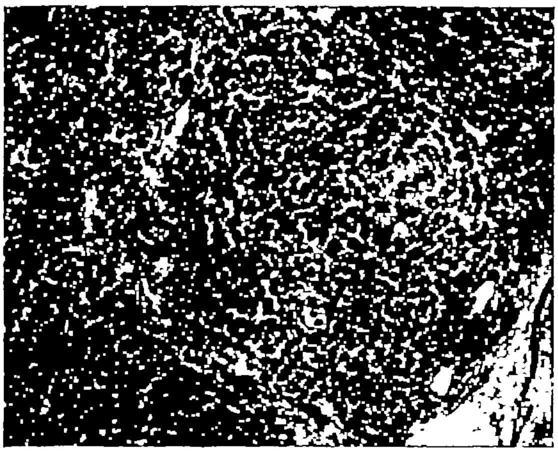

Fig. 3. Lymphoid follicles in the rhesus monkey thymus on the eighteenth day following injection of a mixture of Freund's adjuvant with the cerebral substance

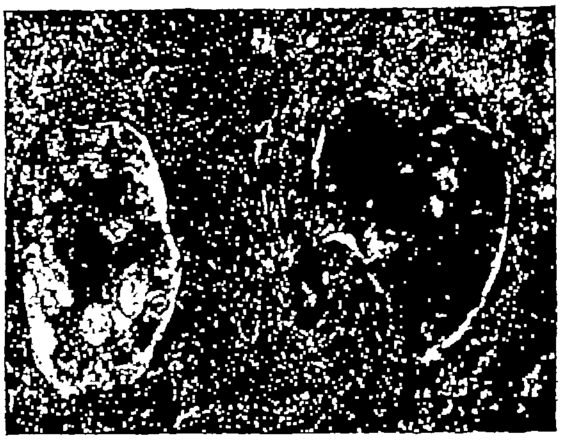

Fig. 4. Giant Hassal body infiltrated with polymorphonuclear leucocytes in the guinea pig thymus on the fifth day following injection of Freund's adjuvant

We suggest that in the thymus–lymphatic nodes system as well as in the system of all mesenchyme derivatives there undoubtedly exist manifold mutual hormonal interactions. In particular, maturation of some cells should be combined with release of substances which stimulate either directly or indirectly the reproduction of immature forms and inhibition of maturation. The study of these interrelationships is now being undertaken.

If the secretion of epithelial structures of the thymus stimulates maturation of plasma cells, mass formation of plasma cells should be associated with the formation of a substance which stimulates reproduction of reticular cells and inhibits reproduction and maturation of lymphocytes of plasma cells and the thymic epithelium.

All these tentative schemes are extremely simplified. In the light of modern evidence[11] as to the primary role of thymus in the development of immunologically competent cells and their repopulation into lymphatic nodes as well as the evidence given here, the question may be raised whether or not there might also occur a reverse repopulation of cells from lymphatic nodes into the thymus. Fontalin's experimental results[12] very likely suggest repopulation of immunologically competent cells from regional nodes into distant ones on conventional immunization.

It might be suggested that in the thymus there functions one of the general forms of information transmission from epithelial structures to the mesenchyme ones which results in the maturation of the latter.

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COMPARATIVE QUANTITATIVE MORPHOLOGY OF THE MAMMALIAN LUNG: DIFFUSING AREA

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THE relationship between structure and function remains a central problem for all students of Nature. Classical comparative anatomy is rich with examples of form uniquely adapted to some circumstance of life; but quantitative morphology from a comparative point of view has been only slightly exploited[1]. The lung is particularly well suited for quantitative study because from only a few elementary considerations of its function there emerge hypotheses about structure which can be tested easily.

Briefly, the lung's function is to exchange gas, and to this end it must meet certain conditions of ventilation and diffusion. Further, the volume of blood-flow through the lungs must be appropriately related, both to these conditions and to the systemic needs of the organism. In the following discussion we shall assume that cardiac output between species is roughly proportional to whole-body oxygen consumption and that the pulmonary capillaries occupy almost the entire surface area of the alveolar wall[2].

Among mammals there is a well-known relationship between body-size and metabolism[3]. The larger the animal, the greater is, in general, its total oxygen requirement; but when oxygen consumption is computed per unit of body-weight, the smaller the animal, the higher is the value.

The availability of oxygen to the organism is dependent on the quantity brought into the lungs per unit of time (ventilation) and, since oxygen is limited by diffusion, on the internal surface of the lungs. Ventilation need not be a function of the lung volume; but it is more reasonable to expect that it will be. Theoretically, it is possible to achieve any ventilation, in any sized lung, by changing the respiratory frequency, but this is most unlikely. Even moderately high frequencies are uneconomical because too large a volume is only washed in and out of the respiratory dead space. On the other hand, very low frequencies will demand a large tidal volume to achieve a given minute volume of ventilation, but in this case there is to be expected an optimal relationship between tidal volume and lung volume. If the tidal volume is too large a fraction of the lung volume, the elastic work which must be performed will be very costly, and furthermore, virtually complete replacement of lung air by fresh air with each breath will cause wide cyclic swings in the gaseous composition of alveolar air and the arterial blood. Conversely, if the tidal volume is too small a fraction of the lung volume, intrapulmonary mixing will be incomplete with each breath. Hence, some relationship between lung volume and tidal volume of ventilation is anticipated.

Intimately related to the lung volume is the consideration of its diffusing surface area. If the internal surface area of the lung is to be a function of the oxygen requirements there are two ways by which animals with different metabolic needs could achieve this relationship. When a larger diffusing surface area is called for, either the lung volume could be increased, or there could be further internal partitioning. The latter choice would indicate an increase in the number of alveoli per unit of lung volume in animals with a high metabolic rate. Therefore, the alveoli in such animals would be smaller than in those animals with a lower metabolic rate. On the other hand, if the first-named alternative were elected, small animals would require lung volumes disproportionately large for their body-size. Lung volume then would not be directly proportional to body mass but rather to body oxygen consumption, which is proportional to body-weight to the 0·74 power[4].

Except for alveolar measurements on a few species[5-8] there are only limited data with which these propositions can be examined. In the present work 26 representative mammals were examined in a search for general rules. The choice of animals included the extremes of body-size, those characterized by sluggish or vigorous locomotion; aquatic and terrestrial forms, and some that walk, swim or fly.

Metabolic data were either taken from the literature[9], or measured on resting unanaesthetized animals by standard techniques. Immediately after death the lungs were removed from the body and kept continuously inflated from a compressed-air source maintained at a constant pressure of 20 cm of water until dry fixation occurred[10]. Volume was then measured by a displacement method. Thin sections (about 100μ) of the surface of the lung were photographed under low-power magnification, enlarged prints made, and the internal surface area computed by the method of Campbell and Tomkeieff[11]. It was assumed for all species that 30 per cent of the area so measured

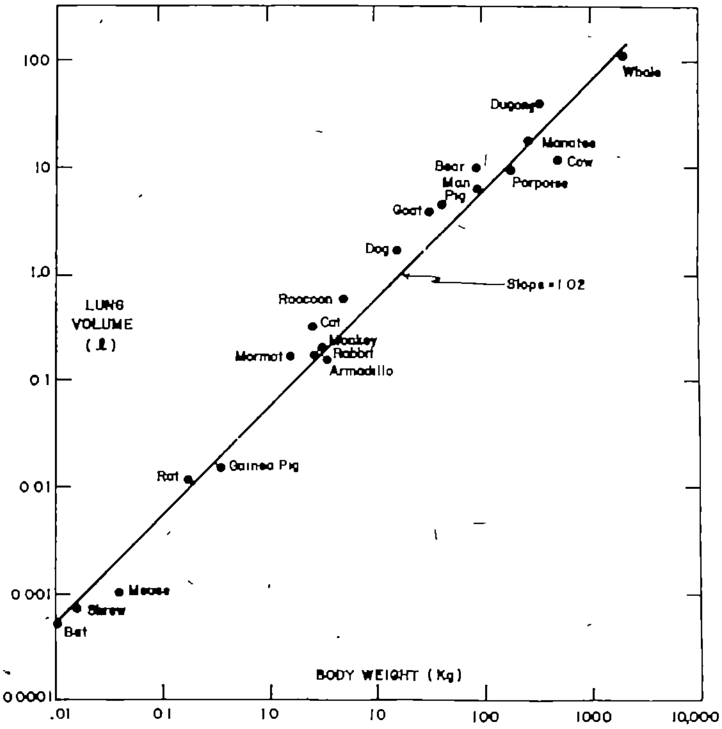

Fig. 1. Logarithmic plot of lung volume as a function of body-weight

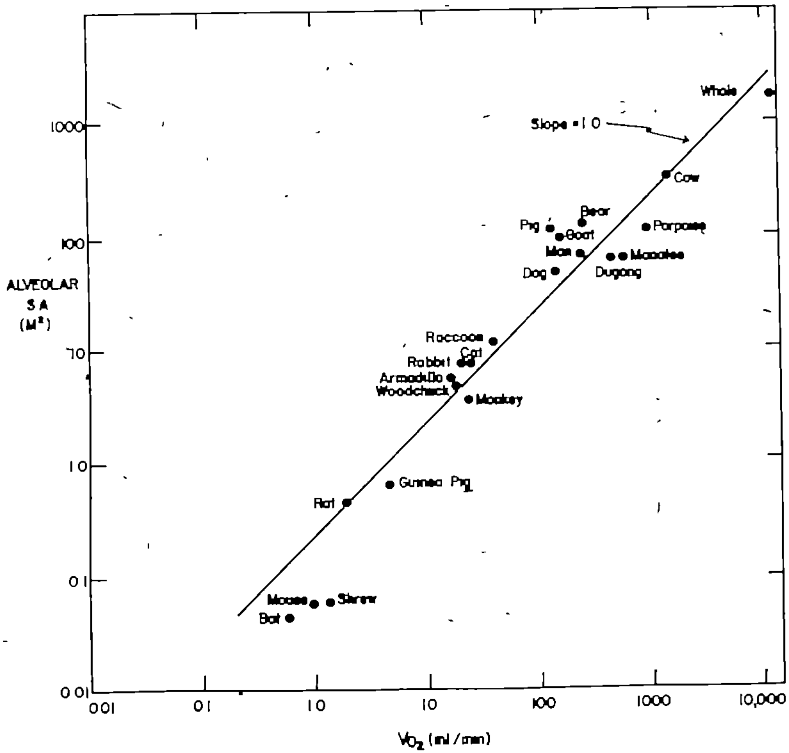

Fig. 2. Logarithmic plot of alveolar surface area a function of whole body oxygen consumption

was alveolar duct area, a fact known for certainty only in the human lung[12].

Fig. 1 illustrates the inter-specific relationship of the lung volume to body-weight. On a log–log plot the points approximate a straight line, and the slope is 1·02: Between species then the lung volume is proportional to the body-weight, and this has also been shown intraspecifically for the dog, cat and rat. Since metabolic rate correlates with body-weight to the 0·74 power it is clear that the lung volume is determined neither by metabolic rate nor body surface area, but is a constant fraction of body mass. But total ventilation is proportional to metabolic rate[4], and respiratory frequency correlates inversely with body mass to the 0·28 power[13]. Hence the tidal volume, which is the quotient of ventilation and respiratory frequency, is directly proportional to the body-weight and is thus a constant fraction of the lung volume.

The speculation about the relationship of the pulmonary diffusing surface area to the metabolic rate of the animal is confirmed to a striking degree (Fig. 2). The internal surface area of the lung correlates linearly and directly with the resting oxygen consumption over the entire range of mammalian size. A correlate of greater evolutionary significance would be maximal metabolic rate not resting; however, there is probably a proportionality between these two values[4].

It is important to note some exceptions to the general relationship between body-size and metabolic activity. The manatee, for example, has about the same body mass as the porpoise, but its metabolic rate per unit of body-weight is much smaller. The lung volumes of these animals are roughly comparable, but the lungs of the porpoise have a larger diffusing surface area. By contrast, a 170-kg manatee and an 80-kg man have about the same resting oxygen consumption and the internal surface areas of their lungs are of the same magnitude. However, the lung volume of the manatee is more than twice that of man. The mouse is larger than both the shrew and the bat, and its lung volume is proportionately larger. The resting oxygen consumption of the mouse is slightly less than the shrew and greater than the bat; and the intrapulmonary surface area is appropriately correlated.

Table 1. VARIATION OF MEAN ALVEOLAR DIAMETER WITHIN SEVERAL SPECIES. NUMBERS IN PARENTHESES REFER TO NUMBER OF ANIMALS EXAMINED. ALVEOLAR DIAMETER CITED ± 1 *S.D.*

Species	Alveolar diameter
Cat (21)	116·9 ± 14·4
Dog (8)	93·9 ± 14·1
Guinea pig (14)	65·4 ± 8·5
Mouse (6)	46·6 ± 2·4
Rat (17)	70·2 ± 6·6
Woodchuck (5)	88·3 ± 5·7

Since lung volume correlates with body-weight, but its internal (alveolar) surface area correlates with metabolic activity, then in animals with the highest metabolic rates there must be increased internal partitioning of the lungs, an arrangement which obviously demands decreasing alveolar dimensions. Table 1 indicates the intraspecific variability of alveolar dimensions in those cases where several animals were available for study. These values differ in no instance by more than two standard deviations from those of any published report[6–9], in spite of considerable differences of technique. By selecting appropriate examples from Table 1, alveolar size appears to be a function of animal size although the cat is clearly out of line. The general relationship emerges when alveolar diameter is plotted as a function of oxygen consumption reduced to unit of body-weight (Fig. 3). The slope of the best-fit regression line is 0·71 for this plot. Inspection indicates that alveolar size is not always related to body-size. The mean diameters of alveoli in whale, porpoise and man are of the same order of magnitude. Those of the racoon are larger than in the cow. The smallest alveoli are seen in the bat and the shrew, while the largest are seen in the dugong and manatee—sluggish animals of low metabolic rate but by no means the largest in the animal kingdom. The sloth lung contains the next largest alveolus. but its body-weight approximates that of a racoon. Those animals the metabolic activity of which is an exception to the body

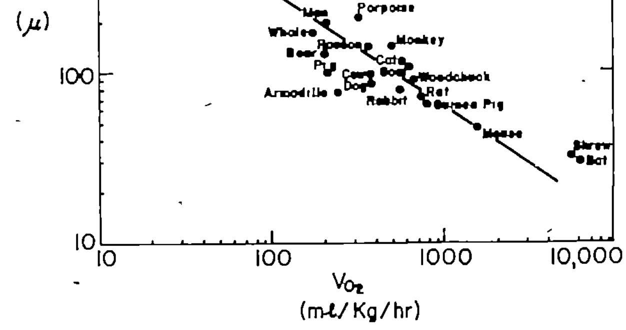

Fig. 3. Logarithmic plot of mean alveolar diameter in microns as a function of metabolic rate per unit of body-weight

surface area law remain consistent with the prediction of pulmonary diffusing surface area and alveolar size.

Summarizing, two animals of equal body-size will have lungs of the same volume, but if one has a higher rate of metabolism its alveoli will be smaller. Stated otherwise, the number of alveoli per unit of lung volume will be greater, the higher is the rate of metabolism. Or, two animals with the same oxygen consumption will have the same pulmonary diffusing surface area; but the (usually) smaller animal, with the higher rate of metabolism per unit of body-weight, will have a smaller lung and smaller alveoli. The absolute number of alveoli in the lung is clearly not a constant for all mammals, nor is alveolar volume proportional to body-weight as recently predicted[14].

The analogy between the respiratory and circulatory systems is of interest. Heart-weight is in general proportional to body-weight[4]; but capillary density in the tissues, where diffusion is important, is better related to metabolic activity[15].

Although these features are consistent with predictions based on optimal design for functional needs, the mechanism through which they are brought about is not clear. Whether or not changes can be induced in the lung's alveoli by environmental stress or whether the morphologic dimensions are fixed genetically and other functional adaptations meet changing physiological needs is a subject of considerable interest.

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A BLOOD-BORNE FACTOR INFLUENCING THE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM OF THE DESERT LOCUST

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THE recent reviews by Kennedy[1,2] have outlined the data available on the differences between the gregarious and solitary phases of locusts and show that until recently little work has been done on the nervous and sensory mechanisms underlying these differences. We have sought to investigate the role of the central nervous system in this context by examining its function in relation to phase differences in reproductive ability and locomotor behaviour. This work has led one of us (P. T. H.) to formulate the hypothesis that the main difference between locust phases is to be found in the manner in which information is utilized by the central nervous system[3].

It is suggested that there is, in fact, no great difference between the sensory receptors of locusts of different phases, that in any given stimulus situation to which both solitary and gregarious locusts are exposed the same information reaches the higher centres of the nervous system and that the observable differences in behaviour between phases results from the different utilization by them of this information. It is further postulated that this utilization is dependent on the influence of a hormone balance on the integrative and other centres in the nervous system; and a corollary of this is that such a hormone balance must be itself at least partially controlled by the sensory receptors and nervous system. For example, the appropriate hormone balance relating to the maintenance of the gregarious phase must depend at least in part on the continuous, quantitatively (and perhaps qualitatively) sufficient inflow of stimuli to the nervous system of an individual locust from its fellows and/or the environment; when such inflow is reduced or terminated by a reduction in locust density, as can happen in the field and can be artificially produced in the laboratory, it may be supposed that the hormone balance changes and it is certainly true that a phase change occurs.

There is now some slight evidence of such a quantitative effect which has been discussed elsewhere[3]. But to substantiate such a hypothesis it is first necessary to demonstrate that hormones capable of influencing the operation of the central nervous system in the expected manner and direction do exist in locusts, and secondly, that interference with the hormone balance alone of a locust of a given phase produces behavioural or other manifestations associated with the opposite phase.

It has already been explained elsewhere[3] why only behavioural and reproductive criteria are utilized in the investigations reported here as phase indicators; one reason is that behavioural differences between phases are most striking, and this particularly relates to the greater locomotor activity and enhanced sensitivity to a variety of stimuli of gregarious as opposed to solitary locusts. Such differences tend to disappear or to become minimal a few days prior to moulting, and since this is a time of great hormonal activity it seemed an appropriate point at which to commence investigations. In the preliminary work described here, all of which was carried out on the desert locust, *Schistocerca gregaria*, a crude estimation of the activity of the central nervous system was made by recording the electrical activity at various points through platinum or silver/silver chloride electrodes and a conventional a.c. amplifier and counting the impulse traffic on a 'Dekatron' counter or an impulse ratemeter.

It is easy to demonstrate[4,5] that the spontaneous locomotor activity of a mid-5th instar locust is higher than that of a 5th instar hopper 12 h before moulting; on the other hand, we found that the electrical activity in the nerve cord of the former is very much less than the latter, both as regards the number of fibres firing and the frequency of the impulses, both for intact animals and for excised nerve cords. To overcome this change of electrical activity near the moult, which, from other experiments, appeared to increase progressively for a few days prior to moulting, mature male *Schistocerca* reared at high density, approximately 4 weeks after their final moult, were used as experimental animals. The insects were opened ventrally and recordings made from various points on the cord, which was not de-sheathed, while the system was irrigated with hæmolymph from locusts and with other preparations. Locusts were also dissected under Hoyle's locust saline[6] and the excised nerve cord pinned in a shallow trough in a paraffin wax block and irrigated with the various solutions; the results described here were obtained with both types of preparation.

On applying the hæmolymph taken from a 5th instar hopper approximately 12 h from the moult to the nerve cord of the experimental insect, and recording at the commissure between the first and second thoracic ganglia, after a delay of between 30 and 90 sec there was a great increase in activity due to several large fibres firing frequently (Fig. 1*A* and *B*); the same result was obtained with at least twelve different animals. Furthermore, this reaction was observed when blood from a moulting 5th instar hopper of *Locusta migratoria migratorioides* was used. On the other hand, when the nerve cord was treated with the blood of a mid-5th instar hopper there was no marked increase in activity or at most a few fibres fired spasmodically. In all cases frequent washing with locust saline restored activity to the pre-treatment-level and the experiment could then be repeated. The factors in the hæmolymph of the moulting hoppers responsible for increasing nervous activity were found to act preferentially on the brain and on all thoracic ganglia; there was some suggestion that different sets of fibres were activated by treatment of these centres. However, no increase in activity could be induced in the ventral cord by treating the sub-œsophageal ganglion.

How can the increased general activity in the nerve cord found in locusts near the moult be reconciled with their greatly reduced locomotor activity at that time? Hoyle has demonstrated[7] that the extensor tibialis muscles in the metathoracic leg of *Locusta* are mainly controlled by fast motor neurones in 3*Nv*5; if the activity in this nerve is monitored a certain level of activity is observed (Fig. 1*C*). On bathing the metathoracic ganglion with blood from a moulting hopper this activity decreases markedly (Fig. 1*D*) but can be restored to its pre-treatment level by washing the ganglion with saline. The same result was obtained when the metathoracic ganglion was isolated from the rest of the nervous system. On one occasion this inhibiting effect was noted when the supra-œsophageal ganglion was treated with moulting blood, but no results were produced by treatment of other ganglia in the cord. As a control, blood from a mid-5th instar was used to bathe the metathoracic ganglion but produced no effect. Treatment of the central nervous system of *Schistocerca* with the blood of a moulting locust therefore produces differential effects, enhanced activity in the ventral cord and depression in the motor system; it seems likely that the latter effect is a synaptic one, but whatever its origin it could explain the reduction of locomotor activity associated with the moult.

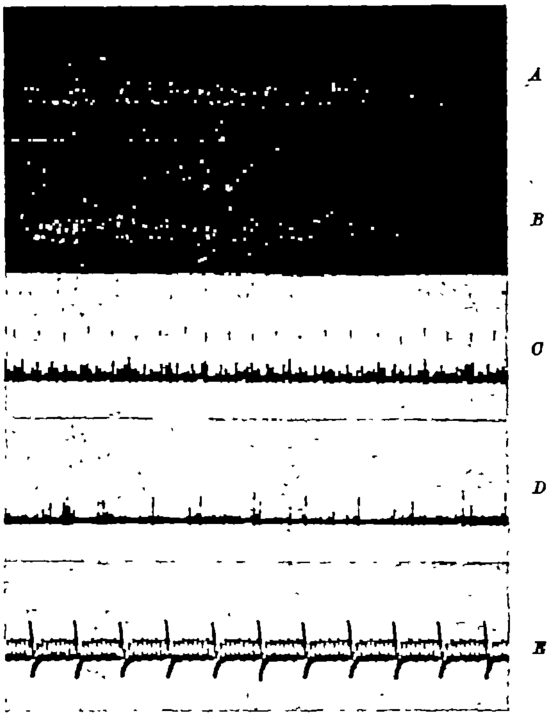

Fig. 1. Electrical activity in various nerves of adult *Schistocerca gregaria*. *A*, spontaneous activity in ventral cord between first and second thoracic ganglia; *B*, same preparation as *A*, 60 sec after treatment with hæmolymph of 5th instar hopper near the moult; *C*, spontaneous activity in 3*Nv*5 of metathoracic ganglion; *D*, same preparation as *C*, 60 sec after treatment with hæmolymph of 5th instar hopper near the moult, *E*, timing trace 1/10 and 1/100 sec

One of the processes which occurs in relation to the moulting of many insects, including locusts, is the production of the moulting hormone ecdyson by the prothoracic glands[8]. It was therefore of interest to see if this substance would bring about changes similar to those produced by blood from moulting hoppers. Accordingly, an extract from *Bombyx* pupæ, which contained a mixture of α- and β-ecdyson, was made up in locust saline in various concentrations and applied to the nerve cord in the same manner as hæmolymph. The results were identical: activity in the cord was increased while motor activity in the metathoracic motor nerve was reduced. There was some difficulty in establishing the minimum effective dose since any given solution of the hormone gave somewhat varied activity-levels, the minimum dose range being 20–100 μg of extract. Although at first sight this dose may seem high from the point of view of physiological levels, it is not unduly so since the extract used was impure and was a lepidopterous hormone; in fact, such levels are of the same order of magnitude as doses found necessary to initiate development in certain Lepidoptera[9].

These findings must be considered in relation to other observations. Carlisle[10] has pointed out that in locusts, unlike other arthropods such as Crustacea, there is no sharply defined 'inter-moult period', but that much of the time between moults may be occupied with physiological processes related to moulting. For example, in locusts, according to Malek[11], half-way through the instar a new pigmented layer has already appeared and has begun to separate from the old cuticle, and if this activity is regulated by prothoracic gland hormone it suggests a build up of concentration of the latter from mid-instar onwards. It is therefore interesting that Edney in his actograph experiments on spontaneous locomotor movement in fifth hoppers of *Locusta*[4] found that activity reached a peak half-way through the instar followed by a steep fall, and 24–36 h before the moult it had practically stopped. Hoyle[12] showed in both *Locusta* and *Schistocerca* hoppers that jumping ability declined progressively from the fourth day after moulting and attributed this to a partial neuromuscular block produced by the changes in ionic concentrations, particularly of potassium, in the hæmolymph which occur in relation to moulting; such changes would accentuate any synaptic block produced by the blood-borne factor. The effects of this factor on motor neurone activity clearly relate to the fall-off in locomotor activity in the later stages of the instar; on the other hand, the increased activity produced in the central nervous system itself may well be related to the intensification of certain physiological and growth processes also necessary at this time, when metabolism needs to be stepped up to cope with the laying down of the new cuticle and other processes involved in the moult. The volleys of impulses in the cord may, for example, be related to the intensification of respiration which normally attends a moult[13].

The foregoing suggests that in *Schistocerca* the prothoracic gland hormone is at least involved in processes which lead to differential effects on the activity of the central and motor nervous system; and the question may now be asked how this is related to phase differences. The link is provided by the finding of Carlisle and Ellis[14] that the prothoracic gland during nymphal life is larger in isolated than in crowded *Schistocerca*, and during adult life persists in the former at least until maturity, while in the latter it atrophies and disappears a few days after the final moult. This latter process is the typical course of events in adult insects since in all groups studied (except the Thysanura, which have an adult moult) the prothoracic glands degenerate after metamorphosis[8]. It is therefore to be expected that the ecdyson titre of the blood of an isolated locust would probably be higher under most circumstances than that of a crowded locust and that if it was used to irrigate a nervous system as in the experiments described here it should give similar effects to blood from a moulting insect; experiment verified this.

The evidence detailed here would constitute strong support for the hypothesis advanced earlier if it could be shown that behavioural differences existed between the phases of the desert locust which might have their origins in the neurohumoral mechanisms postulated and which could be altered in the expected direction by blood transfusion experiments. Now, although the exact significance of the reduction in motor nerve activity by the blood-borne factor is not clear, it might be expected that in isolated locusts this would have the result of reducing spontaneous activity or of raising the threshold reaction to a stimulus which could produce locomotor activity among crowded locusts. Haskell, Paskin and Moorhouse have described experiments[15] in which the locomotor reactions of hoppers of the desert locust to wind and olfactory stimuli were recorded; and Haskell and Roscow[16] have used the behaviour pattern described to determine the differences in behaviour between crowded and first, second and third generation isolated hoppers of *Schistocerca*. The results have been partially described elsewhere[3], and they confirm that isolated hoppers are less reactive or have higher thresholds than crowded hoppers when both are presented with identical stimuli. In fact, isolated hoppers for the most part could not be induced to move at all in response to stimuli which caused rapid oriented locomotor reactions in crowded hoppers; if the isolated hoppers did respond their speed of movement was lower than that of the crowded hoppers. If, however, the isolated hoppers were injected with blood from crowded hoppers then, about 24 h after the operation, they responded to stimuli to which previously they were unresponsive and also their speed of movement was increased.

Because of the time-lag before the appearance of the enhanced reactivity in these latter experiments it may be suspected that some metabolic or chemical process was involved which either removed, destroyed or stopped the production of the hormone; if it was merely that dilution of the solitary blood by the gregarious blood had reduced the concentration of the blood factor below the level at which it could inhibit activity the resultant increase in reactivity would be expected to become apparent much more quickly.

The converse experiment to that described here, that is, the injection of prothoracic gland hormone into gregarious locusts, has been carried out by our colleagues, Carlisle and Ellis (personal communication). They have injected ground-up prothoracic glands taken from crowded 5th instar hoppers of *Schistocerca* into crowded 4th instar hoppers and found that there was a significant decline in the locomotor activity of the latter which lasted for a few hours. In this case the comparatively rapid but transient effect of an increase in the prothoracic gland titre of the experimental animals argues a direct effect depending on concentration, agreeing with the experimental findings detailed here relating to the effect of ecdyson on the motor system in locusts, which can be contrasted with the slower reaction noted when solitary locusts were injected with gregaria blood.

Thus, while the evidence detailed here suggests that the hypothesis enunciated earlier in this article may be useful as a guide to the further work required, there is already in these preliminary findings a hint of the complexity which must obtain in this system.

We thank Prof. P. Karlson, of Munich, for supplying us with an extract of ecdyson.

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ULTRACENTRIFUGAL CONSTITUENTS OF HUMAN AND CANINE PROSTATIC FLUID

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THE biochemical characteristics of prostatic fluid can be described through a number of methods of investigation. For this purpose the electrophoretic patterns of human samples have been investigated, using both the filter-paper[1] and agar-gel[2] methods. As part of this programme of studies on the protein components of normal and abnormal prostatic fluid, preliminary investigations have now been made on such fluids by means of ultracentrifugal examination. Samples of human prostatic fluid were obtained by the standard urological procedure of rectal massage[3]. These were obtained both from normal individuals and those with prostatic disease. The canine samples were obtained through intravenous administration of 'Pilocarpine' to a dog in which the prostate and its urethra had been surgically isolated from the rest of the urological system. This involved the diversion of urine by connecting an isolated loop of ileum to the bladder and skin. The prostatic urethra was closed so that only uncontaminated fluid was collected on demand from the penile urethra, following the procedure described by Mason *et al.*[4].

The human sample was obtained from a 16-year-old boy (D. H.), with no evident signs of prostatic illness. The fluid was only slightly turbid and had a protein concentration of 1·12 per cent, as measured by the biuret method of Gornall *et al.*[5], using an extinction coefficient

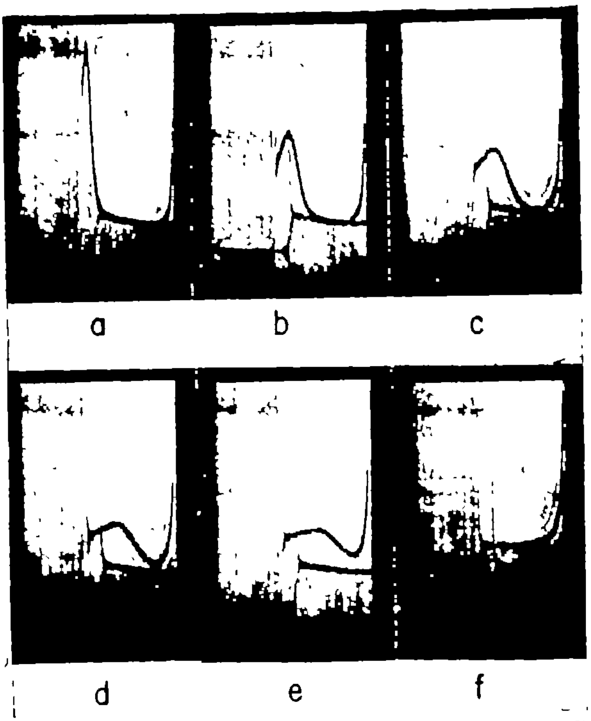

Fig. 1. Ultracentrifugal pattern of human prostatic fluid (undialysed). Concentration of protein, 1·12 per cent. Photographs at 12, 36, 60, 84, 100 and 156 min. Rotor speed, 42,000 r.p.m.

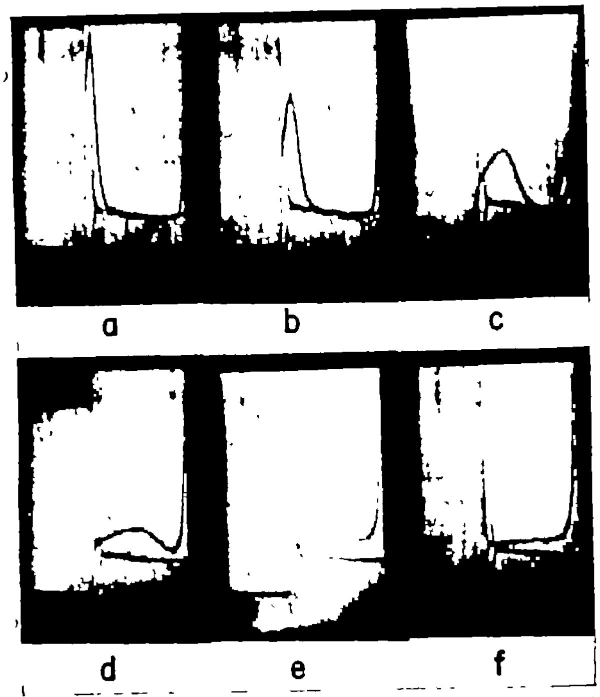

Fig. 2. Ultracentrifugal pattern of human prostatic fluid (dialysed). Concentration of protein, 0·80 per cent. Photographs at 12, 24, 60, 118, 150 and 214 min. Rotor speed, 41,200 r.p.m.

of 2·8, determined in our laboratory for pooled human serum. The volume was 0·5 ml. It was examined, without dialysis, in a first experiment in the ultracentrifuge, using a double-sector cell, with saline (0·15 M sodium chloride) in the solvent sector. Although the speed was thus limited to 42,000 r.p.m., with some consequent reduction in resolution of the components, it was felt preferable to have the advantage of the simultaneously photographed base line. In this way, even the most minor components would be clearly discernible. The schlieren patterns revealed a slowly sedimenting single boundary with a sedimentation coefficient, $s_{20,w}$, of 3·28 *S*. This value is subject to some error, since the sample had not been dialysed to provide a known solvent composition.

The pictures are illustrated in Fig. 1. There was only the slightest trace of a faster moving peak. It was not possible to measure a rate for it. After the peak had travelled far enough in the cell, one could see that a low plateau extended all the way back to the meniscus of the solution. This presumably represented a group of constituents with slower sedimentation rates. To make certain, however, that this did not actually represent some artefact resulting from the lack of dialysis preparation of the sample, a second sample of fluid was obtained from the same individual and was dialysed against 0·15 M sodium chloride. The sample, 0·7 ml., was again somewhat turbid. It was centrifuged for 10 min at 16,000 r.p.m. in a Servall refrigerated centrifuge, giving a small residue and a clear supernatant. The concentration of this sample was 0·80 per cent. In the ultracentrifuge it showed the patterns illustrated in Fig. 2. The picture is similar to that seen before, but the plateau region is somewhat less prominent. The small, faster peak is somewhat more conspicuous now, and its rate could be measured. The calculated sedimentation coefficients were 3·78 and 15·6 *S*.

Canine samples were also examined in the double-sector cell of the ultracentrifuge. The material for the first investigation was obtained intermittently for a period of 26 min. The collecting vessel was changed at various times during this procedure. It was of interest to follow the appearance and concentration of these successive samples in order to determine: (*a*) the magnitude of the initial concentration of protein in this type of prostatic fluid; (*b*) the degree to which this concentration falls as more of the sample is elicited, thus reflecting the extent of dilution of the material, as it continues to be produced (Table 1). The various samples in this group were only slightly opalescent in appearance. They were spun for 10 min at 16,000 r.p.m. in a Servall refrigerated centrifuge and gave a very slight residue. The supernatant liquids

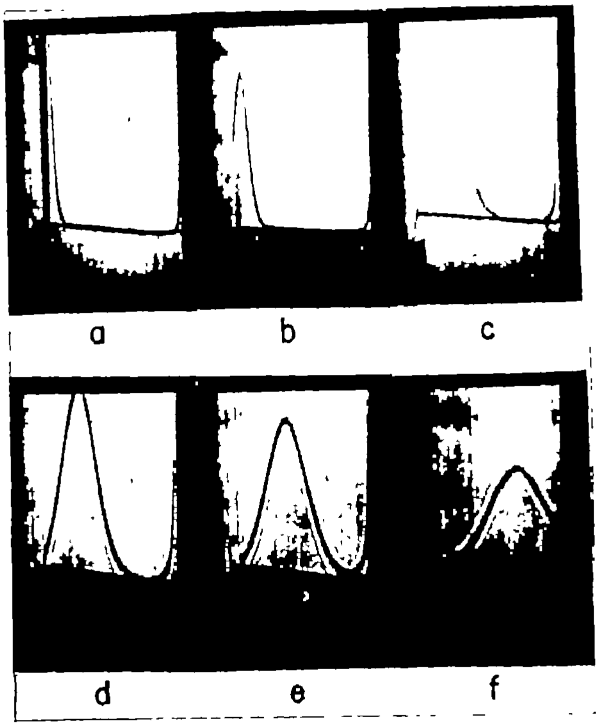

Fig. 3. Ultracentrifugal pattern of canine prostatic fluid (undialysed). Concentration of protein, 2·20 per cent. Photographs at 12, 28, 88, 120, 160 and 256 min. Rotor speed, 41,900 r.p.m.

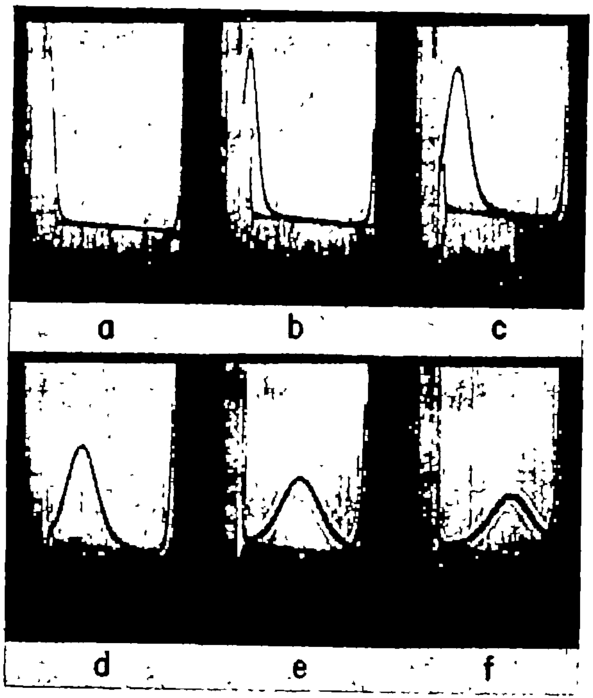

Fig. 4. Ultracentrifugal pattern of canine prostatic fluid (dialysed). Concentration of protein, 1·12 per cent. Photographs at 14, 34, 82, 156, 236 and 300 min. Rotor speed, 42,000 r.p.m.

were examined again by the biuret method and revealed no change in concentrations. Therefore, only one set of figures is given in Table 1. The first two portions of fluid were then pooled. A portion was examined directly in the ultracentrifuge, while the rest was set to dialyse against 0·15 M sodium chloride. The undialysed material was 2·20 per cent in concentration, and showed the patterns seen in Fig. 3. It is of interest to note the similarity to the human fluid. There is, however, no slow plateau region. The sedimentation coefficient was 3·12 *S*.

Table 1. Protein Concentration of Successive Samples of Canine Prostatic Fluid

Sample	Collection duration (min)	Volume (ml.)	Concentration (per cent)
1	3	3	2·94
2	3	4	1·69
3	10	10	0·40
4	10	6	0·32

The dialysed sample of canine prostatic fluid had a concentration of 1·86 per cent, and this was examined in the ultracentrifuge as such, as well as after being diluted to several lower concentrations, in order to examine the sedimentation rate as a function of concentration. The patterns showed a single sharp peak with a sedimentation coefficient in the range 2·7–2·9 *S*. Typical pictures from the examination at a level of 1·12 per cent protein are shown in Fig. 4. It is again seen that in contrast to the human material there is no slow group of components. There is also no trace of a discrete faster boundary. The sedimentation coefficients at these five different concentrations are listed in Table 2. When plotted against concentration, an extrapolated value of 2·70 *S* is obtained, with a slight positive slope. Since it is much more common to observe a negative, if not a zero, slope, it may be just as well to anticipate that experimental error conceals a zero-value slope, and thus the extrapolated figure would properly be the average value of 2·78 *S*.

Table 2. Sedimentation Coefficients of Various Concentrations of Dialysed Canine Prostatic Fluid

Ultracentrifuge run No.	Protein concentration (per cent)	$s_{20,w}$
3680	1·86	2·79
3681	1·49	2·87
3682	1·12	2·78
3683	0·74	2·71
3684	0·38	2·73

Further work is in progress to examine the ultracentrifugal patterns of pathological human fluid, as well as to obtain more precise data concerning the concentration dependence of the main component, and the content of minor constituents. Parallel investigations are being made to examine the electrophoretic patterns of these various canine and human samples. These various investigations will be reported elsewhere.

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IDENTIFICATION OF AN ORGAN SPECIFIC LIPID HAPTEN IN BRAIN

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THE existence of one or more organ specific lipid haptens in brain has been recognized for more than thirty years[1,2]; but little progress has been made in elucidating the chemical nature of these substances since Rudy[3,4] concluded in 1933 that the active fraction was insoluble in water, relatively stable to alkali, and phosphorus free, but not identifiable with cerebroside; Schwab[5] then showed that at least two substances must be involved since alcoholic brain extracts and an alcohol-insoluble fraction called 'protagon' reacted differently in tests with antisera to brain-lipid or brain-tissue fractions.

The criteria for brain specificity are based on the reactivity of a particular antiserum and, according to the classical definition[6], such specificity requires that: (*a*) reactions of a similar order be obtained with alcoholic extracts of brain of several different species; (*b*) that analogous extracts of a limited number of other organs be unreactive.

It is the purpose of this article to show that an anti-brain serum which satisfies the two criteria defining brain specificity reacts with pure galactocerebrosides when these are properly combined with auxiliary lipids. When compared with an established pure lipid hapten system

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(cytolipin *H*), the degree of reactivity is such as to leave little doubt that galactocerebroside (cerasine, phrenosine) is one of the organ specific lipid haptens of brain. Since individual antisera may display unique reactivities, it is not possible to state categorically that Rudy misinterpreted the observations that led him to eliminate cerebrosides, but our results strongly suggest that his conclusion was incorrect. The chief sources of error lay primarily in the failure to appreciate the magnitude of activation (and inhibition) attributable to lipid–lipid interactions[7], and also in the immunological tests, the design of which obscured the quantitative aspects of complement-fixation measurements[8].

Because of the ready availability of galactocerebrosides in pure form and their presence in the brain of all species, the results of this study are of considerable importance for further investigations of the immunological behaviour of lipids, for the identification of other lipid haptens in brain, for the immunological study of membrane architecture, and for examination of the possible role of immunological mechanisms in various pathological conditions of the nervous system.

Rabbits were immunized by intraperitoneal injections with a particulate fraction from human or bovine brain sedimenting from a 10 per cent homogenate in 0·72 M sucrose between 800 and 20,000*g*. Injections were made three times weekly for a total of nine, each injection containing material representing 0·5 g of brain tissue. Antisera were obtained by cardiac puncture 4 days after the final injection. Individual sera were tested by complement fixation with lipid extracts of whole brain. Antibody response was variable: 3 sera from 9 rabbits were reactive. Results to be described were obtained with the most reactive antiserum. All antisera were afterwards re-tested with pure lipids.

Lipids were extracted from brain and other tissues with chloroform–methanol and then washed with water according to the method of Folch *et al.*[9]. Reactivity of these lipids with antiserum was determined by complement fixation[10] and referred to the weight of total lipid in the case of crude fractions, and to the weight of 'essential lipid' in the case of pure substances requiring the addition of auxiliary lipids. Table 1 shows that the antiserum, prepared against a particulate fraction from bovine brain, reacted to approximately the same degree with total brain lipid from ten different mammalian species. It also shows that the antiserum did not react with crude lipid extracted from bovine heart, liver, lung and kidney, and gave only a minimal reaction with lipid from spleen. Thus, this antiserum satisfies the criteria established by earlier workers for organ specificity, and the reactions of this serum recapitulate the problem of 'brain-specific lipid haptens' set forth in the literature[8].

Initial efforts to isolate the active substance sought to determine whether it would be restricted to one of the classes of lipids that can be separated by current techniques of adsorption chromatography. Accordingly, 1 g of ox brain lipid was fractionated on 100 g of alumina at 5° C using the elution solvents of Davison and Wajda[11] to give 4 fractions: I, neutral lipids, chloroform–methanol, 98 : 2 v/v (275 ml.), 225 mg; II, choline phosphatides, chloroform–methanol, 1 : 1 v/v (1,400 ml.), 250 mg; III, cerebrosides, chloroform–methanol–water, 7 : 7 : 1 (1,500 ml.), 204 mg; and IV, ethanolamine phosphatides, chloroform–ethanol–water, 2 : 5 : 2 (1,800 ml.), 230 mg. Tests for haptenic activity were performed after adding 1 part by weight of pure egg lecithin to suppress anticomplementary activity. Only fractions III and IV (cerebroside and ethanolamine phosphatide) were active. Since each of these fractions contained several components, they were subjected to chromatography on silicic acid, eluting with a continuous gradient of methanol in chloroform in a modification of the procedure described by Weiss[12]. A total of 74 mg of lipid was placed on 40 g of adsorbent in chloroform. The mixing chamber contained 325 ml. of chloroform, and the reservoir 400 ml. of methanol. Fractions of 10 ml. volume were collected, examined by thin layer chromatography on silicic acid, and recombined appropriately. Complement-fixation tests showed that activity was associated exclusively with cerebrosides. From fraction III above, 40·8 mg of cerebroside was collected in tubes 13–15, whereas fraction IV gave 3·4 mg of cerebroside in tubes 13–17.

Accordingly, pure cerebrosides were obtained from various sources and tested for their reactivity after preliminary investigation to determine the most suitable combination of auxiliary lipids. Lecithin–cholesterol mixtures of weight proportions 1 : 1 were more effective than those of either 2 : 1 or 1 : 2. With auxiliary lipid of this composition, an increase in activity of the hapten was found up to 150 parts by weight; however, the intensity of reaction (efficiency of complement binding) decreased between 100 and 150 parts by weight. The substances tested were pure cerasine and phrenosine from ox brain, a highly purified galactocerebroside from ox spinal cord, synthetic phrenosine, synthetic behenyl glucocerebroside, and a pure glucocerebroside from ox spleen. In the presence of auxiliary lipid (100 parts by weight of a mixture of equal weights of lecithin and cholesterol), galactocerebrosides all showed activity ranging from 200 to 250 units/μg whereas glucocerebrosides were unreactive at 1 μg. The antiserum was equally unreactive with cytolipin *H* (ceramide lactoside).

Isofixation curves obtained with the galactocerebroside preparations are shown in Fig. 1. The reactivities of the three natural products are indistinguishable. The synthetic phrenosine shows slightly weaker activity in the zone of excess antibody; however, two of the points in antigen excess are within the range of values obtained with the other galactocerebrosides, thus confirming the identity of the antigen in all four preparations[8]. It is probable that the small discrepancy between synthetic and natural products results from an impurity in the synthetic compound. Most of this impurity had been removed by a single recrystallization from pyridine-acetone[13]. Further evidence to support the identification of the active lipid with galactocerebroside was obtained by isolating the cerebroside fraction from the brains of five different species whose crude brain lipids were initially tested with antiserum. These fractions were recrystallized from 95 per cent ethanol, analysed, and tested. The results are shown in Table 2. All preparations were found to be free of contaminants when examined by thin layer chromatography, were found to contain galactose

Table 1. REACTIVITY OF RABBIT ANTI-BOVINE BRAIN SERUM NO. 766 (0·005 ML.) WITH ORGAN LIPIDS

Species	Organ	Units* of activity per mg of total lipid
Dog	Brain	530
Goat	Brain	560
Hamster	Brain	540
Man	Brain	600
Monkey	Brain	650
Ox	Brain	510
Pig	Brain	780
Rabbit	Brain	580
Rat	Brain	600
Sheep	Brain	770
Ox	Heart	<10
Ox	Liver	<10
Ox	Lung	<10
Ox	Kidney	<10
Ox	Spleen	20

* A unit of activity is arbitrarily defined as the quantity of lipid producing an end point of 50 per cent hæmolysis in a complement-fixation test using 6 units of complement (ref. 10).

Table 2. ACTIVITY OF RECRYSTALLIZED GALACTOCEREBROSIDE PREPARATIONS WITH ANTI-BOVINE BRAIN SERUM 766

Species of origin	Per cent hexose	Per cent N	Activity units/μg
Man	—	1·70	190
Ox	21·4	1·75	190
Pig	21·7	1·68	170
Rabbit	20·6	1·68	190
Sheep	20·8	1·74	200

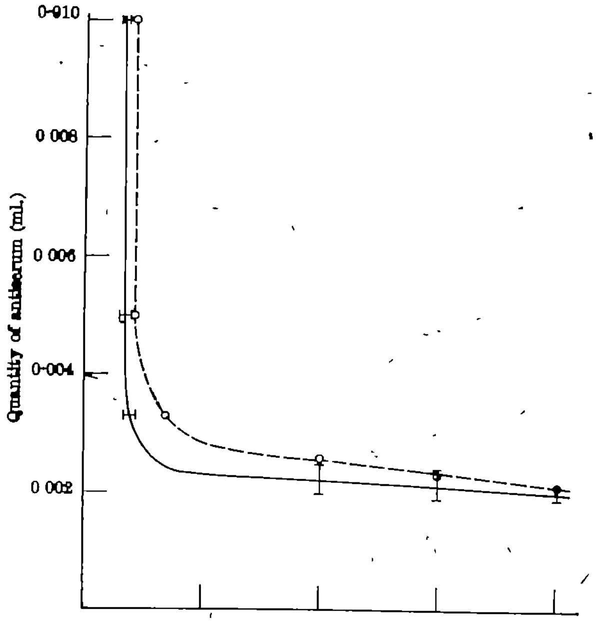

Fig. 1. Isofixation curves (ref. 10) of galactocerebrosides with antiserum to bovine brain. The solid line is drawn through points obtained by averaging the three values found with pure phrenosine and pure cerasine from ox brain, and highly purified galactocerebroside from ox spinal cord. The range of these values is shown by the short horizontal or vertical lines. The dashed line shows the values found with synthetic phrenosine

exclusively on examination of the hydrolysis products by paper chromatography, and had less than 0·1 per cent phosphorus. It is thus clear that a substance can be isolated from the brains of these species that is identifiable as galactocerebroside and reacts with exquisite sensitivity to form an immune complex with antibody prepared against brain fractions. The close correspondence of the activity of these fractions practically eliminates the possibility that the active substance is some unknown contaminant. The inactivity of glucocerebroside and cytolipin H not only attests to the specificity of the antiserum but serves as an effective control for the auxiliary lipid mixture of lecithin and cholesterol.

The knowledge that even the simplest ceramide glycoside can display haptenic function under proper conditions lends strong support to the thesis that this property is common to all glycosphingolipids[14]. Since several other substances of analogous structure are present in brain (for example, sulphatide, ganglioside), it is probable that more lipid haptens will be found in this organ. Identification of galactocerebroside as one of the brain haptens makes it possible to reinvestigate the observations of Schwab[5] in a systematic way; such investigations are in progress.

We thank Dr. David Shapiro of the Weizmann Institute for the specimens of synthetic phrenosine and behenylglucocerebroside and Dr. W. T. Norton for samples of pure phrenosine and cerasine.

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REACTION OF SULPHOQUINOVOSE FROM RED-CLOVER LIPIDS WITH ANTHRONE

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BY means of radiochromatography, the sulpholipid, 6-sulpho-α-D-quinovopyranosyl-(1–1′) glycerol dioleate has been shown by Benson *et al.*[1] to be present in the lipids of many green plant species. These workers measured the concentration of the intact lipid by neutron activation analysis[2]. In this laboratory a sulpholipid has been found in the acetone-insoluble fraction from red-clover (*Trifolium pratense*) leaves and, in the course of analysis, the acid hydrolysis product, sulphoquinovose, was observed to react with anthrone in a manner markedly different from simple hexoses. It is considered that the reaction of sulphoquinovose with anthrone could be developed to give a quantitative method for the determination of this sugar, and thus a quick and specific method of estimating the sulpholipid would be available. It is of interest, however, to report at this stage on the reaction of sulphoquinovose with anthrone as it could prove to be a useful means of detecting this carbohydrate in lipid hydrolysis products.

Red-clover lipids were extracted with boiling 95 per cent ethanol and the acetone-insoluble fraction was obtained as described previously[3]. This fraction (1·4 per cent phosphorus, 0·58 per cent sulphur) was chromatographed on silicic acid to obtain a further concentration of the sulpholipid. Fraction 10 (2·66 per cent phosphorus, 1·41 per cent sulphur), eluted from the column in chloroform–methanol (1 : 1 v/v) contained approximately 40

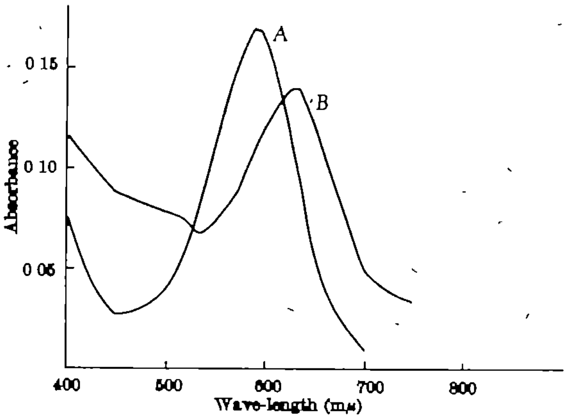

Fig. 1. Spectra of anthrone complexes. *A*, sulphoquinovose; *B*, galactose

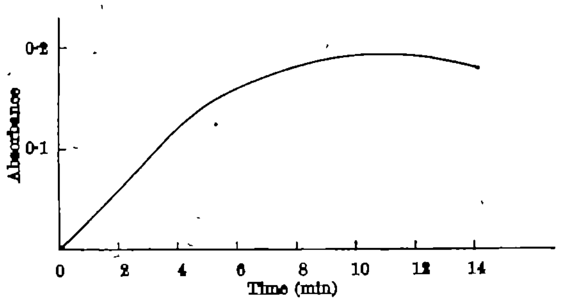

Fig. 2. Rate of colour formation of sulphoquinovose with anthrone at 100°. Conditions are those described by Bailey (ref. 5)

per cent sulpholipid. Small portions of this fraction were subjected to methanolysis[4], and the water-soluble products were acid hydrolysed for 1 h in 1·0 N hydrochloric acid at 100°. Sulphoquinovose was isolated from these hydrolysates by streaking suitable amounts along the origin of paper-chromatograms and eluting the appropriate region of the developed chromatogram (water-saturated phenol) with warm water. The eluted material was shown to be identical with synthetic sulphoquinovose by paper chromatography in six different solvent systems.

Under the conditions described by Bailey[5], the reaction of sulphoquinovose with anthrone produced a different coloured product from that normally encountered with simple hexoses. Sulphoquinovose produced an intensely blue solution with an 'Emax' at 592 mμ as against the green colours of the hexoses which have 'Emax' at 625 mμ.

A plot of optical density against wave-length is shown in Fig. 1.

Compared with galactose, the wave-length of maximum absorption has been shifted about 33 mμ towards the shorter wave-length region of the spectrum. In addition, it has been found that the heating time required to produce maximum colour formation is longer than for hexoses, 10–12 min being needed for sulphoquinovose as compared with 7 min for the simple hexoses[5]. This is illustrated in Fig. 2.

The colour intensity of the anthrone–sulphoquinovose complex has been found to change considerably even after the 30 min ageing period in the dark. In one set of experiments a solution which at 625 mμ had an extinction of 0·613 directly after ageing, increased to 0·700 in 20 min and to 0·845 after 18 h. A maximum was reached in 26 h after which time the colour gradually faded.

This behaviour of sulphoquinovose with anthrone has a parallel in hexuronic acids inasmuch as Helbert and Brown[6] have noted that these carbohydrates react in such a manner as to produce an anthrone complex with an 'Emax' at 540–550 mμ and that the colour intensity also increases on standing. These results would indicate that an abnormal anthrone reaction may result if carbon atom 6 of hexoses has acidic characteristics.

The present results indicate that the anthrone reaction could be used as a means of detecting the presence of sulphoquinovose in lipid hydrolysis products. Even in the presence of other carbohydrates it is possible to detect a small shoulder at 592 mμ thus indicating the presence of the sulphosugar. A rapid and specific chemical means of estimating the sulpholipid in plant lipid extracts would be of considerable use in this field.

I thank Dr. A. A. Benson for a gift of synthetic sulphoquinovose (barium salt) used in the chromatographic work.

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RELATIONSHIP BETWEEN CROP GROWTH RATE AND LEAF AREA INDEX IN THE OIL PALM

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DONALD[1] has recently described some theoretical considerations of leaf area in relation to crop growth which are at variance with earlier published work[2,3]. He also points out that only two published accounts exist of work showing the existence of an optimum leaf area index (leaf area/ground area) for crop growth and stresses the need for more work on other crops and other environments.

Work on the growth of oil palms at the nursery stage has confirmed the presence of an optimum leaf area index with a crop very different from others studied, and in a very different environment, providing further confirmation that an optimum leaf area index is likely to be a widespread occurrence.

Donald[1] follows Davidson and Donald[4] and Iwaki[5] in postulating the existence of a ceiling leaf area index at which the net rate of growth of leaves is zero, new leaf production being balanced by the death of old leaves at the base of the canopy, and the rate of production of non-photosynthetic parts falling to zero with time.

Results from a nursery oil palm spacing experiment are shown in Fig. 1. Seedlings were transplanted at the four square-arrangement spacings of 24, 12, 9 and 7·5 in., and were gathered after 6 months growth on three sampling occasions 27 days apart. Net assimilation rates (rate of increase of dry weight per unit leaf area) were determined in the usual way, using a regression of plant dry weight on leaf area from one set of plants at one sampling to estimate the dry weight of the plants to be sampled 27 days later. This method, first used by Goodall[6], allows greater precision which is necessary with genetically heterogeneous plants. For the estimation of the relative contributions of leaf and non-photosynthetic parts to the total crop growth rate (net assimilation rate × leaf area index) the increase of photosynthetic material as a percentage of total increase for each period was obtained from the plants gathered at each sampling. No leaf weight estimates were available for the plants at the start of each period, and sufficient precision was achieved from these percentages which give results more consistent than using the mean weights from 5 plants as an indication of the population mean.

The similarity between Fig. 1 and the data of Davidson and Donald[4] (Fig. 1*b* of Donald[1]) is remarkable, virtually the only difference being that in the oil palm the photosynthetic/non-photosynthetic ratio approaches closer to unity. There is a difference in scale, however; the optimum leaf area index in the oil palm is about 3, compared with 4·5 in subterranean clover, and the ceiling leaf area index is about 5 compared with 9.

At the highest leaf area index observed, 5·2, leaf growth rate is zero. This leaf area index had been reached by the second sampling, however, and the crop growth rate for the second period occurred at the ceiling leaf area index.

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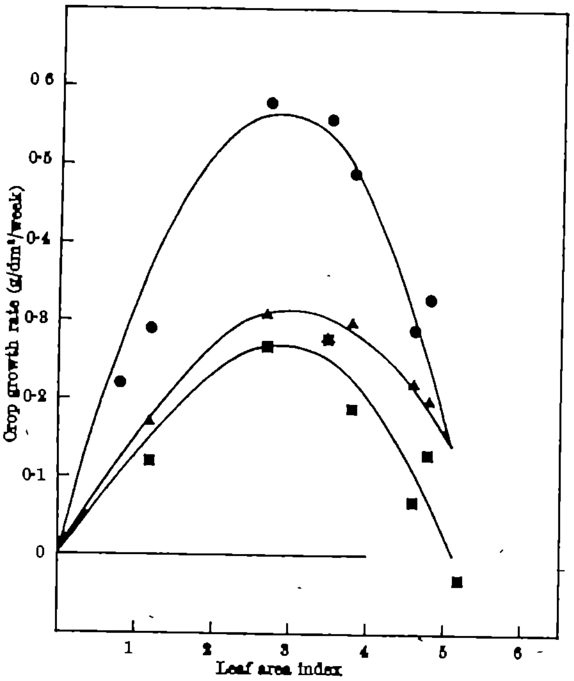

Fig. 1. Crop growth rate of nursery oil palms in relation to leaf area index ■, Leaves; ▲, non-photosynthetic parts; ●, total

The mean values for both non-photosynthetic tissues and crop growth rate for the period were just below zero (−0·02 and −0·05 g/dm^{2}/week respectively) suggesting that the fall with time shown by Fig. 1*b* of Donald[1] may be rapid, resulting in a graph similar to the curve of Davidson and Philip[2] (Fig. 1*a* of Donald[1]).

Growth analysis techniques have not been widely applied to tree crops although the work of Ovington[7] has been used by Donald[1] to demonstrate ceiling leaf area index. Somewhat similar work on plantation oil palms, Rees and Tinker[8], indicate a ceiling leaf area index of about 5 developed by the 17–20-year (age since planting in the field) stage, although the crop growth rate shows very little sign of a decline from 17 to 22 years. For a number of reasons, such as difficulties in harvesting and the availability of higher yielding material, the economic life of a plantation is not much more than 25 years, so there is little available information on older plants, although it is known that old, wild plants have a lower rate of leaf production.

The plantation is a special type of plant association where spacing is intended as the optimum for the duration of the economic life of the plantation. Although young plants have a lower respiratory load, the leaf area index is also low, and maximum crop growth rate occurs when a considerable amount of non-photosynthetic tissue has accumulated, and the leaf area index is near the ceiling value.

Accumulation of dry matter by the plants after 20–22 years occurs in the trunk material only, and a greater part of the dry-matter production is concerned with the replacement of photosynthetic tissues (apart from replacement of roots, about which very little is known, and fruit bunches). If crop growth rate is considered as the rate of increase in dry weight of the standing palms, even a 20-year-old plantation has a very low rate. There is a limit to the growth in height of wild palms; very few palms more than 80 ft. high survive, probably because the respiratory load (particularly of the trunk, which contains a high percentage of living material) increasingly tends, with age, to balance photosynthesis. The plantation palm generally has a trunk of greater diameter, so the ceiling dry matter yield would occur with palms less tall, especially as competition for light in a plantation is severe.

Determination of ceiling dry matter yield is, however, complicated because *Ganoderma* sp. attack palms, causing a trunk rot culminating in the death of the palm[9]. This fungal attack is common, particularly in palms of the height group 40 ft. and above, and in young palms, but not in the intermediate groups, and the disease is often regarded as the sole cause of death of the old palms. It is possible that the activity of the fungus is affected by the balance of photosynthesis and respiration, and that attack by the fungus is a symptom of senescence and decline in vigour.

Increasing crop production. The oil palm is similar to many other plantation crops in that establishment and early growth of seedlings (or cuttings, budwood, etc.) may be treated in a similar way to a pasture although the yield required is the maximum yield per plant not, as in a pasture, the standing crop. Here the principles described by Donald apply. For the most economic return, which is the maximum growth rate, the plants at harvesting, that is, transplanting, should be just at the optimum leaf area index. The unfavourable effects of transplanting on the palms must be minimized, especially as it has been shown that the net assimilation rate of the oil palm appears very susceptible to poor transplanting and takes a period of many weeks to recover[10]. The third consideration, important for the oil palm, is a nursery root disease, 'blast', which decimates nurseries during October–November, particularly if the plants are below a minimum size, when their susceptibility is very high.

For maximum growth rate, the optimum spacing must be worked out commensurate with increased costs of land clearance, planting costs, mulching and irrigation. With 2-ft. square planting, leaf area index reaches a value of 2·0 by early February and 3·0 a month later. Maximum growth per plant occurs at the first level and maximum crop growth at the second. For optimum plant growth, leaf area index should be about 2·0 by about mid-April, when the plants are taken to the field. For this to occur, maximum spacing should be approximately 2·8 ft. square, that is, 8 ft.^{2} land area per plant. This is very similar to recent recommendations (2·5 ft. square) by Gunn, Sly and Chapas[11].

For adult palms, problems of maximum production are more complex. The commercial yield is not the standing crop as in the case of a pasture or of a forestry plantation, and the ceiling yield concept (which applies to the standing crop) is inapplicable. The important value is the crop growth rate or annual dry matter production, part of which represents the economic yield. The commercial yield may be increased in two ways. First, the crop growth rate may be increased, and so increase the quantity of fruit without change in proportion, or, secondly, the proportion of fruit may be increased without change of crop growth rate. The latter would appear to be more promising, and is in fact what is now being achieved by selection. A decrease in stem and leaf production would allow a greater proportion of the dry matter production to be diverted to fruit bunches. The rather unexpected situation would then arise of an increase in fruit production from a decreased rate of leaf production (which would also result in a lower rate of stem formation) and focuses attention on the single growing apex of the palm as of great importance in yield determination. A study of growth rates in the apex is in progress.

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LETTERS TO THE EDITOR

PHYSICS

Multiple Flash-gap Technique for measuring Shock Wave Velocities

A NEW technique has been developed for the recording of the progress of a shock wave through a transparent material. The present communication gives a brief account of the technique and its application to the study of shock initiation of detonation in a solid explosive.

In the photographic flash-gap technique first described by Walsh[1], the arrival of a shock wave at the surface of a metal is indicated by the intense flash of light emitted from a small quantity of gas trapped in the narrow gap between the metal surface and the surface of a block of transparent material such as 'Perspex'. The light flash is thought to be caused by the rapid compression of the gas between the approaching surfaces, rapidly followed by quenching through the loss of heat to the walls, as the separation becomes small.

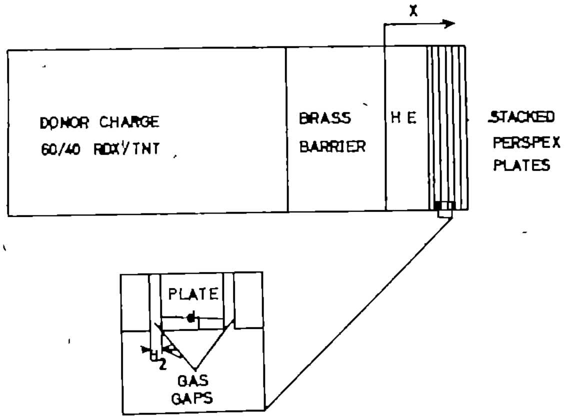
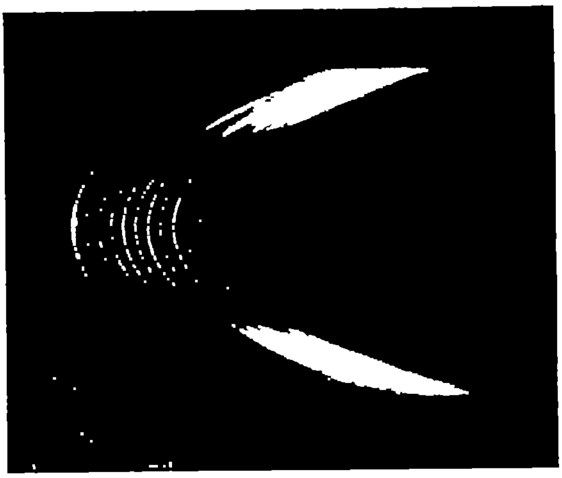


Fig. 1. Experimental arrangement. Charge diameter, 7·5 cm

By using a stack of several 'Perspex' plates separated by narrow gas-filled gaps, we have produced a multiple flash-gap arrangement which has proved valuable in the study of accelerating or decaying shock waves. The progress of the shock wave through the stack gives a series of light flashes as consecutive gaps are closed, so that the shock wave velocity across each 'Perspex' plate can be found from a time-resolved photographic or photoelectric record of the light from the gaps.

Fig. 1 shows the multiple flash-gap arrangement used to study the accelerating shock wave in the receptor charge in a shock initiation experiment. The light flashes have been recorded by means of a 5·4 km/sec streak camera, looking through the 'Perspex' along the charge axis towards the approaching shock wave. Fig. 2 shows such a record.

In calculating the shock wave velocities from the recorded time intervals between flashes, we have assumed the time between two consecutive flashes to be composed of two parts: 1, the time for the shock wave to traverse the plate, and 2, the time needed for closing the gap. We have assumed the gap to be closed with a velocity equal to twice the particle velocity behind the shock front in the 'Perspex'. For the relation between particle velocity and shock wave velocity in 'Perspex' the data by Buchanan, James and Teague[2] have been used.



2 μsec 18 < x < 28 mm

Fig. 2. Streak camera record of light flashes produced in the experimental arrangement of Fig. 1

With plate thicknesses of 1–3 mm and gas-gaps of 0·06 mm, the time for closing the gap was from 7 per cent of the recorded time between flashes at the highest shock wave velocities measured to 26 per cent at the lowest. The uncertainty in the shock wave velocities obtained in the present experiments is about 5 per cent. Of this, 1–2 per cent is due to the time measurement. The rest is due to variations in plate thickness, which was not measured for each plate separately.

Fig. 3 shows the results of a series of 6 experiments with the charge assembly shown in Fig. 1. The thickness of explosive in the receptor charge (60/40 RDX/TNT) between the barrier and the stack of 'Perspex' plates was varied from 0 to 30 mm. The thickness necessary for transition to detonation at the charge axis, as determined in a separate series of experiments from the appearance of detonation light at the surface, was 25 mm.

Each experiment gives a record of the velocity of the shock wave as it proceeds down the 'Perspex'. As conditions behind the front determine the way in which the front velocity changes, it should be possible from such a record to find the pressure profile of the wave transmitted into the 'Perspex' if one knew the velocities of sound at different states. The front pressure of the original wave just before it reaches the first 'Perspex' plate can be

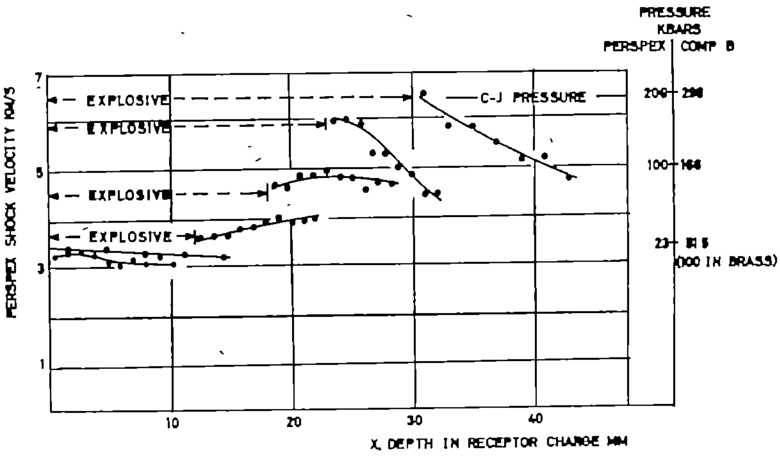


Fig. 3. 'Perspex' shock velocities versus depth in receptor charge assembly

determined directly from the record provided that shock wave data for the two materials are known. (Using two air-gaps only, Paterson and Lamb[3] have measured the Chapman–Jouget pressure in commercial explosives in a similar manner.)

In principle, one should also be able to find the whole of the original pressure profile from an analysis of the record. Even without an elaborate analysis, however, the record can often give a valuable qualitative picture of the pressure profile.

From the records in Fig. 3, one finds: first, that along the axis of the receptor charge the shock wave velocity and amplitude increase quite steadily towards detonation, and not at all so abruptly as one might be led to believe from an observation of the surface of the charge.

Secondly, the shock wave velocity in the 'Perspex' seems to increase as the wave travels into the 'Perspex' in such cases where the receptor charge has a thickness less than the expected depth of initiation. The experiments indicate that there is, in that part of the receptor charge, a region behind the shock front where the instantaneous pressure is greater behind the front than at it.

Thirdly, this region of greater pressure appears to contract and draw closer to the front as the wave moves farther into the receptor charge.

The pressures in the explosive were estimated using the Hugoniot data for detonation products of composition *B* (64/35/1 RDX/TNT/wax) given by Deal[4]. The uncertainty in this estimate is undoubtedly greater at low pressures than at pressures near the *C–J* pressure, where the chemical reaction is closer to completion.

The estimated front pressure in the experiment with the thickest receptor charge (30 mm) agrees well with the *C–J* pressure given by Deal[4].

No sign of a von Neumann spike can be observed in these records. We intend to carry out experiments with very thin 'Perspex' plates in order to look specifically for this phenomenon.

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Effect of Grinding on Quartz Particles

WHETHER a vitreous or otherwise 'disturbed' layer forms on particles of quartz produced by size reduction processes has been the subject of a controversy for some time. In an earlier communication[1] we reported evidence for the absence of a vitreous or mosaic sub-surface layer. A recent paper by Sakabe *et al.*[2] leaves little doubt that, in their experiments, a thick vitreous layer was formed by the grinding procedure used. Samples were ground in a mechanical agate mortar for varying times up to 300 h and fractions of mean particle size of approximately 0·5μ were prepared by centrifuging. Some of their observations are summarized here.

(1) The initially angular particles became rounded, almost spherical, after 24-h grinding.

(2) The initial rate of solution in 10 per cent sodium hydroxide was greatly enhanced by grinding and in all cases diminished to the same steady value after about 10 h.

(3) The intensity of the (101) X-ray Bragg reflexion diminished rapidly with increasing grinding time. After 300 h of grinding the peak disappeared. Little or no broadening of the peak was observed.

(4) In the electron diffraction patterns increased grinding time resulted in a diminution in the number of spectra observed and an increase in the background intensity. No arcs or rings were observed.

We interpret these observations as indicating that the change from the quartz to the vitreous phase occurs directly without passing through a mosaic or other semi-crystalline state, as suggested by King and Nagelschmidt[3].

Samples ground under acetone in our own laboratory, either for 44 h by hand in an agate mortar or for 10 h in an agate phial using a 'Wig-L-Bug', failed to exhibit any of the foregoing phenomena suggesting the formation of vitreous material. In both cases marked broadening of the (101) X-ray reflexion was observed, but only a very slight diminution in the integrated intensity. The particles were observed to retain their angular shapes and efficient size reduction was achieved.

The difference between our results and those of Sakabe *et al.* obviously lies in the method of grinding. It is possible that in dry grinding with the high pestle pressures usual with mechanical mortars, the friction between the particles and the mortar or pestle may at times become so great that a region of the particle near the surface melts momentarily and assumes the vitreous phase on cooling.

Further investigations are necessary to determine the exact conditions under which a vitreous layer will form. From our own experiments it would seem that efficient size reduction of quartz particles can be achieved without the formation of a relatively thick vitreous sub-surface layer.

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Bond Energy of Vacancies and Interstitial Atoms in Graphite

Baker and Kelly[1] have estimated that the energy to form a vacancy in graphite (that is, the energy to remove an atom from the lattice and place it on the free surface) is $U_f = 3{\cdot}3 \pm 0{\cdot}9$ eV. (The symbol ± refers to maximum limit of values.) They comment that this is less than the heat of sublimation, $U_s = 7{\cdot}5$ eV per atom, indicating an energy-relaxation of $4{\cdot}2 \pm 0{\cdot}9$ eV at the vacancy. Equality should occur[2] if the broken lattice bonds around the vacancy remain broken. In graphite, however, all the lattice bonds are unsaturated, and we would expect that the unpaired electrons of the three atoms around the vacancy would form covalent bonds with electrons derived from unsaturated bonds of adjacent atoms (cf. ref. 3). These we will call partial bonds. The net energy associated with their formation (denoted by $3U_1$ per vacancy) we identify with the required energy relaxation. Thus:

$$U_1 = 1{\cdot}4 \pm 0{\cdot}3 \text{ eV} \qquad (1)$$

An interstitial atom should likewise form partial bonds with adjacent lattice atoms, the net energy of formation of which will be denoted by $3U_2$ per interstitial. Strain energy around a vacancy or interstitial in graphite is small, and is neglected, as is also the energy associated with any electron-trapping processes. The energy, U_p, of a well-separated vacancy-interstitial pair is therefore equal to the energy to break all three lattice bonds around a lattice atom, $2U_s$, less the energy-relaxation around the vacancy and the interstitial, $3(U_1 + U_2)$. However, U_p is greater than U_f, as otherwise the surface atoms will spontaneously take up interstitial positions. From this we obtain:

$$U_1 + U_2 < 3{\cdot}9 \pm 0{\cdot}3 \text{ eV} \qquad (2)$$

We will call $U_p/3$ the effective bond energy, U_e. This represents the energy absorbed when one lattice bond between two atoms is broken and converted into two partial bonds.

The activation energy for the motion of a vacancy is $U_m = 3{\cdot}15 \pm 0{\cdot}55$ eV (ref. 1). This involves the counter-motion of a lattice atom. In its rest position this atom has two lattice bonds. We assume that at the mid-point of its jump, these are both broken (each absorbing $2U_e/3$) and each half of each bond forms a partial bond with adjacent lattice atoms, each partial bond emitting energy U_s. Thus $U_m = 4U_e/3 - 4U_s$ or:

$$U_s = 1{\cdot}71 \pm 0{\cdot}16 \text{ eV} \qquad (3)$$

The values of U_1, U_2 and U_s are probably close to each other. For present purposes we will take them to be equal and estimate their mean value U_s. Equations (1), (2) and (3) then lead to $U_s = 1{\cdot}5 \pm 0{\cdot}4$ eV. The stored energy of a vacancy-interstitial pair, U_p, is therefore $6{\cdot}0 \pm 2{\cdot}4$ eV. Half of this is to be associated with the interstitial and half with the vacancy. Experimentally the saturation value of the stored energy in graphite at reactor temperatures is about 600 cal/g (ref. 4) or 0·3 eV per lattice atom. If the damage configuration consists of single vacancies plus clustered interstitials, then the stored energy will be due principally to the bonds around the vacancies, and the saturation vacancy concentration is of order $0{\cdot}3/3{\cdot}0 = 1/10$. This is a very reasonable value[5].

We may estimate the activation energy, U_c, to evaporate interstitial clusters in graphite. The most firmly bound atoms on the edge of the cluster are attached to the edge of the cluster by two lattice bonds. U_c is thus the energy to convert these two bonds to partials (which is also the activation energy U_m for vacancy diffusion) plus the activation energy for diffusion of interstitial atoms, U_i. This latter is at least 0·4 eV (ref. 6), and is less than the 1·2 eV observed for annealing processes at higher temperatures[4], so we will put $U_i = 0{\cdot}8 \pm 0{\cdot}4$ eV.

Thus $U_C = 3{\cdot}95 \pm 0{\cdot}95$ eV. The disappearance of an interstitial cluster as the temperature is raised will thus be due first to erosion by any vacancies initially present in the lattice (U_m), followed closely by the evaporation of the interstitials remaining in the cluster (U_C). If the atoms on the edge of the cluster each evaporate in 10 sec, the value 3·95 eV corresponds to a temperature of about 1,200° C. If no vacancy clusters, dislocations or other suitable sinks are present, these interstitial atoms will condense into fresh clusters on cooling. This mechanism may contribute to the large cluster motion observed at 1,200° C (ref. 6).

The activation energy for the diffusion of a singly bonded interstitial atom along the edge of a cluster will similarly be of the order of $U_s + U_i$ or $2{\cdot}8 \pm 1{\cdot}2$ eV, which on the basis of 10^{-7} jumps per sec corresponds to a temperature of 450 ± 300° C. This implies that at the lowest power-reactor temperatures, the clusters will contain a significant number of vacant (interstitial) sites due to imperfect filling, while at sufficiently higher temperatures the atom-density in the cluster will be normal.

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GEOPHYSICS

Annual Change in the Declination ('Variation') in Western Scandinavia and the North Sea

WITH reference to the letter in *Nature*[1] from Guro Gjellestad and Helge Dalseide it should be noted that the last published British Admiralty chart of declination (epoch 1960.0) does show a decrease in the annual change over the area in question, when compared with the previous chart. The isopors (lines of equal annual change) shown on the 1960 chart are given in Fig. 1 (full) together with those found for 1958 by Gjellestad and Dalseide (dotted).

Isopors appearing upon a declination chart are of necessity compiled in advance of publication of the chart, from data extending backwards and seldom fully up to date. The information deduced from these data are extrapolated values of the annual change which must be used for correcting the chart values of the declination for a period of several years subsequent to the epoch of the chart. No extrapolation formula has yet been devised to cope with the vagaries of secular change, and experience has shown that, even in the best-determined regions, extrapolation over a matter of several years is liable to errors of 1 or 2 min per year in the predicted annual change.

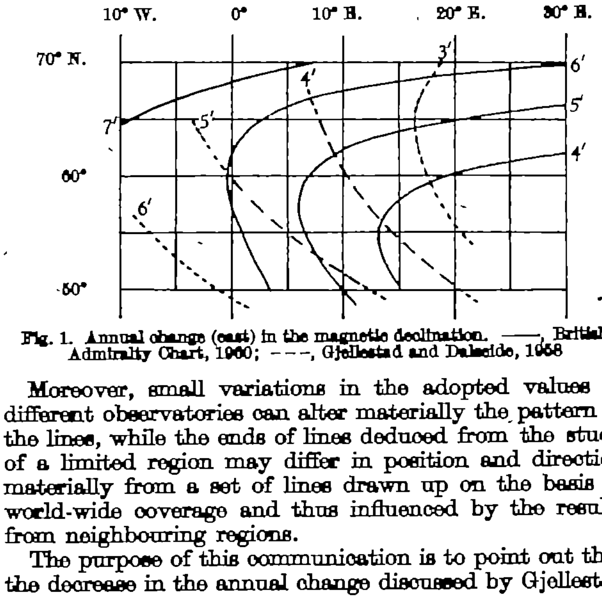

Fig. 1. Annual change (east) in the magnetic declination. ——, British Admiralty Chart, 1960; – – –, Gjellestad and Dalseide, 1958

Moreover, small variations in the adopted values at different observatories can alter materially the pattern of the lines, while the ends of lines deduced from the study of a limited region may differ in position and direction materially from a set of lines drawn up on the basis of world-wide coverage and thus influenced by the results from neighbouring regions.

The purpose of this communication is to point out that the decrease in the annual change discussed by Gjellestad and Dalseide is in fact shown in the last Admiralty world charts, and that the differences of the two sets of isopors should be treated with reserve.

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WE were not referring in our communication to the last published British Admiralty chart (1960.0) which we did not know about, but to several Scandinavian charts and to other sea maps apparently using information from older charts from the Admiralty. We fully agree with Finch in his remarks. It is unfortunate that no theory of the secular variation has been developed that might be generally accepted, and that no empirical formula is known for extrapolating for the secular variation.

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GEOLOGY

Geometrically Complicated Monogenetic Flow Folds

COMMON phenomena observed in gneissic terrains include the remarkable contortions shown by the compositional layers within the gneisses. Some of the more simple deformations have been analysed and accounted for adequately[1], but the more complicated contortions have been dismissed as 'flow folding', 'plastic folding', etc., with no further note.

Folded gneissic bands and enclosed mafic layers (for example, amphibolites) are often very impersistent, and show little regularity in the disposition of fold axes (crest and trough lines) and axial planes. Such folds have been considered to comprise the class of flow folds[2]. However, I have maintained that the geometrically simple folds resulting from a laminar type of plastic flow more or less perpendicular to marker layers should also be included in this class, and suggested that the more complicated flow folds in both hard rock and soft sediment result from unusual patterns of laminar flow[3]. Thus flow folds were redefined in more general terms as "contortions affecting only incompetent beds, which have thickened and thinned without the aid of ruptures above microscopic levels".

In recent years many studies have been made of superimposed fold systems[4–8], and characteristics of such systems include the development of 'hooks' in the rock distribution, and irregularities in the disposition of the fold axes and axial planes.

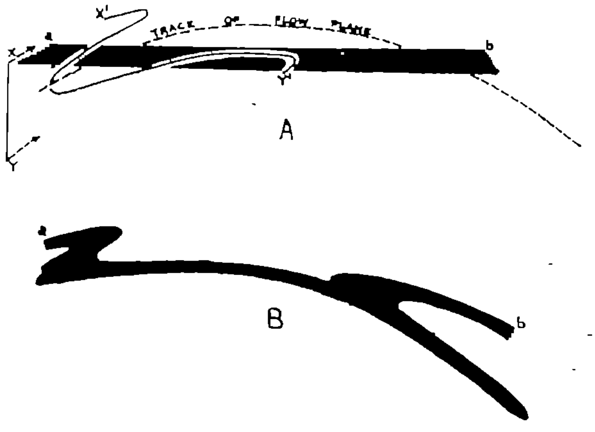

Fig. 1

In amplification of my earlier statements and to introduce a note of caution in concluding multiple folding for geometrically complicated folds, Fig. 1 shows how monogenetic folds may have peculiarities hitherto often taken to suspect the presence of superimposed folding. In Fig. 1*A* let deformation proceed by a laminar-type flow along involuted planes parallel to the broken line in the general direction of the marker layer *ab* (solid shading), so that the imaginary line *XY* is displaced to *X'Y'*. Involution of the flow planes may result from slight variations in pressures, temperatures and chemical compositions. The resulting contortion of the layer *ab* in a by-and-large isotropic plastic medium is shown in Fig. 1*B*, where 'hooks' and folds facing opposite directions have been developed.

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Recrystallization of Zircon as an Indication of Contact Metamorphism

ZIRCON ($ZrSiO_4$) is generally regarded as the most refractory accessory mineral present in the common rock types[1]. According to Poldervaart[2], incipient granulation and recrystallization of zircon when exposed to ultrametamorphic processes can be expected only in a stage as high as the sillimanite grade. Investigations in quartzite xenoliths in the southern norite belt of the Bushveld Igneous Complex of the Transvaal (South Africa), however, indicate definitely that these processes can be caused by usual contact metamorphism and that the foregoing conceptions should be revised.

A zircon concentrate from a thermally reconstituted quartzite slab floating in the Bushveld norite north-west of Pretoria shows a characteristic preponderance of recrystallized zircons, that is, crystals displaying a rounded or fractured core enveloped in a mostly idiomorphic, transparent shell (Fig. 1). Statistical evaluation of the material revealed that from 300 grains counted 137 (45·66 per cent) show clear signs of recrystallization. In a number of others the core is not so easily recognizable, but appears as a cloudy spot, surrounded by a somewhat 'ghostly' outline. A comparison of these metamorphosed quartzites with the 'normal' quartzites of the Pretoria series outside the Bushveld aureole shows that the latter contain scarcely any zircons displaying recrystallization phenomena (< 2 per cent).

The results thus obtained are not difficult to explain. It is self-evident that the rounded or fractured kernels of the recrystallized zircons represent minute zircon 'pebbles' in all stages of attrition, originally representing the bulk of the heavy mineral fraction of a sedimentary rock. Accordingly, these zircons did not originate in this rock; they were merely deposited after having undergone transport, during which they developed their characteristic pebblish or fragmental shape (cf. Fig. 1). Speaking in genetic terms, they cannot be referred to as primary constituents of the rock formed here *in situ*, but only as secondary or allogenic components derived from outside sources and dumped together with other sedimentary detritus finally constituting an arenaceous rock.

Their host-rock later being subjected to contact metamorphism, these zircons suffered thermal recrystallization, that is, a stage of secondary growth resulting in the development of an outer shell which is mostly water-clear and idiomorphic, showing often a model-shaped crystal form with sharply pointed pyramidal terminations (Fig. 1). In contrast to the parent detrital grains, these outgrowths were formed in place; hence they are primary or authigenic constituents of the rock. The cores enclosed

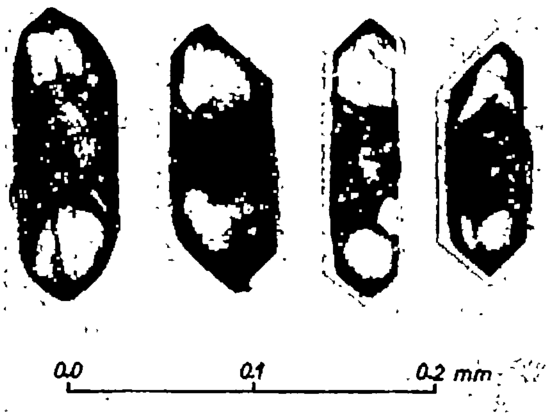

Fig. 1 Recrystallized zircons from a metamorphosed quartzite in the Bushveld norite north of Vissershoek (north-west of Pretoria)

in these shells now represent sedimentary relict structures betraying the original sedimentary character of the reconstituted rocks.

As particularly emphasized by Poldervaart[2], elongation frequency curves usually yield valuable criteria for a quantitative description of a given zircon concentrate. It may be noted, therefore, that the elongation frequency curve of the zircons from the metamorphosed quartzite shows two maxima separated by a somewhat irregular gap (Fig. 2A), being proof that this crop is 'bigenetic', containing a sedimentary fraction with a length/breadth maximum at 1·5 and a metamorphic one with a length/breadth maximum at 2·0. It seems noteworthy that curves displaying this general set-up were already inferred from theoretical considerations by Poldervaart[2], although in connexion with magmatic rock types. Zircons from the common quartzites of the Pretoria series show the usual elongation frequency distribution characteristic of well-sorted sediments with only one distinct maximum (Fig. 2B).

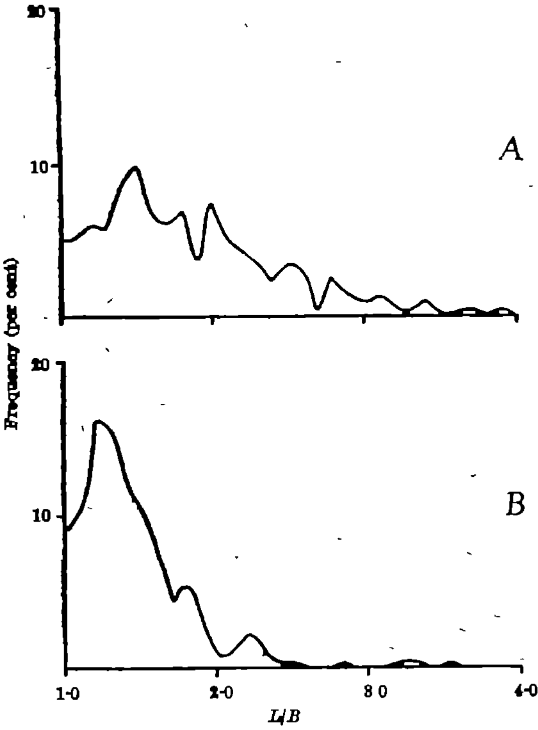

Fig. 2. Elongation (*L*/*B*) frequency curves of: *A*, zircon crop from reconstituted quartzite, as mentioned in Fig. 1; *B*, zircon crop from usual quartzite of the Pretoria series (Magaliesberg quartzite, Silkaatsnek)

Although it seems to be established from these findings that the refractory properties usually ascribed to zircon with regard to recrystallization are less significant than conceived by previous investigators, there can be no doubt about it that this mineral is actually capable of surviving metamorphic processes as a solid body, forming relict structures in newly developed crystals. As has been pointed out convincingly by Poldervaart and Eckelmann[3] and Hoppe[4], these relict bodies are still preserved in zircons of completely reconstituted 'ultra-metamorphic' rocks of sedimentary derivation. Accordingly, the new observations as recorded in this communication in no way restrict or even invalidate the petrogenetic significance of morphological zircon studies. It seems to be of particular interest, therefore, to apply the zircon method in connexion with a transformistic conception of the Bushveld norites advanced by S. van Biljon[5], explaining these magmatic rocks as transformation products of the upper part of the Pretoria series and the afore-mentioned quartzite xenoliths as 'undigested' relics of the latter.

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ASTRONOMY

Determination of Relative Ages of Lunar Craters by Albedo and Polarization Measurements

One of the most important tasks in selenology is to establish the relative ages of different parts of the lunar crust. It may be argued that, during the lifetime of a crater, its floor would be darkened at a uniform rate by the destructive effects of ultra-violet light on the rock crystals. The empirical relationship:

$$2 \log \rho + \log \Delta = 0 \qquad (1)$$

may be fitted to the albedo (ρ) measurements of Markov and Fedorets[1], where Δ is the diameter of a crater in kilometres.

Solid materials polarize less light if they are fragmented, but there comes a point when the degree of pulverization is such that the polarization again increases due to the change in the scattering properties of the material. On the Moon, there is a natural agency for breaking down the rocks in the form of meteorite collisions. The degree of roughness will vary from one region to another, and this may be expected to bear some relationship to the age of the rocks in that region. A freshly formed impact crater will have some covering of rock dust, and a young igneous crater will probably have a covering of volcanic ash. In either case, the microstructure of the floor will be comparatively smooth. As time elapses, the roughness of the floor of a crater may be expected to pass through a maximum, since the fragmentation process will yield large rock blocks at first and these themselves will be subject to gradual pulverization. Hence the maximum proportion $P_{\max}$ of light polarized by an average region should pass through a minimum. Measurement of $P_{\max}$ of a given region would thus yield two possible ages. Dzhapiashvili's[1] measurements of $P_{\max}$ may be used to show that this is the case, and that ($P_{\max}$ expressed as a percentage):

$$3 \log (P_{\max} - 7) = 4 \log (\Delta \sim 170) - 6 \qquad (2)$$

Because of intrinsic differences between the albedo of rocks at different points of the Moon and because the initial degree of roughness will certainly differ from point to point, equations (1) and (2) are essentially statistical. Improved relationships may be obtained as a greater quantity of fundamental data become available, for only then will the number of measurements be sufficient to warrant segregating the points according to whether they lie in the dark maria or in the brighter continental regions. However, interpreted broadly, both relationships point to the fact that the larger lunar craters are the older. In particular, on the theory outlined here, the rocks of the maria would have to be the oldest on the Moon. Yet, considerations of the relative frequency of occurrence of continental and post-mare craters indicate that the continents are some six times older than the maria. This difficulty may be resolved if the continents and maria differ in chemical composition.

I thank Imperial Chemical Industries, Ltd., for a research fellowship.

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CRYSTALLOGRAPHY

Refinement of the Crystal Structure of Antlerite

THE crystal structure of antlerite, $Cu_3(SO_4)(OH)_4$, was determined by Araki[1] while J. J. Finney was also working on the structure. This refinement represents the final results of the two independent determinations.

A specimen of antlerite from Chuquicamata, Chile, was used for the refinement. Data from nine reciprocal levels, zero, one and two for each of the a, b and c axes, were collected by means of multiple film pack Weissenberg photographs. Molybdenum $K\alpha$-radiation was used throughout the investigation. Of a possible 991 unique reflexions, 723 were observed. Intensities were obtained by visual comparison with a known scale.

Antlerite is orthorhombic, space group *Pnma*. The cell dimensions, $a_0 = 8·226$, $b_0 = 6·046$, $c_0 = 11·978$ Å, determined from precession photographs agree with those determined by Araki. The cell formula is $Cu_{12}(SO_4)_4(OH)_{16}$.

Refinement of the antlerite structure was effected by the method of least squares. Parameters obtained by the senior author's structure determination were used as input for the refinement and the final parameters are listed in Table 1.

Table 1. PARAMETERS OBTAINED BY LEAST SQUARES REFINEMENT

	Cu_1	Cu_2	S	O_1	O_2	O_3	OH_1	OH_2	OH_3
x	0·0052	0·2897	0·1304	0·262	0·195	0·033	0·285	0·701	0·046
y	0·2500	0·0022	0·2500	0·250	0·250	0·047	0·250	0·250	0·503
z	0·0015	0·1259	0·3641	0·283	0·476	0·348	0·026	0·779	0·102
B	0·65	0·65	0·49	0·80	1·00	1·17	0·58	1·39	0·90

Table 2. RESIDUALS OF THE ANTLERITE REFINEMENT

Level	Reflexions	R
$0kl$	65	0·092
$1kl$	75	0·107
$2kl$	55	0·099
$hk0$	54	0·120
hk1	54	0·086
$hk2$	38	0·107
$h0l$	194	0·089
$h1l$	50	0·124
$h2l$	138	0·087
Total observed	723	0·096
Unobserved	268	
Total	991	

For the refinement, the entire matrix was computed and inverted. Twenty-one positional parameters, nine temperature factors and nine scale factors were allowed to vary. Only isotropic thermal parameters were used. The residuals for each reciprocal level are given in Table 2. The correlation matrix was calculated for the last cycle, but no large interactions between parameters were observed.

There are two crystallographically distinct copper atoms in the structure each of which has a somewhat distorted octahedral co-ordination. Cu_1 has four hydroxyl groups (OH_3) nearly coplanar at distances of 1·97 Å and one hydroxyl (OH_1) and one oxygen atom (O_2) at 2·32 and 2·56 Å respectively. Cu_2 has three hydroxyl groups (OH_1, OH_2, OH_3) and one oxygen atom (O_3) also nearly coplanar at distances of 1·91, 1·91, 2·02 and 2·05 Å respectively and two oxygen atoms (O_1, O_2) at distances of 2·37 and 2·41 Å. Co-ordination about the sulphur atom is four-fold with the average bond-length being S—O = 1·46 Å. The average bond-angle O—S—O = 109·5°. Bond-lengths are considered accurate to ± 0·02 Å.

The structure consists of systems of connected Cu_1 and Cu_2 polyhedra extending parallel to b-axis. The Cu_1 and Cu_2 polyhedra are linked to each other by one OH_3 group and also by OH_1 and O_2. However, one system is linked to another by only two weaker bonds, Cu_2—O_1 (2·41 Å), in the c-direction. This accounts for the perfect cleavage parallel to (001).

We thank Dr. A. Rosenzweig of the University of New Mexico, Albuquerque, New Mexico, for supplying the sample of antlerite, and Dr. S. W. Bailey, of the University of Wisconsin, Madison, Wisconsin, for use of the X-ray equipment. Computations were carried out on the *CDC* 1604 computer at the University of Wisconsin Numerical Analysis Laboratory.

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Determination of Space-Groups using Anomalous Dispersion

IN the usual method of determining space-groups, the presence of face or body centring and of screw axes and glide planes is detected by examining the systematic absences of reflexions. However, it is not possible to establish by such a procedure the presence or absence of a centre of inversion, a rotation axis or a mirror plane. This is because the translation-free symmetry elements do not lead to any characteristic absences for the reflexions; also, the symmetry of the X-ray diffraction pattern of a crystal is not exactly the same as that of the point group of the crystal itself, but only the corresponding Laue group which has an additional centre of inversion added to it.

In order to test whether the crystal is centrosymmetric or not, statistical methods have geen developed by Wilson *et al.*[1–4] and by Ramachandran and Srinivasan[5]. However, considerable doubts have been expressed about their practical applicability as distributions having properties in between the two limits have often been encountered, and the distributions are also affected by the presence of (a small number of) heavy atoms. It would therefore be worth while examining a more straightforward method of testing the presence or absence of centrosymmetry. This seems to be possible making use of anomalous dispersion.

When anomalous dispersion effects are taken into account, it is found that the symmetry of the X-ray diffraction pattern of a crystal is exactly the same as that of the point group symmetry of the crystal itself. Also, the translation-free symmetry elements, such as a centre of inversion, a rotation axis or a mirror plane, lead to the Bijvoet difference $\Delta I (= I_H - I_{\bar{H}}$, where I_H and $I_{\bar{H}}$ are the intensities of the direct and inverse reflexions including anomalous dispersion effects) being systematically zero along certain rows and planes in the reciprocal lattice. Thus, $\Delta I = 0$ for all reflexions for a centrosymmetric crystal. Similarly, $\Delta I = 0$ for all $0k0$ reflexions if a mirror plane is perpendicular to the b axis and for all $h0l$ reflexions if a 2-fold axis is parallel to the b axis. The systematic absences of the Bijvoet difference have been worked out for all the point groups, and it is found that an examination of these extinctions can reveal the presence or absence of the translation-free symmetry elements in a crystal.

Thus, the systematic extinctions of the Bijvoet difference can give uniquely the point group of a crystal. On the other hand, the usual systematic absences give the elements of the space-group which involve translational symmetry. Combining these two types of information, it is possible to write down a diffraction symbol for all the 230 space-groups, similar to that adopted by Buerger[6], except that the new symbols will contain the point group of the crystal, the lattice type and the translational elements of the space-group which are present[7]. It is found that all the space-groups except $I222$, $I2_12_12_1$ and $I23$, $I2_13$ can be uniquely determined excluding, of course, the enantiomorphous pairs.

However, even the enantiomorphous pairs can be identified in a certain sense. Although it would not be possible to say from the symmetry of the Bijvoet difference whether the space-group is one or the other (say $P4_1$ or $P4_3$), the two crystals which are enantiomorphous to each other can be distinguished by examining the Bijvoet differences. In this sense, it may be said that the method discussed here can differentiate also between the enantiomorphous space groups.

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CHEMISTRY

Thin-Layer Graphited Carbon Black as the Stationary Phase for Capillary Columns in Gas Chromatography

GOLAY pointed out in his early papers[1,2] that excellent capillary columns could be produced by replacing the smooth inner wall area of a tubular column by a uniform porous layer of the support material. Petitjean *et al.*[3] and Mohnke *et al.*[4] were the first to use adsorption-type tubular columns. Both of them invented special methods for preparing alumina and silica adsorption layers from the materials of the walls by chemical reactions. The following general method can presumably be used for depositing any solid substance on the walls of open tubular columns. Here we report our results using carbon black graphited at 3,000° C (surface area $72 \cdot 8$ m^2/g)[5]. 15 g of this carbon black was suspended by rapid stirring in a mixture of 220 ml. trifluortrichlor-ethane and 30 ml. carbon tetrachloride. The carefully cleaned copper tube (in Fig. 2 silvered copper) was filled with this suspension. One end of the tube was closed and the open end was fed cautiously and slowly in a special oven to evaporate the liquid. After this procedure the carbon black remains on the wall of the column in the form of an adherent porous thin layer.

The gas chromatographic apparatus was similar to that already described. A flame ionization detector was used with a fast recorder (full scale time 0·3 sec). The carrier gas was hydrogen, the sample size (syringe) was always 0·2 µl. The columns are identified by the original inner diameter of the metal tube and with the weight of carbon black covering the wall per metre of column-length.

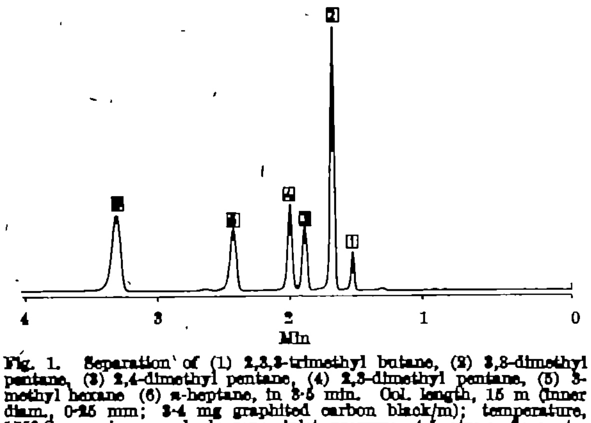

Fig. 1. Separation of (1) 2,2,3-trimethyl butane, (2) 3,3-dimethyl pentane, (3) 2,4-dimethyl pentane, (4) 2,3-dimethyl pentane, (5) 3-methyl hexane (6) *n*-heptane, in 3·5 min. Col. length, 15 m (inner diam., 0·25 mm; 3·4 mg graphited carbon black/m); temperature, 175° C; carrier gas, hydrogen; inlet pressure, 4·5 atm.; flow-rate, 3·2 ml./min (room temperature); sample size, 0·2 µl.; bypass ratio 1 : 1,070. Flame ionization detector with electrometer amplifier and quick recorder

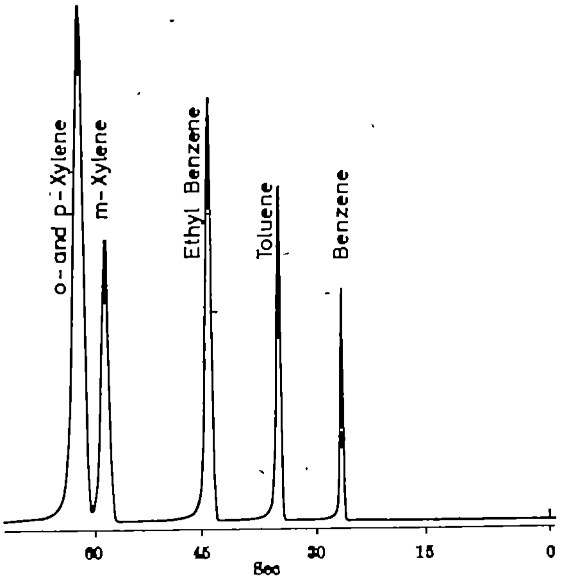

Fig. 2. Separation of (1) benzene, (2) toluene, (3) ethyl benzene, (4) *m*-xylene, (5) *o*- and *p*-xylene in 70 sec. Col. length, 15 m (silvered copper; inner diam., 0·25 mm; 5·4 mg graphited carbon black/m), temperature, 245° C; carrier gas, hydrogen; inlet pressure, 2·7 atm.; flow rate, 2 ml./min (room temperature); sample size, 0·2 µl.; bypass ratio 1 : 1,050. Flame ionization detector with electrometer amplifier and quick recorder

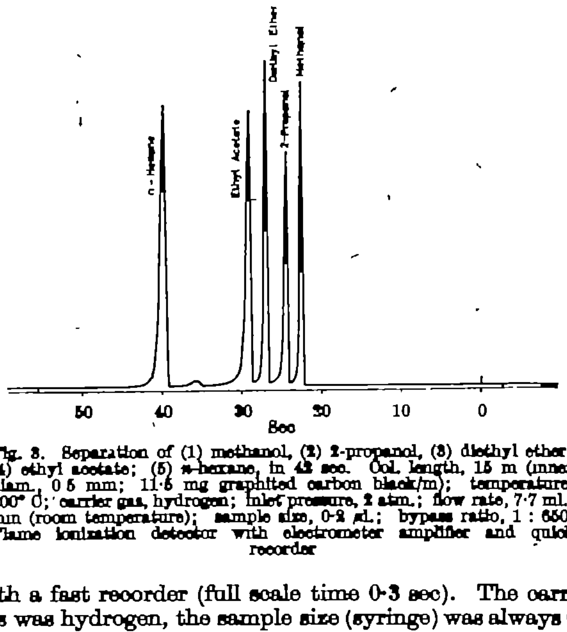

Fig. 3. Separation of (1) methanol, (2) 2-propanol, (3) diethyl ether; (4) ethyl acetate; (5) *n*-hexane, in 42 sec. Col. length, 15 m (inner diam., 0·5 mm; 11·5 mg graphited carbon black/m); temperature, 200° C; carrier gas, hydrogen; inlet pressure, 2 atm.; flow rate, 7·7 ml./min (room temperature); sample size, 0·2 µl.; bypass ratio, 1 : 650. Flame ionization detector with electrometer amplifier and quick recorder

Fig. 1 shows a relatively slow analysis of six heptane isomers available in our laboratory, run at a column temperature of 175° C with a 15 m column (inner diam. 0·25 mm and 3·4 mg carbon black/m). With a similar 30-m column we have made routine analyses of C_1–C_7 paraffins for three months without any change in retention times.

The quick and unusual separation of the xylene isomers is illustrated in Fig. 2. *m*-xylene and *p*-xylene are perfectly resolved in 70 sec with a 15 m column (inner diam. 0·25 mm and 5·4 mg carbon black/m) at 245° C, while the *o*-xylene and *p*-xylene remain unresolved.

In Fig. 3 the separation of four oxygenated compounds is shown in 42 sec at 200° C on a 15-m column (inner diam. 0·5 mm and 11·5 mg carbon black/m). Tailing is relatively small. The retention times of the oxygenated compounds, relative to those of hydrocarbons, are remarkably short.

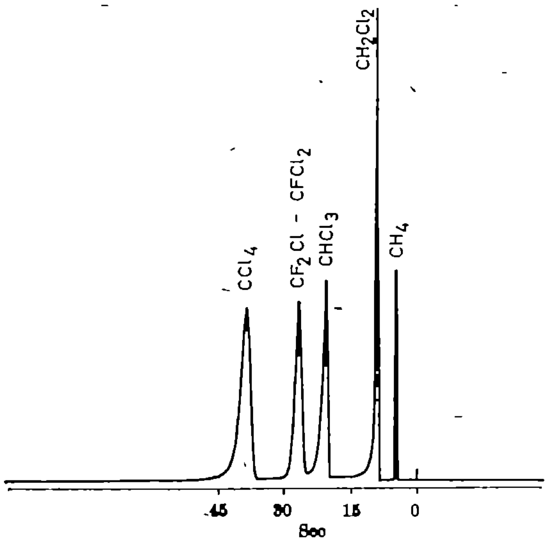

Fig. 4. Separation of (1) methane, (2) dichlor-methane, (3) trichlor-methane, (4) CF$_2$Cl—CFCl$_2$, (5) tetrachlor-methane, in 45 sec. Col. length, 8 m (inner diam., 0·5 mm; 12·8 mg graphited carbon black/m); temperature, 80° C; carrier gas, hydrogen; inlet pressure, 3·8 atm.; flow rate, 35 ml./min (room temperature); sample size, 0·2 µl.; by-pass ratio, 1 : 200. Flame ionization detector with electrometer amplifier and quick recorder

In Fig. 4 the analysis of four chlorinated paraffins in 45 sec at 80° C is shown on an 8-m column (inner diam. 0·5 mm and 12·8 mg carbon black/m). The flow rate of the carrier gas was 35 ml./min, extraordinarily high for capillary columns. We carried out rapid quantitative analyses of halogenated paraffins on similar columns with relative errors less than ± 2 per cent.

The quantity of sample actually reaching the column was $0{\cdot}12 - 0{\cdot}15 \times 10^{-4}$ g in Figs. 1–3 and $1{\cdot}4 \times 10^{-4}$ in the last chromatogram.

We used the same method with some success in preparing thin layers of alumina and silicon carbide on capillary columns for the quick separation of low-boiling hydrocarbons.

We thank Prof. U. Hoffmann and H. P. Boehm for discussions and for providing samples of graphited carbon black.

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A Reliable System for the Introduction of Samples to Gas Chromatographic Columns, particularly suited for Quantitative Capillary Column Analysis

During the past few years a number of different injection systems have been used with capillary columns, but these have either been restricted to favourable samples or have given quantitative results of doubtful validity. The system described here enables the composition of any sample to be evaluated quantitatively.

If a sample of convenient size is placed in a vessel containing a suitable involatile solvent through which an inert gas is continually passed, then the concentrations of the various components will decay exponentially with time and at rates determined by their partition coefficients with respect to the involatile solvent.

A small sample of the exit gas from the vessel can be easily admitted to a capillary column by means of a gas sampling valve. If the gas sample is taken shortly after the admission of the bulk sample to the dilution system then the chromatogram which is obtained is relatively richer in the components of lower partition coefficients than was the original bulk sample. Samples of gas taken later will show progressive reduction in the ratio of low to high partition coefficient components.

If the whole of the original bulk sample could be chromatographed as a series of small samples from the dilution system, each small sample being of a size commensurate with the column requirements, then, although no single chromatogram would represent the original mixture, the sum of all the chromatograms would give the peak ratio which would have been obtained from the chromatogram of the original mixture.

In practice, it is unnecessary to take an infinite number of samples, providing the detector used has a linear response and providing the solvent used in the dilution system is the same as that used for the column stationary phase and is at the same temperature. In this case two samplings will suffice and the following equation may be applied to calculate the mass ratio of any pair of components:

$$\frac{M_1}{M_2} = \frac{R_1}{R_2} \times \frac{K_1}{K_2} \times \frac{A_1\,(t_0)}{A_2\,(t_0)}$$

where R_1 and R_2 are the response factors of the two components; K_1 and K_2 are the respective partition coefficients; $A_1\,(t_0)$ and $A_2\,(t_0)$ are the areas of the peaks obtained at time (t_0), (t_0) being the time at which the sample starts to decay from the system. Such values of A are produced by the linear extrapolation of any two log area measurements against decay time, back to time (t_0) (Fig. 1).

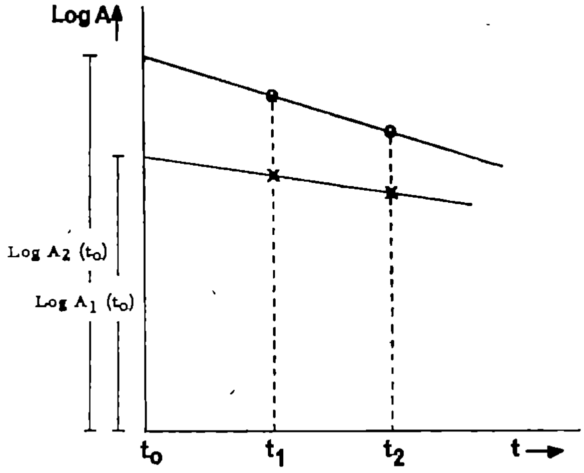

Fig. 1

K_1/K_2 may be obtained from the ratio of the retention times of the two peaks measured from the air peak and R_1/R_2 may either be known or be determined from a similar run where M_1/M_2 is known.

A further interesting aspect of the system is that original samples may be diluted in this manner and the exit gas sampled at times so chosen that the two chromatograms are preferentially enriched in any minor constituent which may be of particular interest; such enrichment obviously results in a more accurate determination of that component. Thus, for the accurate evaluation of trace amounts of substances with low partition coefficients the two gas samples should be taken after small values of t, and the size of the gas sample taken from the dilution system should be small. Conversely, for determining trace amounts of substances with high partition coefficients the values of t should be large, and the size of the gas sample taken from the dilution system should be increased.

The system described here has been developed as a result of a recent improvement in the original vapour dilution technique described by Dr. J. E. Lovelock[1]. Such improvements are described in a paper to be published shortly by I. Fowlis and R. P. W. Scott.

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Effect of Fluoride Ion on the Activation of Molecular Hydrogen by Silver(I)

ACCORDING to Halpern[1] the effect of complex forming ligands on the activation of molecular hydrogen by metal ions can be explained by the following scheme:

$$\begin{array}{cc} M^+ & \!\!—X^- \\ \vdots & \vdots \\ H^- & \!\!—H^+ \end{array}$$

Thus the heterolytic splitting of hydrogen is the consequence of the concerted action of the central metal ion and the ligand bond. Great activation effect can be expected from the ligands which, although weak acids, form unstable complexes with the metal ion, that is, which do not screen the positive charge partly responsible for the heterolytic splitting. These requirements are contradictory in general, being a parallelism between the proton affinity and the complex forming tendency of the ligands. In the case of silver(I), the reduction of which by hydrogen was thoroughly investigated by Halpern *et al.*[2-4], the fluoride ion fulfils the requirements mentioned: although hydrogen fluoride is a rather weak acid ($pK = 3{\cdot}4$), the fluoride ion forms an unstable monofluorosilver(I) complex[6,7].

So the effect of fluoride ion on the reduction of silver(I) by molecular hydrogen seemed worthy of investigation.

The reaction was studied in an all-glass apparatus. Hydrogen was bubbled through the thermostatically controlled neutral solution (pH 6) of silver nitrate (up to 0·1 mole/l.) and potassium fluoride (up to 2·0 mole/l.). The reaction was followed by the titrimetric determination of non-reduced silver(I).

The fluoride ions increase dramatically the velocity of reduction. The results can be described by the following expression:

$$-\frac{d[H_2]}{dt} = \frac{1}{2}\frac{d[Ag^+]}{dt} = v = k.C_{Ag}\frac{K[F^-]}{1+K[F^-]}.[H_2]$$

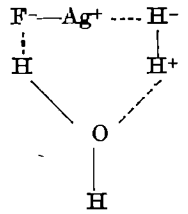
where K is the stability constant of the silver fluoride complex[7]. Light has a small but definite enhancing effect. The velocity constants were calculated at different temperatures in the range of 30–70° C. The results are summarized in Table 1. For comparison some literature data referring to the aquo-complex and to the most effective amine complexes are also included.

Table 1

Ligand	log k (70° C)	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
H_2O	−3·2	23·3	− 6
CH_3–$N(CH_2$–$CH_2)N(CH_3)_2$	0·17	15·1	−14
$N(CH_2CH_2)_3N$	0·26	17·1	− 8
F^- (in dark)	0·79	18·3	− 3
F^- (in diffused daylight)	0·88	20·3	+ 4

Thus the fluoride ion is the most effective ligand so far found.

Considering that the configuration of silver(I) complexes is linear, and that the co-ordinated F^- and H_2 do not touch each other even being in adjacent position, the following scheme for the activated complex must be assumed:



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Steric Effect in Mass Spectra of *cis–trans* Disubstituted Ethylenes

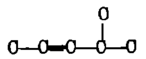
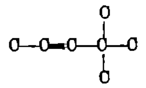
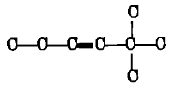
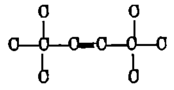
PREVIOUSLY[1-5] we reported results concerning the behaviour under electron impact of *cis* and *trans* isomers of disubstituted cycloalkanes. The steric effect in such compounds shows up by the fact that the ionized *cis* isomer is less stable than the corresponding *trans* one in derivatives of *cyclo*propane[1], *cyclo*butane[2], *cyclo*pentane[3] and *cyclo*hexane[4]. Those results are summarized in a recent paper[5].

In the same way we have investigated several pairs of *cis* and *trans* ethylenic isomers in order to check whether the same kind of relationship exists between mass spectra and stabilities of those isomers. Looking up the few data published in the literature[6] we found six pairs of *cis–trans* derivatives of butene with an increasing number of methyl groups on the two end carbon atoms.

We calculated the abundances I_{cis} and I_{trans} of molecular ions in percentage of total ionization. The results are listed in Table 1. These can be considered as a measure of the stability of the ionized compounds[7].

The figures in the last column of Table 1 indicate that within experimental uncertainties there is no difference between the stabilities of *cis* and *trans* 2-butenes. The same situation occurs in derivatives of butene substituted by one methyl group (2-pentene) or by two methyl groups,

Table 1. RELATIVE ABUNDANCES OF MOLECULAR IONS IN *cis–trans* DISUBSTITUTED ETHYLENES (PERCENTAGE TOTAL IONIZATION)

Pair No.	Name of the compound	Formula*	I_{trans}	I_{cis}	I_{trans}/I_{cis}
1	2-Butene	C—C=C—C	17·98	18·11	0·99
2	2-Pentene	C—C=C—C—C	9·71	9·62	1·01
3	4-Methyl-2-pentene		8·08	7·98	1·01
4	3-Hexene	C—C—C=C—C—C	6·97	6·85	1·02
5	4,4-Dimethyl-2-pentene		6·52	5·96	1·09
6	5,5-Dimethyl-3-hexene		4·19	3·42	1·22
7	2,2,5,5-Tetramethyl-3-hexene		1·08	0·16	≃ 7

* H atoms omitted.

either on the same end carbon atom (4-methyl-2-pentene) or on both of them (3-hexene).

If one of the end carbon atoms of butene is substituted by three methyl groups (4,4-dimethyl-2-pentene), the abundance of the *cis* isomer becomes 10 per cent smaller than that of the *trans* isomer. A further substitution by a methyl group again decreases the abundance of the *cis* isomer by about another 10 per cent (5,5-dimethyl-3-hexene). In the last butene derivative which has six methyl groups on the two end carbon atoms (2,2,5,5-tetramethyl-3-hexene) a tremendous effect shows up since the *trans* isomer molecular ion is about 7 times more abundant than the *cis* isomer.

As a result, one must emphasize that the substitution of ethylene by a tertiary butyl group has an obvious effect on the stability of the *cis* isomers, especially in the last compound. This has to be related in some manner to the steric hindrance provided in the *cis* molecule when such a close-packed group exists in it.

It is likely that the ionization is produced by removal of one of the non-bonding π electrons of the double bond. Consequently the isomerism should disappear in the ionized molecule and the energy provided by steric hindrance would be released and used for further decomposition. Therefore, in the *cis* isomer of the last three pairs where steric hindrance is especially large because of the tertiary butyl group, the stability of the ionized molecule has to be smaller than that of the *trans* isomer.

This result is quite similar to that found in the case of cyclanic isomers previously investigated.

It may also be interesting to notice that there is a regular decrease in the abundance of molecular ions, in either *cis* or *trans* isomers, when the number of methyl groups fixed on the end carbon atoms of butene is increasing. The same rule was observed in the alkane series.

I thank Prof. L. D'Or for his interest in this work. I also thank the Fonds National de la Recherche Scientifique for supporting this work.

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Hydrogen and Chlorine Hyperfine Splittings of Several 2-Hydroxy-1,4-naphthosemiquinones

RECENTLY, Hollocher and Weber[1] published electron spin resonance spectra of alkaline aqueous systems containing partially reduced 2-hydroxy-3-methyl-1,4-naphthoquinone (I), but did not identify the radical responsible.

We now give evidence that the semiquinone of (I) is responsible for the observed spectra. Comparisons among the spectra obtained with the foregoing system and with similar systems containing other 2-hydroxy-1,4-naphthoquinones indicate that the apparent complexity of the hyperfine structure arises from a non-equivalence of the four benzenoid ring protons to give three different splittings with consequent overlapping of individual lines.

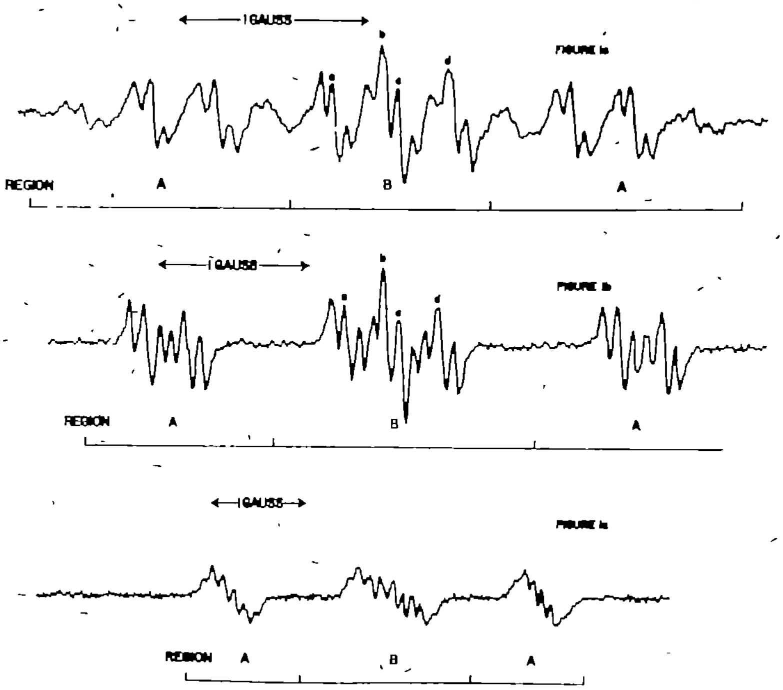

Fig. 1. First derivative electron spin resonance spectra in alkaline aqueous media of the semiquinones of: *a*, 2-hydroxy-3-methyl-1,4-naphthoquinone; *b*, 2-hydroxy-1,4-naphthoquinone; *c*, 2-hydroxy-3-chloro-1,4-naphthoquinone. Field increases from left to right

The striking differences between the electron spin resonance spectra of the semiquinones of 2-hydroxy- and 2-hydroxy-3-chloro-1,4-naphthoquinones (II and III) are interpreted as proof of resolved chlorine hyperfine splitting in the latter compound.

Electron spin resonance observations were made at room temperature using a Strand Lab 602 spectrometer with *2K25* klystron operating at the cavity resonance frequency of 9·5 kMc/s and with modulation frequency of 6 kc/s. The field was generated by a Harvey Wells system including *L*-128 12-in. magnet and model *HS*-1050 power supply.

Field positions and separations were obtained using sodium carbonate solutions of potassium peroxylamine disulphonate or calcium xanthon in tetrahydrofuran.

Modulation amplitudes were 20 milligauss or less at a non-saturating microwave power-level at the sample of 1 mW or less. Field was swept at a rate of 1–2 gauss/min using a time-constant of 0·15 sec. Sample dimensions were 0·8 mm diameter × 35 mm long, and the apparent field inhomogeneity over the sample was about 3 parts in 10^6.

Compounds (I) and (II) were commercial preparations repurified before use. (III) was prepared after the method of Zincke[2] by heating naphthoquinone epoxide with 4 N hydrochloric acid, m.p. 216° C.

For preparation of the naphthosemiquinones, the naphthoquinones were dissolved in 0·1 N potassium hydroxide to give a final concentration of 10^{-3} M or less. Following reduction by sodium dithionite, the excess dithionite was removed and the compounds were partially re-oxidized by contact with air. Once formed, the radicals were found to be stable indefinitely at room temperature in an oxygen-free environment.

All hyperfine splittings reported are estimated to be ± 5 per cent of the value.

Figs. 1*a*, 1*b* and 1*c* show representative first derivative electron spin resonance spectra of partially reduced (I), (II) and (III), respectively, in alkaline aqueous solution at room temperature. Observed line widths are 0·02 to 0·04 gauss and the *g* values are 2·004 ± 0·0005 in each case.

In region *A* of Fig. 1*b* we observe a pair of triplets with splitting of 0·10 gauss and 0·28 gauss and intensity ratios of 1 : 1 for the pair of triplets and 1 : 2 : 1 within each triplet. Ten lines are found in region *B* of Fig. 1 and the splitting of 0·10 gauss is exactly or closely maintained between the lines. Considering the four strongest lines, *a*, *b*, *c*, and *d*, in this region we find the distance *a–b* and *c–d* to be 0·28 gauss.

The basic structure of this spectrum is interpreted to consist of four lines and the distance *a–c* or *b–d* (0·39 gauss) is a measure of the failure of the middle two of these lines to superimpose to form a triplet. The splittings of the two protons responsible for this basic structure are 1·48 and 1·87 gauss. These splittings and those above account for all five protons of the semiquinone of (II).

In (I) a methyl group replaces a hydrogen to give two additional hydrogen atoms to participate in hyperfine splitting. In Fig. 1*a* we observe a spectrum[1] closely analogous to that of Fig. 1*b* showing the additional hyperfine splitting due to the methyl hydrogens. In region *A* of Fig. 1*a* there is a quartet of triplets with the innermost member of the quartet partially obscured by overlapping minor lines extending outward from region *B*. The splittings are 0·35 gauss for the quartet and 0·08 gauss for the triplets. Intensity ratios for the quartet are 1 : 3 : 3 from the outermost inward, neglecting the obscured innermost member on each side, while those for the triplets are 1 : 2 : 1.

For the ten clearly observable lines in region *B* the separation of 0·08 gauss is closely or exactly maintained. The four strongest lines *a*, *b*, *c* and *d* are so disposed that *a–c* and *b–d* are 0·35 gauss. Thus, by analogy with Fig. 1*b*, the distance *a–b* or *c–d* (0·25 gauss) is a measure of the failure of the two middle lines of the basic four-line spectrum (the splittings for which are 0·98 and 1·23 gauss) to superimpose. This interpretation accounts for all seven hydrogen atoms of the semiquinone of (I) and give one set of three equivalent protons (methyl) and, as with (II), another set of two equivalent protons.

The spectrum depicted in Fig. 1*c* shows an unexpected detail. By analogy with (I) and (II) and assuming no observable hyperfine splitting by the chlorine atom, we anticipated with (III) a triplet in region *A* and up to six lines in region *B*. The actual spectrum shows six lines in region *A* with separations of 0·12 gauss and nine lines in region *B* with the same separations. For the six lines, the integrated intensity ratios of 1 : 2·8 : 4·1 : 4·1 : 2·8 : 0·9 approximate the ratios of 1 : 3 : 4 : 4 : 3 : 1 expected of a 3 × 4 splitting of a proton triplet by a chlorine atom to an amount equal to the proton splitting. This observation, and other features of the spectrum, are interpreted to evidence the existence of resolved chlorine hyperfine splitting in the semiquinone of (III). The magnitude of the splitting indicates that the spin density at the chlorine nucleus in the case of (III) is of the same order of magnitude as that at the corresponding methyl or ring proton in the case of (I) and (II). To our knowledge this is the first observation of resolved chlorine hyperfine splitting in organic radicals. We attribute the successful resolution to the low radical concentrations utilized since the lines broaden considerably at higher concentrations.

The remainder of the spectrum is interpreted in the manner described here and the hyperfine splittings for all three spectra are summarized in Table 1.

Table 1. SUMMARY OF HYPERFINE SPLITTINGS

Nuclear species	No. of equivalent nuclei	Ring designation	Splittings in gauss ± 5 per cent (I) 2-Hydroxy-3-methyl-1,4-naphthoquinone	(II) 2-Hydroxy-1,4-naphthoquinone	(III) 2-Hydroxy-3-chloro-1,4 naphthoquinone
H	2	benzenoid	0·08	0·10	0·12
Cl	1	quinoid 3-	—	—	0·12
H	1	quinoid 3-	—	0·28	—
H	3	quinoid 3-methyl	0 35	—	—
H	1	benzenoid	0·98	1·48	1·56
H	1	benzenoid	1·23	1·87	1·90

It will be noted that the hyperfine splittings for the 2-hydroxy-naphthosemiquinones studied are quite different from those reported for methyl and alkyl substituted 1,4-benzo- and naphthosemiquinones[2–7]. For these latter compounds the splittings are: quinoid ring and methyl protons about 2·2 gauss and methylene protons about 1·1 gauss; benzenoid ring protons about 0·5 gauss. The presence of the ionizable hydroxyl function in the 2-position decreases the spin density at the 3-proton or 3-methyl protons by a factor of 6–8 and renders the 5-, 6-, 7- and 8-protons of the benzenoid ring non-equivalent, giving one nearly equivalent pair with strong splitting of order 1–2 gauss and one equivalent pair with weak splitting of order 0·1 gauss.

Work is continuing to confirm the placing of these two pairs of protons in the benzenoid ring.

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BIOCHEMISTRY

Enzymatic Behaviour of the Product of Coenzyme A–Norepinephrine Reaction

REACTION of the sulphydryl group of coenzyme A *in vitro* with oxidation products of epinephrine and norepinephrine has been demonstrated by chemical measurement of the residual —SH groups[1]. The coenzyme A–catecholamine compounds may well represent an important stage in intermediary metabolism, since their breakdown may lead to regeneration of coenzyme A and formation of physiologically active catecholamine derivatives. Furthermore, if coenzyme A is inactivated by these reactions, an explanation may be at hand for some of the physiological effects of the catechol amines. The work reported here evaluates the reactivity and stability of the coenzyme A–catecholamine derivatives in the light of their enzymatic activity *in vitro*.

The reduced form of coenzyme A comprised 75–80 per cent of the preparation obtained from the Nutritional Biochemical Corporation, according to the catalogue, while approximately 10 per cent consisted of glutathione and the remaining 10 per cent of oxidized coenzyme A. The preparation was described as hygroscopic. During experimental manipulations, the coenzyme had an opportunity to bind atmospheric water. The sulphydryl group found in the weighed preparation by the method of Ellman[2] was equivalent, disregarding the glutathione, to 44 per cent of reduced coenzyme A. Tyrosinase, phosphotransacetylase, and dilithium acetyl phosphate were obtained from the Worthington Biochemical Corporation, Freehold, New Jersey.

The Lipmann technique[3] for determination of coenzyme A was used. Separate solutions contained 0·31 × 10^{-3} M coenzyme in water and 1,170 μg of tyrosinase per ml. of 0·1 M *tris* buffer of *p*H 7·4. Tubes containing 0·5 ml. aliquots of phosphotransacetylase were stored in a freezer. Immediately before use, the contents of one tube were diluted 1 : 10 with water. A tube containing 60 μM of dilithium acetyl phosphate in 1 ml. of water was simultaneously removed from the freezer. The concentration of

Table 1. ABSORBANCY AT 510 mμ OF SOLUTIONS CARRIED THROUGH THE PROCEDURES INDICATED IN THE TEXT. 0·28 × 10^{-6} MOLES OF COENZYME A WERE IN EACH TUBE INITIALLY

No Co A	0·570
Co A alone	0·167
Co A, 0·30 × 10^{-6} moles norepinephrine	0·556
Co A, 0·20 × 10^{-6} moles norepinephrine	0·425
Co A, 0·10 × 10^{-6} moles norepinephrine	0·305

norepinephrine in 0·01 N hydrochloric acid was 0·30 × 10^{-3} M. The reaction between coenzyme A and an oxidation product of norepinephrine proceeded at 28° C in separate tubes, each of which ordinarily contained 0·9 ml. of coenzyme A, norepinephrine, 0·6 ml. of tyrosinase, and water to a total volume in each tube of 2·1 ml. Six min. later, 0·5 ml. were removed from each tube and started through the rest of the Lipmann procedure[3]. The absorbancy was measured at 510 mμ in the Beckman spectrophotometer, with water as the blank.

Typical results are presented in Table 1. The decrease in the colour produced from acetyl phosphate was directly proportional to the amount of coenzyme A present. Tyrosinase had no effect in the absence of the catecholamine. The relatively large amount of tyrosinase oxidized the norepinephrine within 5 min. The reaction of coenzyme A with an oxidation product of norepinephrine led to an increase in the residual amount of acetyl phosphate. The amount of coenzyme A activity left after reaction on an equimolar basis with various concentrations of the norepinephrine derivative was closely consistent with the initial amount of the coenzyme calculated from the measured sulphydryl group.

The inability of the coenzyme A which had reacted with an oxidation product of norepinephrine to combine with the acetyl phosphate in the Lipmann technique confirmed that the enzymatically active sulphydryl group was the reaction site. It demonstrated also the stability of the coenzyme A–catecholamine product in an enzymatic environment *in vitro*.

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Carbonic Anhydrase in Bacteria

THE richest source of carbonic anhydrase is mammalian erythrocytes. It is also found in the pancreas, gastric mucosa, and kidney in fairly high concentration. Carbonic anhydrase has also been found localized in the cytoplasm of plant leaf tissue[1–4]. A survey of the literature revealed no report of the occurrence of microbial carbonic anhydrases. Main and Lock[5] reported that Type I *Pneumococcus* and *Staphylococcus aureus* did not produce carbonic anhydrase.

One of us (F. P. V.) had noted some carbonic anhydrase activity in the nasal exudates of persons suffering from respiratory infections. This carbonic anhydrase activity could have been from several sources: (1) from erythrocytes arising from ruptured capillaries; (2) from the tissues involved; (3) or possibly from bacteria of the normal naso-pharyngeal flora. It therefore seemed of interest to examine some of the ubiquitous oral and naso-pharyngeal micro-organisms for carbonic anhydrase activity.

Nine cultures of *Neisseria* (*N. catarrhalis*, strains No. 8716 and *N*9; *N. flava N*8; *N. perflava* 231*D*; *N. subflava N*15; *N. sicca*, strains Nos. 6021, 22, 19 and *N*5), a species of *Lactobacilli* (*L. acidophilus* No. 926), and a strain of *Streptococcus salivarius* were examined for carbonic anhydrase activity. (All *Neisseria* cultures were given by Prof. Michael J. Pelczar, Department of Microbiology, University of Maryland, and the *Lactobacilli* and *Streptococcus* were obtained from the stock culture collection of the Department of Microbiology, University of Maryland.)

The *Neisseria* and *Streptococci* were grown on trypticase-soy agar (B.B.L.) whereas *Lactobacilli* were grown in deep broth cultures of trypticase-soy broth to which was added 0·5 per cent (w/v) yeast extract (B.B.L.), 0·05 per cent (w/v) cysteine hydrochloride, and sufficient 1·0 N hydrochloric acid to adjust the *p*H to 6·5. All media were sterilized by autoclaving at 121° C, 15 p.s.i. for 20 min. All cultures were incubated at 37° C for 48 h.

Cells were collected from agar plates by washing off the growth with distilled water; broth cultures were collected by centrifugation. All cell crops were washed three times with glass distilled water and finally suspended in sufficient distilled water to make a dense suspension. Fresh suspensions were prepared for each day's experiment. Dry weight of the various suspensions was determined by drying 3 ml. of each in small beakers in an oven for 24 h at 95° C, followed by a 30-min cooling period in a desiccator prior to weighing.

A crude chloroform enzyme preparation was produced from horse erythrocytes according to the method of Meldrum and Roughton[6]. This preparation will be referred to as 'horse enzyme', and was used for comparison with bacterial cell suspensions and evaluation of the assay method. It was stored in a stoppered Erlenmeyer flask at 3° C. Dry weight was determined in the same manner as outlined here for bacterial cell suspensions.

Enzyme activity was measured manometrically as evolution of carbon dioxide resulting from dehydration of carbonic acid after the method of Krebs and Roughton[7]. Reaction flasks were charged as follows: 1·0 ml. 0·10 M K_2HPO_4–KH_2PO_4 *p*H 6·8 buffer, and 1·0 ml. appropriate enzyme preparation (or 1·0 ml. distilled water in buffer controls) in the main compartment; 1·0 ml. 0·05 M sodium bicarbonate (substrate) in arm 1. In flasks utilized to determine endogenous oxygen uptake 0·2 ml. 20 per cent potassium hydroxide was included in the centre well. A 10-min temperature equilibration period was followed by a 5-min leak-check period before the reactions were begun. Flasks were shaken at the rate of 120 strokes/min. All reactions were carried out at 24·9° C for 4 min and observed at 30-sec intervals. Samples of *N. sicca* suspensions were heated in test-tubes for 10-, 15-, and 20-min periods at 100° C in a boiling water bath to determine if observed activity was enzymatic.

The activity of each preparation expressed in terms of carbon dioxide evolved per mg dry weight per 60 sec is given in Table 1. *N. sicca* strain No. 6021 showed activity which was almost as high as the horse enzyme. Other strains of *N. sicca* also had measurable activity. Other bacteria tested showed little or no activity.

Table 1. A SUMMARY OF BACTERIAL AND HORSE ERYTHROCYTE CARBONIC ANHYDRASE ACTIVITY BASED ON DRY WEIGHT AND CORRECTED FOR BUFFER CONTROL

Preparation	μl. CO_2 evolved per mg dry weight per 60 sec = $Q_{dw}CO_2$*
Horse enzyme	8·31, 8·79
N. catarrhalis 8716	0·75
S. salivarius	0·69
*N. flava N*8	0·75
*N. catarrhalis N*9	0
N. perflava 231*D*	1·74
N. sicca 6021	7·90, 7·86, 8·05, 7·70
*N. subflava N*15	0
L. acidophilus 926	0
N. sicca 22	2·84
*N. sicca N*5	0
N. sicca 19	3·00
N. sicca 6021 Boiled 20 min	1·12

* Values are averages of two or more trials.

Flasks shaken in 24·9° C water bath contained 1·0 ml. 0·1 M K_2HPO_4–KH_2PO_4 *p*H 6·8 buffer and 1·0 ml. appropriate enzyme preparation (1·0 ml. water in buffer control) in the main compartment, 1·0 ml. 0·05 M sodium bicarbonate in side-arm 1.

The established role of carbonic anhydrase[4] in fluid and ion exchange processes tempts one to speculate on a possible relation between the presence of this enzyme in the ubiquitous microbial inhabitants of the naso-pharynx and the symptoms of the common cold. Two possibilities immediately present themselves. (1) Carbonic anhydrase is present in these organisms and plays a part in the excretion of certain products of microbial metabolism: that is, acids, and/or the ill-defined slimes known to be produced[9]. These excretory products could in turn irritate the mucous membranes of the naso-pharynx altering their permeability and susceptibility to invasion. (2) The presence of extra-cellular carbonic anhydrase could disturb the normal excretory mechanism of the mucous membrane tissue cells themselves, leading to changes in permeability and resistance to invasion. We find these two postulated mechanisms intriguing and intend further studies in this area.

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Unusual Behaviour of a Mammalian Collagen in Deuterium Oxide

GROSS[1] observed that heating a solution of collagen in phosphate buffer to 37° C resulted in the formation of an opaque gel of collagen fibres. That this occurred at 37° C, but not in the cold, suggests that some unfolding of the collagen molecule is required to form fibres or gels, and that newly synthesized collagen molecules, *in vivo*, are also not as highly ordered as possible, but are somewhat unfolded in order to form connective tissue fibres. To test this idea *in vitro* we proposed to conduct the Gross heat gelation in deuterium oxide. Harrington and von Hippel[2] showed that in the case of ichthyocol collagen in deuterium oxide, the helix→coil transition occurs at a temperature 4° C higher than in water. If heat gelation of collagen in deuterium oxide were to require a higher temperature than in water, the hypothesis would be supported. When we attempted to carry out the experiment, unexpected results were obtained, of which a preliminary report is presented here.

Soluble collagen was obtained from rabbit skin by extraction at 5° C with two changes of 0·5 M sodium acetate, followed by citrate buffer of *p*H 3·5. The citrate solution was dialysed against *p*H 7·6 phosphate buffer and distilled water to yield collagen fibres which were collected by centrifugation, washed three times with distilled water and freeze-dried for storage.

Solutions of collagen were made and treated as shown in Table 1. Electron micrographs of the fibres *D* showed them to be about 500 Å wide and unstructured. It is of interest that after dialysis against water–phosphate fibres did not form on dialysis against deuterium oxide–phosphate (*C*), although in the original preparation the collagen had been dialysed against aqueous phosphate, although of a different strength and *p*H.

When *B* was slowly warmed the opaque gel of Gross[1] began to form at 35° C, and finally disappeared at 52° to give a clear gelatin solution. When *C* was heated, the opaque gel began to form at 25° C and finally disappeared at 56°. The range of existence of the deuterium oxide gel is thus 14° larger than that of the water gel, a remarkably large protein deuterium effect. Solutions *B* and *C* gave single hypersharp peaks in the ultracentrifuge.

Table 1. BEHAVIOUR OF COLLAGEN IN DEUTERIUM OXIDE AND WATER BUFFERS (0·04 M SODIUM DIHYDROGEN PHOSPHATE, 0·09 M DISODIUM HYDROGEN PHOSPHATE, *p*H 7·2) AT 5° C

0·2 per cent collagen in 0·5 M acetic acid in water or deuterium oxide, solution *A*	
A dialysed against water-phosphate buffer to yield clear, viscous solution *B* *B* dialysed against deuterium oxide–phosphate to yield clear, viscous solution *C*	*A* dialysed against deuterium oxide–phosphate buffer to yield fibres, *D*. Larger yield of fibres when *A* is made in deuterium oxide

When collagen in aqueous citrate buffer (0·1 M sodium citrate, *p*H 4·3) was heated, it merely became converted to gelatin on reaching about 40° C, as indicated by a large decrease in viscosity. However, in deuterium oxide–citrate the solution became turbid at 25° C, then opaque at about 29° C and deposited fibres.

Possible slight differences of acidity between the water and deuterium oxide solutions have been ignored so far. Both heat gelation[1] and denaturation in acid citrate[3] are known to be insensitive to such small *p*H differences. After the initial observations were made, we carried out the next experiments with deuterium oxide double-distilled from permanganate; identical results were obtained. Nevertheless, we are using purified deuterium oxide in all the present work.

We are carrying on an extensive investigation of the chemical and physical properties of various collagens in deuterium oxide, and of fibres conditioned with deuterium oxide. This solvent is also being used to extract collagen and other macromolecules from biological sources.

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Cholesterol Esters of the Cockroach *Eurycotis floridana*

INVESTIGATIONS of the metabolism and occurrence of sterols in insects have led to several reports of the presence of sterol esters in these organisms[1–3], and some observations concerning the characteristics of a sterol esterase in at least one species of cockroach have been recorded[1]. Up to the present time, however, no work on the constitution of the sterol esters of an insect has been described. We have undertaken such a study in relation to a broader investigation of the functions of sterols in the cockroach, *Eurycotis floridana*, and some of our preliminary findings are reported here.

The cockroaches used in this work were reared aseptically by the technique which has been described[4]. Materials from insects reared on three diets of distinctly different fatty acid content were analysed. Diet 1 was that which is used routinely in our laboratory and consisted of the semi-synthetic diet of Noland and Baumann[5], in which the corn oil was replaced by commercial sodium oleate. Diet 2 was identical with diet 1, except that the corn oil was replaced by a mixture of saturated fatty acids. Diet 3 was a commercially available dog food. The individual fatty acid components of these diets are given in Table 1 as percentages of the total fatty acid present.

Table 1. STEROL ESTERS OF THE COCKROACH, *Eurycotis floridana*, REARED UNDER VARYING DIETARY CONDITIONS

Diet	Percentage composition* of dietary fatty acids		Percentage composition of cholesterol esters of the insect Saturated	Mono-unsaturated	Di-unsaturated
1	Palmitic	9·9	1·3	82·0	16·7
	Stearic	1·9			
	Oleic	79·0			
	Linoleic	2·9			
	Others	6·3			
2	Palmitic	29·9	8·8	81·4	9·8
	Stearic	26·4			
	Arachidic	24·8			
	Docosanoic	12·2			
	Others (saturated)	6·7			
3	Palmitic	26·8	3·8	82·3	13·9
	Stearic	12·3			
	Oleic	39·3			
	Linoleic	11·6			
	Others	10·0			

* Calculated as: $\dfrac{\text{Peak height} \times \text{distance of peak from injection point}}{\text{Sum of such products for all peaks detected}}$

These values were determined by gas-liquid chromatographic analysis of the methyl esters prepared from the fatty acid additives of diets 1 and 2, and from the saponifiable fraction of diet 3. Identification of the peaks in these gas-liquid chromatograms and in others to be described was based on retention times and comparison with known standards. Diets 1 and 2 each contained 0·1 per cent 4-[^{14}C]-cholesterol, specific activity 4,140 d.p.m. per µg. 4-[^{14}C]-cholesterol was added to diet 3 to give a final specific activity greater than that of the sterol in diets 1 and 2.

As a general procedure for the isolation of sterol esters, the insects were chilled in ice and dissected as rapidly as possible into cuticle and soft tissues, from which the gut contents were carefully excluded.

The hard and soft tissues were separately extracted to remove the total lipids, and a crude sterol ester-fraction was isolated in each case by chromatography of the lipids on silicic acid, according to Barron and Hanahan[6]. This material was then fractionated into its component esters by a second chromatography on silicic acid by the method of Klein and Janssen[7].

Gas-liquid chromatography[8] of the methyl ether of the crude sterol isolated from the tissues of insects reared aseptically on diet 1 showed cholesterol to be the only sterol present. Attention was therefore focused on the identification of the fatty acid moieties of the sterol esters.

By the chromatographic procedure of Klein and Janssen, the sterol esters are eluted in order of increasing desaturation of the fatty acid chain and may be tentatively identified on the basis of their elution volumes. When chromatographed in this way, sterol esters of the insects were always eluted as three peaks, corresponding in relative elution volume to the esters of saturated, mono-unsaturated and di-unsaturated fatty acids, respectively, which together generally accounted for more than 90 per cent of the radioactive material applied to the column. These tentative identifications were confirmed with insects raised on all three diets by co-chromatography of their crude [^{14}C]-sterol esters with trace amounts of synthetic 3α-[^{3}H]-cholesteryl oleate and linoleate, followed by simultaneous assay of the eluant fractions for tritium and carbon-14. The two isotopes in the mono-unsaturated and di-unsaturated fractions were consistently recovered as coincident peaks extending over approximately 40 and 60 fractions respectively, indicating the possible identity of the main biological [^{14}C]-esters with the two added tracer substances.

The relative abundance of each type of ester found in the tissues of the insects is shown in Table 1. These values were calculated from the ^{14}C content of individual peaks as a percentage of the total carbon-14 recovered in all three peaks in the chromatography according to Klein and Janssen. This chromatographic evidence suggests that a similar pattern of cholesterol esters occurs in the insects reared on all three diets. The appearance of a large proportion of unsaturated esters of cholesterol in insects reared on diet 2, in which the fatty acids are entirely saturated, is consistent with our finding that unsaturated fatty acids are synthesized in this insect under the experimental conditions used[9].

Since our primary object was to identify the sterol esters present in the tissues of cockroaches reared under our routine conditions with diet 1, a more searching analysis of the sterol esters isolated from these insects was undertaken. Soft tissues weighing 2·8 g from twelve insects reared on diet 1 yielded 4·16 mg crude 4-[^{14}C]-cholesterol esters, the carbon-14 content of which indicated the presence of approximately 3·5 mg cholesterol esters, calculated as oleate. This material was divided into two portions, each of which was separated into its three component ester fractions in the manner indicated here. The fractions corresponding to the mono-unsaturated and di-unsaturated esters from one portion were combined with pure synthetic unlabelled cholesteryl oleate and linoleate, respectively, and recrystallized repeatedly with measurement of the specific activity after each crystallization. The oleate showed no loss of radioactivity after 5 recrystallizations (found 34·8; calc. 34·2 d.p.m. per µg), indicating the presence of 100 per cent of the label of the biological material in cholesteryl oleate. The linoleate, on the other hand, lost 26 per cent of the original activity in a similar series of crystallizations (found 8·2; calc. 11·1 d.p.m. per µg), indicating a maximum of 74 per cent cholesteryl linoleate in the di-unsaturated cholesterol esters of the insect.

The three ester fractions isolated from the second portion of this material were separately saponified and the fatty acids extracted, converted to their methyl esters by treatment with diazomethane, and analysed by gas-liquid chromatography under conditions which have been described[10]. All reagents and equipment used in the initial chromatography of this material and in the preparation of the methyl esters were specially treated to eliminate contaminants which otherwise produced spurious peaks in the gas-liquid chromatograms.

The results of these analyses supported the evidence of co-crystallization in showing that 99·5 per cent of the acid from the mono-unsaturated ester peak could be accounted for as oleate and that the major component (90·6 per cent) of the di-unsaturated ester fraction could be accounted for as linoleate. Other acids detected in the latter fraction were stearate (3·1 per cent), oleate (3·4 per cent), and possibly traces of palmitate, palmitoleate, myristate and other unknown materials. The 'saturated' ester fraction contained palmitate (43·1 per cent) and stearate (10·8 per cent) as the principal saturated acids and oleate (23·6 per cent) and palmitoleate (19·3 per cent) as mono-unsaturated contaminants.

The discrepancy between the values for the linoleate content of the di-unsaturated fraction, as shown by co-crystallization and by gas-liquid chromatography, may be due to varying degrees of cross-contamination of the major sterol ester fractions which we have observed to occur under some conditions during chromatography by the technique of Klein and Janssen. The appearance of unsaturated acids in the 'saturated' fraction may be due to the same cause. Whether these contaminants are always derived from the corresponding sterol esters is not known.

Further evidence for the identity of the oleate and linoleate esters was obtained by oxidative degradation of the acids isolated after saponification of the appropriate sterol ester fractions, which were obtained in this instance from the cuticle of insects raised on diet 1. The acids were oxidized by a micro-modification of the method of van Rudloff[11] to yield, in each case, a dicarboxylic acid representative of that part of the fatty acid chain lying between the carboxyl group and the double bond closest to it. Gas-liquid chromatography of the methyl esters of the oxidation products showed that a C9 acid was almost the sole dicarboxylic acid formed, a finding which is consistent with the proposed structures of the fatty acid moieties of the sterol esters.

The results so far obtained in this work leave little doubt that the major sterol ester of *E. floridana* reared on diet 1 is cholesteryl oleate. The remaining sterol esters appear to consist principally of linoleate and a small saturated ester fraction in which palmitate is the principal component. Further work is in progress to determine whether there is any consistent influence of the diet on the proportions of the various cholesterol esters in this insect.

We thank Prof. Konrad Bloch, in whose laboratory this work was carried out, for his continued interest and encouragement. One of us (R. B. C.) is an established investigator of the American Heart Association. This work was supported by grants from the American Heart Association and the U.S. National Institutes of Health.

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Phospholipid Composition and Turnover in Rat Intestinal Mucosa during Fat Absorption

SINCLAIR[1] suggested in 1929 that phospholipids are obligatory intermediates in the absorption of fat. Later work by Zilversmit, Entenman and Chaikoff[2] indicated, however, that there is little or no increase in turnover of the gross phospholipid fraction during absorption. Johnston and Bearden[3] recently obtained evidence that a minor component, phosphatidic acid, has increased turnover during fat absorption *in vitro*. It seemed important, therefore, to examine the turnover of individual mucosal phospholipids during fat absorption *in vivo*, with special reference to phosphatidic acid.

Rats, previously starved for periods of about 15 h, were fed with 0·75 ml. of olive oil, by stomach tube. A period of 2 h was allowed for absorption to proceed and the animals were then injected intravenously with 1 mc. carrier-free NaH$_2$^{32}PO$_4$ in 1 ml. of 0·9 per cent sodium chloride. Half an hour after the injection of the isotope, the animals were killed, the intestine removed and the contents of the small intestine, stomach and caecum irrigated with 0·9 per cent sodium chloride. The excess fat was extracted from these washings and related to the amount fed. The mucosal cells were then scraped off and immediately homogenized in ice-cold 10 per cent trichloroacetic acid solution. Phospholipids extracted from the precipitated material were subjected to mild alkaline hydrolysis and the water-soluble phosphate esters were separated by two-dimensional paper chromatography[4]. The presence of phosphatidic acid was confirmed by silicic acid chromatography. The phosphatidic acid-containing fraction was then hydrolysed by mild alkali and the resulting phosphate esters separated by ion-exchange chromatography[5]. Specific activities were calculated for each phospholipid and related to the specific activity of the acid-soluble phosphate fraction. All results were related to results from animals fed with 0·75 ml. of a 25 per cent glucose solution, but treated in the same way in all other respects.

The chromatograms showed a phospholipid pattern similar to that seen in liver, but the amount of inositol phospholipid was low. Phosphatidic acid was present in small amounts (approximately 2 per cent of the total lipid phosphorus) but had a higher specific activity than any other lipid; this did not appear to change during fat absorption. The high turnover of phosphatidylinositol in both control and fat-fed animals is noteworthy. The specific activity of the gross phospholipid fraction increased slightly during fat absorption, confirming the studies of Zilversmit *et al.*[2]. This increase appeared to be due chiefly to a more rapid turnover of phosphatidylcholine. The results are summarized in Table 1.

Table 1. RELATIVE SPECIFIC ACTIVITIES* OF RAT MUCOSAL PHOSPHOLIPIDS

Phospholipid	Control rats†	Fat-fed rats†
Phosphatidylcholine	21 (3)	93 (3)
Phosphatidylethanolamine	21 (3)	22 (3)
Phosphatidylserine‡	18 (2)	24 (2)
Phosphatidylinositol	175 (3)	183 (3)
Diphosphatidylglycerol	35 (2)	27 (2)
Phosphatidic acid	429 (3)	442 (3)
Total phospholipid fraction	44 (3)	60 (3)

* Expressed as: $\dfrac{\text{counts per 100 sec per 100 }\mu\text{g lipid P} \times 10^{4}}{\text{counts per 100 sec per 100 }\mu\text{g acid-soluble P}}$

† Average values, number of rats in parentheses.

‡ Since both counts and P content were very low, the difference between control and fat-fed rats is probably not significant.

The increased turnover of phosphatidylcholine during fat absorption may be connected with its requirement in stabilizing chylomicrons. The failure to find an increased specific activity of phosphatidic acid during fat absorption appears to conflict with the results of Johnston and Bearden[3]. There are, however, several important differences in experimental conditions since they studied hamster intestine *in vitro*, absorbing fatty acid rather than partially hydrolysed triglycerides. Phosphatidic acid is an intermediate in triglyceride biosynthesis when L-3-glycerophosphate is the glycerol precursor, but the resynthesis of triglyceride can also take place with monoglyceride as glycerol precursor[6]. This latter pathway, which does not involve phosphatidic acid, could well be more important when triglyceride is fed than when fatty acid is given, since lipolysis would provide monoglyceride.

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PHYSIOLOGY

Participation of Hepatic Glucoreceptors in the Control of Intake of Food

IN a previous communication[1], it was shown that anorexia produced by adrenaline is easily conditioned, while anorexia produced by the injection of glucose, instead of becoming conditioned, disappears with repetition. It was also observed during this work that intraperitoneal injection of glucose had a stronger and more persistent anorectic effect than intravenous.

After intraperitoneal injections of glucose (300 mg/kg) the venous and arterial glycaemias started to fall, reaching a minimum of 5–20 mg/100 ml. under the basal 8 min after the injection. Only 5 min after the injection the glycæmia started to rise, reaching a maximum increase of 15–60 mg/100 ml. above the basal, 10–20 min after the injection. On the other hand, anorexia began 1·5 min after the injection, being maximal at the moment of maximal hypoglycæmia, and ended in about 10 min, during the period of maximal hyperglycæmia. Therefore, it seemed that the hypothalamic centres are receiving hypoglycæmic blood during the maximum of the anorexia caused by glucose injection and, on the contrary, they are receiving hyperglycæmic blood just when the anorexia is over. Moreover, in the portal blood, as would be expected, the glycæmia rose immediately after an intraperitoneal injection of glucose, coming to an asymptotic maximum at about 3 min. The basal portal glycæmia was lower than the arterial one; that is, the arterio-portal glucose difference is positive. After an injection of glucose, when arterial glycæmia is decreasing and portal glycæmia increasing, there is a great reduction (or inversion) of the arterio-portal glucose difference, and this coincides with the time of maximal anorexia.

Based on these facts and assumptions, the following hypothesis is propounded:

There should be some 'information' about the difference in arterio-portal glucose, entering the central nervous system, and this could be achieved by a hepatic receptor 'informing' about the intracellular concentration of glucose (or any related metabolite). Therefore, the 'tonicity' of the 'feeding centre' would be the result (at least partially) of a tonic information coming from the liver that will be reduced or suppressed by the increase of glucose intracellular concentration in the hepatic cells. This would explain the stronger effect of adrenaline as compared with that of glucose injection. It will also explain the paradox of hunger in the diabetic, without the assumption that the hypothalamic glucoreceptors need insulin: in the absence of insulin, there will be a great increase on the tonic information from the liver and perhaps also muscle which could not be inhibited in spite of a possible rise on the satiation centre activity.

Further support to this hypothesis comes from the fact that, after glucose has lost its anorectic effect due to repetition, the glycæmic curve does not start any more with a hypoglycæmic phase, but takes a similar time course as the portal curve. Therefore, the arterio-portal difference is preserved all through and no anorexia is produced. Moreover, the hypothesis allows the prediction that small doses of insulin, by increasing intracellular glucose in the hepatic cells, should produce a reduction of food-intake, which was actually observed by Bach[2] and Anand[3].

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Increase of Sodium Efflux induced by Epinephrine in the Heart of the Frog

The sinus venosus or the atrium of the frog heart was dissected, cut open, and bathed in Ringer's solution containing sodium-24. When equilibrium had been reached between intracellular and extracellular activity the preparation was mounted in a small chamber through which a rapid stream of inactive Ringer's solution flowed. The activity of the preparation was determined by a scintillation counter in short intervals. After elimination of the extracellular activity the curve which represents the remaining activity consists of two exponential components with time constants of about 10 min and 100 min, respectively[1]. The slow component can directly be determined at the end of a loss curve, and the fast component is evaluated by graphical analysis (Fig. 1). The sum of the two components at zero time is equal to the intracellular sodium content as determined by flame photometry[2]. The flux Φ through the cell membranes is defined by the initial values A_1, A_2 and the time constants τ_1, τ_2 of the two components:

$$\Phi = \varphi_1 + \varphi_2 = A_1/\tau_1 + A_2/\tau_2 \qquad (1)$$

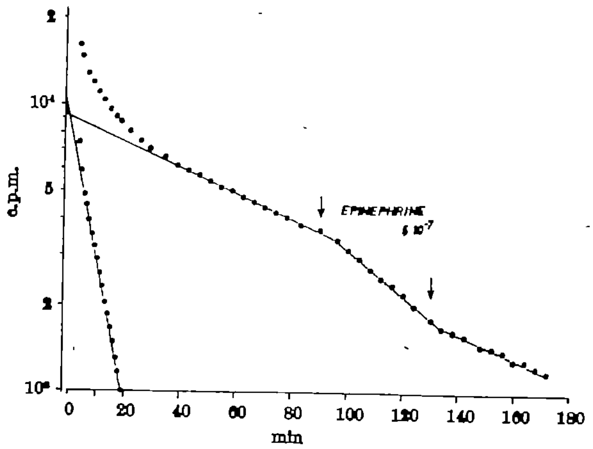

Fig. 1. Effect of epinephrine on the loss of sodium-24 in a frog sinus (open circles). The spontaneous rate was 26 min, but the sinus was driven electrically with 80 stimuli/min throughout the experiment. Temperature, 20° C. Wet weight, 1·1 mg. From min 40 to 90 the loss curve is a straight line (slow component). The fast component is represented by the filled circles. The numerical values of the initial activities and time constants are for the fast component A_1 = 6·2 m.equiv./kg, τ_1 = 9·1 min; corresponding values for the slow component are A_2 = 6·5 m.equiv./kg, τ_2 = 96 min. The fluxes are φ_1 = 0·68, φ_2 = 0·068, Φ = 0·748 m.equiv./kg tissue (wet weight) min. From min 90 to 130 epinephrine causes a decrease of the time constant of the slow component from 96 to 53 min. The slow flux component φ_2 is increased by 82 per cent, the total flux Φ at least by 7·4 per cent. After min 130 epinephrine is omitted, τ_2 increases again to 98 min

The beating rate of the preparation was kept constant throughout the experiment by electrical stimulation at 2–3 times the spontaneous rate of the preparation. Moreover, the effect of changes in frequency on the loss curve was tested and was found to be minimal for the slow component, but an increase in outflow of sodium could clearly be shown for the fast component[3].

When epinephrine was added to the perfusing Ringer's solution (final concentration 5 × 10^{-7} g/ml.) for 10–30 min an increase in the rate of loss of sodium-24 of both sinus and atrial preparations occurred. The time constant of the slow component can increase up to 100 per cent. When epinephrine is omitted the original rate of loss of sodium-24 is re-established (Fig. 1). The effect of epinephrine on the fast component of the loss curve seems to be small; the time constant of the fast component, however, cannot be determined with the same accuracy as that of the slow component. In a few experiments epinephrine caused only a transient increase in the loss of sodium-24 : 10–15 min after application of epinephrine the effects disappeared although the drug was still present. The increase in sodium outflow effected by epinephrine was found to be very much reduced or completely suppressed in presence of 2,4 DNP (1 m.mol/l.) or IAA (m.mol/l.) In test experiments with pericardium or adjacent connective tissue epinephrine did not increase the sodium outflow.

In similar experiments using potassium-42, epinephrine caused a comparatively small increase in the loss of activity for both components of the loss curve of atrial preparations.

Assuming that only the time constant of the slow component is affected by epinephrine according to equation (1) the effect of this drug amounts to an increase of total outflow Φ of sodium-24 by 7–10 per cent in our experiments. It cannot be decided whether an increase in sodium permeability and/or increase in the active transport is the basis of this increase in sodium outflow. The effect might be related to the marked tendency of epinephrine to produce depolarization of the membrane potential as observed in the sinus venosus[3,4]. It seems improbable that changes of the action potential produced by epinephrine are responsible for the increase in sodium outflow, since the beating rate of the preparation does not affect the time constant of the slow component of the loss curve.

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Piezoelectric Effect in Bone

THERE is convincing evidence that bone has an orderly morphological and microscopic structure. This evidence is derived mainly from electron microscopy, ordinary histological preparations, and microradiography[1]. Such a structure, consisting essentially of apatite crystals embedded in an organic matrix, might be expected to exhibit piezoelectric properties, as in the case of many other multicrystalline structures.

This has been conclusively demonstrated by Fukada and Yasuda[2], who measured the piezoelectric constants of small specimens of compact bone cut from human and ox femurs. These authors conclude from the observed relationship between polarization and stress that the effect is truly piezoelectric, and they suggest that it results from the slipping of collagen fibres past one another.

We have observed the same stress-induced electrical effect in a number of whole bones from different anatomical sites and species, both in bending and compression modes. The bones were cleaned of soft tissue and periosteum, then placed in an ultrasonic cleaner with detergent and finally dried. Electrodes were painted on the bones with conductive paint (DuPont silver paint No. 4817), leads were attached to these electrodes and connected to a vibrating reed electrometer (Applied Physics Corporation model 31), the output of which was displayed on a chart recorder. To minimize electrical pickup the bone being tested was kept in a shielded box and stressed from the outside. Bones which were stressed by bending, such as human ribs or rabbit femurs, were clamped at one end and bent by applying a force normal to the other end. Shorter bones, such as a human toe phalanx, were stressed by applying known compressive forces lengthwise along the bone axis.

Our results may be illustrated by the following example: the ends of a human toe phalanx were squared off with a saw to permit uniform application of the compressive force. Two silver bands, spaced 12·7 mm apart, were painted completely around the mid-portion of the bone. The d.c. resistance between these electrodes was approximately 10^9 ohms. Sudden application of a static force resulted in a potential difference between the electrodes proportional to the stress and with a decay time of about 0·5 sec; the latter appeared to be characteristic not only of the electrical circuit constants but also of some mechanical property (relaxation phenomenon) of the bone. On releasing the stress the same voltage pulse appeared with opposite polarity. The magnitude of the effect was of the order of 0·3 mV/kg of applied force.

It has long been known that the architectural structure of bones depends to a large extent on the mechanical forces acting on them. The fact that surface charges appear on stressed bone may be the controlling factor in bone formation. The local electric fields resulting from such surface charges might be expected to influence the orientation and deposition of ions or polarizable molecules. As evidence for the effect of electric fields on bone formation, Yasuda *et al.*[3] showed that the development of callus in living bone could be induced by electrical energy. Similarly, Bassett[4] has influenced the direction of bone growth in tissue culture by means of electric fields, as well as by inhomogeneous magnetic fields.

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A Possible Mechanism of Neurosecretion: Release of Vasopressin by Depolarization and its Dependence on Calcium

IT is recognized that the various influences, nervous and osmotic, which bring about release of the hormones vasopressin and oxytocin from the neurosecretory terminals in the posterior lobe of the pituitary gland act through the parent cells in the supra-optic and paraventricular nuclei. Such a scheme demands that these cell bodies in the hypothalamus regulate release of hormone from their terminals a considerable distance away and it is simplest to suppose that this regulation is effected by action potentials propagated down the supra-opticohypophysial tract. In this view the cells of the supra-opticohypophysial system possess the characteristic property of neurones—the propagation of action potentials—as well as the characteristic property of endocrine organs—the liberation of hormones into the blood stream[1,2].

The purpose of this communication is to give a preliminary account of experiments indicating that depolarization of the endings of the supra-opticohypophysial tract, such as might be caused by action potentials, is an adequate stimulus for the release of vasopressin and suggesting an explanation of the way in which depolarization may act.

The experiments were performed on rats weighing about 150 g. The infundibular stem was cut and the posterior pituitary glands rapidly removed, halved and suspended *in vitro* in Locke's solution at 37·5° C. Five glands were used in each experiment. At intervals of 20 min the incubation medium was drawn off and replaced with fresh medium of the same or different composition. The pressor activity of the incubates was then assayed against pitressin using the method described by Dekanski[3]. After 20–40 min incubation in Locke's solution the resting output of vasopressin was generally about 20 μU per gland per min. A much higher rate of secretion occurred when the potassium concentration was raised ten-fold (to 56 mM). A typical effect is shown in Fig. 1*a*. This powerful stimulant effect of excess potassium was found to be dependent on the presence of calcium; there was no increase in vasopressin output when excess potassium was given in calcium-free Locke's solution after a period of 40 min incubation with the calcium-free medium (Fig. 1*b*). The inhibitory effect of calcium omission was readily reversible and potassium regularly regained its effectiveness as a stimulus for vasopressin release after calcium had been restored to the incubation medium. Finally, it was found that calcium itself (2 mM) caused a powerful release of vasopressin when added to a preparation exposed to

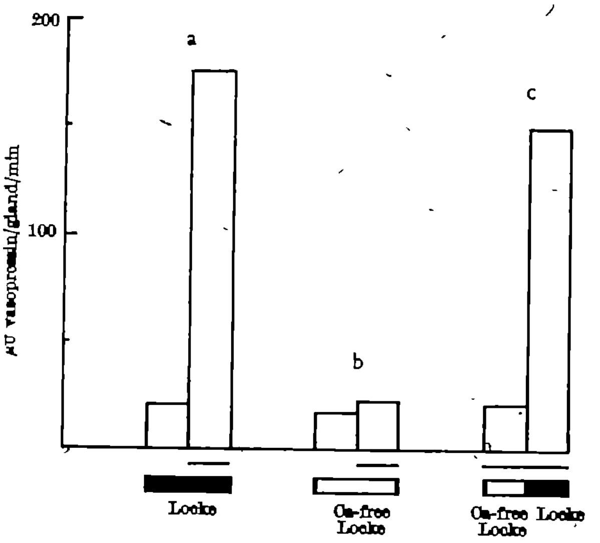

Fig. 1. Vasopressin output from the rat's posterior pituitary gland incubated *in vitro*. Each vertical column indicates the rate of vasopressin release in a 20-min period by a group of five hemisected glands. *a*, shows the stimulant effect of raising potassium from 5·6 to 56 mM; *b*, shows that 56 mM potassium is without effect in calcium-free Locke's solution. Records *a* and *b* are from the same experiment. *b* was obtained first after a total of 40-min incubation in calcium-free medium and *a* was obtained 40 min later after the re-introduction of calcium. *c* shows, in another experiment, the stimulant effect of re-introducing calcium during exposure to 56 mM potassium in a calcium-free medium. The thin horizontal bars indicate the presence of 56 mM potassium. The thick horizontal bars indicate the nature of the basic perfusion medium

calcium-free high-potassium Locke (Fig. 1*c*). The re-introduction of calcium in the absence of excess potassium did not have such a stimulant effect.

Unless the fibres of the neurohypophysis are quite different from peripheral nerves it may be assumed that an extracellular potassium concentration of 56 mM results in a large reduction in membrane potential. For example, in the small nerves of the mammalian vagus which, like those of the pituitary, are non-myelinated, such a concentration of potassium lowers the resting potential to about 1/3 of its normal value[4]. The stimulant effect of excess potassium would therefore suggest that depolarization of the endings of the supra-opticohypophysial tract is an adequate stimulus for vasopressin release. The additional observations on the inhibitory effect of calcium omission and the stimulant effect of adding calcium during exposure to excess potassium indicate that calcium has a key role in the release process. In interpreting these results it would seem most profitable to consider the existing evidence on the role of calcium in the secretory activity of another endocrine gland of neural origin, the adrenal medulla. The medulla, too, is stimulated by excess potassium, and calcium is required for the response[5]; moreover, the addition of calcium during exposure to excess potassium increases the rate of secretion[6]. It is further known that adrenal medullary secretion varies directly with the extracellular calcium concentration over a wide range of concentrations above as well as below the normal[5], that calcium itself is a secretogogue in several different situations known to increase membrane permeability[5,6] and that potassium increases the rate of uptake of calcium-45 by the medulla[7]. The conclusion which has been drawn from these experiments on the adrenal medulla is that calcium acts as a crucial link in the process of 'stimulus-secretion coupling'[5-8]. Although the present observations on the neurohypophysis are comparatively limited, they suggest a similar mechanism of secretion. As a working hypothesis it may therefore be proposed that the release of vasopressin by the neurohypophysis is effected by the arrival of impulses from the supra-optic and paraventricular nuclei which, by depolarizing the endings of the supra-opticohypophysial tract, promote calcium influx (as is known to occur in peripheral nerves[9]) and that the appearance of free calcium ions somewhere in the endings then causes the release of the stored hormone.

A more complex mechanism of release of neurohypophysial hormones has been postulated by Abrahams, Koelle and Smart[10] and Gerschenfeld, Tramezzani and De Robertis[11], who have speculated that acetylcholine may have some physiological role in the release of the hormones from the endings of the supra-opticohypophysial tract. The first group base their conjecture on histochemical evidence that cholinesterase is present in this system, and the second on the finding of 'typical synaptic vesicles' in the endings. In some preliminary experiments with Dr. A. M. Poisner, no release of vasopressin was found when the posterior pituitary was exposed to acetylcholine 10^{-4} g/ml. with or without eserine 10^{-5} g/ml., carbachol 10^{-4} g/ml. or nicotine 10^{-4} g/ml.

When considered along with the existing evidence that calcium is involved in the release of catecholamines from the adrenal medulla[5-8] and of acetylcholine from cholinergic neurones[9,12] the present findings suggest that calcium may have a general role in 'stimulus-secretion coupling' in neurones and endocrine glands of nervous origin.

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PHARMACOLOGY

Role of Species Specificity in Failure of Inhibition of Guinea Pig Anaphylactic Sensitization with Cancer Chemotherapy Drugs

Three years ago Schwarz and Dameshek showed that 6-mercaptopurine could block the formation of antibodies to foreign protein in the rabbit[1]. This phenomenon has been verified by some, and its existence questioned by others. To understand better the mechanisms involved in the drug-induced antibody blockade, it is necessary to examine those variables which enhance or prevent this blockade of antibody formation. The work reported here gives evidence that the effect of the cancer chemotherapy agents in blocking primary antibody formation depends, at least in part, on the species studied.

The techniques used have been described in detail elsewhere[1,2]. Briefly, approximately 400-g stock albino guinea pigs were given daily intraperitoneal injections of the test drug according to the following schedule: (1) 6-mercaptopurine, 50 mg/day; (2) vincaleukoblastine, 10 γ/day; (3) actinomycin-*D*, 10 γ/day; and (4) *cyclo*-phosphoramide, 10 mg/day. The drugs were given daily for ten days.

10 mg of purified ovalbumin were given intraperitoneally to the experimental animals on the second day of administration of the drug. Control animals received only ovalbumin. Three weeks after the initial injection of ovalbumin, an intracardiac eliciting injection of 2 mg of ovalbumin was given. Immediate death in anaphylactic shock provided the end point.

The dosage schedule used was determined in preliminary toxicity studies in our laboratory. It is seen from Table 1 that 6-mercaptopurine and *cyclo*phosphoramide were less toxic than the remainder of the drugs studied, even though the former were dosed in 1,000-fold greater quantities.

Table 1

	Total animals	Drug toxicity deaths
6-Mercaptopurine	30	3
Vincaleukoblastine	30	12
Actinomycin-*D*	30	16
*Cyclo*phosphoramide	30	9
Controls	30	0

Table 2

	Animals elicited	Fatal anaphylaxis	Survivors
6-Mercaptopurine	27	25	2
Vincaleukoblastine	18	15	3
Actinomycin-*D*	14	14	0
*Cyclo*phosphoramide	21	1	20
Controls	30	27	3

The anaphylaxis rate in the several groups are summarized in Table 2.

*Cyclo*phosphoramide prevented fatal anaphylaxis in 20 of 21 animals. This verifies our previous work in which we showed that in the guinea pigs the drug prevented development of anaphylaxis[2] and of precipitin formation as measured by the Preer agar diffusion technique[3].

6-mercaptopurine, actinomycin-*D* and vincaleukoblastine were completely ineffective in preventing anaphylaxis; the survival rate with the drugs was not significantly greater than that of the control group which received ovalbumin without drug therapy.

The finding that *cyclo*phosphoramide blocks anaphylaxis in the guinea pig, while 6-mercaptopurine, vincaleukoblastine and actinomycin-*D* do not, we interpret as evidence that species specificity plays a significant part in the action of these drugs. A similar interpretation has been suggested by Genghof and Battisto, who showed no inhibition of precipitin formation in guinea pigs receiving 6-mercaptopurine[4]. Frisch and Davies examined the inhibition of hæmagglutinin formation in mice by purine and pyrimidine analogues and noted great variation in the effectiveness of the drugs tested in this species[5].

It has been stated[1] that immunological unresponsiveness produced by the cancer chemotherapy agents may be due to a general inanition of the animals. Our data would not support this concept; the animals with the greatest weight loss and highest death rate were those receiving vincaleukoblastine and actinomycin-*D*. Neither drug evidenced the ability to inhibit antibody formation.

In summary, *cyclo*phosphoramide blocks the development of anaphylaxis to ovalbumin in the guinea pig. In equally or more toxic doses, 6-mercaptopurine, vincaleukoblastine and actinomycin-*D* do not afford protection. We interpret this as evidence of the species specificity of this class of drugs in inhibiting antibody formation.

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Estimation of Methylpentynol

Methylpentynol, a central nervous system depressant, is a tertiary carbinol. Previous methods for its estimation consist of distilling the drug which is volatile out of the tissue or fluid sample into ammoniacal silver nitrate. By virtue of its acetylinic bond methylpentynol combines quantitatively with the silver to form a precipitate. Since two moles of silver react with each acetylinic group[1] the quantity of methylpentynol present can be detected by either colorimetric determination of the amount of silver in the precipitate[2] or by back-titration of the uncombined silver in the supernatant with potassium thiocyanate[3], recovery curves for methylpentynol being obtained by the addition of known quantities of methylpentynol to ammoniacal silver and titration with potassium thiocyanate. The former method is sensitive to 1·0 mg and the latter to 0·1 mg methylpentynol.

Present work on the recovery of the drug from the brains of small animals necessitated the refinement of the technique and a potentiometric method was developed. The specimens containing methylpentynol were heated in a water-bath at 90° C and the volatile drug drawn by an air-stream through 0·0125 M alkaline silver reagent with the formation of an insoluble silver derivative. The excess silver in the supernatant was titrated against 0·0125 M potassium iodide solution. The equivalence point was taken as the centre of steepest portion of the titration curve (Fig. 1), usually between 350 and 400 mV. This was measured with a *p*H meter using a silver electrode (20 s.w.g.) and a reference sodium sulphate glass electrode connected to the solution through a potassium nitrate agar bridge. The end point was plotted graphically so that inaccuracies due to drop size delivered from the 5 ml. burette were not crucial.

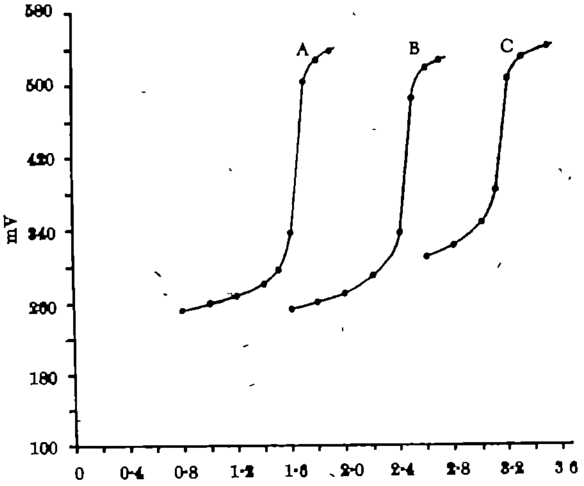

Fig. 1. Titration curves for: *A*, distilled standard of 1·0 mg methylpentynol; *B*, drug sample distilled from cat brain; *C*, control specimen of 0·0125 M ammoniacal silver nitrate

The method enabled 0·01 mg methylpentynol to be detected. Examination of the reliability of the technique revealed that the correlation between the amount of methylpentynol and its estimation varied between 0·92 and 0·96 ($P < 0{\cdot}01$), whereas for the chemical method[3] r varied between 0·64 and 0·93, the former value not being significant. The standard error of the titration of 0·5 mg methylpentynol was 0·012 mg (12 estimations); mean recoveries of 0·5 mg methylpentynol from distilled water were 99, 99 and 102 per cent. Drug recoveries were not affected by the presence of brain tissue ($P > 0{\cdot}1$).

This sensitive and reliable method for the estimation of a central depressant drug made the technique of considerable value in correlating drug concentration in the brain with cerebral function.

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Increased Uptake of Mercury in Mouse Brain caused by 2,3-Dimercaptopropanol

2,3-DIMERCAPTOPROPANOL (BAL) is generally used in the treatment of mercury poisoning. In the case of acute mercury poisoning there is evidence to indicate that such treatment is beneficial[1]. Experiments on animals[2,3] have shown that BAL effects a redistribution of the mercury in the body and enhances the urinary excretion of the former, thereby mobilizing mercury accumulated in the kidney, which is the critical organ in acute mercury poisoning. In chronic mercury poisoning, however, the brain is the critical organ, because of the slow elimination of mercury from some of its parts[4]. It was therefore considered important to learn how BAL therapy affects the uptake of mercury by brain tissue. In one approach to this problem mice were given mercury-203 (0·5 mg mercury/kg body-weight), together with BAL in about equivalent amounts (0·4 mg BAL/kg). Controls were given only the mercury compound. The animals were killed at various intervals after the injection. Autoradiograms of whole-body sagittal sections were made as described earlier[5]. The order of isotope concentration in the organs was determined by densitometric comparison with an isotopic standard 'staircase'.

The autoradiograms revealed that the uptake of mercury in the brain was greater when phenyl- or methyl-mercuric compounds were given together with BAL than when they were given alone. Fig. 1 shows the autoradiograms of sagittal sections of mice injected with phenyl-mercuric acetate (upper), and with phenyl-mercuric acetate + BAL (lower). The animals were killed 8 days after the injection. The increased uptake of radiomercury in the brain of the BAL-treated mouse is well illustrated. The standard 'staircase' below each section shows the density caused by decreasing concentrations of the isotope in geometric series (ratio ½). In the case of injection of inorganic mercury compounds the autoradiograms did not provide any conclusive evidence as to how BAL treatment affects uptake in the brain. However, further experiments are being made in this regard. Detailed

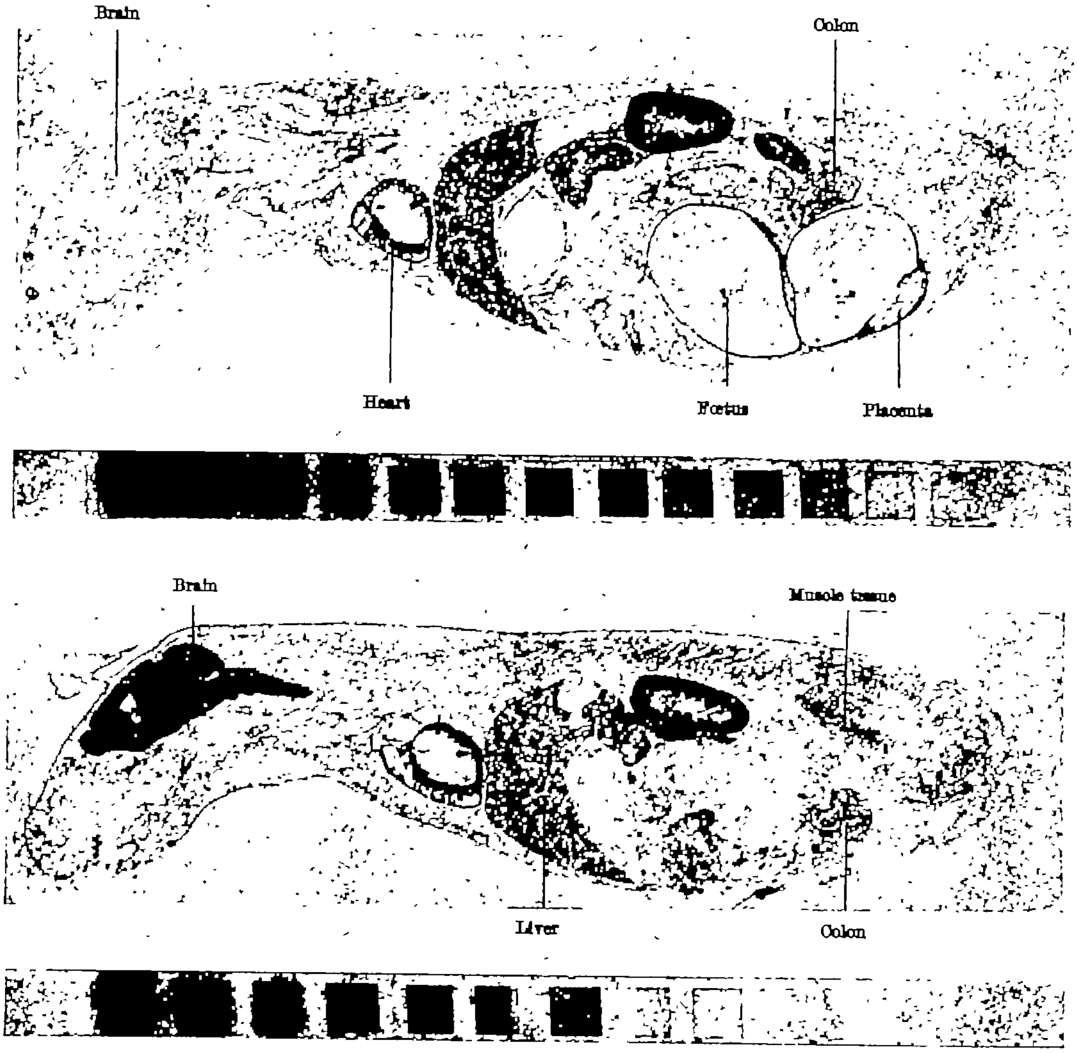

Fig. 1. Autoradiograms of distribution of mercury in whole-body sagittal sections of mice 8 days after injection of phenylmercuric acetate (upper), and of phenylmercuric acetate and BAL (lower). Below each section is the isotope standard 'staircase' autoradiographed on the same film

reports of the studies referred to here will be published elsewhere.

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HÆMATOLOGY

Decreased Erythrocyte Survival in Alloxan Diabetic Rat

Although numerous metabolic activities have been investigated in the alloxan diabetic rat, the red cell survival has not been reported. Derangement in the metabolism of the human erythrocyte in diabetes mellitus was shown recently by Long and Carson[1]. The present communication reports a reduction in the red cell life-span in the alloxan diabetic rat.

Albino rats were rendered diabetic by the intravenous injection of 40 mg alloxan/kg body-weight. The diabetic animals (blood sugar-levels more than 300 mg per cent) were tagged with 10 μc chromium-51 three weeks after alloxanization in order to minimize the possibility of a direct effect of alloxan on the erythrocytes. The time in days between tagging and the disappearance of the radioactivity from the blood is taken as the maximal survival time. The details of the procedure have been published elsewhere[2].

Table 1. Maximal Survival Time of Erythrocytes of Normal and Alloxan Diabetic Rats*

	Normal		Alloxan diabetic	
	Body-weight (g)	Max. survival (days)	Body-weight (g)	Max. survival (days)
Mean (± *S.D.*)	206 (± 18)	68 (± 3) $P < 0·01$	146 (± 18)	56 (± 1)

* Nine rats in each group.

Table 1 shows that while normal rats had an average maximal survival time of 68 (±3) days, the alloxan diabetic rats had an average value of 56 (± 1) days. These results indicate clearly that alloxan diabetes reduces significantly the survival of red cells in the rat. Although three weeks were allowed between alloxanization and tagging, one cannot exclude the possibility that the observed effect might be, at least in part, due to the direct action of alloxan on the erythrocytes. However, of 11 diabetic rats, 2 recovered spontaneously after tagging, and their erythrocytes were found to have maximal survival times of 60 and 68 days, values which approach those of the normal animals. This tends to provide evidence against the direct effect of alloxan on the red cells. Moreover, György and Rose[3] showed that although alloxan hæmolyses red cells from vitamin E-deficient rats, it had no such effect on those from normal animals.

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PATHOLOGY

Effect of a New Anticancer Agent, 1,4-Dimethyl-1,4-Diphenyl-2-Tetrazene, on a Transplantable Mammary Adenocarcinoma (72*j*) and Spontaneous Mouse Breast Tumours

1,4-Dimethyl-1,4-diphenyl-2-tetrazene[1] (MPT) inhibits the growth of transplanted 72*j* mammary adenocarcinoma carried in C_3H mice. Its activity was found during the screening[2,3] of compounds structurally related to the antineoplastic agent 3,3-dimethyl-1-phenyltriazene[4]. The corresponding 1,4-diethyl[5] and 1,4-di(*p*-nitrophenyl) analogues of MPT were also found active against this tumour. Extensive laboratory evaluation of MPT and synthesis of additional compounds are in progress and will be reported in detail[6,7]. The following biological findings were considered to be of immediate interest.

MPT given subcutaneously or intraperitoneally over a wide range of doses (that is, 2–50 mg/kg/day) prolonged the median survival-time of mice bearing the 72*j* mammary adenocarcinoma to about 60 days, from an average of 30 days in control animals. (Treatment was started 17 days following transplantation. Experimental design in both the transplanted and spontaneous tumour test systems was that introduced by Goldin[8].) In contrast, THIO-TEPA, a known clinically useful anticancer agent, was active over a much narrower range of doses (that is, 0·8–2 mg/kg/day), and had an estimated maximal median survival-time of 50 days. MPT fed in the diet resulted in prolongation of the median survival-time to about 100 days (500, 1,000 or 2,000 mg/kg/day). This median survival-time is the longest observed in these laboratories with any anticancer agent given to the 72*j* mammary adenocarcinoma bearing mouse.

MPT was also interesting because of its apparent ability to prolong significantly the median survival-time of spontaneous tumour-bearing C_3H mice. MPT given subcutaneously (6 or 20 mg/kg/day) to C_3H mice bearing spontaneous mammary tumours increased the maximum median survival-time from 35 days in control mice (starting from the time the mice were placed on test) to 68 days. The compound showed effects over a wide range of doses: that is, 2–150 mg/kg/day. MPT was less effective when given intraperitoneally. By the oral route the median survival-time was prolonged to 70–80 days (500, 1,000 or 2,000 mg/kg/day) versus 37 days for control animals (see Table 1 for results of diet administration).

Table 1. Survival Data of C_3H Mice bearing Spontaneous Mammary Tumours

Compound	Estimated daily dose (mg/kg)	No. of mice/group	No. of exp.	Mean median survival (days)
MPT (in diet)	2,000	5	5	77*
	1,000	5	5	78*
	500	5	5	73*
THIO-TEPA (in drinking water)†	6·7	5	2	42
	4·5	5	2	37
	3·0	5	2	39
	2·0	5	2	43
	1·3	5	2	46
	0·9	5	2	34
Controls (no treatment)	—	5	7	37

* Significant prolongation of median survival $P = 0·01$.
† Solutions were made up fresh daily.

In contrast, THIO-TEPA (by any route of administration) had no effect on the median survival-time of spontaneous tumour-bearing mice.

An earlier report indicated that marked bone marrow destruction was found concomitant with antitumour activity in the laboratory evaluation of alkylating agents and many antifolic compounds[9]. In similar experiments MPT caused no depression of the marrow concomitant with tumour inhibition; moreover, this compound was not identified as an alkylating agent when tested chemically by the Friedman and Boger method[10]. MPT was completely ineffective against the $6C_3HED$ lymphosarcoma and the sarcoma 180 tumour when given by any of the routes or dosage schedules mentioned here.

It should be noted that in none of these reported investigations was destruction of tumour tissue (neither spontaneous nor transplanted 72*j* mammary adenocarcinoma) observed: that is, growth was apparently held in abeyance to size tolerated by the host. Short-term tumour growth investigations (1 week of therapy) did not reveal, as well as did the survival examination cited here, the interesting biological aspects of this compound.

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Effects of Corticosteroids on Experimental Amœbiasis

The examination of the influence of drugs on amœbiasis has depended in large part on results obtained from experimentally infected laboratory animals. Conventional methods for the establishment of experimental amœbiasis have involved administration of amoeba *per os* or *per anum*, or the injection of trophozoites into the cæcal lumen.

The most widespread method is that of Jones[1] for the intracæcal inoculation of amœbic trophozoites, which produces multiple, disseminated lesions, of different sizes in different locations, and great diversity in the pathological progress of the experimental disease. The unpredictable degree of infection makes it impossible to standardize lesions so that they will be suitable for critical studies. Similarly, *per os* or *per anum* administration of amoebae is unsuitable for critical evaluation of the complexities in amœbiasis because uniformity of infection cannot be established.

Recently, an intramural inoculation technique has been developed involving the injection of amoebae into the wall of the intestine[2]. Inoculation is made just below the serosa of the cæcum with negligible injury to the tissue at the site of injection and mixing of the injected amoebae with the contents of the cæcal lumen is avoided. The most important advantage of the intramural technique is that it provides a single-lesion infection, standardized in its nature, characteristics and development, always located at the spot of inoculation, from which the pathological process proceeds toward the mucosa. In about one week, a typical amoebic abscess forms, and there is active migration of amoebae towards the mucous membrane, and also through the physically intact mucous membrane into the lumen of the cæcum.

Some time between the fifth and tenth days, the abscess perforates through the mucous membrane and the lesion is converted to a typical amœbic ulcer with central erosion, deep necrosis and a widely undermined mucosal margin. The mass of the amoebae are now spilled into the intestinal lumen and marks the end of the period of existence of the experimental model since multiple, secondary lesions can now develop.

This communication is concerned with the influence of corticosteroids on the rate of pathological development in the early, single-lesion phase of the disease. The decision to test corticosteroids in this model disease was based on: (1) the general knowledge that infectious disease is often a contraindication for therapy with corticotropin or cortisones; (2) the fact that there are clinical reports which attribute the exacerbation of latent amœbiasis to the use of cortisone[3,4].

The dose of corticosteroid used in this work was 25 mg/kg. In the first and second experiments the drug was given *per os* the day before infection, repeated on the day of infection and for three consecutive days after infection. In the third experiment, hydrocortisone was given by intraperitoneal injection the day before infection and every other day thereafter. In the fourth experiment, hydrocortisone was given as in the first two, but medication was continued until the time the animal died or was killed. Controls were handled in the same manner as treated animals, and were given equivalent volumes of vehicle.

The infection was initiated as already described. Male guinea pigs weighing about 250 g were placed under hexobarbital anæsthesia and inoculated subserosally in the cæcal wall with approximately 10,000 trophozoites of a virulent strain of *Entamoeba histolytica* which had recently been isolated from an active clinical human case.

Evaluation of the pathological process was based on observations of all animals with single lesions.

The extent of the hyperæmic zone surrounding the lesion was scored on the following basis: 0, no inflammation; 1, slight hyperæmia; 2, moderate hyperæmia; 3, pronounced hyperæmia. Direct smears of the mucosa in the vicinity of the lesion were made at the time the cæcum was excised and opened, and examined microscopically for the prevalence of amoebae. The scoring system for microscopic index of abundance of amoebae was as follows: 0, none present; 0·5, less than 1 per field; 1, 1–5 per field; 2, 6–30 per field; 3, 30 or more per field. A minimum of 20 fields was examined with the 10 × objective and a 10 × eyepiece.

In the first experiment, cortisone and hydrocortisone treatment were compared, and results are shown in the upper part of Table 1. On the basis of objective measurement, it is seen that in this series the lesions were larger in corticosteroid-treated animals than in untreated controls. When cortisone-treated and hydrocortisone-treated animals are considered together, their mean combined lesion size is 12·79 ± 1·44 mm^2, with $P < 0{\cdot}02$ for significance of difference from control value. Perforations were more frequent in corticosteroid-treated animals. Prevalence of amoebae in the intestinal lumen was most marked in animals which had been given hydrocortisone.

Table 1. Some Influences of Corticosteroids on Experimental Single-Lesion Amœbiasis in the Guinea Pig

Treatment	No. infected	No. surviving on fifth day with single lesions	Microscopic index (No. of amoebae)	Hyperæmic index	Lesion area (mm^2 ± *S.E.*)	Perforation
Control	5	4	0·75	1·75	7·17 ± 0·97	2/4
Hydrocortisone acetate	4	3	1·33	1·67	12·96 ± 3·34 $P > 0{\cdot}1$	2/3
Cortisone acetate	4	4	0·33	1·50	12·06 ± 1·28 $P < 0{\cdot}02$	4/4
Control	6	6	0·50	2·17	24·32 ± 4·47	3/6
Hydrocortisone acetate	6	6	0·67	3·00	26·17 ± 3·20 not. sig.	2/6

Subjectively, from the appearance of the animals, it was felt that both drugs were having an adverse effect on host resistance, and the work was extended with hydrocortisone. Repeating the initial experiment with hydrocortisone alone gave results which are shown in the lower part of Table 1. Once again the prevalence of amoebae in the intestinal lumen was greater in the hydrocortisone-treated animals than in controls, and surrounding tissue reactions were more marked. All treated animals showed a highly hyperæmic response.

In a third experiment, 14 guinea pigs were given a light amœbic infection by intracæcal inoculation. Half were given hydrocortisone every other day, and all survivors were killed and autopsied on the 11th day after infection. The gross appearance of hydrocortisone-treated animals was far worse than that of controls. Treated animals invariably showed massive hæmorrhagic and hyperæmic involvement of the entire cæca while control animals showed only occasional scattered hyperæmic patches.

A final experiment was carried out with guinea pigs which had received an intramural inoculation of slightly more than 13,000 amoebae each. Half the animals received hydrocortisone daily. The majority of the animals in both treated and control groups were killed on the fifth day after infection. Again, hydrocortisone-treated animals showed hyperæmia, hæmorrhage and occasional metastasis of amœbic foci while such findings were rarely found in control animals, and then only to a minor degree. Four treated and four control animals were not killed on the fifth day, but were maintained with the intention of killing them on the tenth day after infection. By the tenth day, three of the four hydrocortisone-treated animals had died spontaneously, while none of the controls had died. Since guinea pigs have a life expectancy of about twenty days after an infection of this magnitude, we attribute these early deaths to the influence of the corticosteroid on the progress of the disease. At autopsy, these animals evidenced a fulminating amœbiasis characteristic of animals dying on or about the twentieth day without treatment.

Thus, we have found that administration of corticosteroids results in marked exacerbation of pathology in animals with amœbic disease. The mode of action for these changes is not yet clear. Our results so far suggest several lines of future investigation. These include the response of the experimental disease to decreased dosages of corticosteroids, and the use of axenic cultures of *Entamoeba histolytica*[5], not available at the time this work was done.

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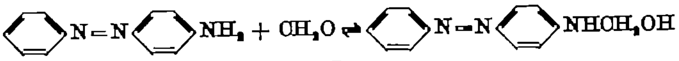
Azo Dye Liver Carcinogenesis: Reaction of Hydroxymethylaminoazobenzene with Nucleic Acids *in vitro*

IN a long series of aminoazobenzene derivatives tested for hepatocarcinogenicity in the rat only those compounds with at least one *N*-methyl group are markedly active[1]. The hypothesis has been advanced that the actual carcinogen is a derivative of the parent dye and that its production may be hindered or enhanced under different dietary conditions, or in different species. The *N*-methyl group is thought to undergo stepwise oxidation in the liver to formaldehyde, the first product being an *N*-methylol derivative, hydroxymethylaminoazobenzene (I) in the case of methylaminoazobenzene[2]. Such compounds are reactive species and are capable of alkylating thiol groups, amino-groups, phenolic residues and so on by a Mannich condensation. It has been suggested that reactions of this type account to some extent for the formation of protein-bound dye *in vivo* in the case of dimethylaminoazobenzene (butter yellow) and certain of its derivatives[1,3,4]. In view of the importance of DNA and RNA in cellular metabolism, an investigation has been carried out to determine whether such methylol derivatives are capable of reacting with nucleic acids *in vitro*.

It is not possible to isolate the *N*-methylol derivative of aminoazobenzene, but at neutrality it will exist in equilibrium with aminoazobenzene and formaldehyde when the latter reactants are mixed at room temperature.



(I)

In the absence of any competing reactant, it condensed rapidly with aminoazobenzene forming di-(4-phenylazophenylamino) methane (II):

$$\text{PhN} = \text{NC}_6\text{H}_4\text{NHCH}_2\text{OH} + \text{PhN} = \text{NC}_6\text{H}_4\text{NH}_2$$
$$\rightarrow \text{PhN} = \text{NC}_6\text{H}_4\text{NHCH}_2\text{NHC}_6\text{H}_4\text{N} = \text{NPh} \quad \text{(II)}$$

but condensation readily occurred with RNA when this was added to the equilibrium mixture.

Yeast RNA (200 mg) in water (10 ml.) was treated with a mixture of 40 per cent formaldehyde (0·1 ml.) and aminoazobenzene (150 mg), and the mixture shaken for 10 min. After standing overnight at room temperature the mixture was extracted many times with ether or benzene until no further colour could be removed from the yellow aqueous solution. RNA, which was coloured yellow, was precipitated with ethanol after adding sodium acetate to a concentration of 2 per cent. Repeated washing of the RNA with ether and benzene failed to remove further colour. The amount of dye bound was 0·0045 mg/mg RNA. In the absence of formaldehyde no dye binding occurred either with aminoazobenzene or the cross-linked compound (II) which was formed to some extent during the reaction.

A second run using RNA (300 mg), aminoazobenzene (150 mg), 40 per cent formaldehyde (0·05 ml.), water (60 ml.) and ethanol (40 ml.) gave a similar product after 10 min at room temperature (0·001 mg dye/mg RNA).

The nature of the dye binding to RNA has not yet been fully investigated but a Mannich condensation involving base amino-groups seems likely. The dye–RNA complex has the properties one would expect from a compound possessing an —NH.CH$_2$.NH— linkage, since it is stable to alkali (0·3 N caustic soda for 12 h at room temperature), but decomposes rapidly in the presence of 6 N hydrochloric acid. After neutralization of the acid solution with ammonia the dye was extractable into benzene, showing that it was no longer bound to RNA. The extracted dye had major absorption peaks at 380 mμ in neutral solution and at 500 mμ in 6 N hydrochloric acid, indicating that it was probably aminoazobenzene. In aqueous solution the RNA–dye complex slowly dissociated and with one sample of the complex, containing 0·0014 mg dye/mg RNA–dye complex at a concentration of 20 mg complex/ml., equilibrium was reached within 2 h.

Further evidence that a covalent bond was formed between the dye and RNA rather than simple adsorption of the dye on to the RNA was obtained by hydrolysing the RNA to its nucleotides with 0·3 N caustic soda for 12 h, when it was still not possible to extract the dye with benzene.

In the case of DNA the situation was complicated by the adsorption of large quantities of free dye, both aminoazobenzene and II, although this could be removed from the more rigidly bound dye by extraction of an alkaline solution of the complex with benzene or ether.

Salmon sperm DNA (40 mg) in water (10 ml.) was treated with formaldehyde (0·05 ml.) and aminoazobenzene (95 mg) and the mixture left at room temperature for 11 h. The mixture was extracted three times with ether, and the DNA–dye complex was then precipitated with ethanol. The yellow DNA was washed many times with ethanol and then with benzene, the final washing being only slightly coloured. It was then dissolved in water and reprecipitated with ethanol. The DNA–dye complex in 6 N hydrochloric acid had an absorption maximum at 500 mμ corresponding to 0·04 mg dye/mg DNA–dye complex.

7 mg of DNA–dye complex in water (20 ml.) were treated with concentrated ammonia (0·15 ml.) and extracted with benzene until no more colour could be removed. The nearly colourless alkaline solution when treated with an equal volume of concentrated hydrochloric acid had an absorption maximum at 500 mμ corresponding to 0·02 mg dye/mg DNA. Treatment of the complex with 6 N hydrochloric acid followed by neutralization and extraction with benzene removed this remaining dye. These experiments suggest that half the dye associated with DNA when first estimated was only loosely bound, whereas the remainder was associated in a manner comparable to that found for RNA in that it was stable to alkali but decomposed in acid solution.

In a second run, DNA (50 mg) in water (50 ml.) was treated with aminoazobenzene (150 mg) in ethanol (40 ml.) and formaldehyde 0·05 ml. After 2 min the yellow fibrous DNA was precipitated with ethanol after adding sodium acetate to a concentration of 2 per cent. The solid was washed many times with ethanol and reprecipitated twice more from aqueous solution, the solid being washed each time. DNA–dye complex isolated under these conditions contained 0·0027 mg aminoazobenzene/mg DNA–dye complex.

The lability of the nucleic acid–dye complexes in aqueous and in acid media will obviously make isolation from whole rat liver difficult, and may account for the failure to find evidence of extensive binding to nucleic acids with methylated *p*-aminoazobenzene dyes *in vivo*[4,5]. Tritiated butter yellow of high specific radioactivity has been prepared and this is being used to investigate the situation *in vivo* with these facts in mind.

These findings suggest an additional way in which methylated aminoazo dyes might interfere with cellular function in the rat liver. The conversion of an *N*-methyl group to a reactive *N*-methylol derivate *in vivo* raises the possibility that other *N*-methylated carcinogens such as dimethylaminostilbene might also be converted enzymatically into alkylating agents. In this connexion it is of interest that reactions of the alkylating type have been previously implicated in liver carcinogenesis. For example, dimethylnitrosamine, which undergoes metabolic modification in the liver, has been shown to methylate nucleic acids and proteins[6], while certain pyrrazolizidine alkaloids which are liver carcinogens and cause other cellular abnormalities, have recently been shown to be alkylating agents[7].

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Failure to protect Mice against Endotoxin with Snake Venom

WHEN bacterial exotoxin, endotoxin, or snake venom is injected intravenously into animals, peripheral vascular collapse occurs. Preliminary investigations demonstrated that the administration of sub-lethal doses of *Escherichia coli*, *Salmonella typhi* or brucella endotoxins protected mice against lethal doses of snake venoms (*Crotalus terrificus* and *Bothrops jararaca*). Since North and Doery[1] have reported that the venom of the Australian tiger snake (*Notechis scutatus scutatus*) protected mice against staphylococcal exotoxin, comparable work was carried out in our laboratory to determine if rattlesnake venom (*Bothrops jararaca*) would protect Swiss–Webster white mice against *E. coli* endotoxin, as well as staphylococcal toxin.

Three methods were used in injecting the materials intravenously into the tail vein in a total volume of 0·5 ml.

Method 1. A needle was inserted into a vein, the venom injected, and then immediately afterwards, with the needle still in place, exotoxin or endotoxin contained in a second syringe was injected.

Method 2. Venom was injected 30 min before either the exotoxin or endotoxin.

Method 3. The venom and staphylococcal toxin or endotoxin were thoroughly mixed at room temperature and then injected. The amounts of venom, toxin or endotoxin remained the same in the mixtures, but were contained in one-half the volume.

Table 1. RESULTS IN MICE RECEIVING LETHAL DOSE OF STAPHYLOCOCCAL TOXIN OR *E. coli* ENDOTOXIN WHEN (*A*) SNAKE VENOM (*Bothrops jararaca*) AND TOXIN OR ENDOTOXIN WERE INJECTED SIMULTANEOUSLY, (*B*) VENOM WAS INJECTED 30 MIN BEFORE THE TOXIN OR ENDOTOXIN

Materials injected	Survivors after 24 h
5 μg venom	0/20
1 : 10 toxin	5/20
5 μg venom followed immediately by 1 : 5 toxin	2/20
5 μg venom followed in 30 min by 1 : 5 toxin	16/20
Endotoxin	2/10
5 μg venom followed immediately by endotoxin	3/10
5 μg venom followed in 30 min by endotoxin	0/10

Table 2. RESULTS IN MICE WITH LETHAL DOSE OF STAPHYLOCOCCAL TOXIN OR *E. coli* ENDOTOXIN WHEN MIXED WITH SNAKE VENOM (*Bothrops jararaca*) PRIOR TO INJECTION

Materials injected	Survivors after 24 h
5 μg venom	0/20
1 : 10 toxin	1/10
Endotoxin	2/10
5 μg venom + toxin	9/10
5 μg venom + endotoxin	1/10

The results presented in Table 1 show that snake venom did not protect against staphylococcal toxin or endotoxin when the two were injected simultaneously. Venom did protect against staphylococcal toxin when the latter was injected 30 min after the venom; but in similar circumstances venom did not protect against endotoxin. Table 2 shows that a pre-injection mixture of venom and toxins protects against a lethal dose of toxin, but no protection against endotoxin resulted from this procedure.

These results confirm the findings of North and Doery[1] that venom will protect mice against staphylococcal toxin. However, such protection is not afforded against endotoxin. Others[2] have shown that pre-treatment of rabbits with venoms accelerates death due to endotoxin. Although exotoxin and endotoxin both induce peripheral vascular collapse, the initial mode of action of each is different. Staphylococcal exotoxin acts directly on the vascular endothelium, whereas the action of endotoxin is mediated through a labile serum factor resulting in the liberation of vasoactive substances[3,4]. Doery and North[5] have suggested that a mechanism whereby venoms inactivate exotoxin involves phosphatidase *A*, an enzyme present in venom, which interacts with phosphatide at the cell surface liberating fatty acid and lysophosphatide. In further observations they have inactivated staphylococcal toxin with unsaturated fatty acids. Preliminary *in vivo* studies in our laboratory with several bacterial exotoxins and with endotoxin have shown that unsaturated fatty acids protect against exotoxins but not against endotoxin.

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RADIOBIOLOGY

A Combined Microphoresis–Autoradiography Technique

BASE ratios can be determined with microelectrophoresis (microphoresis) in as little as 5×10^{-10} g RNA[1], a sensitivity which permits intracellular analysis in suitable material. It would be of interest for turn-over studies to evaluate radioactivity in the separated RNA components. Autoradiography is the simplest tool for this purpose, both because of its sensitivity and high resolution.

Tests were performed with graded applications of tracer on microphoresis fibres and with separations of yeast RNA hydrolysate mixed with tracer. These indicated that autoradiograms of individual bands could be resolved and that the fibre did not interfere with the performance of the emulsion.

A biological test was conducted on middle-late larvæ of a local chironomid in Gothenburg, probably *Chironomus thummi*. Whole salivary glands were dissected and cultured *in vitro* in medium '199' (Glaxo Laboratories)[2], containing 11·4 μc. (8-^{14}C) adenosine-5′-triphosphate per ml. (The disodium nucleotide from Schwarz Bioresearch, New York, was 77 per cent pure by ultra-violet analysis, and was used at 5 mM.) The glands were cultured at room temperature for 6 h, during which time they remained viable as judged by the extrusion of abundant secretion. They were fixed for 1 h in Carnoy (chloroform/ethanol/glacial acetic acid 6 : 3 : 1, by volume), a procedure which involves no measurable loss of RNA[3], and then embedded in paraffin and sectioned at 25μ. Individual nucleoli from sections hydrated with 0·01 N acetic acid were isolated intact in an oil chamber using a de Fonbrunes's micromanipulator fitted with two glass needles. RNA was extracted with a buffered ribonuclease solution and hydrolysed in a micropipette as previously described[1]. Aliquots of the hydrolysates corresponding to 6–8 nucleoli were subjected to microphoresis and the amount of bases measured photometrically. (Although base ratios can be determined accurately, absolute amounts can only be determined with an estimated error of about ±25 per cent.) For autoradiography the fibres were taken off the quartz glass on which they had been photographed in ultra-violet light of 257 mμ, and placed on a glass slide which was then exposed to an atmosphere of ammonia for three minutes. Immediately thereafter the slide with the fibre was pressed on to dried Kodak *AR*.10, previously coated emulsion-up on subbed slides. Autoradiograms were obtained of the separations and grains counted at the centre to calculate (in first approximation) a relative electron flux. The results are given in Table 1.

All the detectable radioactivity corresponded with a single band, that is, adenine, except in preparation 2 where also traces were in guanine. For evaluation of autoradiography it should be considered, first, that a compressible fibre of 25–30μ diameter is the actual source and, second, that the label in it remains during exposure in a free-diffusion system, that is, the microphoresis buffer. Allowing for electron scattering, the length of the bulk of the autoradiograms in preparations 3 and 4 compares well with the length of the initial adenine bands (columns *a* and *b*, Table 1). This suggests that appreciable diffusion was prevented by an increased viscosity at 4° C of the buffer; that there is some diffusion is shown by the tailing-off of the autoradiograms (not included in the estimates). A greater compression could largely explain the less favourably comparable lengths in preparations 1 and 2, since the lower temperature of exposure would have further increased the viscosity (preparation 1 showed an autoradiogram excessively broad as well as long). Excluding preparation 1, the specific activity of which would be the most decreased for the previous reasons, the specific activities of preparations 2–4 (column *f*) are in reasonable agreement (for comparison with the other preparations, the differences indicated in footnote 2 for preparation 4 roughly cancel each other). The reproducibility of the determinations is satisfactory within a single fibre, that is, in preparations 2 and 4.

From the present tests the technique would seem useful in the examination of turn-over of RNA components in individually isolated cell constituents. The present needs were to obtain a high labelling of RNA and to apply microphoresis on larger aliquots of this than necessary; that is, the RNA of about two of these nucleoli. It is considered that substantially less radioactivity (as encountered in turn-over work) can be measured, since short exposure times were used and these still resulted in grain densities far above the lower limit for grain counting. Thus, the technique can be coupled with direct autoradiography of nucleoli to encompass the topography of turnover. Improvement in the quantitative aspects can be expected from including known reference amounts of tracer in the microphoretic separations. A single fibre can accommodate 6–7 separations from different cell constituents.

Table 1. MICROPHORESIS–AUTORADIOGRAPHY OF NUCLEOLAR RNA ADENINE

Preparation	Autoradiograph exposed at (°C)	Length of adenine band by ultra-violet light before autoradiography (μ)	Estimated length of autoradiogram (μ)	Adenine by ultra-violet photometry* (μμg)	Net grain count per unit area in autoradiogram of adenine†		Relative specific activity $\frac{(d) \text{ or } (e) \times 100}{(c)}$
		(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
1	−20	180	475	124		44·2	36
2*a* 2*b*		180 225	295 323	150 153 151·5		122·9 134·1 128·5	85
3	4	235	285	155		96·3	62
4*c* 4*d*		215 215	228 228	100 118 109	76·0 76·0 76·0		70

* Average molar ratio of adenine was 34·9 per cent of the sum.

† Exposure and development times of autoradiographs : (*d*) 4·8 days, 30 min. ; (*e*) 6 days, 15 min. The developer was commercial Kodak *D*–19 at 18° C.

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Restoration of X-irradiated *L*-cells by Means of Highly Polymerized Isologous Deoxyribonucleic Acid

L-CELLS were treated with sub-cellular fractions after X-irradiation and an appreciable restorative effect was noted after addition of nuclear fraction and to a lesser extent with mitochondria. The results will be presented elsewhere. However, the results of these experiments varied from experiment to experiment. In some experiments significant restorative effect was noticed but in the other additions unirradiated nuclei had no effect.

In order to establish the nature of the restorative agent as well as the cause of variability of our results we have made several analyses. First of all, the effect of isologous macromolecules such as deoxyribonucleic acid have been examined, because some evidence concerning the restorative effect of nucleic acids was reported by others[1-4].

In most experiments a clear restorative effect was noticed after addition of highly polymerized isologous DNA to irradiated culture of *L*-cells under our conditions. This effect also varied from experiment to experiment.

For each experiment 40 Carrel flasks were inoculated with *L*-cells in Eagle's growth medium[5] supplemented with 20 per cent calf serum and 0·8 mg/l. cholesterol. After 14 h of incubation at 37° C, 20 flasks were irradiated with 500 r.; 20 flasks were kept as control. To 5 irradiated flasks DNA was added in final concentration of 35 μg DNA per ml., and to 5 other flasks DNA was added 3 μg per ml. of medium only. With non-irradiated control flasks the same treatment was applied: to 5 control flasks 35 μg DNA/l. was added, and to another 5 flasks 3 μg DNA/ml. After two weeks of incubation at 37° C, colony count was made.

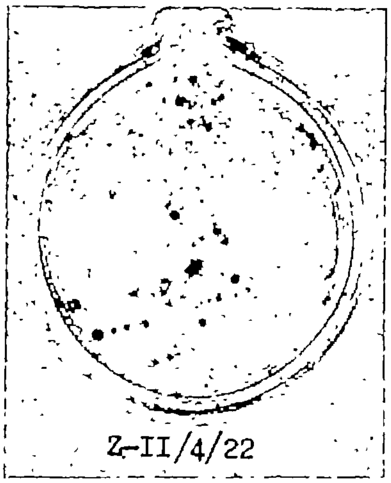

Fig. 1. Colonies of *L*-cells in a Carrel flask. Each flask was plated with 2,000 cells and irradiated with 500 r.

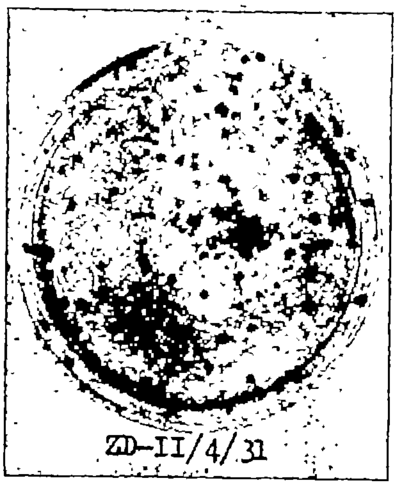

Fig. 2. Colonies of *L*-cells in a Carrel flask plated with 2,000 cells irradiated with 500 r. and treated with 35 μg DNA/ml. medium

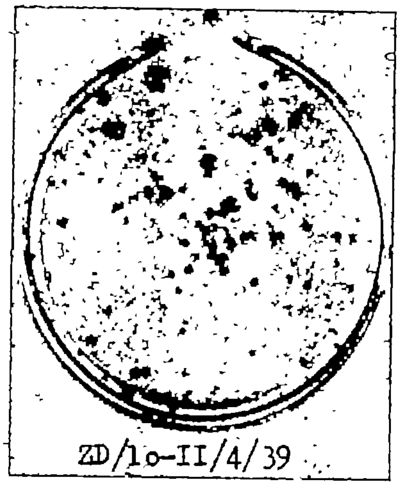

Fig. 3. Colonies of *L*-cells in a Carrel flask plated with 2,000 cells, irradiated with 500 r. and treated with 3 μg DNA/ml. medium

DNA isolation was carried out with a phenol method out of 10^{9}–10^{10} *L*-cells. Such isolated DNA was contaminated with appreciable amounts of RNA (16 per cent) and proteins (6·1 per cent).

Survival for 500 r. under our experimental conditions was 8·8 ± 0·6 per cent. Addition of 35 μg per ml DNA to the irradiated cells increased survival up to 14·8 per cent with respect to the unirradiated control treated with the same amount of DNA. When 3 μg/ml. of DNA was added after irradiation, survival was increased to 13·6 per cent. In unirradiated flasks, plating efficiency was increased by addition of 35 μg/ml. DNA from 56 to 65 per cent. After addition of 3 μg/ml. of DNA, plating efficiency was increased up to 66 per cent. Only colonies with more than 50 cells were counted.

It is evident from these results that addition of isologous highly polymerized DNA leads to restoration of irradiated cells. It is interesting to note that the degree of restoration is the same whether 3 μg DNA/ml. or almost 12 times more DNA was added.

In Figs. 1, 2 and 3 colonies formed from irradiated cells are shown, to be compared with samples treated with DNA.

In some experiments with the same DNA kept in a freezer, results varied to a greater extent.

On the basis of preliminary experiments undertaken to analyse the survival curves of DNA treated and non-treated cells, it seems likely that restoration is connected with an increase of the extrapolation number.

Experiments with denaturated, hydrolysed and heterologous DNA and with purified DNA are in progress.

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Maximum Protection of Mice against 8-MeV Electron Irradiation

Mice can be protected from the effects of whole-body irradiation by a variety of different ways. Sulphydryl compounds given immediately before[1,2], hypoxia induced during[3], or infusion of syngeneic bone marrow given after[4] irradiation all afford major degrees of protection. If each of these agents acted by an independent mechanism, then the $LD_{50/30}$ days dose might be expected to be increased by as much as six to eight fold when all were used together. We now report the results of combining these factors.

Male and female inbred *C3H* mice, 15 weeks old, were irradiated with 8-MeV electrons at a dose rate of 40,000 rads/min. All the mice were anaesthetized with 65 mg/kg 'Nembutal' (Abbott) 30 min before irradiation. The protective measures used were: intraperitoneal cysteamine 200 mg/kg 15 min before irradiation; hypoxia, produced by breathing pure nitrogen for 45 sec (the irradiation being given during the last 5 sec of the nitrogen administration); and intravenous injection of bone marrow cells taken from littermates of the same sex 20 h after irradiation. Antibiotics ('Crystamycin', Glaxo) were injected daily for 30 days—50,000 I.U. sodium penicillin *G* and 50 mg streptomycin per kg mouse body-weight.

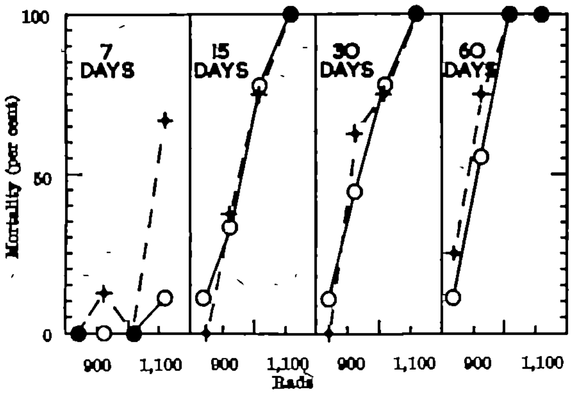

Fig. 1. Percentage mortality of irradiated *C3H* mice at different time intervals after irradiation. The dose received by the protected mice (black crosses) has been divided by 3·8 to compare with the dose received by the unprotected mice (open circles)

The results are shown in the Figs 1 and 2. It is seen (Fig. 1) that a dose reduction factor of approximately 3·8 is observed over a wide range of mortalities when measured at 15, 30 and 60 days after irradiation. From Fig. 2 the $LD_{50/30}$ for the unprotected animals is seen to be 940 rads, while that for the protected animals is 3,520 rads. To compare these figures with equivalent doses of 250-kVp X-rays we have used a relative biological effectiveness of 0·82 for the 8-MeV electrons. This is reasonable when the polarization correction is included[5,6]. The $LD_{50/30}$ unprotected becomes 770-rads X-rays, and the $LD_{50/30}$ protected becomes 2,890-rads X-rays. We believe this latter dose to be the highest $LD_{50/30}$ that has ever been recorded, and it is probably very nearly the highest dose that is possible with present methods of protection. (Burnett and Doherty[7] reported that some survivors had been obtained in mice given up to 2,600 r. γ-radiation when aminoethylisothiouronium bromide hydrobromide protection was followed by treatment with bone marrow and streptomycin. This might be equivalent to about 2,130 r. 250-kVp X-rays[8].) Although the degree of protection achieved here is very large, it falls far short of the product of each factor multiplied together. These results will be published more fully elsewhere.

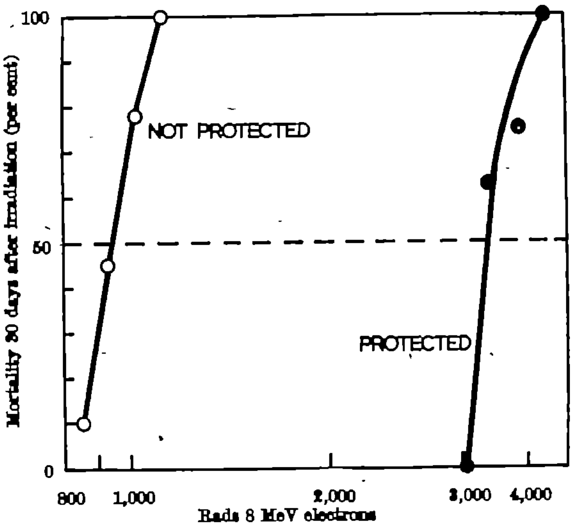

Fig. 2. Percentage mortality of protected and unprotected *C3H* mice at 30 days after irradiation. Dose of radiation on log scale

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BIOLOGY

Effect of Air Drying and Dressings on the Surface of a Wound

It has been pointed out that the normal dry scab on a wound exposed to the air includes a superficial part of the dermis, and it was suggested that this is because the exposed dermal tissue is dehydrated[1]. Epidermis migrates below the dehydrated fibrous tissue where there is sufficient moisture for the cells to live. If the surface of the wound is deliberately kept moist by covering the wound with an occlusive film, the epidermis will migrate over the surface of the dermis. In this latter event migration of the epidermis is twice as rapid as when it is forced to pass through the fibrous tissue.

The opposite effect has now been demonstrated, namely, that if a wound surface is artificially dried more extensively than by mere exposure to the atmosphere, then more of the dermis is incorporated in the scab.

For these experiments superficial wounds were made in the skin of the backs of two female Large White pigs. Rapid drying of the wounds was achieved by floating the pigs on a cushion of air on the 'levitation' apparatus described by Scales[2]. In the first experiment, the air temperature was 20° C (pressure, 25 cm water; flow-rate, 1,250 ft.³/min; blowing time, 1 h). In the second experiment, the air was heated to 40° C (pressure, 29 cm water; flow-rate, 1,350 ft.³/min; blowing time, 1 h). After the fresh wounds had been blown on and dried, the pigs were returned to their pens wearing a cage to protect the wounds from damage. Biopsy specimens were taken after three days.

Histologically, it was found that the tissue in the floor of the wound had been affected to a greater depth than normal. Within the affected layer the fibrous bundles were closely packed and there appeared to be no living cells. Epidermis was migrating through the dermal fibrous tissue below the altered layer. The scab therefore was similar to that which forms on a wound exposed to the air, but thicker, incorporating more of the superficial dermal tissue.

Epithelization was measured from serial sections. In the first experiment, in which the wounds were dried by air at room temperature, 34 per cent of the wound area had been re-populated by new epidermis at three days (Table 1). Where warmed air was used to dry the wounds, only 18 per cent of the wound surface was covered by new epidermis at three days (Table 2).

Table 1. EPITHELIZATION AFTER AIR BLOWING

Time	Wound	Total length of epidermis (mm × 10^{-3})	Total length of section examined (mm × 10^{-3})	Percentage of epidermis
3 days	5	17,428	55,012	32
	6	20,687	66,511	31
	7	16,310	40,888	40
		54,425	162,411	34

Pig. L5. Superficial wounds, 2·5 cm². Hair was clipped short three days before wound making. Air at 20° C blown over wounds for 1 h after wound making. Serial sections at 10µ every fifth section measured.

Table 2. EPITHELIZATION AFTER AIR BLOWING

Time	Wound	Total length of epidermis (mm × 10^{-3})	Total length of section examined (mm × 10^{-3})	Percentage of epidermis
3 days	7	11,525	58,788	20
	8	13,363	72,510	18
	9	13,892	67,546	21
	10	13,233	63,992	21
	11	7,524	60,263	12
		59,537	323,099	18

Pig L8. Superficial wounds, 2·5 cm². Hair was clipped short three days before wound making. Air at 40° C blown over wounds for 30 min after wound making and a further 30 min, 5 h later. Serial sections at 10µ, every fifth section measured.

Thus the effect of blowing on an exposed superficial wound was to create a thicker than normal scab and delay epithelial migration. The results of these experiments support the view that the incorporation of part of the dermis into the scab is due to dehydration of that tissue.

A diagram summarizes the findings on the formation of the scab and epithelization under normal and experimental conditions (Fig. 1).

From a practical point of view, dry or moist conditions of the wound surface may prevail, or very frequently some intermediate state, depending on the type of dressing. Ventilation is obviously a significant feature of dressings in the treatment of wounds. Under the favourable conditions of animal experimentation, a moist wound heals most rapidly; but in human clinical practice a moist wound surface may not be desirable because of the risk of infection.

Many clinicians advocate the exposure treatment of wounds, particularly burns, where the aim is to encourage the formation of a natural protective scab. The experiments reported here suggest that the benefits of the natural scab can be achieved rapidly with air at a suitable temperature and pressure.

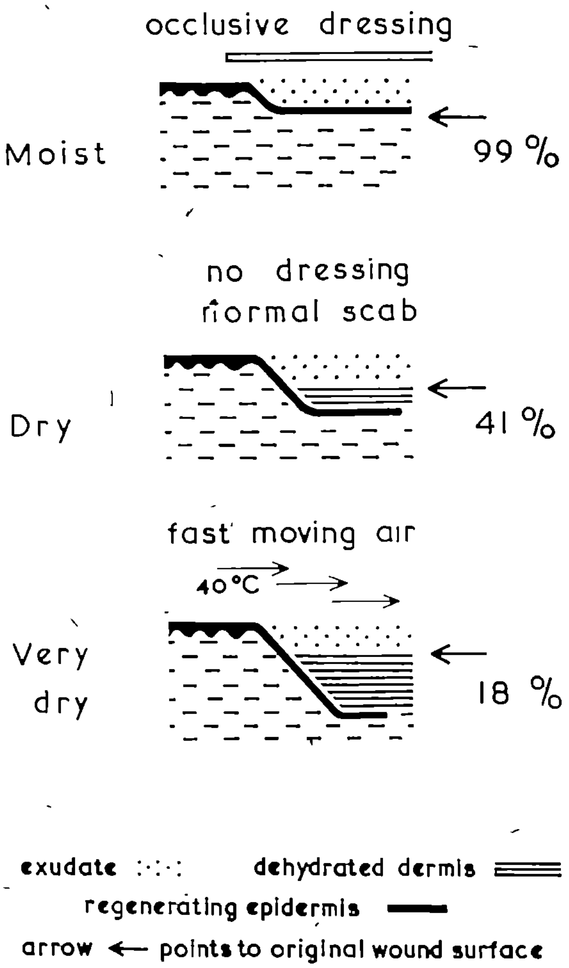

Fig. 1. Formation of the scab and the level of migration of epidermis in superficial wounds of the skin. Percentages represent the proportion of the wound area covered by new epidermis three days after the surface epidermis and the papillary layer of the dermis were removed from a 2·5-cm square of skin in young domestic pigs

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Measurement of Length of the Fore-Cannon Bone in Live Sheep

MANY workers have sought to describe the size and shape of farm animals objectively by making measurements on live animals, while a number of others have proposed various combinations of measurements as a measure of body size and/or conformation, for example, Lush[1] (beef cattle), Cook *et al.*[2] (dairy Shorthorns), Berges[3] (beef cattle), Rae[4] (New Zealand Romney Marsh), Turner *et al.*[5] (Australian Merino), Phillips and Dawson[6], Hazel and Kline[7], Price *et al.*[8] (pigs). While the significance of cannon bone measurement as an index of total bone-weight of the carcass has been reported for sheep

(Hammond[9], Verges[10]; Palsson[11,12]) and pigs (McMeekan[13]), there are no reports of cannon bone measurements for live sheep being used as an index of carcass characteristics.

This communication describes a technique for measuring the fore-cannon bone length in live sheep. Its relation to the dressing percentage of lambs from Border Leicester × Merino ewes sired by Dorset horn rams is reported.

In 1961, during the course of investigations of grazing management practices by the C.S.I.R.O. Division of Plant Industry at the Dickson Experimental Station, Canberra, 66 August-drop lambs were reared. All the lambs were slaughtered at a mean weight of 70·6 lb. body-weight. (*S.D.* ± 2·89.) Carcass-weights ranged from 25 to 40 lb. Twenty-one of these lambs reached slaughter-weight on pasture, but owing to drying-off of the feed, 45 had to be finished by hand-feeding in a yard.

All measurements were made 24 h prior to slaughter. The foreleg is flexed to the maximum degree and one point of a steel caliper is placed on the lateral condyles of the distal end of the metacarpus while the other point is rested on the proximal end of the metacarpus at the knee joint. The distance between the caliper points is read off on a steel tape measure graduated in centimetres. All measurements were read to the nearest 0·1 cm and the mean of three consecutive readings was taken. Repeatability of these measurements for an observer was within 0·1 cm and between observers within 0·2 cm. The technique is simple and quick, taking, with two operators, less than 3 min for three consecutive readings.

After slaughter the left fore-cannon bones were collected, cleaned and oven-dried for 2 h at 100° C and their length measured to the nearest 0·1 cm with a vernier caliper. The correlation coefficient between live–dead cannon bone length for 61 observations is 0·80 ($P < 0{\cdot}001$). The regression equation expressing the relationship is $Y = 0{\cdot}9446X + 45{\cdot}8123$, *S.E.* 2·91 mm, where X = dead cannon bone length (mm) and Y = live cannon bone length (mm.).

For the 21 lambs finished on pasture the correlation coefficient between the live cannon bone length and dressing percentage is $-0{\cdot}55$ ($P < 0{\cdot}01$).

These are preliminary findings for the dressing percentages of pasture finished lambs. The relationship for the remaining 45 carcasses finished on a hand-feeding regime will be the subject of a later communication.

We thank the C.S.I.R.O. Division of Plant Industry, Canberra, for making available the material and actively assisting in the field aspects of this work. We particularly thank Dr. F. H. W. Morley, head of the Pastoral Agronomy Section of this Division, for advice, and Mr. H. W. Chapman, also of this Division, and Mr. A. Redpath, of Dickson Station, and the Canberra Abattoirs, for facilities.

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Sound Reception in the Dogfish

Kritzler and Wood[1] succeeded recently in training a bull shark (*Carcharhinus leucas*) to respond to sine-wave sound signals emitted from a submerged loudspeaker. Responses occurred to frequencies between 100 and 1,500 c.p.s. A threshold curve shows that the animal was most sensitive to frequencies of 400–600 c.p.s. The sound source was (acoustically or visually) localized.

These experiments, affording valuable new evidence about the sensitivity of elasmobranchs to sound, lent new weight to the question how sound stimuli are perceived in these animals. On comparative physiological grounds, the participation of two groups of sense endings seems likely: (1) otolith organs and possibly other labyrinthine sense endings; (2) general cutaneous sense endings (touch or vibration receptors) in the outer skin. The lateral line organs and the ampullæ of Lorenzini have other special tasks; their participation in the reception of propagated sound waves seems unlikely, although stimulation may occur by local phenomena at short range of the sound source[2].

Up to now, there is no direct proof for sound reception by cutaneous sense endings in any fish[3]. As to the labyrinthine endings, Lowenstein and Roberts[4] found in electrophysiological experiments with *Raja clavata* that parts of the maculæ sacculi and utriculi as well as the macula neglecta responded to vibrations of the solid substratum on which the preparation was mounted. The highest effective frequency was found at about 120 c.p.s. Furthermore, Vilstrup[5] conditioned *Acanthias vulgaris* to the sound of a submerged motor horn and reports that the reactions were completely abolished after bilateral section of the acoustic nerve. Frequency and intensity of the sound used are not indicated. The sacculus is regarded as the main sound receptor. Previous experiments of Parker[6] on *Mustelus canis* had led to the conclusion that sounds would affect both skin and labyrinths but that the latter organs were the more sensitive of the two.

During a stay at the Zoological Station of Villefranche-sur-Mer (France) several dogfishes (*Scyliorhinus canicula*) were trained to respond to the tone of a submerged speaker by combining the sound stimulus with an 8-V alternating current from a submerged pair of electrodes as an unconditioned stimulus. Several click-free tone pips[7] of 1 sec duration each were given with intervals of about 0·5 sec before the unconditioned stimulus was added. The frequency of the tone used (resonance frequency of the speaker with its enclosing air-filled box) was about 180 c.p.s.; when picked up from the water via a barium titanate hydrophone, the tone appeared as a pure sine wave on the oscilloscope screen. The maximal intensity used initially was such that the vibrations could be felt by the submerged hand up to about 5 cm from the speaker; it was determined at this distance with the aid of a calibrated hydrophone at somewhat more than 500 dynes/cm^2. In order to keep the distance between fish and speaker constant, the animal was enclosed in a cylindrical, wide-mesh gauze cage made of plastic-covered wire. The cage measured 9–10 cm in diameter and was as long as the animal (48 cm). It was suspended horizontally in the midst of a large aquarium, the head of the dogfish pointing towards the speaker, which was suspended at the same depth at a distance of about 5 cm from the tip of the animal's snout. One eye of the fish was 'blinded' by covering the cornea with an oval piece of soft and thin opaque plastic, over which the eyelids were closed and sewn together with operation silk. The covered eye was at the side of the experimenter. The aquarium containing the fish was put on bicycle tubes to reduce penetration of sounds from the solid substratum.

After a number of trials, relatively weak but unmistakable sound reactions were observed. Sometimes there were certain fin movements but the most regular response was a short interruption of the respiratory rhythm, visible at the gill-slits. Now the sound intensity was reduced from trial to trial. Even at the lowest intensity used, when nothing could be felt by the submerged hand, still regular responses occurred. This intensity of about 90 dynes/cm² at the distance of 5 cm from the speaker or 45 dynes/cm² at the region of the animal's labyrinths was probably still considerably above threshold strength.

A trained dogfish was anaesthetized with 1·5 per cent urethane in sea-water. The root of the right acoustic nerve was dissected free from the ventral side via the mouth and cut near the medulla. After recovery, responses to the lowest sound intensity used previously were easily obtained. Now the left acoustic nerve was cut as described here. After this second operation, responses occurred only at definitely higher intensities and even then the reactions were irregular and very weak. The threshold intensity was determined at about 400 dynes/cm² when measured at a distance of 5 cm from the speaker. Before and after the operations short periods of training on lighting a nearby lamp (combined with the same electrical unconditioned stimulus) were inserted in order to test whether the animal was fit enough to respond to training stimuli in general. Light reactions occurred always regularly both before and after section of the second acoustic nerve.

These preliminary results—they are mainly based on experiments with a single dogfish, because the other specimens did not keep quiet long enough within their narrow cage—seem to indicate that perception of a tone of 180 c.p.s. occurs predominantly in the labyrinth. Only with great intensities do other sense-endings participate. The results are in accordance with those of Parker and Vilstrup.

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Experimental Lathyrism in the South African Toad

EXPERIMENTAL lathyrism was produced originally more than a quarter of a century ago[1] by feeding rats seeds of the flowering sweet pea—*Lathyrus odoratus*. Since then a number of compounds, including semicarbazide, have been tested to determine their lathyrogenic activity. This experiment was to show the changes produced in larvæ of the South African toad (*Xenopus laevis*) when semicarbazide was added to the aquarium water.

Once the larvæ had reached developmental stages 48–58, that is, approximately 10 days before the break-through of the fore-limbs[2], they were divided into a control (250 larvæ) and test (350 larvæ) series. At this time, 4 µl. of a 0·005 per cent (w/v) solution of semicarbazide hydrochloride was added to each litre of aquarium water in which the test animals were. They were kept in this concentration until metamorphosis occurred, and both series were fed liver powder as required. Only 230 control animals and 280 of the test group survived until metamorphosis. Once metamorphosis was complete the toads were killed by adding excess ether to the aquarium water. There was marked retardation in body-growth in all test animals and they were much paler and weaker than the control animals. No gross dislocations, as described by Chang *et al.*[3,4] when using *Lathyrus odoratus*, were observed, although 76 test animals showed a compression fracture dislocation of the spine with resulting paresis. In 75 per cent of the test toads, histological examination revealed clefts between the connective tissue and the epiphysis of the tibio-tarsal joints when compared with the control animals (Fig. 1). The hip joints of 20 per cent of the test animals showed a degree of flattening of the upper femoral epiphysis with widening of the joint space when compared with the control (Fig. 2). This finding was constant on repeated sections and was not an artefact.

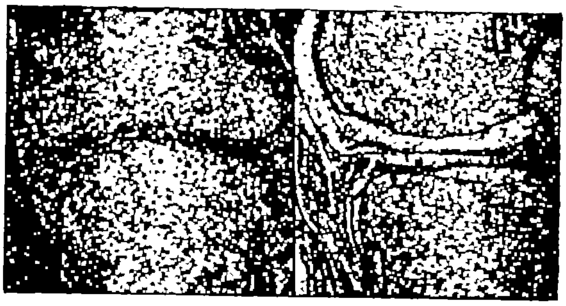

Fig. 1. Composite photomicrograph showing a tibio-tarsal joint of a normal toad (left) and a similar joint of a test animal (right). The presence of clefts between the connective tissue and epiphysis can be seen in the test toad. (picro-Mallory, × c. 85)

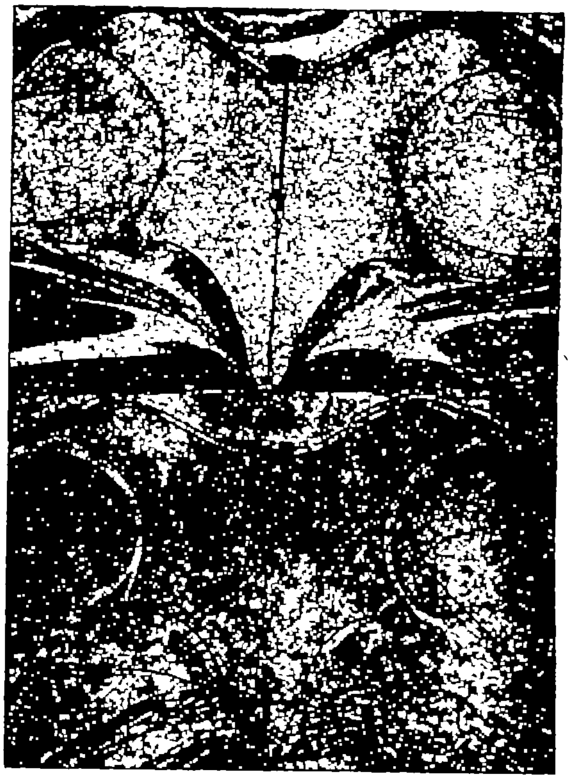

Fig. 2. Composite photomicrograph showing the pelvis of a normal toad and of a test toad in which is seen flattening of the upper femoral capital epiphysis on the right side. There is also widening of the joint spaces. The appearances are suggestive of what one would expect to see in humans with Perthes' disease. (picro-Mallory, × c. 45)

Previous workers, when dealing with *Xenopus laevis*, have used *Lathyrus odoratus* or β-aminoproprionitrile as their lathyrogenic agent. Here semicarbazide hydrochloride, a completely unrelated chemical compound, has been used with the production of virtually identical lesions. However, with semicarbazide, changes in the hip joints are reported with flattening of the upper femoral epiphysis and widening of the joint spaces. These changes could be similar to the appearances one would expect to find in humans suffering from Perthes' disease[5]. A Perthes-like appearance has been produced in rats on a lathyrogenic diet[6].

I thank Prof. T. Symington, Dr. A. Dick and Dr. B. M. Hobson for advice, and the last-named for supplying the larvae.

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Root Parasitism in Santalaceae

THE Santalaceae are a family of some 250 species of mostly root semi-parasites. Although parasitism has been known in some species of this family, the existence of parasitism in the New Zealand representatives, *Exocarpus bidwillii* Hook. f. and *Mida salicifolia* A. Cunn., was not definitely known until recently[1]. Since then the parasitism of *E. bidwillii* has been examined in greater detail. The investigation has revealed many features not previously known concerning the parasitism of the plant and of the structure and development of its haustorium.

Like many santalaceous parasites, *Exocarpus* attacks a wide range of host plants and even roots of the same species. Occasionally also young haustoria are found attached to dead plant remains such as leaves, present in the surface soil layers. In the structure of its haustorium *Exocarpus* displays the general features of santalaceous haustoria, namely, cortical folds, collapsed layers and inverted conical flask-shaped form of the vascular tissue. Other characters it shares with only one or two other santalaceous haustoria. The presence of granules in the tracheary elements of the body of the haustorium is a feature which *E. bidwillii* only shares with *E. cupressiformis*[2] in so far as the Santalaceae are concerned. These granules only occur in the body of the haustorium and are not found elsewhere in the plant or in the vessels of the sucker. Their presence in the expanded part of the vascular tissue of the body suggests they may possibly be connected with the mechanism of the haustorium.

With the possible exception of *Buckleya quadriala*[3] and a few other santalaceous haustoria, the mature haustorium of *Exocarpus* differs from most haustoria in that it is a long-lived organ which exhibits secondary growth. According to Rao[4] most santalaceous haustoria are short-lived and seldom develop secondary tissues to any extent. In one striking feature the haustorium of *E. bidwillii* is distinct from all described haustoria of the Santalaceae. Whereas in santalaceous haustoria the sucker is usually described as a simple wedge or disk-like organ embedded in the tissue of the host root, that of *Exocarpus* is complex, consisting of a number of finger-like sucker branches extending along within the host root. In cross-section each sucker branch is wedge-shaped and deeply embedded in the host xylem. The development of the haustorium from shortly after initiation to the establishment of the mature organ was also followed. Haustoria arise laterally on *Exocarpus* roots in the primary state of growth, and develop chiefly from cortical tissue. They are exogenous in origin, there being no rupture of cortical tissue as with lateral roots.

Apart from ascertaining the nature of parasitism in *E. bidwillii*, the results of this work also suggest that some of the earlier work on santalaceous parasites was incomplete and that the conclusions and comparisons drawn may not be valid. This applies particularly to what is described as the mature haustorium. Where penetration is reported to be only superficial, as was originally described for *E. bidwillii*, further investigation might show that the haustorium investigated is merely a stage of development. Since young haustoria are usually more numerous and conspicuous than old, an insufficient examination of the root system has probably been responsible for immature haustoria being described instead of the mature organ. The assumption that most santalaceous haustoria are short-lived organs developing little secondary tissue could also be re-evaluated. Most santalaceous parasites are woody plants, and since their nutrition is apparently dependent in part on parasitism it seems peculiar that this demand should be satisfied by short-lived organs which seldom become intimately associated with host tissue for any length of time. Such a condition would scarcely appear to be successful parasitism.

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Application of Gibberellic Acid to Strawberry Plants at Different Stages of Development

GIBBERELLINS are known to play an essential part in the metabolism of the strawberry plant from which they have been isolated chromatographically[1]. By spraying plants with gibberellic acid in the winter Singh *et al.*[2] advanced harvesting times by three weeks and increased yields. In the United States, Smith *et al.*[3] gave autumn applications which advanced fruit ripening by several days without affecting yields. Guttridge[4], however, reported that when gibberellic acid was administered in the autumn, fruit yields were reduced in Scotland.

To examine further the effect of different application times solutions of 12·5, 25, 50 and 75 p.p.m. gibberellic acid, to which had been added 'Agral 90' wetter, were sprayed over triplicate randomized plots of one-year old Royal Sovereign plants on March 6, April 13, or May 30 and at all combinations of these times. Plants were sprayed to run-off with volumes varying from 7 to 20 ml. of solution depending on the size at time of application.

On May 18 all treatments had accelerated flowering to an extent increasing with concentration and at the higher concentrations and multiple applications marked changes had occurred in the growth habit of the plants, the flower clusters being more numerous and borne on elongated peduncles extending well above the foliage. As 1962 was a year of late frosts many of these early flowers were killed.

The crop was harvested by picking three times weekly from June 27 until July 20 and numbers of fruits and their total weights were recorded. Total crop weights per treatment for the first two pickings and for the total harvest of 11 pickings are shown in Table 1.

Ripening was accelerated by several days, particularly at the higher concentrations of gibberellic acid, and the effect was greater with repeated applications. Total yields were not significantly improved but the number of berries increased with increasing concentrations up to 50 p.p.m., but tended to decrease with falling yields at 75 p.p.m. Sizes of fruits were normal with single applications of 50 p.p.m. or less and with the 12·5 p.p.m. concentration in the March–April and April–May applications. With the higher doses fruits were undersized, and, at the double and triple applications of 50 and 75 p.p.m., were noticeably elongated and substandard.

Table 1. EFFECT OF GIBBERELLIC ACID ON EARLY AND TOTAL WEIGHTS OF ROYAL SOVEREIGN STRAWBERRIES (KG FROM 18 PLANTS)

Application date	Gibberellic acid concentration (p.p.m.) 12½	25	50	75
March	0·72 (11·20)	0·68 (11·40)	0·83 (9·78)	1·05 (11·13)
April	0·32 (10·59)	0·49 (13·39)	0·74 (13·50)	0·80 (11·99)
May	0·45 (11·88)	0·50 (10·78)	0·52 (11·00)	0·60 (9·86)
March and April	0·32 (11·43)	0·76 (12·57)	1·15 (11·22)	1·53 (9·12)
March and May	0·36 (11·69)	0·78 (10·92)	1·05 (10·48)	1·44 (8·55)
April and May	0·34 (10·69)	0·81 (12·53)	0·78 (14·10)	1·10 (10·02)
March, April and May	1·05 (10·88)	1·24 (11·44)	1·58 (10·38)	1·69 (8·00)
Untreated		0·27 (10·30)		

Note. Complete harvest weights given in parentheses.

At the optimum treatment-levels of 25 or 50 p.p.m. in April or April–May the financial return from the crop exceeded that of untreated fruit by up to 25 per cent. A commercial grower could expect under these conditions an additional profit of £200 per acre at a cost which is only a fraction of this. The exact cost of chemical will depend on the spray technique involved, and it may be preferable to give an all-over spray at twice the experimental rate to ensure adequate cover rather than to attempt application to individual plants as was done in this experimental trial.

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Nucleic Acids and Other Characteristics in Biotin-deficient and Normal *Protomyces inundatus* Cultures

IN the course of a taxonomic investigation into the genus *Protomyces*[1], it was found that in the absence of biotin the fungus grows much slower than the control, and the budding spores have a clumpy appearance. In view of the recent demonstration that biotin deficiency has been found to have a profound effect on the synthesis of nucleic acids and proteins in *Saccharomyces cerevisiae*[2], it was thought worth while investigating the effect of biotin deficiency in *P. inundatus*.

Diploid cultures of *P. inundatus* were grown either on Lilly and Barnett's[3] basal semi-synthetic liquid medium or on the same medium minus biotin. The same methods used by Valadon *et al.*[4] were used for the estimation of nucleic acids, metaphosphate, insoluble nitrogen, dry weight and volume in this experiment. Samples were removed for analysis at intervals between 8 and 18 days' incubation. The results were converted on a per cell basis[4] by counting the cells in a small aliquot by means of a hæmocytometer.

The results obtained are presented in Table 1. The values per cell of all the characteristics investigated are higher in the cultures with, than in those without, biotin. It has been shown[4] for a normal culture that from 14 days onwards there is a decrease in DNA and RNA per cell; after 8 days'-growth the values per cell are low, gradually increase until they reach a maximum on the fourteenth day and then fall off. In the cultures without biotin DNA and RNA per cell increase up to the tenth day and then rapidly decrease; the cells are not observed to divide, they increase in weight until the twelfth day and after sixteen days the number of living cells decrease rapidly. In the normal cultures, however, cell number per ml. increases up to the fourteenth day when the cells stop dividing but are growing and are still growing at the end of the experiment.

Metaphosphate per cell goes down rapidly when cell division has stopped in the normal cultures after 14 days. Metaphosphate per cell seems to be correlated with the rate of cell division especially as metaphosphate per cell stays at a relatively constant low level in the cultures without biotin in which no cell division is observed. Further investigation of this relationship seems worth while.

Volume per cell increases in step with dry weight and insoluble nitrogen in the normal cultures while they increase up to the fourteenth day in cultures without biotin.

The ratio RNA/DNA was found to be constant[4], and the same constancy is found in the ageing normal cultures but in those without biotin the ratio RNA/DNA is slightly smaller. In agreement with Ahmad *et al.*[2] a decrease is found in the values of nucleic acids per cell in biotin-deficient cultures and these differ significantly from those in normal cultures. Total insoluble nitrogen per cell, on the other hand, is shown to increase up to the twelfth day in the biotin-deficient cultures and is still increasing after 18 days in the normal cultures. This is comparable with the results obtained by Valadon *et al.*[4], who showed that insoluble nitrogen per cell was increasing even though RNA and DNA per cell were decreasing.

The fact that RNA per cell in *P. inundatus* decreases after 12 days in normal cultures while total insoluble nitrogen still increases is not inconsistent with the well-known statement that RNA takes part in protein synthesis[5]. The biotin-deficient cultures, too, show the same thing, RNA per cell decreases after 10 days while insoluble nitrogen per cell is increasing. It was not possible to estimate the various characteristics of very young cultures as the fungus does not grow fast. Attempts are being made to obtain a large number of cells as soon as possible after the cultures are established and it will be interesting to find out if there are any relationships between nucleic

Table 1. NUCLEIC ACID CONTENTS AND OTHER CHARACTERISTICS OF BIOTIN-DEFICIENT AND NORMAL CELLS OF *P. inundatus*

Values per cell		Age of cultures in days 8	10	12	14	16	18
DNAP ($\times 10^{9}$ μg)	+biotin −biotin	4·58 3·28	5·19 4·59	5·76 4·30	6·02 3·34	5·69 3·12	4·79 2·83
RNAP ($\times 10^{8}$ μg)	+biotin −biotin	38·29 28·43	37·54 32·13	46·81 28·74	39·16 20·84	39·90 17·93	33·15 14·03
Meta-P ($\times 10^{8}$ μg)	+biotin −biotin	196·93 18·30	174·70 19·71	159·21 17·80	100·24 15·62	31·49 16·24	26·78 15·45
Insoluble N ($\times 10^{9}$ μg)	+biotin −biotin	96·99 83·69	137·37 103·37	156·71 119·60	174·96 117·44	255·79 115·74	308·12 90·72
Dry weight ($\times 10^{8}$ μg)	+biotin −biotin	1589·2 1433·3	2568·9 2463·9	2796·2 2636·3	3035·4 2625·2	3326·3 2255·3	3413·8 2304·1
Volume (μ^{3})	+biotin −biotin	36·33 32·80	39·37 33·45	41·52 34·21	44·26 36·62	45·36 36·84	47·52 36·80
Cell No./ml. ($\times 10^{5}$)	+biotin −biotin	160·4 151·8	179·3 152·6	193·5 150·1	272·4 156·6	216·0 106·8	194·7 82·2

acids, metaphosphate, insoluble nitrogen and cell division in young biotin-deficient *P. inundatus* cultures.

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Nitrogen Source in Fungal Decomposition of Wood

It is a well-known fact that the nitrogen content of the medium is one of the limiting factors in fungal decomposition. Two experiments were carried out in order to determine whether the effective destruction of wood by *Merulius lacrymans* is sustained solely by the amount of nitrogen originally present in the wood, or whether any fixation of nitrogen from the atmosphere might possibly occur. Small cubes of wood were used in Exp. 1 and wood shavings in Exp. 2; the procedure, largely the same in both, was as follows:

A heap of cubes or shavings from pine sapwood, placed on a table and protected from dust, was left in the atmosphere of an ordinary room until, after about a week, constant weight was reached. Samples of 5 or 10 g air-dry cubes or shavings were transferred to Kjeldahl flasks and sterilized. After inoculating every second flask with *Merulius*, all flasks were stored, necks horizontal, at 24° in a constant-temperature room. During storage both test and control flasks received at intervals the same amounts of sterile water to keep them moist. At the conclusion of the experiments all the control flasks were still quite free from fungal growth and the cubes or shavings seemed quite fresh. The inoculated wood was fully decomposed leaving only some brown powder in the mycelium.

After 2 (in Exp. 1 only), 4, 6 and 8 months of storage the contents of three control flasks and three inoculated flasks were subjected to the Kjeldahl procedure. As no clear influence of storage time could be detected in the nitrogen figures, the mean values are given in Table 1.

Table 1

	No. of flasks (excluding failed analyses)	Average N-content mg/air-dry wood	Deviation $\pm\sqrt{\frac{\Sigma x^2}{n}}$
Exp. 1, wooden cubes:			
Inoculated	14	0·71	0·13
Controls	13	0·57	0·07
Exp. 2, wood shavings:			
Inoculated	8	0·48	0·05
Controls	8	0·445	0·05

The difference in the final nitrogen content in Exp. 2 is not significant, and in Exp. 1 only barely so; thus scarcely lending much support to any theory of nitrogen fixation. The weak point of the method, however, is obviously the heterogeneity of the wood samples.

Before proceeding further with these long-term experiments we would like to invite criticisms of the method. Apart from greatly increasing the number of trials, there are perhaps alternative procedures which might be adopted. The possibility of using sawdust, which might be more homogeneous, has been considered but we have noticed that the growth of *Merulius* was better on the cubes than on the shavings but worse in sawdust. A method of promoting faster growth on woody materials is desirable, thereby decreasing the experimental time.

(Drying the material at higher temperatures was avoided, owing to irreversible drying-swelling hysteresis which was apparent in some preliminary experiments.)

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ENTOMOLOGY

Origin of Wings in Insects

The wings of insects are outgrowths of the thorax, quite independent of the limbs. The difficulty of picturing their origin in evolution is that of imagining what selective value they could have had during the primitive stages of their development.

The view that flight started from the surface of the sea by the use of fin-like appendages adapted for swimming[1] has found little support. By analogy with the evolution of wings from the fore-limbs of vertebrates, the wings of insects are commonly supposed to have arisen as fixed organs used for gliding[2,3]. The occurrence of fixed paranotal lobes in fossil Palaeodictyoptera is often held to support this speculation.

Walton[4] claimed that the wings of insects originated from basal parapodial appendages of Polychaetes; and Raw[5], in proposing likewise their evolution from the dorsal swimming lobes of the parapodia, pointed out that if that were so they would be already furnished with the muscles used for swimming. Pringle[6], on the other hand, suggests that flight was first evolved in an insect of large size, perhaps a leaping insect, which developed muscles that could control the inclination of the paranotal planes used for gliding; only at a later stage were muscles developed which could move the wings up and down and thus lead to the evolution of slow flapping flight.

The purpose of this communication is to put forward a third suggestion—that the wings may have been evolved neither in aquatic ancestors, nor in large earth-bound insects, but in small aerial species.

As is well known, there is a large aerial fauna of insects and other arthropods extending up into the sky for some thousands of feet[7]. Tetranychid mites make regular migrations into the orchards during the summer months, on days when there is a gentle breeze, by means of silken threads; spiders are carried great distances in the same way. Among the insects it is mainly winged forms which make these aerial migrations; but small hairy caterpillars, such as those of the gypsy moth, can be carried many miles by the wind[8,9]; one human flea, *Pulex irritans*, was taken at a height of 200 ft.; and wingless Thysanura, Collembola, young stages of Hemiptera and Orthoptera. larvae of Coleoptera, Lepidoptera and Diptera, and great numbers of wingless ants have been captured at heights up to 5,000 ft.[7]. Such flights must have been common among the earliest terrestrial arthropods.

Although many arthropods possess notal extensions, it is only in the wings of insects and the 'pteromorphs' of certain Oribatid mites that these extensions are hinged[10]. Oribatid mites figured among the arthropods collected in the sky by Glick[7], but they were not abundant.

These aerial migrations are of undoubted selective value in securing the dispersal of many of the species concerned. It is not difficult to imagine conditions under which the provision of light cuticular expansions from the thorax would facilitate 'take-off'. The existence or appearance of muscles which could twist these light planes, as suggested by Pringle[6], would increase their efficiency in take-off and might even afford some control during landing. Flapping muscles, even of the most primitive type, would increase the efficiency of both take-off and landing still further—although, of course, the greater part of the flight would be wholly passive. In short, very small steps towards the evolution of completely functional wings could at once have selective value.

It is worth pointing out that among the Aphids, which are highly efficient fliers and which migrate in vast numbers, often for hundreds of miles[11], it is only at the point of departure and at the point of landing that their movements are under their own control. Once they become well and truly air-borne the Aphids, and even locusts, are at the mercy of convection currents and winds[12].

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Vitamin Requirements of the Silkworm

KNOWLEDGE of the nutritional requirements of the silkworm, *Bombyx mori* L., has recently been accumulating by the use of artificial diets[1,2], and more recently successful rearing on synthetic diets[3,4] and the establishment of aseptic culture[4,5] have been reported. Artificial diets have been used for the demonstration of the requirements for ascorbic acid in this insect[3]; but so far not for the compounds of vitamin B group. From the experiment on growth inhibition with antimetabolites it has been shown that nicotinic acid and pyridoxine are necessary for the silkworm[6]. In this experiment either antimetabolite or this plus vitamin was supplemented on fresh mulberry leaves to induce growth retardation by antimetabolite or growth resumption, but never completely, by supplementing vitamin. This preliminary communication reports the requirements for vitamin B compounds, demonstrated by means of aseptic culture on synthetic diets.

The composition of synthetic diets, somewhat modified from previous ones[3,4], is given in Table 1. The diets contained 0·8 per cent of mulberry leaf extracts, which had been proved to be required for good growth and feeding[7]. The procedure of separation of leaf extracts is outlined in Fig. 1. Each of the diets lacked one of the vitamins listed in Table 2. The preparation of diets has already been described in detail[3,4]. The larvæ were reared aseptically by the use of Erlenmeyer's flasks from hatching. The methods of autoclaving of diets and sterilization of egg surface have also been reported previously[4,5]. After 25-day culture, the flasks were opened and the number of surviving larvæ, by each instar, was counted. The instar progression of larvæ, both surviving and dead, was determined by observing head-capsule width.

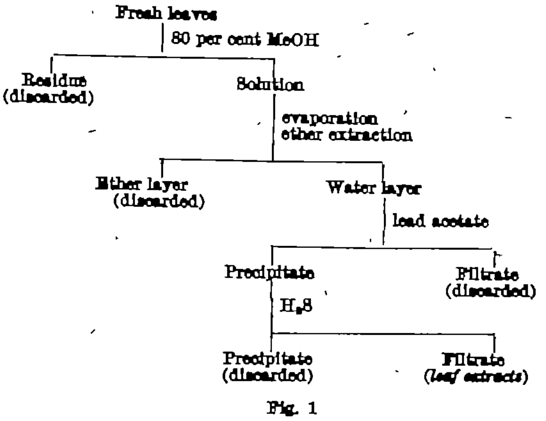

Fig. 1

Table 1. COMPOSITION OF THE DIET

Substance	Dry diet %
Starch (potato)	15
Glucose	15
Soybean meal*	25
β-Sitosterol	1
Ascorbic acid	1
Wesson's salt mixture	1
Cellulose powder	41·2
Mulberry leaf extracts	0·8
	100·0

* Hexane-extracted soybean meal (Ajinomoto Co., Ltd., Yokohama Factory) was washed first with ethyl ether, then with ethyl alcohol, respectively for 24 h.

1·5 ml. of distilled water were added per 1 g of the dry diet.

Table 2. COMPOSITION OF THE VITAMIN MIXTURE

Vitamin	Amount added to 6 gm. dry diet (mg)
Biotin	0·02
Calcium pantothenate	0·2
Choline chloride	2·0
Folic acid	0·02
Inositol	2·0
Nicotinic acid	0·2
Pyridoxine hydrochloride	0·1
Riboflavin	0·1
Thiamine hydrochloride	0·1
Total	4·74

Table 3. GROWTH AND DEVELOPMENT OF THE SILKWORM ON DIETS LACKING VARIOUS VITAMINS

Diet	Initial No. of larvæ	Number of larvæ reaching through various instars during 25 days				No. of surviving larvæ when flask opened	Mean body-weight (mg)
		Second instar	Third instar	Fourth instar	Fifth instar		
No vitamin	21	—	—	—	—	0	—
	20	—	—	—	—	0	—
	19	5	—	—	—	0	—
All vitamins	18	13	12	11	5	12	126·8
	20	11	10	8	4	9	101·7
Biotin-free	20	10	9	8	—	8	117·6
	21	14	13	12	3	12	117·8
Folic acid-free	22	13	13	10	3	13	124·1
Inositol-free	19	16	14	11	2	12	76·6
Nicotinic acid-free	19	15	2	—	—	1	2·9
	20	13	—	—	—	0	—
Pantothenic acid-free	17	16	—	—	—	1	Not weighed
	23	20	—	—	—	2	
	22	16	—	—	—	0	—
Pyridoxine-free	22	11	—	—	—	0	—
Riboflavin-free	20	18	14	12	3	11	120·8
	17	8	7	4	1	7	57·4
Thiamine-free	20	17	12	10	1	12	69·7
	19	15	14	8	1	12	44·7
	19	19	18	14	2	16	89 6

Table 3 shows one of the results obtained. On the diet without any added vitamin most of the larvæ died before reaching the second instar and a few reaching the second instar died shortly after moulting. The addition of all vitamins resulted in fairly good growth and development and some of the larvæ reached the fifth instar during 25 days. Even on such diets a considerable number of larvæ died by the end of the experimental period. A relatively high mortality in the aseptic culture has been recognized even on diets containing leaf powder or leaf juice[4,5]. The omission of nicotinic acid, pantothenic acid, or pyridoxine affected growth adversely. In these cases the larvæ reached the second instar but did not develop to the third instar, except for only a few in the case of nicotinic acid. On the diet lacking thiamine weight increase was somewhat reduced, but not necessarily decisive. Lack of either biotin, folic acid, riboflavin, or inositol did not result in any noticeable effect during the present developmental period. No significant result was obtained when choline was omitted.

Thus, it is concluded at present that nicotinic acid, pantothenic acid, and pyridoxine are essential dietetic constituents for normal growth and development of the silkworm and the effect of the lack of one of them appears in the younger larvæ. The omission of riboflavin or biotin did not suppress the growth (Table 3), which, however, are almost commonly required by insects[8]. Whether or not the lack of those vitamins might affect the older larvæ or in later stages beyond 25-day culturing period must be determined in future. Hexane-extracted soybean meal, one of the possible sources of vitamin contamination, was washed with ether and afterwards with alcohol, as stated here. There is almost no possibility of vitamin contamination from leaf extracts.

When the larvæ were reared under non-sterile conditions by means of Petri dish, larval growth and development were somewhat improved even on vitamin-deficient diets, that is, a few larvæ reached the third instar, but did not develop further and finally all died. Even in the case of the omission of pantothenic acid or pyridoxine, larvæ grew better than in the case of aseptic culture. It is thus concluded that micro-organisms are partly the source of vitamin supply, as has been suggested[6,4].

Further experiments on vitamin requirements are being carried out and the details will be published in the *Bulletin of the Sericultural Experiment Station*.

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Tests for Multiple Insemination in *Anopheles gambiae* Giles

IT has been shown that in *Aedes aegypti*[1,2], three forms (*pipiens*, *fatigans* and *molestus*) of the *Culex pipiens* complex[3], and *Anopheles gambiae*[4], one male is capable of inseminating more than one female. On the other hand, in *C. fatigans* and *C. molestus*, one female is fertilized by only one male[5]. Multiple mating of the female has been reported in *Aedes aegypti*[2,6] and inferred in *Anopheles albimanus*[7]; but it is not certain whether multiple *insemination* was consequently accomplished. Mating in mosquitoes may sometimes be ineffective, resulting in no discharge of spermatozoa into the spermatheca[4]. The following observations were undertaken to test whether one *Anopheles gambiae* female may be inseminated by more than one male.

The observations were made on two genetically distinct laboratory strains of *A. gambiae* maintained at the Ross Institute in London, namely: (1) 'Kano *RR*', which is homozygous 'Dieldrin'-resistant; and (2) 'Lagos *SS*', homozygous insecticide-susceptible. The adults of Lagos *SS* are killed by exposure for 1 h to 0·4 per cent 'Dieldrin'; while those of Kano *RR* survive 4·0 per cent. Cross-fertilization between the two strains produces hybrids which are killed by exposure to 4·0 per cent 'Dieldrin', but survive 0·4 per cent. However, a very small percentage of the hybrids may sometimes survive exposure to 4·0 per cent 'Dieldrin', while experimental handling may account for mortalities of 1·0–2·0 per cent. In the present observations mortalities and survival rates of less than 5·0 per cent are believed to have been due to this. Insecticide-resistance was used as a genetic marker. The experimental results were then compared with the expected distribution of this marker characteristic in the progeny of individual females.

Lagos *SS* females were caged together with Lagos *SS* and Kano *RR* males, in a ratio of one female to two types of males. After mating and feeding the females were isolated for oviposition. The progeny of each female was bred out separately and the resulting adults then tested for resistance to 0·4 per cent 'Dieldrin'. The results are shown in Table 1.

Table 1. LAGOS *SS* ♀♀ × (LAGOS *SS* + KANO *RR*) ♂♂

No. of parent females	Progeny (both sexes) exposed to 0·4% 'Dieldrin' Total	Alive	Dead	Mortality (%)
6	222	222	0	0·0
1	132	131	1	0·8
1	64	60	4	6·3
1	35	32	3	8·6
1	15	4	11	73·3
8	170	0	170	100·0

In 14 out of 18 females, all progeny from a single female were clearly of one type or the other, never mixed. But the progeny of 3 (those between the dotted lines) of the remaining 4 females was distinctly mixed, indicating multiple fertilization.

Kano *RR* females were caged together with Kano *RR* and Lagos *SS* males. The foregoing procedure was followed and the progeny was tested for resistance to 4·0 per cent 'Dieldrin'. The results are shown in Table 2. The progeny of 6 females out of 22 was not mixed. However, in only 8 (those between the dotted lines) of the rest was the mixed progeny thought to have been due to multiple fertilization.

Table 2. KANO *RR* ♀♀ × (KANO *RR* + LAGOS *SS*) ♂♂

No. of parent females	Progeny (both sexes) exposed to 4·0% 'Dieldrin' Total	Alive	Dead	Mortality (%)
4	178	178	0	0·0
1	102	100	2	2·0
1	101	99	2	2·0
1	48	47	1	2·1
1	40	39	1	2·5
1	81	79	2	2·5
1	27	26	1	3·7
1	17	16	1	5·9
1	102	94	8	7·8
1	51	47	4	7·8
1	91	83	8	8·8
1	10	9	1	10·0
1	46	38	8	17·4
1	77	59	18	23·4
1	30	2	28	93·3
1	21	1	20	95·2
1	91	3	88	96·7
2	65	0	65	100·0

Kano *RR* females were first caged together with their own males only. Those which had been fertilized, as shown by the production of viable eggs, were afterwards placed in another cage with virgin Lagos *SS* males in a 1 : 1 ratio. After sufficient contact between the sexes, the females were allowed to oviposit separately again and their adult progeny tested for resistance to 4·0 per cent 'Dieldrin'. The results are shown in Table 3. The progeny of all the females was undoubtedly of one kind, indicating lack of re-insemination by the Lagos *SS* males.

Table 3. KANO *RR* ♀♀ PREVIOUSLY INSEMINATED BY THEIR OWN MALES × LAGOS *SS* ♂♂

No. of parent females	Progeny (both sexes) exposed to 4·0% 'Dieldrin' Total	Alive	Dead	Mortality (%)
6	245	245	0	0·0
1	154	153	1	0·6
1	127	126	1	0·8
1	116	115	1	0·9

These observations clearly indicate that multiple insemination of females is rare in *Anopheles gambiae*. It is also clear that even when this occurs the fertilization of the ova of any one batch is unequal, the progeny being preponderantly of one kind, never on a 50 : 50 basis. This may well indicate possible differences in the fertilization mechanisms of spermatozoa produced by different

A. gambiae strains. It has been suggested[4] that the inhibition of multiple insemination by the female mosquito could operate as an effective isolating mechanism, barring hybridization between closely related sympatric species, races or strains. On the other hand, having once mated with a 'foreign' male, the potentiality of the particular female to contribute to the next generation of her own kind will be inhibited.

I thank Mr. G. Davidson for advice.

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Function of the Areæ Porosæ of Ixodid Ticks

The function of the areæ porosæ of the dorsal side of the basis capituli of the female ixodid tick was unknown for a long time. The tradition of considering it as an organ of unknown function has apparently been so well accepted by acarologists that, for at least the past 50 years, no attempt seems to have been made to elucidate the function. The interest of most acarologists in the porose area seems to be restricted to using its size and shape as an auxiliary character for specific diagnosis.

It can now be shown that the areæ porosæ are the outlets of the glands recently discovered and described[1].

Feldman-Muhsam and Havivi reported on two previously undescribed groups of accessory glands in the vicinity of Gené's organ, in gorged females of *Hyalomma excavatum*, *Rhipicephalus sanguineus* s.l. and *Boophilus annulatus*. The glands become evident soon after the onset of feeding, and attain full development by the time oviposition begins. It was also noted that these glands were not found in *Ornithodoros tholozani*.

It should be added that the glands were also found in *Ixodes ricinus* and *Dermacentor albipictus*. These additional observations suggest that these glands are present in all Ixodidae, and are absent in Argasidae.

Further examination of the morphology and development of these glands showed that the very thin and elongated ducts of these glands terminate in the areæ porosæ. Each gland is apparently connected to a separate pore of the area porosa; the number of glands corresponds to the number of pores. The relationship between the areæ porosæ and these glands explains why there are no areæ porosæ in Argasidae.

Experimental interception of the flow of the glandular secretion by obstruction of the orifices of the areæ porosæ interfered with the normal functioning of Gené's organ and consequently with oviposition.

During oviposition Gené's organ comes in close contact with the basis capituli; it is protruded and retracted for the laying of each egg. Thus, during oviposition Gené's organ is protruded and retracted on the average about 2,000 times in *Boophilus annulatus*, about 3,000 times in *R. sanguineus* s.l., about 5,000 times in *D. andersoni*, and 8,000–15,000 times in some species of *Hyalomma*. It is quite obvious that such a mechanism needs a lubricating system to function smoothly.

The obliteration of the areæ porosæ showed clearly that Gené's organ is incapable of functioning without the secretion of the recently described glands.

When the orifices of the areæ porosæ are destroyed, Gené's organ stops its movements and often remains wholly or partly stuck to the basis capituli. It is therefore assumed that the main function of the glands is most probably the lubrication of the evagination–invagination mechanism of Gené's organ.

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MICROBIOLOGY

'Endogenous Factor' in Sporogenesis in Bacteria

Extensive investigations on the morphological changes during sporogenesis have afforded valuable information about this phenomenon[1]. The initiation of sporulation can be better understood if the metabolic events during the transition from a vegetative cell to its sporulating phase are known. Several attempts have been made to investigate the physiology of growth distinct from sporulation. Foster *et al.*[2] found that when vegetative cells of *B. mycoids* were removed from the complex growth media before the point of maximal growth and transferred to distilled water, they formed spores. This was termed 'endotrophic sporulation'. But experiments with *B. cereus* strain *T* (phage-resistant organisms) showed that 'endotrophic sporulation' can be observed only when granulated cells were suspended in a medium containing sufficient calcium ions[3]. (The shortening and granulation of cells are usually noticed only long after the cessation of growth.) Non-granulated vegetative cells lysed on transfer into a non-growth supporting medium. In this communication, we report observations which lend evidence to the presence of an 'endogenous factor' involved in the initiation of the morphogenesis in the vegetative cell leading to the formation of a granulated cell (Fig. 1) possessing the ability to sporulate in a medium which does not support growth.

B. cereus strain *T* (phage-sensitive) was used throughout these investigations. The cells were grown by the active culture technique[4] from a heat-shocked spore inoculum in *G* medium (see legend for Table 1) and collected when they appeared granular under the phase contrast microscope (Fig. 1*b*). They were washed with 0·01 M *tris* *p*H 7·4 suspended in 0·01 M potassium phosphate *p*H 6·8 (1/50 vol. of original medium) and sonicated in the Raytheon sonicator for 10–12 min. The broken cells and cell debris were removed by centrifugation at 15,000*g* for 15 min. Most of the protein in the clear supernatant was removed by acidifying with 1 N hydrochloric acid and centrifuging off the precipitate. The 'active principle' was

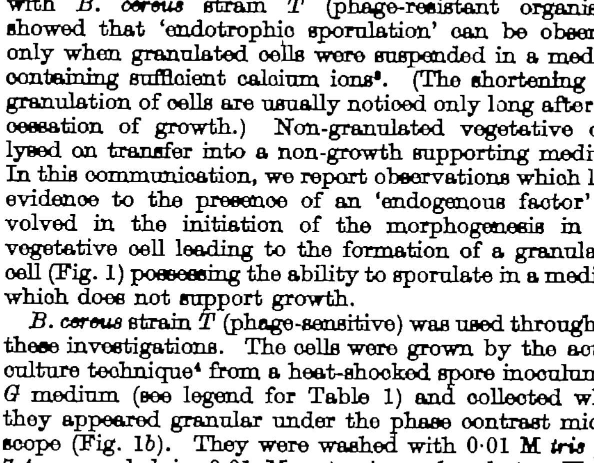
a *b*

Fig. 1. Phase contrast micrograph of (*a*) vegetative cells; (*b*) granular cells

Table 1. THERMAL STABILITY OF SPORES OBTAINED UNDER VARIOUS CONDITIONS: ANALYSES AFTER 24 H

No.	Suspending medium	% Spores (microscopic)	Viable cells/ml. before heating*	Viable cells/ml. after heating	% Survival
1	*G* medium	99	$2 \cdot 1 \times 10^8$	$2 \cdot 3 \times 10^8$	100
2	*A* medium	5	$2 \cdot 1 \times 10^6$	—	—
3	*A* medium + active principle	95	$6 \cdot 5 + 10^7$	$5 \cdot 4 \times 10^7$	83

Composition of the *G* medium in percentage. $(NH_4)_2SO_4$, 0·2; K_2HPO_4, 0·05; $MnSO_4.H_2O$, 5×10^{-3}; $CaCl_2$, 8×10^{-3}; $ZnSO_4.7H_2O$, 5×10^{-4}; $CuSO_4.5H_2O$, 5×10^{-4}; $FeSO_4.7H_2O$, 5×10^{-5}. Yeast extract, 0·2, and glucose, 0·1.

A, medium consists of all the minerals in *G* medium and the following (percentage solution): sodium citrate, 0·05; sodium acetate, 0·05; and sodium glutamate, 0·05.

* The number of cells was the same in all flasks at the start of the experiment. The low viable counts in No. 2 denote extensive lysis and loss of viability. The difference between No. 1 and No. 3 is due to the ability of *G* medium to support growth.

found to be present in non-protein fraction and was tested in the following manner. Vegetative cells (Fig. 1*a*) grown as usual in *G* medium were transferred to a non-growing medium (medium *A*) and allowed to sporulate in the presence of aliquots of the cell extract. The total solids present in the cell extracts added to a 10 ml. of test culture varied between 100–200 μg. The results obtained in a typical experiment are shown in Table 1. The vegetative cells in the absence of the active principle had a tendency to remain vegetative for a few hours and eventually lysed, whereas in the presence of the extract they were converted into granulated cells within 2–3 h and later formed spores. The activity of the extracts was relatively heat-stable but labile under alkaline conditions. Our experiments offer support for Murrell's[5] speculation on the initiation of sporogenesis by a "specific endogenous substance having a hormone-like activity".

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Temperature-dependent Variation in the *Vi*-typing Reactions of Strains of *Salmonella typhi*

IT has been found that the specific *Vi*-phage sensitivity patterns of several of the types belonging to the *Vi*-phage typing scheme[1] are easily modified by changing the temperature of incubation of the typing plates. The types so far found to display this phenomenon are: *D*1, *D*4, *D*7, *D*8, *D*9 and *D*10. In general, each type exhibits its maximum specificity at 38·5° C (the standard temperature for *Vi*-phage typing). As the temperature is lowered, this specificity is progressively reduced. The diminution in the specificity of a given type is shown by a broadening of the sensitivity spectrum of the strain to the *Vi*-typing phages. This broadening is maximal at 36·5° C. All the types concerned are known to be lysogenic, and their structural formulæ[2] and sensitivities to a range of typing phages at 36·5° C and 38·5° C are shown in Table 1.

The current type strain of *D*1 (which was supplied by Dr. P. R. Edwards, and which we call *D*1-E) is fully sensitive to all the phages shown over a wide range of temperatures. This culture was substituted for the original type strain of Craigie and Yen[1] (called here *D*1-C) because of the relative insensitivity of the latter to several of the typing phages of this group, especially phage *D*6. It has been found that the difference in the *Vi*-typing reactions of these two strains are due to differences in the type-determining phages[3] which they carry. These two phages, called *d*1-E and *d*1-C, respectively, are serologically identical, and a culture of type *A* lysogenized with either phage becomes resistant to the other. They differ only in their type-determining properties. The type-determining phages isolated from types *D*1-C, *D*4 and *D*7 are indistinguishable from each other and we use the symbol *d*1-C for all of them.

Read and Ferguson[4] have shown that the typing reactions of types *D*8, *D*9 and *D*10 are determined by double lysogeny and they give their structural formulæ as *A*(*d*8α)(*d*8β), *A*(*d*9α)(*d*9β) and ?(*d*10α)(*d*10β), respectively. I have confirmed the double lysogeny, but find that the three so-called 'α' phages are indistinguishable from *d*1-C. I therefore propose to abandon the 'α' designations and to call each of these phages *d*1-C. It then becomes unnecessary to use the 'β' symbol to distinguish the second temperate phage of each type, and I suggest that this, too, should be dropped. Phages '*d*8β' and '*d*9β' are unrelated to each other or to *d*1-C and *d*1-E, but '*d*8β' and '*d*10β' appear to be identical. I shall therefore call the last two phages *d*8. The structural formulæ which I propose for types *D*8, *D*9 and *D*10 are, therefore, *A*(*d*1-C) (*d*8), *A*(*d*1-C) (*d*9) and ?(*d*1-C) (*d*8), respectively, in place of those published earlier[4,5].

The temperature dependence of the specificity of most of these *Vi*-types seems to be largely due to the phage *d*1-C which they all carry. Phages *d*8 and *d*9, however, also play some part in determining the temperature sensitivity. Cultures with the structures *A*(*d*8) and *A*(*d*9) have been made, and it has been found that the typing reactions of these are also sensitive to temperature variation.

The increased resistance at 38·5° C of type *D*1-C, or *A*(*d*1-C), to several of the *Vi*-phages is the result of a reduction in both size and numbers of the plaques formed. The temperature of incubation during the first 4 h after the typing phages have been applied determines the final reactions. Thus a culture incubated for 4 h at 38·5° C followed by 16 h at 36·5° C will react as if it had been incubated at the higher temperature throughout. A change of temperature as small as 0·5° C during the first 4 h, or a shortening of this initial incubation period by as little as ½ h, is reflected in the final typing reactions.

One-step growth experiments have been performed using type *A* and cultures *A*(*d*1-C) and *A*(*d*1-E) as hosts

Table 1. EFFECT OF TEMPERATURE ON THE SENSITIVITY OF CERTAIN TYPE-STRAINS OF *Salmonella typhi* TO THE GROUP *D* *Vi*-TYPING PHAGES AT ROUTINE TEST DILUTION (REF. 12)

Vi-type	Structural formula	Temperature (° C)	*Vi*-typing phages *D*1	*D*2	*D*4	*D*5	*D*6	*D*7	*D*8	*D*9	*D*10	*D*11
*D*1-C	*A*(*d*1-C)	36·5	CL	CL	CL	CL	+ + +	CL	CL	CL	CL	+ + +
		38·5	+ + +	+ + +	SCL	+ + +	–	CL	CL	CL	CL	+
*D*1-E	*A*(*d*1-E)	36·5	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
		38·5	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
*D*4	?(*d*1-C)*	36·5	+ +	–	CL	–	–	–	–	–	–	–
		38·5	–	–	CL	–	–	–	–	–	–	–
*D*7	?(*d*1-C)	36·5	–	+	SCL	–	–	CL	+ +	–	CL	–
		38·5	–	–	–	–	–	CL	–	–	CL	–
*D*8	*A*(*d*1-C) (*d*8)	36·5	CL	CL	CL	CL	–	CL	CL	+ +	CL	–
		38·5	–	–	–	–	–	–	CL	–	SCL	–
*D*9	*A*(*d*1-C) (*d*9)	36·5	+ +	+ +	SCL	+ +	+	CL	CL	CL	CL	+ +
		38·5	–	–	+ + +	–	–	CL	CL	CL	CL	–
*D*10	?(*d*1-C) (*d*8)	36·5	–	–	+ + +	–	–	CL	–	+ +	CL	–
		38·5	–	–	–	–	–	–	–	–	CL	–

* The use of the '?' symbol signifies that the non-lysogenic precursor is not known.
CL, confluent lysis; SCL, semi-confluent lysis; +, + +, + + +, increasing numbers of discrete plaques.

Table 2. RESULTS OF ONE-STEP GROWTH EXPERIMENTS WITH *Vi*-PHAGE *D*1 IN DIFFERENT HOSTS AND AT DIFFERENT TEMPERATURES

Host culture	Growth temperature (° C)	Latent period (min)	Rise period (min)	Burst size
Type *A*	35·0	38	15	100
	39·5	37	17	160
A(*d*1-E)	35·0	37	18	40
	39·5	35	18	65
A(*d*1-C)	35·0	37	35	12
	38·0	36	40	4
	39·5	?	?	1–2

for *Vi*-typing phage *D*1. The burst size in *A*(*d*1-C), which is considerably lower than that in type *A* and *A*(*d*1-E) at all temperatures, is progressively reduced as the temperature is raised from 35·0° C to 39·5° C. In *A* and *A*(*d*1-E) the burst size of phage *D*1 increases with the rise in temperature. The latent period at all temperatures is the same in each of the cultures, but the rise periods with *A*(*d*1-C) are longer than with the other two cultures (Table 2).

Temperature sensitivity of *Vi*-typing reactions was first observed by Craigie, who found that type *N* of *Salmonella typhi* cross-reacted with several *Vi*-phages of the *D* group. The cross-reactions were abolished by incubation above 38·0° C (ref. 6).

The resistance to viral infection of several plant species is increased by raising the temperature at which the plants are grown[7] and it is possible to cure some virus-infected plants by heat therapy[8]. A similar phenomenon has been observed in animal viruses. For example, it has been shown that the likelihood of survival of mice infected with the MEF_1 strain of poliovirus is increased when the environmental temperature is raised[9]. The Lwoffs[10] have found that raising the temperature of incubation reduces the viral yield of tissue cultures infected with attenuated vaccine strains of poliovirus more so than that from cultures infected with a highly neurovirulent wild strain. Mutants of this virus have been isolated which develop preferentially at either a high or a low temperature, and it has been found that the former are usually the more virulent[11a,b].

The phenomenon reported here resembles that found in plants and animals but differs from them in that it is not the intrinsic phage resistance of the bacterial host which is modified, or the virulence of the infecting phage. It is essential that the bacterium carries a prophage such as *d*1-C which confers, on the phage-host cell complex, temperature sensitivity in relation to multiplication of the superinfecting *Vi*-phage.

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Minimum Requirements for Heterotrophic Growth, and Reserve Substance in *Beggiatoa*

CATALDI[1] has shown that *Beggiatoa*, instead of living autotrophically on the oxidation of hydrogen sulphide, can grow heterotrophically on proteinacious substances. Faust and Wolfe[2] observed stimulation by acetate.

We found that most of our 14 strains isolated bacteria-free thrive on acetate, and some other organic salts, as the only organic nutrients, provided the necessary chemical elements are supplied. They utilize inorganic nitrogen and do not require growth substances. This appears to be the simplest medium imaginable for a chlorophyll-free organism.

Beggiatoa is much favoured by aeration. The growth is then dense enough to collect ample material for biochemical purposes. In such circumstances the cells are eventually stuffed with small spherical to oblong bodies slightly less refractive than the sulphur droplets in natural material. These bodies turned out to be poly-β-hydroxybutyric acid.

The utilization of acetate occurs with the help of isocitratase, the main enzyme of the glyoxylic acid cycle. This enzyme is produced not only when the organism lives on acetate but also when aspartate or glutamate is the only substrate.

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CYTOLOGY

A Ring Chromosome in Man

THE known chromosomal abnormalities in man comprise mainly whole chromosome and segmental monosomy and trisomy. Ring chromosomes have been described in tumour stem lines[1,2] and in normal tissues following irradiation[3]. Recently a ring *X*-chromosome has been reported in a female with gonadal dysgenesis[4]. A self-perpetuating ring chromosome is now described in a mentally and physically retarded but pubertal male. A full clinical description of the case is being published elsewhere[5].

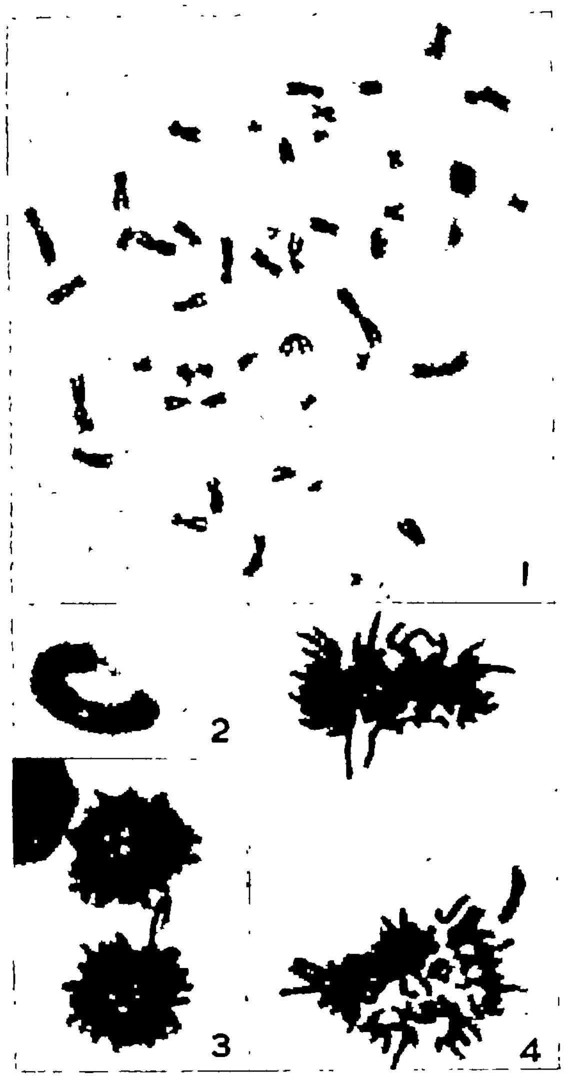
Cytological investigations have been made of cultured and uncultured peripheral blood, cultured skin tissue, and bone marrow. All mitoses in the bone marrow and most in the cultured leucocytes and skin were found to contain 45 rod chromosomes plus a monocentric ring (Fig. 1). In the cultured tissues a proportion of mitoses with 45 rod chromosomes only were observed (Table 1). Karyotype analyses indicated the absence of one member of the 6–12 chromosome group (Denver classification) in cells possessing the ring chromosome. The size of the ring was found to be consistent with the assumption that it represents this missing chromosome.

Table 1. KARYOTYPE FREQUENCIES

Tissue	Karyotypes	
	45 rods only	45 rods + ring
Cultured leucocytes	39	104
Skin	5	31
Bone marrow	0	19

The ring is generally synchronous with the rest of the chromosome complement in mitosis, but shows a significant variation in mean length (Table 2). At late prophase and at metaphase the two chromatids of the ring usually lack relational coiling (Fig. 2), and at anaphase the two daughter chromosomes separate without interlocking. In occasional anaphases interlocked or dicentric rings are present, and these may bridge the anaphase or may lag and be excluded from the daughter nuclei (Figs. 3 and 4). Conformably, micronuclei have been observed in both cultured and uncultured leucocytes.

The observed variation in the size of the ring must result from breakage and re-union of bridged dicentric or interlocked rings. Lagging and exclusion of such rings must also generate the nuclei with 45 rod chromosomes only,



Figs. 1–4. 1, Colchicine metaphase showing 46 rod chromosomes and the monocentric ring (× 1,050); 2, monocentric ring at prophase showing absence of relational coiling (× 2,300), 3, dicentric ring chromosome bridge at anaphase (× 1,300); 4, non-disjunctional interlocked rings at anaphase (× 1,300)

and the micronuclei. The absence of cells with two ring chromosomes, which would be expected from non-disjunction, may indicate either regular double exclusion or else lethality of the trisomic condition. The latter assumption is concordant with the fact that trisomy for the larger autosomes in man is unknown, and so it may be further suggested that the ring is an autosome rather than the X-chromosome.

The dicentric and interlocked rings derive from relational coiling, which must be limited to one 180° half twist or to one 360° full twist since knotted dicentrics and doubly interlocked rings have not been observed. Relational coiling in ring chromosomes has been attributed alternatively to rotation of the plane of chromosome splitting[6] or to sister chromatid breakage and re-union (mitotic sister chromatid crossing-over) combining with uniplanar chromatid replication[7,8]. In the former hypothesis breakage and re-union are necessary for free ring separation. Superficially, the behaviour of this ring chromosome favours the second hypothesis. However, such a hypothesis provides no implicit cause of breakage, whereas rotation of the plane of split could lead in a ring chromosome to torsions which would cause breakage in each mitotic cycle, allowing the relational coiling to be undone.

Table 2. Mean Relative Chromosome-Lengths (Arbitrary Units)

Chromosome	1	2	3	Ring
Mean length	18 8 ± 0·6	18 3 ± 0·6	14·8 ± 0·5	6·2 ± 0·3
Variance of log transforms	0 0103	0 0103	0·0097	0 0184

$F_{1}/_{2(47,47)} = 1\cdot06$ Non-significant

$Fr/_{3(47,47)} = 1\cdot79$ Significant at 5% level

Since the ring chromosome is present in all the tissues which have been studied, and since the cells which lack it can be accounted for by non-disjunction, the individual does not appear to be a primary mosaic. It is therefore inferred that the ring was present in the original zygote. Both the parents, however, have normal 46 chromosome karyotypes, so that the ring must have been formed at or shortly before gametogenesis in one parent. Alternative methods for its origin are: (*a*) somatic breakage and re-union, which would require two simultaneous breaks and would necessarily involve segmental deficiency; (*b*) meiotic crossing-over in a parent heterozygous for an inverted intrachromosomal translocation, such as $\frac{ABCDE.FGHIJ}{ADE.FGHBCIJ}$. Since a maternal uncle is known to have been mentally retarded and to have died in a mental institution, the second hypothesis is favoured, the mother then being the heterozygote.

The syndrome of mental and physical abnormalities shown by the individual may be a consequence of the mitotic instability of the ring chromosome and the secondary mosaicism for monosomic cells, or may be due to genic deficiency in the ring. The latter is not an obligatory assumption, since on the basis of the second hypothesis of ring formation it would result from interstitial but not from terminal translocations.

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An Optically Efficient Method for observing Single Plant Cells from Shake Cultures during Growth and Division

The ease with which the individual cells of callus cultures of tissues of higher plants separate from each other has led to several successful methods of growing them singly. Muir, Hildebrandt and Riker[1] grew a single crown-gall callus cell on a piece of filter paper over a large callus of the same strain. Torrey[2] placed many separate cells around a nurse callus; some of the former divided once or more during the subsequent two weeks. Bergmann[3] made a considerable advance by utilizing a plating method for individual cells of tobacco and of bean. Of the numerous separate cells in a thin plate of agar medium, individuals were followed by time-lapse photography during their division. Optical quality of the pictures was poor due to the necessity of observation through the bottom of a Petri dish and a layer of agar. A perusal of these methods or an attempt to duplicate them makes the investigator conscious of their deficiencies; there is no possibility of observing the single cell in the first and growth is recognized only when it has formed a callus that is visible to the unaided eye, the second has most of the disadvantages of the hanging-drop, and the third suffers from poor optical quality.

In attempts to gain a cellular picture of the changes involved in the callus of *Sequoia sempervirens*[4] grown in a wide variety of media, I have worked out a method that appears to yield high optical quality and to permit prolonged observations in sterile conditions without loss of volume of the medium. The individual cells in suspension were obtained by shaking the tissue in Erlenmeyer flasks containing the standard medium without agar at 160 r.p.m. for 10 days. The observation chamber to be used on the stage of the inverted phase contrast microscope was made by grinding two half-overlapping holes 2·5 cm in diameter near the centres of glass plates 2 mm thick, 6 cm wide and 10 cm long. These plates and cover-glasses 48 mm × 60 mm in size and No. 1 in thickness were cleaned by the method of Rappaport and Bishop[5] and after drying by evaporation were sterilized at 175° C for 4 h. After cooling, an oval line of sterile Dow Corning silicone stopcock grease was placed on the cover-glass *c.* 5 mm back of the top and side margins. Cover-glass was then pressed securely on the slide so as to force a minimum of the grease into the chamber and none at all beyond the edge of the cover-glass on to the slide. Resting the slide with the open chamber up, 0·5 ml. of the suspension of individual cells was added. With some practice, it has been found possible to judge the necessary quantity of sterile liquid culture medium to be added next. Usually it amounts to about 4 ml., and brings the meniscus of the liquid to a slight bulge above the edges of the cavity. Next, a second cover-glass is prepared with the stopcock grease and pressed down over the liquid-filled cavity. A proper preparation can be considered one that has a small gas bubble trapped above the liquid and which loses no liquid from the edges of the cover-glass when the latter is pressed down, effectively sealing the contents from evaporation and entrance of micro-organisms.

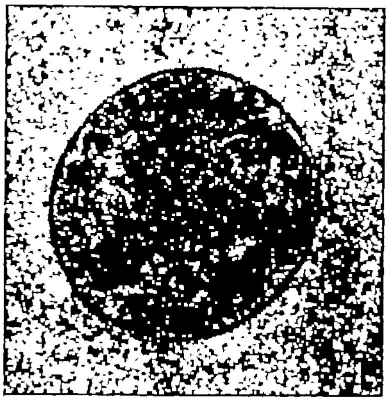
Fig. 1. Single cell of callus of *Sequoia sempervirens* resting on the lower cover slip at the time of inoculation in the observation chamber. (× *c.* 220)

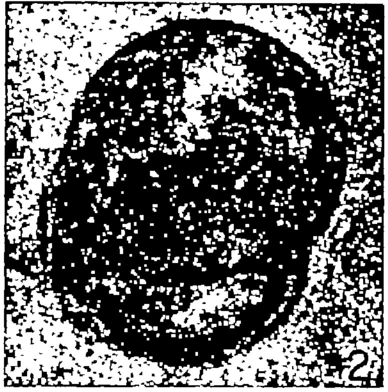

Fig. 2. The two derivative cells produced by the division of the cell of Fig. 1. The lower cell was attached to the glass surface. The division figure was produced at right-angles to the glass surface; the upper derivative cell was almost directly above the lower. (× *c.* 220)

The Dow Corning silicone stopcock grease was found ideal for the purpose outlined above. It is inert chemically, and appears to be sterile bacteriologically as it comes in the tube. Precautionarily, it was autoclaved at 120° C for 15 min.

The preparation, now containing scores of freely floating individual cells, was placed on the stage of the inverted phase-contrast microscope. All the cells rested at the bottom of the liquid on the specially cleaned glass surface of the lower cover slip and could be viewed as images of excellent optical quality through the objectives. An appropriate cell was chosen, centred in the field and time-lapse pictures at intervals of 2 min were taken. Fig. 1 shows such a cell at the time of inoculation, and Fig. 2 shows the two derivative cells 36 h later. Attachment of the cell to the glass surface had occurred prior to the division and the mitotic figure was oriented at right angles to the surface. Subsequent growth during a period of 7 days produced five additional cells all resting upon the one anchored to the cover-glass. Attachment of multicellular callus to glass surfaces was reported by Ball[6] and by Gadgil[7]. It would appear in the present work that the attachment of the single plant cell to the glass surface is comparable to that considered normal in culture of animal cells[3].

The negatives for Figs. 1 and 2 were taken through a × 20 phase objective and a × 5 ocular on to 35-mm motion picture film. This work was supported by National Science Foundation grant No. 4394.

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Induction of Partial Synchrony in Mammalian Cells by Sub-culturing Procedures

THERE are many advantages of working with synchronized cell cultures and, indeed, much work has been done in this field[1]. However, the interpretation of many kinds of experiments requires either that the cultures be not synchronized, or that the investigator be aware of the possibility of inadvertent synchrony[2].

This communication reports instances of such unintentionally induced partial synchrony of human amnion[3] and kitten lung[4] cells grown in Rose chambers and examined by means of time-lapse microcinematography.

Both the medium used and general culture methods have been described[5]. Cells were removed from the glass surface of the stock culture bottle by gentle scraping with a rubber policeman, separated by passage through a 15-gauge needle, and seeded into the Rose chambers (approximate capacity, 2 ml.). In one case, *HA*36, a pre-adapted medium was used, that is, one in which cells had previously been grown for approximately 24 h. Photography began 24–30 h after seeding the chambers with 5,000–10,000 cells at *p*H 7·8 and was continued for periods of 100–122 h at 37·2 ± 0·5° C. A thermistor oiled to the coverslip of each chamber regulated the temperature by controlling a continuously variable voltage fed to an infra-red heating lamp. The chamber itself was shielded from direct infra-red rays by a strip of aluminium foil. The sensitivity of the system was such that in any given experiment the temperature of the cells varied no more

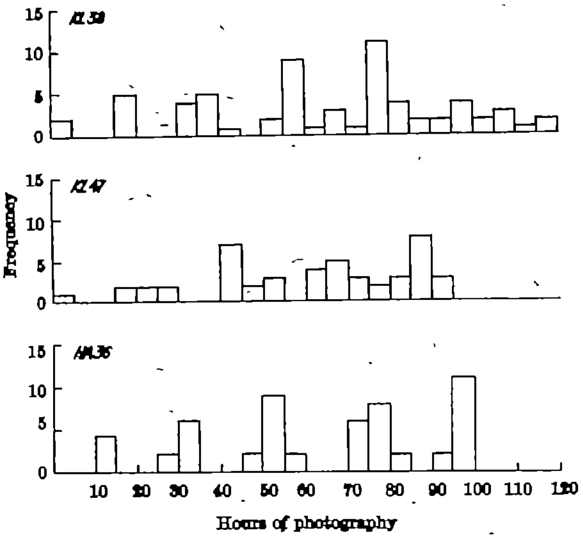

Fig. 1. Frequency of mitoses plotted as a function of time since the beginning of the photography

than 0·1°–0·2° C, although the differences between experiments might have been as great as 0·5° C due to calibration problems and changes in instrumentation.

The only mitoses recorded were those of cells which were traceable from generation to generation. Pedigree diagrams showing generation times of individual cells will be reported in detail elsewhere.

Fig. 1 is the frequency distribution which shows that peaks of mitoses were repeated for at least four cell generations. In *HA*36, synchrony was somewhat better preserved despite the fact that this was the only culture prepared with pre-adapted medium. In these experiments, we were dealing with the offspring of only a few cells in a sparsely populated chamber. To determine whether the synchrony could be due to these unique conditions, a single exploratory experiment was carried out using cells seeded on coverslips in Leighton tubes. While the results in Fig. 2 indicate a periodicity of mitoses for the first 45 h, the degree of synchrony was not great and appeared to have been soon lost.

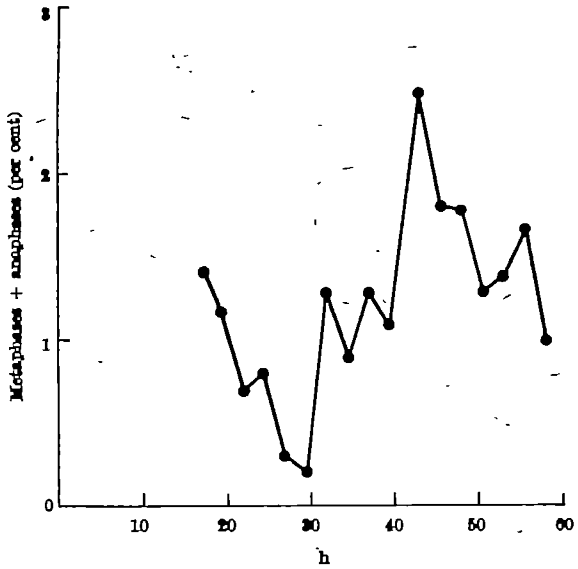

Fig. 2. Frequency of metaphases + anaphases in Leighton tube coverslip cultures of kitten lung cells as a function of age of the culture. Original inoculum: approximately 26,500 cells/ml.

The reasons for this synchrony are unknown. The experiment in which a pre-adapted medium was used appears to rule out the possibility that subculturing into fresh medium could be an only cause of synchrony. The other possible causes could be either the cooling of the cultures to room temperature during sub-culturing, although this is a gentler treatment than that deliberately used by Newton and Wildy[6] to induce synchrony, or the trauma produced by scraping the cells off the surface of the glass bottle.

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GENETICS

Assortative Mating in producing New Species

Thoday and Gibson[1] have recently reported an experiment with *Drosophila* where assortative mating occurred in a small population artificially selected to remove the central portion of the distribution for number of chæta. This assortative mating was so complete that 12 generations of such selection produced two distinct lines with no overlapping.

Parker[2] reported a fertility problem with the Cornish breed of poultry. Six Cornish males, mated to a flock of 90 females of the New Hampshire breed, gave a much lower average fertility than the same flock with New Hampshire males. Repeated trials gave the same result.

Various tests finally disclosed that both sets of males were equally fertile. However, with Cornish males up to 14 per cent of the hens gave all infertile eggs, but with New Hampshire males only 2 per cent of the hens gave this result. Some of the hens were infertile in two different trials.

Since this was a flock mating it would appear that mating preference was being exercised by males and/or females and that such preference was common to all the males of the Cornish breed and/or several of the New Hampshire females.

This would seem to be further evidence of the possibilities of assortative mating in producing new species.

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Balanced Polymorphism in *Dactylis glomerata* Sub-species *woronowii*

A case of balanced polymorphism, involving a recessive gene causing albinism, in *Dactylis glomerata* sub-species *judaica* has been reported by Apirion and Zohary[1]. What appears to be the same phenomenon is now reported for another diploid sub-species, *woronowii*, a seed-sample (No. 24171) of which was made available to me by the Division of Plant Industry, C.S.I.R.O., Canberra. Albino seedlings appear with a frequency of 13·5 per cent in this sample, and it may be taken that one is dealing with a recessive allele which, because of the lethality it confers in the homozygote, is present in unexpectedly high proportion. Evidently some superiority is attached to the heterozygous condition, which probably displays itself through a greater competitive ability in the seedling stage or a greater reproductive capacity when mature. However, because of the changed environmental circumstances in which the material is now being grown this selective advantage may be altered or lost. These possibilities are being investigated.

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PSYCHOLOGY

Learning Deficits of Offspring after Administration of Tranquillizing Drugs to the Mothers

IN previous reports[1-4] from this laboratory, various psychotropic drugs administered to gravid rats resulted in increased neonatal mortality and altered the development and behaviour of the surviving offspring. On the basis of observations[1] that learning ability was not altered in offspring after prenatal administration of drugs that affected diencephalic centres, it was suggested that particular drugs may have a specificity of action on developing fœtal brain centres and would alter behavioural processes controlled by these centres. This communication compares three commonly used tranquillizing drugs (reserpine, chlorpromazine and meprobamate) with different pharmacological properties and sites of action, administered to gravid rats for their effect on maze learning ability of the offspring.

Subjects were 89 gravid (primiparous) albino rats of the Sprague–Dawley strain (Holtzman Co.). Breeding procedures have been described in detail elsewhere[1-4]. The drugs administered were reserpine, chlorpromazine and meprobamate. Reserpine was prepared from reserpine phosphate powder in a 1·0 per cent solution with distilled water. Chlorpromazine was prepared from chlorpromazine hydrochloride powder in a 0·1 per cent solution with distilled water. Meprobamate was prepared from meprobamate powder in a 0·5 per cent solution with distilled water heated to 80° C and cooled at room temperature to retard recrystallization. All drugs were unbuffered with *p*H in the range of 5·8–6·4. The drugs were prepared every 48 h. The daily dosages were: reserpine (0·1 mg/kg), chlorpromazine (6·0 mg/kg), and meprobamate (60·0 mg/kg). A volume control of distilled water was also administered. These daily dosages were divided in three equal amounts and injected every 8 h at 8 a.m., 4 p.m., and 12 midnight in order to maintain blood-levels. All injections were administered subcutaneously in either the right or left dorsal area with the side of injection alternated. The drugs were administered during one period of gestation on either days 5–8, 11–14, or 17–20.

Of the 690 offspring that survived through weaning (21 days of age), a random sample of at least 10 males and 10 females from pooled litters from each period of administration was selected from each of the drug and control groups. Learning ability measures were obtained from 232 animals on the Lashley III maze for an 8-day period starting at 82 days of age and after 7 days pretraining on a straight alley and acclimatization to a 23-h food deprivation schedule. The apparatus and procedure for this test have been previously described[5].

Table 1 gives the number of animals evaluated in each group on the Lashley III maze with the mean and standard deviation for the number of trials required to reach the criterion of learning. The mean score includes the criterion trials. The criterion of learning was defined as two out of three errorless trials run in 20 sec or less per trial. The score for each drug and control group is a combined score of males and females and combined periods of administration. This was done because no significant or consistent differences were found between males and females or between periods of administration. Statistical analysis among groups was done with the *t*-test. In comparing each of the drug groups to the control group, only the offspring of the meprobamate-treated mothers required significantly more trials to reach the criterion of learning. This difference is significant at the 0·01-level with a *t* score of 4·13. In comparing the drug groups between each other the meprobamate group is found to be significantly higher than the reserpine group ($t=2·30$, $P=0·05$) and the chlorpromazine group ($t=3·96$, $P=0·01$). No significant difference between the reserpine and chlorpromazine groups is noted.

Table 1. TRIALS TO CRITERION ON LASHLEY III MAZE OF OFFSPRING OF MOTHERS ADMINISTERED TRANQUILLIZERS DURING GESTATION

Group	No.	*M*	*S.D.*
Reserpine	56	10·7*	4·6
Chlorpromazine	57	9·4†	4·1
Meprobamate	60	12·7	5·0
Control	59	9·5†	3·4

Difference between this score and meprobamate is significant at the * 0·05- and † 0·01-levels respectively.

The finding that only meprobamate of the three tranquillizing drugs administered to gravid rats detrimentally altered maze learning ability of the offspring was anticipated. Meprobamate, in contrast to the other tranquillizing drugs (reserpine and chlorpromazine), is known to have a depressing effect on the cerebral cortex[6]. In this respect and in other ways, meprobamate is considered to have pharmacological properties of a barbiturate[7]. Other investigations, in which barbiturates[8] and other potent central nervous system drugs such as sodium bromide[9] and alcohol[10] were administered to gravid rats, also report deficits in the learning ability of the offspring.

Although no complete explanations are available to account for these results, several possibilities must be considered. The first possibility is that the prenatal administration of meprobamate altered the animal's physical condition and impaired its performance. Consistent and significant differences in physical condition as measured by gain in weight between the various groups were noted at all ages from weaning until the termination of the experiment with meprobamate and reserpine offspring weighing less than controls and chlorpromazine offspring weighing more than controls[3]. Although the meprobamate and reserpine offspring weighed less, they also were significantly less active on two separate activity measures[4]. If decreased activity accounted for poor performance on the Lashley III maze, then both the reserpine and meprobamate offspring should have been detrimentally affected. However, this was not the case and only the meprobamate offspring showed the learning impairment. The criterion for learning depended not only on speed of running but also on making correct responses.

A second consideration is that the learning impairment of the meprobamate offspring reflects altered development and/or function of the cerebral cortex. Whether this is a direct consequence of meprobamate on the fœtal central nervous system or whether meprobamate created metabolic changes in the gravid animal which indirectly caused alteration of the fœtal central nervous system through such mechanisms as anoxia has not been determined in the present experiment. The fact that these drugs do pass the maternal–fœtal placental barrier[11] suggests that meprobamate could have exerted a direct influence on the central nervous system of the developing fœtus.

The findings from the present investigation support the hypothesis that pharmacological agents may have a specificity of action on developing brain areas so that tranquillizing drugs such as meprobamate can produce learning deficits in offspring of mothers administered such drugs.

We acknowledge the support of the following companies for supplying the drugs used in this experiment: Ciba for

reserpine, Smith, Kline and French for chlorpromazine, and Wyeth for meprobamate.

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Intermodality Transfer of Specific Discriminations in the Monkey

WE wish to report evidence that monkeys trained to discriminate between two forms using only tactual cues are affected by such experience when the same forms are presented visually with tactual cues excluded.

Six rhesus monkeys were used in two experiments. All testing was done in a Wisconsin general testing apparatus, with food reward for the correct choice of two objects presented simultaneously. Forty (non-correction) trials were given daily. All objects to be discriminated (cross, square, *S*, etc.) were cut from ¼ in. plywood, painted black, and attached to food-well covers. For visual discriminations, transparent plastic cylinders covered the objects; tactual discriminations were performed in the dark with the objects uncovered.

In the first experiment, all monkeys learned to discriminate between 11 pairs of objects, both visually and tactually. Each problem was learned to a criterion of 27 correct responses out of 30. The first pair of objects given to an animal (and the third, fifth, etc., pairs) was learned first visually and then tactually; the even-numbered pairs were learned first tactually and then visually.

Comparisons were made of average performance on tactual problems presented after visual learning of the same objects (transfer problems) with performance on those problems for which tactual learning was the initial experience with the object pair. Neither trials to criterion nor errors in the first 30 trials of each problem disclosed a significant transfer effect. Furthermore, similar analyses of visual-problem performance provided no evidence for tactual-visual transfer.

During the tactual problems animals were observed (with a snooperscope) and it was noted that only rarely did they palpate the full surface of the stimuli before making a choice. Usually some aspect of the near edge of the stimulus, or the distance of some part of it from an exposed edge of the testing board, appeared to be the effective cue; and it is well known that not all aspects of a stimulus object presented visually enter equally into the discrimination which is learned. It is possible that our subjects (and those of other experiments) did not always 'see' the same part or aspect of the object as the part that they 'felt'. Stimulus objects for the second experiment were designed so that there would be more certainty as to the nature of the discrimination being formed.

Pieces of ¼ in. plywood, ½ in. in width, and either 1 in. or 2 in. in length, were used. The wooden strips were attached to food-well covers, and were so oriented that they differed in length in the near–far dimension of the animal. Three short stimuli were made, with the near edge of the wooden strip ¼ in., 1¼ in., or 2¼ in. from the near edge of the food-well cover; two long stimuli were made, with the near edge of the strip ¼ in. or 1¼ in. from the near edge of the cover. On each trial one short and one long stimulus were presented, with balanced series determining the combination to be used and the right–left placement of the positive stimulus.

The subjects were divided randomly into two groups of three monkeys each, which received the sequences of problems shown in Table 1. The first two discriminations were run to a criterion of 38 correct out of 40 trials, plus six consecutive correct trials on each of the six combinations of stimuli. On the day after an animal reached criterion on a problem, 10 additional trials were given on that problem followed immediately by 30 trials on the next problem.

Table 1. SEQUENCES OF LENGTH DISCRIMINATIONS, AND PERFORMANCE ON TRANSFER TESTS

Group	Tactual (1) Positive stimulus	Visual Positive stimulus	Visual Errors in first 30 trials	Tactual (2) Positive stimulus	Tactual (2) Errors in first 30 trials
I	Short	Short	9	Long	28
	Short	Short	13	Long	24
	Short	Short	14	Long	16
II	Long	Short	16	Short	16
	Long	Short	18	Short	20
	Long	Short	21	Short	27
			$P < 0.05$		$P > 0.05$

Evidence for intermodality transfer is shown on the first 30 trials of the visual discrimination (Table 1). All animals that had previously learned the same discrimination tactually (Group I) made fewer errors than all animals that had had the opposite tactual discrimination ($t = 3.01$, $P < 0.05$). Furthermore, the two groups of scores are placed appropriately on either side of the chance value of 15. (The effect is apparently weak in comparison to other influences, for the groups did not differ in trials-to-criterion on the visual task nor on performance on the second tactual task, where the strong intramodal effect of the first tactual task apparently overwhelms any possible intermodal effect.)

We conclude, then, that a specific intermodality transfer effect can be demonstrated if we require that the 'same' discrimination be made in the two modalities. It can be suggested that in our first experiment there were some problems on which individual animals happened to make use of the same cues in the two modalities: each animal met criterion immediately (in the first 30 trials) more frequently on transfer problems than on non-transfer problems—for visual and tactual problems combined, or for tactual problems alone. Perhaps the one monkey of Ettlinger[1] which showed what may have been tactual-visual transfer happened to attend to the same cue in the two modalities.

In this experiment we have studied transfer between touch and vision when the same objects are presented to the two modalities. This must be distinguished from transfer between analogous auditory and visual discriminations[2], evidence for which has come primarily from an experiment[3] the results of which might possibly be explained as due to an even more general effect, the intermodality facilitation of learning sets[4]. These may prove to be three different kinds of transfer in discrimination problems, which must be studied separately.

In any event, a method for demonstrating specific intersensory transfer opens the way to investigations of the role of early sensory experience and of various neural structures in the normal development of such transfer.

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Influence of Hunger on Olfactory Acuity

A HUNGRY animal is considered to be in a state of high nervous irritability leading to a heightened level of sensory and motor excitation. As glycogen stores are depleted, blood glucose-levels decrease, circulating epinephrine rises and stimulates the reticular formation of the brain, which in turn produces cortical arousal and facilitation of motor and sensory activity, lowering the threshold for all external sensory stimuli[1]. A satiated animal, by contrast, is relaxed, disinterested in the outside world, sleepy, and consequently should have a high sensory threshold.

There are conflicting opinions on whether olfactory acuity is a reliable, reproducible index of appetite, hunger, or satiety. According to Goetzl[2], freely selected meals, as well as administration of sucrose solutions, were preceded by increased olfactory acuity and followed by decreased acuity. Janowitz and Grossman[3] were unable to duplicate Goetzl's results. Subsequent investigators, however, have reported that olfactory acuity was higher before lunch than after lunch[4]. Coffee and citral, respectively, were used by the first two investigators, whereas isoamylacetate, *n*-butanol, and oil of cloves were tested by the third. Their respective testing methods were the Elsberg blast-injection technique, sniffing odour-saturated air in an olfactorium, and sniffing from a series of test-tubes.

An olfactometer designed to deliver calculated amounts of odour in purified air, at controlled flow rates, temperatures, and humidities, and utilizing natural breathing, was used[5]. Experimental subjects consisted of University personnel, 11 males and 1 female, 30–58 years of age, selected on the basis of their proved olfactory acuity and reproducibility of judgment. Citral was found to deteriorate rapidly, changing its odorous characteristics, therefore 2-heptanone, a highly stable compound which smells like mild blue cheese, was substituted. The panel was divided into two groups which were tested alternately just before lunch (11 a.m.–12.15 p.m.) and just after lunch (1 p.m.–2.15 p.m.).

Table 1. OLFACTORY RESPONSES PRECEDING AND FOLLOWING LUNCH

Subject	Sex	No. of judgments a.m.	No. of judgments p.m.	Correct response (per cent) a.m.	Correct response (per cent) p.m.
A	M	400	480	65	70
B	M	370	350	62	75
C	M	400	490	71	71
D	M	290	420	69	75
E	M	380	380	66	80
F	M	330	490	77	72
G	M	290	190	69	73
H	F	450	420	72	72
I	M	270	260	65	67
J	M	350	490	70	76
K	M	450	470	68	74
L	M	450	440	77	80
Average		369	407	68·1	73·7

Since all the standard testing procedures had one or more serious defects, a constant stimulus method was developed which would eliminate the fatiguing effect of a strong concentration preceding a weaker one and would place each subject on an equal sensitivity basis. Ten samples were administered at each daily session. Each sample was presented for 15 sec with a 15-sec interval of pure air between subsequent presentations. The number of samples containing odour and the order of presentation were determined by chance. The concentration of odour given was slightly above threshold for each subject as determined by previous testing. Subjects were requested to indicate the presence or absence of the test compound. Over a period of 5 months, an average of 369 and 407 separate judgments was obtained from each subject before and after lunch, respectively.

As shown in Table 1, only subject *F* was more acute before lunch, subjects *C* and *H* showed no difference, whereas the remaining nine were more acute after than before lunch. The *t*-test for variance indicated the difference between morning and afternoon panels was significant at the 0·1 per cent level of probability. Thus, using an olfactometer-presentation of 2-heptanone by a constant stimulus methodology, olfactory acuity was higher after lunch than before.

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A Vertical–Horizontal Illusion for Movement perceived tactually

THERE is a well-known visual illusion in which a vertical line is judged as longer than a horizontal line of the same length. The illusion also holds, though attenuated, when lines are perceived tactually, even in blind subjects[1]. This communication is concerned with a similar error which is made when speed of movement is assessed tactually, but which does not show when the judgment is made visually.

The apparatus consisted of two 6-in. drums the speed of rotation of which could be varied. One was mounted with its axis vertical, the other horizontal. The drums were covered with paper embossed by means of Braille writing equipment with a pattern of dots in triplets, 1 mm across, and with an interval of 12 mm between triplets of dots. In both experiments the subject rested his finger-tips against the dots on the surfaces of the drums as they rotated. Thus one hand was held in a vertical plane and the other horizontally.

In the first experiment, subjects were required to adjust the speed of one drum so that it appeared tactually (with eyes shut) to rotate at the speed of the other. All 12 subjects adjusted the speed so that the drum with vertical axis was objectively rotating faster. This means that the speed of the dots moving vertically past the fingers was overestimated.

The finding was tested in another way. The two drums were rotated at the same speed with dots passing the fingers at 27·5 mm/sec, and subjects were each required to make a single tactile judgment as to which drum was rotating faster (subjects were not allowed to say 'equal'). Of 24 subjects, 19 reported that the drum with horizontal axis (vertical movement) was the faster. On a χ^2 test this result is expected in less than 1 per cent of cases by chance. Another 24 subjects made the judgment visually. Thirteen reported the drum with horizontal axis as faster, 11 the drum with vertical axis. In the tactile judgments the effect did not depend on whether the dots were moving away from or towards the body, nor did it depend on handedness.

The results suggest a vertical–horizontal asymmetry in tactile perception. The most plausible explanations of visual illusions[2] are in terms of eye-movements, or configurational effects or depth perception. None of these appears to explain the present illusion.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 7

British Institution of Radio Engineers, Research Committee at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 2 p.m.—Symposium on "Radio and Electronics Research in Great Britain".

University College, London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. Harland G. Wood (U.S.A.): "The Problems of Determining Pathways of Carbohydrate Metabolism using Labelled Glucose".* (Further lecture on January 10.)

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Testing of Modern Large Steam Generating Plant".

Royal Institute of Chemistry, London Section (joint meeting with the London Section of the Society of Chemical Industry, at the Society of Chemical Industry, 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. Alexander King: "Scientific Research in Europe in Relation to Economic Development".

Tuesday, January 8

Institution of Chemical Engineers, South-Eastern Branch (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4 p.m.—Annual General Meeting. 6 p.m.—Discussion Papers.

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. R. E. Hayes: "Cryogenics".

Institution of the Rubber Industry (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. G. F. Bloomfield: "Recent Developments in the Malayan Rubber Industry". 7 p.m.—Prof. C. E. H. Bawn, F.R.S.: "Some Aspects of Stereospecific Polymerization".

Wednesday, January 9

Society for Applied Bacteriology (in the Barnes Lecture Theatre, Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 10.30 a.m.—Winter Meeting. 2.15 p.m.—(joint meeting with the Microbiology Group of the Society of Chemical Industry)—Symposium on "Applied Aspects of Mixed Microbial Populations".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. J. C. Duckworth: "Progress and Innovation" (Peter Le Neve Foster Lecture).

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. R. V. Beavon: "The Succession and Structure East of the Glaslyn River, North Wales" (to be read by Prof. Alan Wood); Mr. N. J. Soper: "The Structure of the Rogart Igneous Complex, Sutherland".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. J. Chalmers: "Asynchronous Performance Characteristics of Turbo-Generators".

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. T. Shore: "Heat Exchange in Profound Hypothermia. Heat Exchanger Design for Blood during External Circulation"; Mr. B. G. B. Lucas and Mr. S. R. Montgomery: "Engineering Aspects of Extra-corporeal Blood Circulation".

Plastics Institute (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. W. S. Callender: "Plastics in the Bakery and Confectionery Industries".

Society of Instrument Technology, Measurement Technology Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. D. H. Desty: "Recent Advances in Gas Chromatography".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m.—Mr. J. M. Low: "Man-made Fibres".

Thursday, January 10

Royal Institution of Naval Architects (at 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Mr. J. W. English: "Hydrodynamic Factors Affecting the Design and Performance of Lateral Thrust Units for Ships".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. M. Waldram: "Some Engineering Aspects of Lighting" (Fifth Hunter Memorial Lecture).

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. C. C. Martin: "Integrating Electronics in Missile and Space Systems".

Friday, January 11

Biochemical Society (at the National Institute for Research in Dairying, Shinfield, Reading), at 10 a.m.—Scientific Papers.

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Effect of Installation on Pump Performance".

Saturday, January 12

Association of Clinical Biochemists, and the Biochemical Society (in the Physics Department, The University, Reading), at 9.40 a.m.—Symposium on "Chromatography in Clinical Biochemistry. Standard Methods and Recent Advances".

Monday, January 14

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 4.30 p.m.—Mr. J. M. Winchester and Mr. R. Pearson: "Development of Packages for Pesticides".

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. B. Braithwaite: "Believing (as Opposed to Considering) Natural Laws".

Institution of Electrical Engineers, Science and General Division P.G.86 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. Thomson: "Modern Transducers with an Electrical Output".

University of London (in the Eugenics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Paul Turan (University of Budapest): "Analysis and Diophantine Approximation".* (Further lectures on January 15 and 16.)

Institution of Mechanical Engineers, Lubrication and Wear Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Oil Mist Lubrication".

Monday, January 14—Thursday, January 17

Institute of Physics and the Physical Society (in the Old and New Halls of the Royal Horticultural Society, Vincent Square, London, S.W.1)—Annual Exhibition.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer and a Lecturer in Applied Mathematics—The Registrar, The University, Nottingham (January 12).

Assistant Plant Pathologist (with an honours degree in botany and preferably postgraduate experience), for duties which will include general advisory work in plant pathology, and opportunities exist for research work—The Secretary, School of Agriculture, West Mains Road, Edinburgh (January 15).

Principal Lecturer in Statistics; and a Lecturer in Mathematics—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4 (January 15).

Lecturer (with training in mathematics and some experience in genetics, not necessarily in the human field) in Human Genetics in the Faculty of Medicine, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 18).

Analytical Chemist (Experimental Officer) (with a degree or H.N.C. or Diploma in Technology in Chemistry, with previous experience) in the Department of Mineralogy, for the analysis of minerals, rocks and meteorites, and research into analytical methods—The Secretary, British Museum (Natural History), Cromwell Road, London, S.W.7 (January 21).

Lecturer in the Department of Mechanical Engineering—The Registrar, The University, Liverpool, quoting Ref. CV/230 (January 26).

Lecturer (with experience in general virology or in allied fields such as biochemistry, microbiology or cytology) in Virology—The Secretary of University Court, The University, Glasgow (January 30).

Lecturer (with special interest in one or more of the following fields soils, conservation, geomorphology) in Geography—The Registrar, University College, Gower Street, London, W.C.1 (January 30)

Assistant Lecturers or Lecturers (3) (with good academic qualifications in pure or applied mathematics or in mathematical statistics) in the Mathematics Department—The Registrar, The University, Hull (January 31).

Senior Lecturer or Lecturer (with an honours or higher degree or other approved higher qualifications, and preferably with teaching experience) in Mathematics at the South Australian Institute of Technology, Adelaide—The Agent-General for South Australia, 50 Strand, London, W.C.2 (January 31).

Reader in Analytical Chemistry at Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 6).

Chair of Biochemistry at the Royal Free Hospital School of Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 7).

Lecturer or Senior Lecturer (with special qualifications in the teaching of science) in Education at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 8).

Reader in Physics—The Acting Deputy Registrar, University Office (University of Durham), 1A Kensington Terrace, Newcastle upon Tyne 2 (February 11).

Chair of Geography at Fourah Bay College (The University College of Sierra Leone)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 15).

Assistant Lecturer or Lecturer in Inorganic Chemistry—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1 (February 15).

Assistant (preferably with an interest in the biochemistry of micro-organisms) in Biochemistry—The Secretary of University Court, The University, Glasgow (February 15).

Chamber of Mines Chair of Mining Engineering in the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, February 15).

Lecturer (graduate of a British Commonwealth University or of equivalent status, preferably a Corporate Member of the Institution of Mechanical Engineers, and with teaching experience at university-level) in Mechanical Engineering at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, February 15).

Lecturer/Senior Lecturer in Biology in the Department of Botany, University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 15).

Lecturer (preferably with interests in the field of political philosophy) in Philosophy at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 24).

Chair of Philosophy—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (March 30).

LECTURER IN EXPERIMENTAL PHYSICS—The Secretary, University College, Cork, Republic of Ireland (March 30).

ASSISTANT or ASSOCIATE PROFESSOR (with a Ph.D. in pharmaceutical or medicinal chemistry) IN THE FACULTY OF PHARMACY, to teach undergraduate courses, conduct research and develop a graduate programme—The Dean, Faculty of Pharmacy, University of British Columbia, Vancouver, B.C., Canada.

DIRECTOR (with suitable academic qualifications, and industrial and research experience) OF STUDIES IN THE DEPARTMENT OF TEXTILE INDUSTRIES—The Registrar, Bradford Institute of Technology, Bradford 7.

EDITORIAL ASSISTANT (with physics and/or mathematics to degree standard, and an interest in scientific publication), for work which will include the preparation of mathematical and scientific material for publication—The Editor and Deputy Secretary, The Institute of Physics and The Physical Society, 1 Lowther Gardens, Prince Consort Road, London, S.W.7.

NEMATOLOGIST (male, with an honours degree in zoology or entomology, and at least one year's postgraduate training or experience, preferably in the study of nematodes) in the Ministry of Agriculture, Uganda, to undertake a survey of the occurrence of harmful nematodes on crops; to develop techniques for testing host reactions and for controlling nematodes of economic importance—The Director of Recruitment, Department of Technical Co-operation, Great Smith Street, London, S.W.1, quoting T/RC. 213/153/07.

ORGANIC CHEMIST (with a Ph.D. degree), for research in synthesis and reactions of organic sulphur compounds—The Professor of Chemistry, Box 1507, Substation B, Vanderbilt University, Nashville 5, Tennessee, U.S.A.

POSTDOCTORAL FELLOW IN INORGANIC CHEMISTRY, for work which will involve the preparation of inorganic polymers, and the investigation of their structure and degradation by various physical methods—The Professor of Chemistry, Westfield College (University of London), Hampstead, London, N.W.3.

POSTDOCTORAL RESEARCH FELLOWS IN THEORETICAL PHYSICS—L. H. H. Trainor, Physics Department, University of Alberta, Edmonton, Canada.

SOIL SURVEYOR (aged 23–45, with a good honours degree in chemistry, botany, geology or agriculture, and preferably some postgraduate experience of soil survey work) in the Ministry of Agriculture, Kenya, to carry out, as a member of a team, the surveying, classifying and mapping of soils for land use purposes—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. C1/RC.213/80/013.

TECHNICAL ASSISTANT (with a G.C.E. "A" level in chemistry and an "A" level in mathematics, physics or biology), for work on biochemical analyses—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Summer Place, London, S.W.7, quoting Ref. 301/B/120.

TECHNICIAN or JUNIOR TECHNICIAN IN THE DERMO-PATHOLOGY LABORATORY at St. George's Hospital, Tooting Grove—The Secretary, Pathology Department, St. George's Hospital Medical School, Hyde Park Corner, London, S.W.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

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Imperial College of Science and Technology (University of London). Code of Practice against Radiation. Fourth Edition. Pp. v+35. (London: Imperial College of Science and Technology, 1962.) [1411

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 61: The Effect of Soil Organic Matter on the Setting of Soil–Cement Mixtures. By P. T. Sherwood. Pp. iv+56+2 plates. 5s. net. Road Research Technical Paper No. 64: Investigations to Assess the Potentialities of Lime for Soil Stabilization in the United Kingdom. By Dr. M. J. Dumbleton. Pp. iv+76+2 plates. 5s. 6d. net. (London: H.M. Stationery Office, 1962.) [1411

The Internal Combustion Engine and Its Effect upon Society. By Lord Brabazon of Tara. (Sir David Russell Memorial Lecture delivered before the University of St. Andrews at Queen's College, Dundee, 3 May, 1962.) Pp. 22. (London: Oxford University Press, 1962.) 3s. 6d. net. [1411

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The Royal Institute of Chemistry. Lecture Series, 1962. No. 1: Nomograms for Chemists. By Dr. A. B. Calder and D. A. Calder. Pp. ii+38. 5s No. 2: Structure and Reactions in Phosphorus Chemistry. By N. L. Paddock. Pp. iii+52. 6s. (London: Royal Institute of Chemistry, 1962.) [1611

Department of Scientific and Industrial Research. Technical Services for Industry: a Guide to Technical Information and other Services offered to Industry by D.S.I.R. and the Grant-Aided Research Associations, 1962.) Pp. 104. (London: Department of Scientific and Industrial Research, 1962.) [1611

Discovery Reports. Vol. 32, pp. 33–464+plate 3: The Natural History and Geography of the Antarctic Krill (*Euphausia superba* Dana). By James Marr. 200s. net. Vol. 33, pp. 55–94+plate 1: Rhizocephala. By H. Boschma. 22s. 6d. net. (Cambridge: At the University Press, 1962.) [1611

Bulletin of the British Museum (Natural History), Zoology. Vol. 9, No. 2: A Review of the Indo-Pacific Gizzard Shad Genera *Nematalosa*, *Clupanodon* and *Konosirus* (Pisces: Dorosomatidae). By P. J. P. Whitehead. Pp. 87–102. 6s. Vol. 9, No. 3: The Pantanodontinae, Edentulous Toothcarps from East Africa. By P. J. P. Whitehead. Pp. 103–137. 12s. 6d. (London: British Museum (Natural History), 1962.) [1611

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A Rational Animal. By Prof. Gilbert Ryle. (Auguste Comte Memoria Lecture, No. 5.) Pp. 25. (London: The Athlone Press, University of London 1962.) 5s. net. [161

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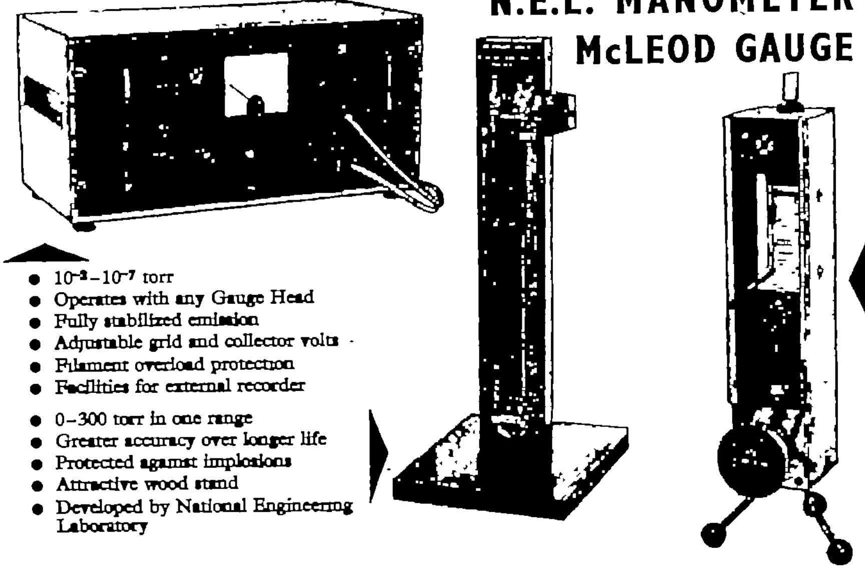

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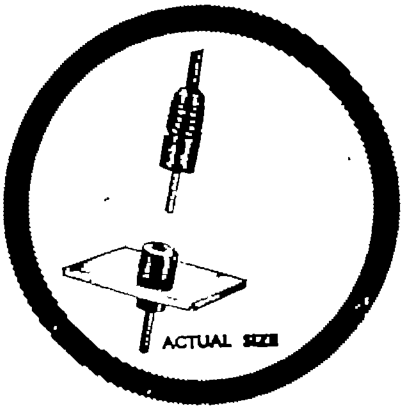

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Applications are invited for appointment to a position of Soil Microbiologist at the Division's Soil Fertility Group in the Cunningham Laboratory, St. Lucia, Brisbane.

The Soil Fertility Group in Brisbane contains workers from the Division of Soils and the Division of Tropical Pastures, and is studying basic aspects of nitrogen transformations in the soil/plant system, with reference to nitrogen balance data, N cycles in soil under pasture, and the effects of either field or fertilizer nitrogen on the distribution of this element in the various components of the system. Certain aspects of this project, which concern simultaneous mineralization-immobilization reactions in soils growing perennial grasses fertilized with nitrogen have been studied in preliminary experiments, but a soil microbiologist is required to amplify this aspect of the programme in some detail.

The group has good facilities for research, including a mass spectrometer for isotope assay, and ready access to equipment possessed by other groups in the Cunningham Laboratory is also possible; this equipment includes facilities for radioactive counting and an automatic amino-acid analyzer.

The appointee will be required to undertake research in transformation of nitrogen in tropical soils under pasture or crop and the decomposition of carbonaceous materials under these conditions.

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Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 270/254) should be addressed by February 2, 1963

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Applications and inquiries, now invited, should be addressed to the Head of the Department of Physics.

SCIENCE AND PRESSURE GROUP POLITICS

THE debate in the House of Lords on the introduction of commercial broadcasting, opened on May 9 by rd Reith, centred largely on the disclosure of the work a political pressure group for the introduction of com- ercial broadcasting given in a book, *Pressure Group*, by of. H. H. Wilson, of Princeton University, published June 1961. It was in view of the disclosures in this ok of methods alleged to be used to secure the intro- uction of commercial broadcasting ten years ago that ncern was expressed in the House of Lords as to attempts discredit in advance the report of the Pilkington Com- ittee and, in particular, to commit local broadcasting commercial use. It was not, of course, argued in the bate that pressure groups are not a normal part of our litical life, but there was some divergence of opinion as the scale and nature of their use, and some support as forthcoming for an inquiry. More especially was this pressed, as Lord Taylor put it, in respect of secret essure groups, which, without clearly identified mem- rs and objects, working underground and out of sight, e a menace to society.

The Lord Chancellor did not reply to Lord Taylor's ecific inquiry on this point, and while the debate con- asted Prof. Wilson's book unfavourably with Dr. B. aulu's *British Broadcasting in Transition*, it did little resolve the doubts which had been expressed or the nxiety with which the Pilkington Committee's report as then awaited. When on July 31 that report was st discussed in the House of Commons, the Postmaster- eneral, Mr. R. Bevins, emphasized with all the force he uld command that the Government had not been fluenced in any of these matters by any pressures or bbies, whatever their nature, nor would the Govern- ent be in taking future decisions. Mr. Bevins was eaking in support of a motion asking the House to take ote of the Government's White Paper on the report of e Committee on Broadcasting.

Nevertheless, there are aspects of this question of essure groups with which the scientist and other pro- ssional men and women are very much concerned. This as brought out by J. B. Sanderson as illustrated by the ork of the National Society for Clean Air, and more nerally is well indicated by Prof. J. D. B. Miller in his ok *The Nature of Politics* (see *Nature*, 196, 301; 1962). ithout using the term, Prof. Miller showed how institu- ons are concerned with the conflict of interests which overnments have to face, the way in which professional stitutions bring diversity and the limits within which eir influence is exerted. This is brought out even more early in an earlier book by Prof. H. Eckstein in hich, under the title *Pressure Group Politics**, he amines the way in which one important professional ody has functioned in the defence of its members as ell as of its conception of the public interest.

The British Medical Association may not have been e best example to choose for an examination of what is sentially a study of the function and locus of power nd influence in a democracy. That Association is scarcely typical professional organization. but the example serves well enough to illustrate both the character and the limits of the influence which professional bodies can exert on public policy. Moreover, Prof. Eckstein refers freely to the study of the professions by Sir Alexander Carr-Saunders and P. A. Wilson a generation ago, and much in this book endorses the soundness of their conclusions.

From the point of view of the professional man generally, the most significant chapters in the book are those which examine the framework within which pressure groups function in Britain's society and which discuss their significance in politics. Prof. Eckstein points out that the formation and activities of pressure groups are determined by the interaction of the structure, activities and attitude of Government itself, and that the growing extent of Government influence in the Welfare State has inevitably led to an increase in the number of active or potential groups. Government decisions have a direct interest on the activities and well-being of more and more groups, professional and otherwise, and this has led them, sometimes reluctantly, to interest themselves as such in politics. So far as scientists and technologists are concerned, it is the counterpart of the increasing extent to which scientific and technical considerations are of dominant importance in determining public policy.

It is thus unfortunate that the term 'pressure group' has acquired some sinister significance, for any abuse of the process does not alter the fact that such groups are an essential feature of the process by which, in the modern democratic State, those with expert and professional knowledge and skill are able to make their full contribution to public policy. We cannot wisely rely on that contribution being made solely through those engaged, for example, in the Scientific Civil Service, quite apart from the persistence of the view that the expert should be on 'top' and not on 'tap'. How a group exerts its influence is determined largely by the structure of the Government itself and the way in which decisions on policy are made. It is also determined by the organization of the group itself, and even more is this true of the effectiveness with which the group is able to influence Government decisions.

What emerges particularly from Prof. Eckstein's study is the desirability of professional organizations looking afresh at their own internal organization, not simply from the point of view of the defence of their own professional interests and the maintenance of professional standards of practice and competence, but more especially from the wider point of view of the appropriateness of that organization to serve the public interest by putting fully at the disposal of the State the collective knowledge and experience of its members. It is not to be forgotten, as well appears in this study of the British Medical Association, that professional experience and knowledge may also be made available to the Government in many informal ways. That, as for example the report of the Zuckerman Committee indicated, is in part a matter of appropriate staffing policy and sound administration. However, more critical and creative thought about the structure of professional bodies and the way in which they can make effective expert representations on matters of public policy is as desirable from the point of view of the public welfare as it is from that of the professional bodies themselves.

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* *Pressure Group Politics: The Case of the British Medical Association.* y Harry Eckstein. Pp. 168. (London: George Allen and Unwin, Ltd., 60.) 16s. net.

This could undoubtedly go far to eliminate any fear that such professional bodies may exert any unwholesome or undesirable influence on public affairs; it is all to the good that there should be free and widespread public discussion of this general question. Not all pressure groups are as responsible as professional associations, and while the existence of pressure groups is no new phenomenon and can easily be illustrated from the nineteenth century, there is undoubtedly growing awareness and concern over the activities of local and political pressure groups. With the widening influence of the Government over the economy, such activities are likely to increase. What is desirable is that the nature of those activities should be clearly understood and the point at which their legitimacy is open to question should be widely recognized. Awareness is, in fact, probably the best safeguard against the abuse of practices which are probably unavoidable, but are undesirable as such only when they are abused or become the accepted mean.

This is the more important because the methods now sometimes used are open to criticism. It will be noted that Prof. Eckstein, like Prof. J. B. Miller, comments that the point at which pressure is exerted is increasingly the administration. "In the age of big administration and crowded legislative time-tables," he writes, "interventions by voluntary organizations are also the chief control over the executive, far more thorough and efficient than question time and adjournment debates in Parliament." Only after failure does a pressure group try Parliament, although often enough it may set out to try to create a climate of opinion favourable to its purpose.

Clearly it is undesirable that Parliament should be by-passed or ignored, though the British Parliamentary organization is not well-adapted to the widening range of its responsibilities. There is, in fact, little direct representation in Parliament of interests which find typical expression in pressure groups, and if major professional and other interests were authoritatively represented at Westminster there might be less danger of abuse. Certainly there should be a more general public understanding of the contribution which professional bodies could bring to bear on particular public issues. As Prof. Eckstein has shown, pressure groups of the professional type can contribute usefully in the formulation and administration of specific policies. Moreover, they constantly define opinion for Government, and on a level on which political parties rarely touch.

There is thus substantial evidence that pressure groups have a definite contribution to make in a democratic system where political parties sometimes fail simultaneously to provide efficient decision-makers and represent accurately opinions. Representative organizations such as professional associations supplement the party system with alternative organizations which can influence decisions without affecting the position of the decision-makers—this, as Prof. Eckstein argues, is probably their pre-eminent function in an effective democratic system. It is worth further consideration, especially by professional bodies of scientists and technologists, how best that function can be discharged.

This is not, of course, the only way in which professional bodies can perform a social function. As Prof. Eckstein also shows in his book, the difficulties of the British Medical Association in regard to the National Health Service arise in part out of the transformation of the profession from one in which the majority of members were self-employed or in independent practice to one in which they are mainly employed by the State. Mo classes of scientists and technologists have indeed alway been salaried employees, and this has from time to tim led to difficulties over professional responsibilities an standards and to the formation of bodies registered a 'Trade Unions' for the defence of their economic interest as, for example, the Association of Scientific Workers an the British Association of Chemists. By and large, thos difficulties, however, do not appear to have been considerable and, so far as public policy is concerned, it seem to have been immaterial whether representations ar made by these latter bodies or by the chartered institutions and qualifying bodies. Each seems to have it specific place and function, though, so far as industry i concerned, the bodies with economic responsibilities unde the Trade Unions Acts may be in the stronger position.

Nevertheless, this aspect of professional organization i nothing like as important—at least from the public poin of view—as Prof. W. Kornhauser suggests in a recen book†. In both this book and in a subsequent article "Strains and Accommodations in Industrial Researc Organizations in the United States", published in th autumn 1962 issue of *Minerva*, Prof. Kornhauser seems t exaggerate the stresses and clashes between professiona interests and standards and industrial objectives an purposes—in any event so far as British industry is concerned. Molehills sometimes become mountains, but, o the other hand, the real difficulties and problems of th structure and place of professional organizations in th technological society in which we live are virtuall ignored.

Both Prof. Kornhauser's book and his article are essentially on the management and control of research and perhaps because he seems to be almost obsessed with th idea that research is the only possible or legitimat occupation for a scientist, Prof. Kornhauser's book even o that theme is scarcely as illuminating as the Zuckerma report. Besides his narrow conception of the vocation o a scientist he shows no clear conception of the limits o professional competence. The admission that th diffusion of scientists and technologists throughout a enterprise with an appropriate organizational structure may diminish what he regards as the inevitabl tension between the primary functions of a professiona organization and the purposes of an industrial enterpris is as near as he gets to any recognition of the need fo a wide understanding in society as a whole and in it industrial units of the nature of scientific activity.

It will thus be seen that Prof. Kornhauser's book i disappointing—this is the more unfortunate in that th respective functions of the learned and scientific society and of the professional association have acquired a new significance and importance to-day for reasons which Prof Miller and Prof. Eckstein have made plain. That th influence of the professional body, on Prof. Kornhauser' evidence, is declining in the United States, and that in Britain the scientific society finds great difficulty in recruiting a reasonable membership from scientists and technologists in industry are beside the point. Both the scientific society and the professional institution have an essential place and an important function to perform among the various means by which expert knowledge is placed at the service of the community and given its full weight in executive decisions on public policy. It is not a healthy sign if the members of a particular profession are reluctant to support their professional institutions which are responsible for the maintenance of professional standards of acceptance or the diffusion of knowledge of advances in that field of knowledge. Neither is it healthy if these institutions are unable, for any reason, to place effectively before the appropriate authority at any level up to the Cabinet itself, as may be necessary, the considered views of their members on questions of public policy in which they have special knowledge. There is more evidence than is discussed by either Prof. Eckstein or Prof. Kornhauser that the time is ripe for a critical review of the structure and functioning of professional institutions in general and of the ways in which they can to-day most effectively and appropriately put their special knowledge and skill at the disposal of the community. Few aspects of this wide problem of power and influence are more important to-day, and once again the question of effective communication is at the root of the whole problem.

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† *Scientists in Industry: Conflict and Accommodation*. By Prof. William Kornhauser, with the assistance of Warren O. Hagstrom. Pp. xii + 230 (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1962.) 48s.

MOLECULAR BIOLOGY AND CANCER RESEARCH

The Molecular Basis of Neoplasia

A Collection of papers presented at the Fifteenth Annual Symposium on Fundamental Cancer Research, 1961. Pp. ix+614. (Austin: University of Texas Press, 1962. Published for the University of Texas M. D. Anderson Hospital and Tumor Institute.) 10.50 dollars.

THIS remarkable book illustrates the striking way in which molecular biology has recently advanced our understanding of fundamental biological processes. That it is a report of the fifteenth annual symposium on "Fundamental Cancer Research" held at the University of Texas shows the importance of understanding biology at the molecular level for gaining insight into the phenomenon of cancer. The volume consists of thirty-four detailed lectures, all beginning with an extensive general introduction which states the problem and is invaluable to the reader who works in a different field. Most articles are by biochemists or biophysicists: but towards the end of the book materials and methods become more complex, and it is often the biologist, particularly the geneticist, who interprets the findings of the biochemist.

The first lectures deal with the building blocks of nucleoproteins and enzymes, starting with the nucleic acids. The enzymatic synthesis of artificial nucleic acids, their denaturation and the restoration of the double helix helps in the understanding of the properties of natural nucleic acids. This is particularly important in view of the fact that an analytical method for determining nucleotide sequences in natural nucleic acids is still missing. In the next group of lectures, which deal with nucleic acid and proteins, we read how analysis in chemical terms of the action of alkylating agents and some other mutagens has become possible. Furthermore, man can cheat growing cells by feeding them halogenated, instead of normal, purine and pyrimidine bases. The increased sensitivity to radiation of cells grown under these conditions finds a chemical explanation, and may one day find clinical application. Lectures on proteins in viruses and in cell nuclei, on the functional role of these proteins and their possible importance in cancer, precede chapters on conformation and on substructure of certain well-analysed protein molecules.

The second part of the book deals with more complex matters, namely, with the control of synthetic processes. The past years have brought fascinating discoveries of homoeostatic mechanisms at the enzyme level able to function in cell-free systems, inhibition being exerted either on the action or, in other instances, on the synthesis of the enzyme. Here again, biochemical progress owes much to the geneticist's techniques, the suppressor genes for example giving an explanation for enzyme induction through loss of a normal suppressing mechanism. The idea that some cancer cells may have lost a suppressor gene is suggestive indeed. Feed-back control of enzyme concentrations, first discovered in micro-organisms, has now also been found in vertebrates. The last lectures deal with the ribosomes, the vital organs for protein synthesis both in cytoplasm and cell nuclei. The precise way in which they obtain and transmit their information is still obscure, and represents one of the most debated questions of the present time.

The book is well produced, and should be of value not only to the specialist but also to anybody interested in recent advances in fundamental biology. MARTHE VOGT

BIOCHEMICAL LABORATORY TECHNIQUES

Methods of Biochemical Analysis

Vol. 9. Edited by Prof. David Glick. Pp. ix+452. (New York and London: Interscience Publishers, a division of John Wiley and Sons, Inc., 1962.) 109s.

A Laboratory Manual of Analytical Methods of Protein Chemistry (including Polypeptides)

Edited by P. Alexander and R. J. Block. Vol. 3: Determination of the Size and Shape of Protein Molecules. Pp. x+286. (London and New York: Pergamon Press, 1961.) 50s. net.

METHODS of Biochemical Analysis (Volume 9) offers the customary diverse collection of topics. Only the first two chapters on deoxyribo- and ribo-nucleases are related, and these deal with the various methods available for the assay of these enzymes. A discussion of plant hormones is mainly devoted to the extraction, purification and assay of auxins, but other plant-growth regulators, such as the gibberellins and ascorbigen, receive mention. An account of the analysis of adrenal steroids in blood by countercurrent distribution is, in my own opinion, tedious to read and overburdened with detail. In a concise survey of techniques for column electrophoresis in granular media, the authors critically consider the relative merits of various inert supports. The description of the spectrophotometry of opaque biological materials by reflexion methods is complementary to that for translucent materials in Volume 7. The remaining half of the book is devoted to magnetic resonance spectroscopy. A theoretical treatment of magnetic resonance and spectral characteristics is followed by sections on experimental techniques and biochemical applications of nuclear magnetic resonance and electron-spin resonance spectroscopy. Many biochemists will find this difficult terrain, but the successful application of magnetic resonance spectroscopy to the study of the catalytic mechanisms of several enzymes should be an incentive to the faint-hearted.

The final volume of *A Laboratory Manual of Analytical Methods of Protein Chemistry* is devoted to techniques for determination of molecular size and shape. As in the earlier volumes, each chapter is written by one or two experts. The volume opens with an account of methods of preparation of specimens for electron microscopy and the use of techniques such as shadowing, staining, and replication. The chapter on osmometry is a salutary reminder of what can be achieved with a combination of experimental skill and simple home-made equipment. One feels that the inclusion of a description of the electronic osmometer is an apologetic afterthought. In complete contrast, there follow reviews on the diffusion and ultracentrifugation of proteins with their heavy reliance on commercial instruments. The principles of the methods, however, rather than detailed descriptions of equipment, are emphasized. An account of the study of non-Newtonian liquids in capillary viscometers pays welcome attention to experimental difficulties and illustrates by numerical examples possible sources of errors.

Research on unimolecular protein films has too few exponents; perhaps the present discussion of techniques and their application to the determination of molecular weights and diffusion coefficients on the microscale will stimulate more interest in this field. The book ends with a description of the measurement of light-scattering by proteins; it emphasizes the particular suitability of this technique for determining the molecular weight and asymmetry of large proteins and also for studying the kinetics of processes such as aggregation and denaturation. Like its two forerunners, this volume contains a wealth of illustrations and tables. Bearing this and the number of contributors in mind, the price is very reasonable. It is a pity that differential ultra-violet spectrophotometry has not been mentioned, but perhaps its value has only been realized since this series was planned.

D. T. Elmore

CHROMOSOME NUMBERS AND TAXONOMY

Chromosome Numbers of Central and Northwest European Plant Species

By Áskell Lövé and Doris Löve. ("Opera Botanica" a Societate Botanica Lundensi, Vol. 5.) Pp. 581. (Stockholm: Almqvist and Wiksell, 1961.) 40 kronor.

THIS latest manual is intended as a reference work to the chromosome numbers so far reported for plant species and subspecies known to occur in central and north-west Europe as circumscribed by the authors. It lists 5,490 species and 1,360 subspecies of Pteridophytes, Gymnosperms and Angiosperms, of which chromosome counts are given for 4,417, that is, 80·7 per cent. Diploid and basic numbers are given: the basic numbers are calculated from the lowest diploid number known in the group in question, or are theoretical when only apparently polyploid numbers have so far been reported. An appendix lists new counts which have not been previously published, together with an indication of the source of the material and the name and institutional address of the worker concerned.

On the critical question of the taxonomic identification of the plants, the numbers of which are listed in the main text, the authors point out that most chromosome numbers are determined either by "cytologists lacking taxonomic training or by taxonomists lacking cytological skill and experience". They have not found it feasible to distinguish between those counts based on cultivated material and those based on spontaneous material.

This compilation must surely be the last of a distinguished series of chromosome lists to reproduce all numbers and counts reported, irrespective of the origin of the material or checks on its identity. What is now needed is much more selective chromosome information—for example, lists of accredited numbers for more restricted regions, discarding earlier or doubtful records and including only counts determined from spontaneous material growing within the region in question.

At the moment most chromosome number compilations, including the present one, serve more as a systematic guide to the relevant literature than as a basis for taxonomic, phytogeographical and similar conclusions. It cannot be stressed too often that reported numbers for species have to be sifted through and verified in perhaps the majority of cases before they can be accepted. If even the most elementary conditions are laid down before numbers are accepted for inclusion in, say, a Flora, it is surprising how few numbers in the literature are found to satisfy these conditions.

Certainly the Löves have performed a signal service in making the source references available to us (the bibliography runs to nearly 200 pages) and this book should find a place on all taxonomists' shelves. The introduction of some 15 pages, in addition to outlining the scope of the work and giving the limits of the area covered and statistical data, includes an essay on the systematic arrangement adopted and a section somewhat ambiguously entitled "Limitation of Categories". The point of view of the authors is summarized by their acceptance of Mayr's definition of species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". As readers familiar with the Löves' work will expect, they adhere to the view that, with certain exceptions, all taxa differing in chromosome number are logically regarded as distinct species. Consequently, they do not recognize infraspecific polyploidy (apart from sporadic variants), an attitude that receives diminishing support to-day.

These views affect, of course, the taxonomic treatment adopted in the text. This would be of little moment but for the fact that they have found it necessary to make a number of nomenclatural changes (the details published in *Botaniska Notiser*, 114: 33, 48; 1961), some concerning the status of the groups concerned, others consequent on the adoption of narrow generic limits, especially in cases where cytological evidence apparently lends support. Thus *Lycopodium* is split into *Lycopodium 'sensu stricto'*, *Diphasium*, *Lepidotis* and *Huperzia;* and *Euphorbia* into *Tithymalus*, *Agaloma*, *Poinsettia* and *Chamaesyce*.

The volume is well produced, as we have come to expect from *Opera Botanica*. There are several misprints and minor errors. The foregoing criticisms apart, the Löves are to be congratulated on their immense industry and patience in producing this work. It must have been indeed a labour of love.

V. H. Heywood

BIOLOGY AND HUMANITY

Biology and the Nature of Man

Based upon the Riddell Memorial Lectures Thirty-third Series delivered at King's College in the University of Durham on 7, 8 and 9 March, 1961. By Dr. W. H. Thorpe. Pp. xii + 122. (London, New York and Toronto: Oxford University Press, 1962.) 12*s*. 6*d*.

THIS collection of lectures by Dr. W. H. Thorpe, the thirty-third series of the Riddell Memorial Lectures, is in the manner and on the scale of the fourteenth series, namely, those delivered by the late Sir Edmund Whittaker. Biologist and mathematician respectively, they discuss the fundamentals of scientific theory and of belief: as a result they confirm the Christian religion in its sacramental aspect as paramount for the Universe and for experience. It is significant that the latter position at least is also reached on archæological grounds in a recent book by Prof. E. O. James. Taken all in all, this is a remarkable degree of agreement between a trio of scholars in such widely different fields of research.

In the chapters now before us, Dr. Thorpe discusses (1) the origin of man, (2) the mind of man and its origin, (3) the origin of man's moral nature, (4) the distinctiveness of man's moral nature, (5) the destiny of man. The terms of these lectures request that they should be given "with particular emphasis on and reference to the bearing of such developments on the ethics and tenets of Christianity". This wish has indeed been faithfully fulfilled. As a result, a distinguished natural scientist finds himself dealing with matters essentially theological and philosophical. As he truly says, it is because life and destiny are the concern of us all that this incursion must be undertaken. It might be added, not only that, but also trespass—if trespass it be—should be greeted as a major contribution to the whole edifice of truth. In classical times this would not have appeared strange: it is a comparatively modern misfortune that philosophy and theology have become 'departments' of knowledge rather than something to live by, and deriving much of their inspiration from every discipline which is "honest and of good report".

Dr. Thorpe's approach leads him to meditate on both metaphysics and mysticism. Concerning the former, there will always be axiological factors in biological systems (as even in quantum physics), associated with vestiges of teleology. In this context, one would expect perhaps some reference to the late Dr. E. S. Russell's 'directiveness': and so far as mysticism is concerned, this has often been the goal of the deepest philosophical contemplation, a curve of awareness tending to the inexpressible as an asymptote. Maybe it is difficult for some minds to admit this; to others it seems obvious and even welcome.

On page 88 is a reference to form, and the possibility of its analysis "into energy *plus* organization" (my italics). Is this quite right? I have investigated an analogous problem myself (1939, 1946) with energy as a *function* of organization. Then the integral is of the nature of a potential, which is suggestive.

We can but thank Dr. Thorpe for a set of essays (as they are now) of such absorbing interest. They contain an element of Spinoza's statement of 1677, which may be translated thus—"The effort by which each thing tries to persist in his own being is nothing but the essence of the thing itself". F. I. G. RAWLINS

HUMAN GROWTH

Growth at Adolescence

By Dr. J. M. Tanner. Second edition. Pp. xiii + 325 + 11 plates. (Oxford: Blackwell Scientific Publications, 1962.) 47*s*. 6*d*. net.

THIS is the second edition of a well-received book on human growth and development which appeared first in 1955. It now comes in an improved and extended form, and Dr. Tanner promises that in the next edition he will cover all aspects of the growth and development of children. Already the title of the book inadequately describes its contents.

There is no other text in Great Britain which gives the same comprehensive survey of human growth—its measurement, its velocity, the factors affecting it and the total aspects of physiological maturation. Dr. Tanner, with the help of his assistant, Mr. R. H. Whitehouse, has himself provided much of the data regarding the velocity of growth in Britain. The Harpenden Growth Society which he initiated, with the help of the Ministry of Health, is well advanced in the longitudinal study of children from birth to puberty and beyond.

He discusses the merits and disadvantages of cross-sectional and longitudinal studies of growth. Cross-sectional measurements can provide information on the distance curve of growth and the mean age of a certain stage of maturation, such as the menarche, but for velocity curves, and particularly for the facts concerning the variation of velocity, longitudinal studies are necessary. Doctors and others concerned with the development of individual children employ a useful convention in assessment which the author discusses fully—it refers to a child's skeletal age, dental age, height age, weight age, and so on. This method requires the provision of accurate standards for development in both sexes and at any age, and for all parameters of maturation these are increasingly available. Clinicians seek to use these conventions both for immediate assessment and for prediction. There are many difficulties in so doing—of which one simple example is provided by the short child. Is the child's linear growth retarded by some ascertainable environmental factor, by the inheritance of short stature, or are both influences at work? There is in the book an interesting example of the necessity and value of prediction. The School of the Royal Ballet admits its pupils at the age of nine years and, having strict limits for the mature height of its ballerinas, now excludes those girls whose chance of ending between the limits is under 5 per cent.

The effects of disease and nutrition, good and bad, on development is a complicated and fascinating subject to which the author gives much attention. For example, can the advancing secular trend in height and the earlier age of sexual development be entirely related to improved nutrition and the reduction of disease? Other additional possibilities are discussed—in environment the rising world temperature and in inheritance that some degree of dominance exists in the genes governing stature and that heterosis is proceeding more rapidly (outbreeding has, in fact, increased at a fairly steady rate ever since the introduction of the bicycle). He is interested, as indeed we all must be, in the implications for our social and educational organization of the physiological fact of the steadily advancing age of sexual maturity, a process which is still continuing.

The author promises us an extension in the content of his next edition. While this will be welcome, he should consider exclusion as well as further inclusion and re-arrangement. It is obviously impossible for him to continue to include psychological and behavioural maturation and he might well decide to limit his book to physical and intellectual growth and development, excluding also the chapter on the physiological changes of adolescence. Excellent and indispensable though this edition is, it bears the marks of a book in transition. It contains an enormous collection of observations which have not always been assessed and digested, and the book would be much improved by a judicial summary of the evidence at the end of each chapter. Consideration, too, should be given to the bibliography, which is valuable but excessive, occupying one-sixth of the book and totalling many more than a thousand references. I myself failed to understand the relevance of the quotation from Edward Lear on the fly leaf, but perhaps the author intended to launch his book on the "sea in a Sieve", hoping that it would return to him with the small amount of dross separated from the preponderant good.

DOUGLAS HUBBLE

HORMONE RESEARCH

Recent Progress in Hormone Research

Vol. 17: Proceedings of the 1960 Laurentian Hormone Conference. Edited by Gregory Pincus. Pp. viii + 600. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 100*s*.

THIS volume contains fifteen papers presented at the Laurentian Hormone Conference during September 4–9, 1960. The aim of the Committee which arranges these conferences is to present a contemporary view of developments in selected topics of experimental endocrinology. Four main themes were covered at the conference, namely, testicular function, hormones and reproduction, aldosterone and electrolyte metabolism, and finally hormones and organic metabolism.

The first section contains two papers, one on endocrinological factors which influence the histology and function of non-neoplastic interstitial cells in the mouse. The other paper in this section deals with fascinating observations on genetic aspects in male hypogonadism.

The subject of hormones and reproduction is dealt with in five papers, of which the first is a nutritional-endocrinological study on the effect of dietary erucic acid on testicular function in the rat. The other four papers in this section are concerned with the endocrinology of female domestic mammals, oestrogen metabolism in the human fœtus and new-born, the transplacental passage of steroid hormones and a paper on the metabolic effects of progesterone in man.

The largest single portion of the volume is devoted to six papers on hormones and electrolytes. The subjects covered include: mechanisms regulating the control of aldosterone, mechanisms of oedema formation, primary aldosteronism, the physiology of the posterior pituitary and its effects on permeability of membranes, etc.

The volume concludes with two papers on the regulation of glucose uptake by rat heart muscle and the effect of insulin and other hormones on the isolated perfused rat liver.

This volume maintains the high scientific standard set by the sixteen previous issues and contains a series of informative chapters by experts in diverse areas of hormone research. Like its predecessors this volume contains an "edited discussion" after each communication. Conferences require discussion, but the value to the reader of the inclusion of discussion in the published text might be reviewed by the organizers or the editorial axe could be used advantageously here and there.

The book is well produced and illustrated and this series is now an obligatory annual reference book for all endocrinologists; in addition, this volume will appeal to many other biologists. G. S. BOYD

PAPER SCIENCE

Formation and Structure of Paper

Transactions of the Symposium held at Oxford, September, 1961. Edited by Francis Bolam. Vol. 1: pp. x + 535 + xliv + 34 plates. Vol. 2: pp. viii + 536–910 + xliv. (London: Technical Section of the British Paper and Board Makers' Association (Inc.), 1962.) £10 10s.

FOR the first twenty-five years after its foundation in 1920 the Technical Section of the British Paper and Board Makers' Association depended, for its publications, on the research efforts of very few men. For a long time there was only one qualified worker in the whole British paper industry and many technical papers were written by busy routine workers in their spare time. Some indeed were officially discouraged from attending meetings of the Technical Section in case they 'gave anything away'. By the end of the First World War the climate of opinion was changing. The British Paper and Board Industry Research Association was established at Kenley and several of the larger companies set up research laboratories.

An international conference on paper technology was successfully convened in Paris in 1955 by the French organization 'L'ATIPE' and increasing numbers of British technologists were able to attend similar meetings in the United States. The fundamental research committee of the Technical Section arranged a successful symposium in Cambridge in 1957 and its proceedings were later published as *Fundamentals of Papermaking Fibres*, edited by F. Bolam, Technical Section, 1958. At the conclusion of that conference it was decided that another symposium on *Formation and Structure* should be held in Oxford in 1961 and the two volumes under review are the transactions of this meeting.

At each symposium the contributors came from the United States, Canada, Australia and several European countries as well as from Great Britain. The Cambridge symposium was to a considerable extent a review of existing knowledge and is very useful to teachers of paper science.

The Oxford symposium, with its subject announced four years ahead, attracted a much larger proportion of new work than the Cambridge meeting and is, probably, of more interest to research workers. In summing up the conference at its conclusion, Prof. Rånby (Stockholm) said, "What has been presented here has, indeed, advanced our knowledge of the structure and basic properties of paper. In particular, I congratulate our British colleagues, who have contributed so beautifully in this field of research, which we previously did not think of as a speciality for this country". The early members of the Technical Section who struggled with what was known, most incorrectly, as "Paper Chemistry" with desperately little equipment and negligible library facilities might feel indeed that "the wheel is come full circle".

Paper is a porous assemblage of fibres, and it is difficult to describe its properties in precise scientific terms. Even its thickness is indeterminate; the value measured depends so much on the pressure used in the micrometer that it is best to avoid quantities like stress which necessitate the use of thickness in calculation. There are numerous empirical physical and chemical tests well used in commercial specifications.

The papers given at the Oxford symposium are a noteworthy attempt to view paper in more fundamental terms than usual. Corte and Kallmes have shown that many properties can be explained by the statistical geometry of random straight lines. For studying the permeation of fluids a paper may be considered to be built up from very thin networks which are themselves composed of random polygons built from these straight lines. There are many excellent photomicrographs of the surface structure of paper from Kenley, electron micrographs of different parts of fibres from Darmstadt and photographs of paper made with the scanning electron microscope in Montreal. Page and Tydeman (Kenley) develop a theory to explain the anisotropic tensile and hygro-expansive properties of paper in terms of structure and show in particular that the compressive forces which occur in drying can cause fibres to contract longitudinally and frequently to exhibit micropuckering. This is confirmed by electron micrographs from Montreal introduced into the proceedings after the conference.

The early Chinese paper-makers discovered that paper could be made from vegetable fibres by suspending them in water and drying them without any other adhesive, but the reasons for the adhesion are still debated. The current hydrogen bonding theory and visco-elastic properties related to it are developed by Nissan. Bonds between fibres can be seen to break (Page) and heard to break (Corte) while the stresses within fibre joints can be treated and clarified mathematically (Van den Akker). There still seems to be some discrepancy between the views of paper scientists and colloid chemists as regards the distance over which bonding forces can act.

A simple but ingenious method for breaking up paper and analysing the clay-fibre ratios at different levels through its thickness is described by Groen. Investigations on uniformity, flocculation and wet end variables are described by Robertson and Mason and by Wrist. Only a few of the many interesting papers can be mentioned here; in the two volumes there is plenty of mathematics and physics, some morphology and scarcely any chemistry. Rånby's summary and Rance's introduction are two of the most valuable chapters and should be read first.

The issue of the main papers as preprints before the conference gave rise to lengthy discussions which are reported in full. A number of contributors succeeded in introducing sizeable papers at this stage. This, and the lack of brevity of some authors, mentioned in Rånby's summary may account, in part, for the high cost of the book. Some of this must, however, be due to the numerous micrographs which are exceptionally well reproduced.

It is difficult to see how more information on structure could have been collected in the present state of knowledge even though some might, perhaps, have been left out. It is expected that the book will sell well all over the world to those interested in advancing the subject of paper science.

F. LYTH HUDSON

Neutron Physics

Proceedings of the Symposium held at Rensselaer Polytechnic Institute, May 5–6, 1961. Edited by M. L. Yeater. (Nuclear Science and Technology, a Series of Monographs and Textbooks. Vol. 2.) Pp. xiii + 303. (New York and London: Academic Press, Inc., 1962.) 86s.

THIS volume reports the Proceedings of the Symposium held at the Rensselaer Polytechnic Institute in May, 1961, to mark the opening of the Neutron Physics Laboratory and its high-intensity linear electron accelerator. The papers presented cover all areas of neutron physics work, including nuclear physics measurements on fissile and non-fissile nuclei, solid and liquid state research and neutron thermalization problems. In addition, there is one paper on the fusion problem. Unfortunately, with such a wide range to cover, some limitations are inevitable. In effect this book presents a review of the resonance region neutron cross-section field and the thermalization field, while the other subjects are only covered in part. But, as a review of the cross-section field, it is marred by the very fact that it is a compilation of papers which present the results obtained in individual laboratories with individual machines, rather than broad surveys. Thus, while this book is quite useful in giving a picture of these fields to a 'new-comer', it is unable to compete with the text-books on neutron physics which are being published by the same publisher.

The style is informal in that the papers were prepared from tape recordings taken at the meeting, and the articles read as though they were being spoken rather than prepared in the manner of a text-book. This fact, coupled with the good layout of the book, makes it very easy to read. On the whole this is an interesting and useful book but one likely to have a rather short life.

P. A. Egelstaff

Brompton Hospital Reports

Vol. 30, 1961. (A Collection of Papers by Members of the Staffs of the Hospitals for Diseases of the Chest (Brompton Hospital; London Chest Hospital); and of the Institute of Diseases of the Chest.) Pp. viii + 287. (London: Lloyd-Luke (Medical Books), Ltd., 1962.) 15s. net.

FOR many years papers published by the staffs of the Brompton Hospital, London Chest Hospital and the Institute of Diseases of the Chest have been brought together in a composite volume which is issued annually. The thirtieth of these is notable for an article by Sir Russell Brock on the surgical treatment of pulmonary stenosis and a series of articles dealing with various aspects of sarcoidonis. There is also a description of a quantitative method of studying tumour cells in blood by Drs. S. W. A. Kuper, J. R. Bignall and E. D. Luckcock as well as an assessment of antitussive drugs in man by Dr. F. J. Prime. Dr. Lynne Reid examines the relationship between chronic bronchitis and the hypersecretion of mucus, while Dr. J. H. Ross reviews the surgery of the thoracic duct. In the main the articles are written in 'popular' style and are well supported by diagrams and photographs.

T. H. Hawkins

The Exploration of Outer Space

By Sir Bernard Lovell. Pp. ix + 87. (London: Oxford University Press, 1962.) 16s.

THIS is an introduction for the layman to some of the recent developments in astronomy, particularly those involving radio methods of investigation. Sir Bernard's skill in popular exposition is well known, and this book bears out his reputation.

It is based on the Gregynog Lectures, given in Aberystwyth in October 1961, and starts with a chapter on techniques followed by further chapters on the solar system, the structure of galaxies and the origin and evolution of the universe. With all this in 87 octavo pages there is obviously no room for details but most of the problems at present engaging attention in the field find at least a mention.

The final chapter is on extra-terrestrial life and the possibility of detecting it. The author gives a strong warning about the danger that the U.S.S.R. or the United States might inadvertently contaminate other planets with organisms from the Earth and perhaps destroy our chance of studying their own life-forms, if any. This warning comes over with particular force when it is remembered that Sir Bernard's criticisms of high-altitude nuclear explosions were 'pooh-poohed' by 'responsible' scientists before the event of July 9, 1962, but are now seen to have been largely justified. The last chapter also describes the recent attempts to receive 21-cm radio waves from intelligent beings on nearby stars. It is ironical that these attempts have already been overtaken by scientific progress, for coherent light from lasers is now considered to be a more effective method of communication over interstellar distances and who can say what will be the best technique available in even ten, let alone a hundred or a thousand, years' time?

J. R. Shakeshaft

The Annual of the British School at Athens, No. 56, 1961

Pp. x + 228 + 31 plates. (London: The British School at Athens, 1962.) 105s. net.

THE 1961 *Annual* maintains the British School's high standard of archæological publication. The weight of this volume is given to two subjects, topography and epigraphy; but numismatics, excavation publication and early Athenian history are also treated and there is an article about ancient Greek kilns and their "double stoking tunnel".

D. M. Metcalf discusses the Byzantine scyphate (saucer-shaped) bronze coins in Greece during the twelfth and early thirteenth centuries. From a study of hoards and site-finds he assigns certain types to areas and suggests possible mints. M. F. S. Hood and J. Boardman publish three early Iron Age tombs at Knossos, Crete; mainland Greece is represented by the publication of evidence for Mycenæan occupation near the Cyclopean Terrace Building at Mycenæ and by two pieces of a chariot larnax from the same site. Prof. Hopper's article on the early history of Athens destroys the thesis that there was a connexion between the three 'regions' of ancient Athens (Plain, Shore, Hill) and political parties later imagined to have been based on them. The author attempts to reconstruct Athenian history in the seventh and sixth centuries B.C. and sees political parties as fluctuating groups supporting particular individuals who were mostly concerned with advancing their own political interests. Thus he expresses a view of ancient Athenian politics coming increasingly to the fore, displacing the accepted idea (based on modern and political theory) of 'conservatives', 'moderates' 'radicals'.

The emphasis on topographical research is a marked feature of Greek archæology to-day. The famous sites are for the most part dug and published and so the topographers have taken the field and are doing valuable work in establishing the relations of small outlying sites to main centres, in working out the chronological patterns of settlement in specific areas and in identifying sites for which literary evidence exists. In this volume Helen Waterhouse and R. Hope Simpson present (at perhaps excessive length) the second part of their exploration of prehistoric Laconia, J. M. Cook argues for identifying four small classical cities near ancient Miletus in Turkey, and Garnett and Boardman publish the results of underwater reconnaissance off the island of Chios in 1954.

For epigraphy, A. Schachter publishes ten small inscriptions from Boeotia and Ursula Duncan writes on lettering by Attic masons. T. B. Mitford gives the formal publication of the 113 Hellenistic inscriptions from Old Paphos in Cyprus. Twenty-one of these are new, and two are important.

Peter Warren

SHAPE DISCRIMINATION AND RECEPTIVE FIELDS

By DR. N. S. SUTHERLAND
Institute of Experimental Psychology, University of Oxford

THE problem of how patterned stimuli are analysed by the nervous system is important but until recent years has been neglected. It is important since unless we know how incoming stimuli are coded, we cannot say anything very helpful about the further processing mechanisms in the nervous system which lead to the overt response. It has been neglected since until recently the tools were not available to attack the problem. Three developments in three different disciplines have brought promise of a solution. Psychologists can apply the 'method of stimulus equivalence' pioneered in the 'thirties by Klüver and by Lashley, and thus can attempt to discover the categories into which incoming information is sorted. Physiologists are able to throw direct light on the coding of incoming information by the newly developed technique of microelectrode recording. Recent developments in electrical engineering suggest that complex recognition tasks can be performed by systems simple enough to be understood and even built. In addition to the contribution made by developments in the foregoing three subjects, important evidence is, of course, also provided by neuro-anatomists using longer established techniques. Developments in other disciplines have made it easier to give functional interpretations of patterns of connectivity in the nervous system, and the direct study of such patterns by anatomical means in turn assumes a new importance.

The work with which this article deals is relevant to one aspect of the general problem, namely, how animals recognize visually presented shapes.

In 1957, I put forward a theory about shape recognition in the octopus[1]. Predictions made from this model were afterwards tested by behavioural experiments and the status of the model was reviewed in *Nature* in 1960[2]: although the theory was not without its critics, it was able to handle most of the evidence then available. More recently collected results suggest that some details of the theory require modification. Deutsch[3] and Dodwell[4] have also proposed theories to account for shape discrimination in the octopus; despite their ingenuity, both theories leave some evidence unaccounted for[5].

The aims of the present article are: (1) to summarize some new results on shape recognition in the octopus and other species; (2) to show where my theory fails to explain these results; (3) to suggest a modification of the theory which both fits with recent physiological and neuro-anatomical findings and helps to explain the recent behavioural data. Before discussing these topics, a brief description of the problem and the methods used will be given, and the original theory stated.

Problem and Method

The problem is to discover what are the physical properties or dimensions in terms of which animals classify shapes and to infer the type of analysing mechanisms at work in the brain. Although it is clearly impossible to specify brain mechanisms with certainty on the basis of behavioural evidence, the attempt is worth making because such models have heuristic value in the collection of fresh behavioural and physiological data. Moreover, if the dimensions of analysis are complex it may be very difficult to specify them without having some model in mind.

Two behavioural methods have been used: (1) animals are trained to give different responses to the members of various pairs of shapes in order to establish which pairs are readily discriminated, which are difficult or impossible to discriminate. (2) After training, animals are given 'transfer tests' with other shapes in order to establish to what extent these new shapes are treated as similar to one or other of the originals. The two methods are complementary.

A simple example will demonstrate the sort of inference it is possible to make by the use of these methods. If the primary property of shapes analysed were the number of sides, then a square and a triangle should be easier to discriminate than a square and a diamond (method 1). Furthermore, after training on a square and a triangle, animals should give the same response to a diamond as that which they have learned to make to a square (method 2)—in practice, octopuses treat a diamond like a triangle not like a square, and this suggests that the number of sides (or angles) present in a shape is not one of the primary properties under analysis.

An attempt has been made to collect data from species widely separated phylogenetically (octopus, rat and goldfish) in the hope of uncovering differences in the way different species classify shapes. If consistent differences emerged this would provide a further clue to the dimensions under analysis and the mechanisms at work, particularly if such differences could be correlated with differences in neuroanatomy or environment[6].

The First Model

In 1957 a model was proposed which accounted well for many early findings[1,2]. It was suggested that in the octopus's visual system shapes were converted into their 'horizontal' and 'vertical projections'. The horizontal projection is arrived at by counting the horizontal extent of a shape at each point on the vertical axis, and the vertical projection by counting the vertical extent at each point on the horizontal axis (for some examples of such projections cf. Fig. 2). To explain the early evidence it was necessary to make the further assumption that behaviour is more readily determined by differences in the horizontal projections of shapes than by differences in their vertical projections.

Among the earlier findings which the model predicted or explained are the following. Octopuses discriminate very readily between a vertical and horizontal rectangle, but scarcely at all between two rectangles, one set at 45° to horizontal, the other at 135°. The discrimination of a horizontal or vertical rectangle from an oblique rectangle is of intermediate difficulty. M and Λ shapes having identical projections are very difficult to discriminate; after learning to discriminate M from >, animals treat Ʒ as equivalent to the original >, and Λ as equivalent to the original M. Shapes reversed along the horizontal axis are less readily discriminable from one another than shapes reversed along the vertical axis. Two horizontal rectangles differing in length are more readily discriminated than two vertical rectangles differing in length. After being trained to discriminate a square and a vertical rectangle there is a tendency to treat a horizontal rectangle as equivalent to the square. A square and a triangle are more readily discriminable than a diamond and a triangle. After training on square and circle there is no tendency to treat a diamond as equivalent to the square. For a fuller description of these results and for references to the experimental papers see ref. 2. All these findings were originally obtained with octopuses, but some of them have since been replicated with other species, namely, rats[7], goldfish[8], monkeys[9], and children[10].

Some Recent Data

It is impossible to summarize fully all the more recently collected behavioural data on shape discrimination. Attention will be focused on data which it is difficult or impossible for the original model to explain.

(1) *Republicated patterns.* The two patterns shown in Fig. 1 should, according to the theory, be impossible to discriminate. Whether the white portions of the patterns or the black are counted, the projections of each pattern are the same and are as shown in Fig. 1. Despite this, the two patterns are very readily discriminated by octopuses[11]. Indeed, they are much easier to discriminate than are a single horizontal and a single vertical rectangle of the same size as each of the elements in the two patterns.

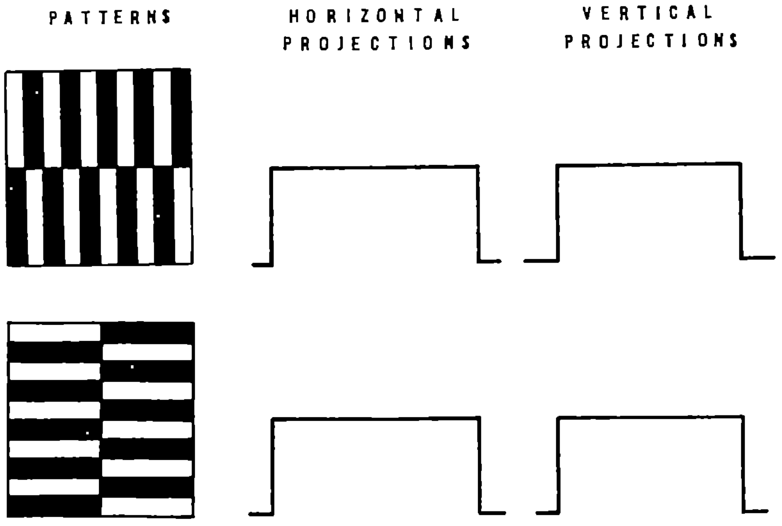

Fig. 1

(2) *Horizontal and vertical projections.* Since the theory assumes that differences in horizontal projections are more readily detected than differences in vertical projections a pair of shapes differing solely in the former projections should be more readily discriminable than a pair differing solely in the latter. From Fig. 2, it will be seen that pair 1, 2 differ only in their vertical projections whereas the members of pair 3, 4 differ only in their horizontal projections. Nevertheless, the first pair was found to be as readily discriminable by octopuses as the second[12]. Pairs 11, 12 and 13, 14 were also found to be equally discriminable although again one pair differs only in vertical projections, the other only in horizontal[13]. The same finding was obtained with pair 5, 6 and the pair made up of the 90° rotations of these shapes[14].

(3) *Open and closed shapes.* If animals are trained to give response x to shape X and response y to shape Y and are then shown several transfer shapes, we can count up how often each response is given to each transfer shape and thus rank order the transfer shapes starting with that to which most x responses are given and ending with that to which most y responses are given. Perhaps the most interesting recent finding is that when the same transfer shapes are presented in different experiments, they are frequently arranged in the same order despite wide variations in the pairs of training shapes used[7,12-14]. Thus after training on any of the seven pairs of training shapes shown on the left-hand side of Fig. 2, octopuses arrange the transfer shapes shown on the right-hand side of the figure in the same order. This suggests that the members of pairs of training shapes producing this ordering of transfer shapes themselves differ along one of the primary dimensions used by the octopus in the analysis of shape. The problem is to specify this dimension by examining the way in which transfer shapes are ordered along it. Early work[7,14] made it appear possible that a shape's position on the dimension might be determined simply by the ratio of its periphery to the square root of its area. Later work[13,15] suggested that this was incorrect since changing the orientation in which a shape was presented altered its position on the scale. Although we cannot define

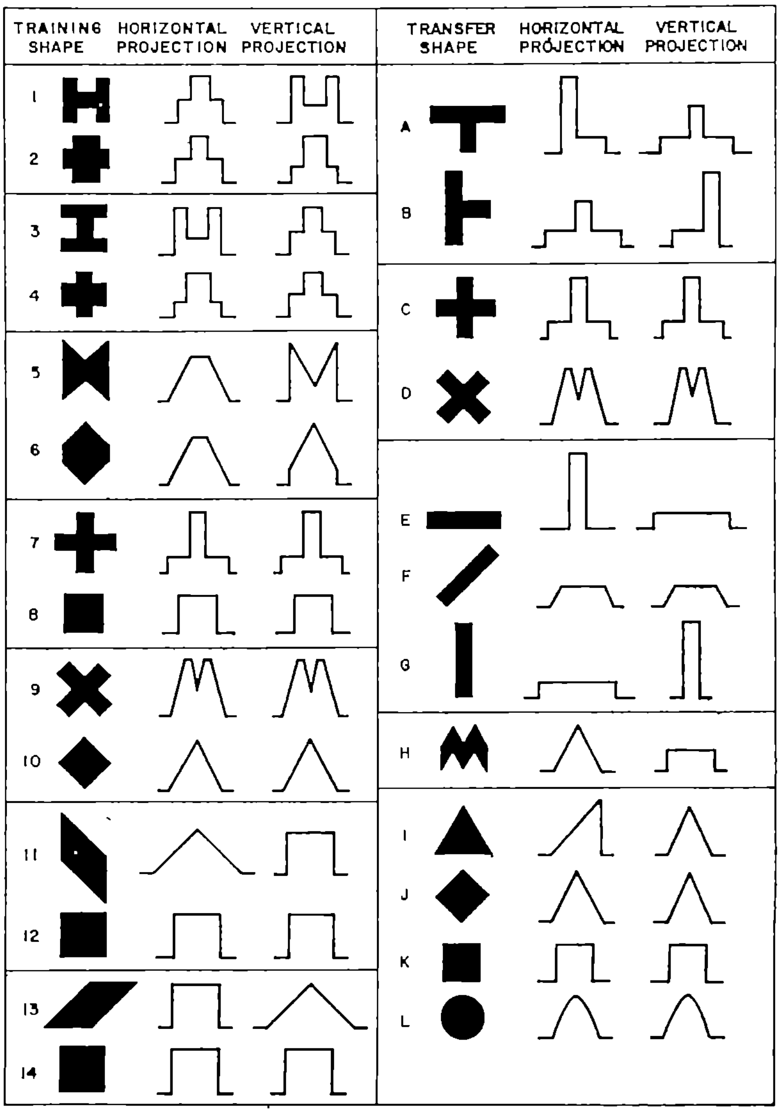

Fig. 2

the dimension in question precisely until we have discovered exactly which physical properties of shapes determine their position on it, it is useful in the meantime to name it. Since shapes at one end of the dimension appear to human beings spread out or open, whereas at the other end they appear compact or closed we shall refer to the dimension as 'open–closed'. Of the training shapes shown in Fig. 2, the odd-numbered member in each pair lies towards one end of the dimension (open), and the even-numbered shapes lie towards the other end (closed). In what follows the words 'open' and 'closed' will be used to indicate the position of shapes on a scale: it is important to remember that their position was determined experimentally—the octopus not the experimenter decides whether one shape is more or less open than another.

Octopuses were trained on each of the training pairs shown in the first column of Fig. 2 (and on several others not shown) and were then presented with various transfer shapes some of which are shown in the fourth column. The more open a transfer shape was found to be (that is, the more nearly it was treated in the way the animal had learned to respond to the open training shape), the higher its position in Fig. 2. Shapes lying inside the same pair of horizontal lines definitely lie along the dimension in the order given—thus *C* is more open than *D*, *E* is more open than *G*, etc. The position of blocks of shapes along the dimension is, however, only approximate. Thus *D* is probably but not certainly more open than *E*. To determine exactly the relative positions of such shapes further experiments are necessary.

One interesting feature of the ordering of the transfer shapes is that the position of a shape on this dimension often depends on its orientation: thus the square and diamond (*J*, *K*) are the same shape shown in different orientations, but the diamond is always found to be more open than the square. Similarly, an upright cross (*C*) is more open than an '*X*' (*D*), and a horizontal rectangle (*E*) than a vertical (*G*). One general finding is not shown in Fig. 2. It will be noticed that the original training shapes were all filled-in block shapes. If, after training, an outline version of any of the original open shapes (odd numbers) is presented, it is always treated in exactly the same way as the animal had learned to treat the original open shape. If an outline version of any of the original closed shapes (even numbers) is presented, there is either no transfer at all, or the animal tends to respond to it in the same way as it had been trained to treat the original open shape.

There is, then, a remarkable consistency in the way octopuses order transfer shapes when different training shapes are used. Even more remarkable is the fact that two other species have been found to order transfer shapes in the same way as the octopus. Rats and goldfish were trained on pair 5, 6 and the 90° rotations of these shapes. Most of the transfer shapes shown here were afterwards presented and within the limits of experimental error they were ordered from most open to most closed in the same way in which they were ordered by octopuses. It therefore seems likely that the results obtained with octopuses have considerable generality, and many of them hold for species widely different phylogenetically.

We must now consider how well the original model explains the ordering of shapes along the 'open–closed' dimension. It will be noticed that the more open member of each pair of training shapes yields at least one projection which contains more or sharper peaks than occur in the corresponding projection of the closed members (Fig. 2). In general, the ordering of transfer shapes agrees well with the number and sharpness of the peaks occurring in their projections; thus, the diamond (*J*) yields more of a peak on both projections than does the square (*K*). Moreover, shapes yielding a high peak on their horizontal projections (*A*, *E*) are more open than shapes yielding the same peak on their vertical projections (*B*, *G*) and this agrees with the assumption that horizontal projections determine behaviour more readily than vertical. However, in three instances this analysis fails to predict the correct order. The circle (*L*) yields more of a peak on both projections than does the square (*K*) but it is a less open shape. The oblique rectangle (*F*) yields no peaks but is a comparatively open shape. It is not clear that the upright cross (*C*) yields more peaked projections than the '*X*' (*D*).

A Revised Model

At the time the original theory was proposed there was little physiological evidence available as to how the visual input was coded. Evidence of quite complex kinds of coding has recently been forthcoming from microelectrode studies on the visual cortex of the cat[16] and the tectum of the frog[17]. Hubel and Wiesel have discovered in the striate cortex of cat neurones which have elliptical receptive fields. One type of receptive field has an excitatory centre strip running longitudinally flanked by two longitudinal inhibitory strips. A neurone having such a field will only give a maximal response when a rectangle is laid along the length of the field covering only the excitatory centre strip. A small rotation of the rectangle abolishes the response, since when the rectangle is not in the same orientation as the elliptical field it covers both excitatory and inhibitory areas. In the cat striate cortex such neurones occur with receptive fields orientated in all visual axes. Hubel and Wiesel have discovered several other types of receptive fields, some of them considerably more complex in their properties than that described: these complex fields probably represent a further stage of analysis. It is unnecessary to describe in detail the other receptive fields discovered in the cat: they all seem to have the property of responding maximally either to a narrow bar or to a straight edge, though some are more affected by movement of the stimulus, etc., than others.

We cannot, of course, infer from the existence of a given type of receptive field in the cat to its existence in a species as phylogenetically different as the octopus. However, at the same time that Hubel and Wiesel were investigating coding in the cat's visual pathways by microelectrode methods, J. Z. Young was studying the neuroanatomy of the octopus's optic lobe. He obtained evidence that the dendritic pick-up fields in the optic lobe of *Octopus* are predominantly elliptical, and that there is a maximum number orientated in a plane which corresponds to the visual horizontal, a second maximum in the plane corresponding to the visual vertical, and fewer in intermediate orientations[18]. It is possible that Young has discovered in the octopus the anatomical basis for a coding arrangement similar to that demonstrated to exist by physiological techniques in the cat. It should also be noted that this type of coding is not dissimilar to that previously suggested by me[1] on the basis of behavioural evidence alone, though there are important differences.

If we assume that in the octopus brain the counting of visual extents is performed by a mechanism similar to that uncovered by Hubel and Wiesel and that (following Young) there are more neurones with horizontal receptive fields than with vertical and more with vertical than with oblique, then most of the behavioural data on shape recognition in the octopus can be readily explained. It is necessary to assume only that an open shape is one which fires many receptive fields and a closed shape one which fires few. Any thin segment of a shape will tend to fire receptive fields of the type described here, and thin segments occur not only where a portion of a shape is rectangular or elliptical but also at any corner.

Fig. 3 shows some shapes superimposed on receptive fields that have a centre excitatory strip flanked by inhibitory areas. It must be remembered that the extent

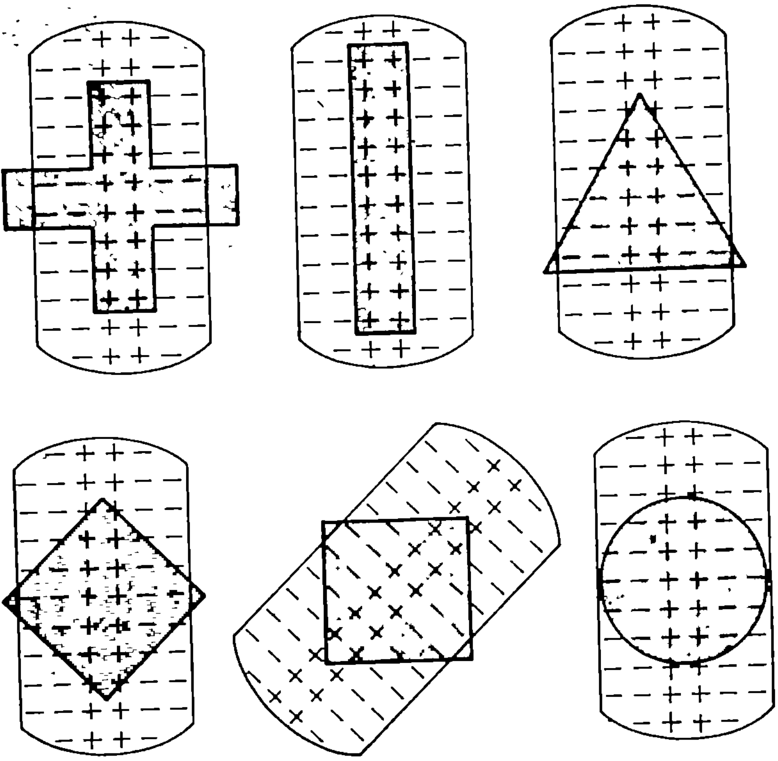

Fig. 3

to which a given neurone is fired depends on the excess of excitatory area over inhibitory area stimulated in its receptive field, that is, firing rate will vary with E–I, where E is the number of excitatory units stimulated, I the number of inhibitory. If we count each 'plus' in Fig. 3 as one excitatory unit, and each 'minus' as one inhibitory unit, then the value of E–I for each shape is cross 10, rectangle 20, triangle 0, diamond 0, square 0, circle -5. However, only the cross and the rectangle have been drawn in such a position as to maximize the value of E–I. If the triangle is superimposed on the field so that the base falls below it and the apex protrudes into it then the triangle yields an E–I value of 4. Similarly, if the diamond and the square are drawn with only one corner sticking up into the receptive field they each give an E–I value of 2. No matter in what position the circle is placed on a field E–I must be zero or less. It is clear that the extent to which a receptive field is stimulated by a given shape depends on the exact position in which that shape is placed on it. Variations in the receptive fields themselves will also result in variations in their tendency to be fired by different shapes. Thus, if we imagine a receptive field consisting of a central column two excitatory units wide flanked by one inhibitory unit on each side (instead of two as shown in Fig. 3), then all the shapes will have an E–I value greater than zero even placed in the positions in which they are shown in Fig. 3. This type of arrangement of excitatory and inhibitory units seems less useful than that shown in Fig. 3, since fields having equal numbers of excitatory and inhibitory units could be strongly fired by almost any shape merely by placing the shape slightly off centre on the field so that two excitatory units were stimulated for each inhibitory unit. Although the change in the arrangement of the field just envisaged results in a change in the absolute value of E–I it does not in fact alter the rank order of the values for the different shapes when placed in such a position as to maximize E–I. As we have seen, this rank order from most to least firing is rectangle, cross, triangle, diamond = square, circle.

It is clear that even if the actual distribution of excitatory and inhibitory units in a field is specified, it is not easy to calculate the total amount of firing each shape would produce since, whatever a shape's position on the retina, it will fall simultaneously on many overlapping receptive fields. Intuitively, however, it seems likely that the total amount of firing produced by different shapes will correlate with the maximum possible value of E–I for each shape.

In order to estimate the relative amount of firing produced by different shapes, we must also consider how far each shape will fire fields in different orientations. Thus, of the shapes drawn in Fig. 3, it is clear that the cross, triangle and diamond would all fire horizontally orientated fields as well as vertical; the square would fire an oblique field orientated at 90° to that on which it is shown superimposed, whereas the vertical rectangle can only fire fields which are themselves vertically orientated. Since we have assumed that there are more horizontally orientated fields than vertically orientated a shape which fired predominantly horizontal fields would actually produce more total firing than one which fired predominantly vertical fields. Similarly, it must be borne in mind that in the octopus there are probably more fields in a vertical orientation than in an oblique orientation. We can now try to estimate relatively how much firing will be produced by different shapes in order to see whether their positions on the 'open–closed' dimension (Fig. 2) can be accounted for in terms of the amount of firing they produce.

It is clear that a circle should be the most closed shape: it has no corners, and no matter how it is placed on a receptive field little or no firing can result. A square will be less closed since some firing of obliquely orientated receptive fields may occur where a corner of the square projects into such a field. A diamond should be less closed than a square since it will fire predominantly horizontal and vertical fields rather than the obliquely orientated fields fired by a square, and we have postulated that there are fewest fields in oblique orientations. For the same reason an upright cross will be more open than an 'X'. Again, a horizontal rectangle will be more open than a vertical since the former shape will fire horizontal fields the latter vertical and it is assumed that there are more horizontal fields than vertical. However, if there are more vertical than oblique fields an oblique rectangle should be less open than a vertical, but the octopus treats it as a more open shape. This discrepancy does not appear in the results from the goldfish. Despite its jagged appearance to us the M shape will not be particularly open since its various arms will tend to excite inhibitory as well as excitatory areas of receptive fields. Outline shapes will be more open than the corresponding filled-in shapes since they contain long, thin segments.

Most of the remaining data on open and closed shapes summarized under 3 above can be accounted for in similar ways. However, it is necessary to assume that as well as a count of the total number of neurones excited (giving the position of a shape on the open–closed dimension), an analysis is also performed of the proportions of neurones fired with fields in different orientations. This second type of analysis would account for much of the earlier data. Moreover, even when training shapes do differ on the 'open–closed' dimension, there is evidence that transfer is determined to some extent by whether the orientation of thin segments in transfer shapes is the same as or different from that of the segments of the open training shape.

The revised model based on Hubel and Wiesel's and Young's data will also allow us to explain the ease with which octopuses discriminate between the reduplicated patterns shown in Fig. 1 (point (1) here). Many more vertical fields will be fired by the first pattern than by a single small vertical rectangle and many more horizontal fields by the second than by a single small horizontal rectangle. Presumably the greater the number of neurones with fields in a given orientation that are fired by a shape, the easier it will be for further detection mechanisms to pick out the firing from a background of noise and therefore the more readily will the discrimination be learned.

The findings reported under point (2) here are not so readily explainable under the revised model. Thus the main difference in firing patterns between shapes 1 and 2 in Fig. 2 will be in the number of neurones fired having vertically orientated receptive fields, whereas the main difference for shapes 3 and 4 will be that 3 will fire more neurones with horizontally orientated fields than 4. It may be that when the difference in the number of horizontal fields or of vertical fields fired is great enough then no effect due to there being more horizontal than vertical fields will be detectable.

There is one further fact which the revised model explains better than the original theory. Sutherland and Muntz[19] investigated the preference of octopuses for different shapes in the absence of any training and discovered that the animals attacked shapes moving along their long axis much more readily than shapes moving across it. Rectangles moving along their long axis should excite the type of receptive field discussed here very strongly, whereas rectangles moved at right angles to their long axis should excite much less strongly since they will move over both excitatory and inhibitory areas of any given field. The octopus's preference for such shapes may be connected with the fact that crabs are its main prey, and crabs move along their long axis. It is likely that one of the features of crabs used in identification by cephalopods is the presence of legs[20]: again the type of mechanism discussed here would be well adapted to detect legs provided sufficiently small retinal fields were present.

The model here proposed raises many further questions. Three of these will be briefly delineated. (i) It is clear that with the type of retinal field arrangement discussed transfer of learning from one part of the retina to another could be achieved by linking up similar units from different parts of the retina to a common unit at a later stage, and Hubel and Wiesel provide evidence that this may occur. (ii) It is not so easy to envisage a mechanism which will result in the same output being given for a shape irrespective of its retinal size (size transfer). It may be that there are receptive fields of different sizes and the size actually used can be altered with the size of the shape under analysis. (iii) Only one type of receptive field found by Hubel and Wiesel in the cat has been considered in the foregoing analysis. This is because the findings on octopus suggest that in this species the length and orientation of thin segments of shapes are of primary importance in shape recognition, and this is precisely the sort of analysis made by the receptive fields discussed here. A second type of field discovered by Hubel and Wiesel seems to detect the orientation of contours rather than of thin segments of shapes. Recently acquired behavioural evidence from goldfish indicates that in this species contour orientation is more important for shape recognition than it is in the octopus. This suggests the exciting possibility that when both neurophysiological and behavioural evidence are available for a variety of species, we may be able to correlate variations in behaviour with variations in the proportions of different types of receptive field present in the brain of different species.

It has only been possible to give the barest outline of a revised model here, and clearly a great deal of further work remains to be done to test and develop it. For example, in order to account in detail for the ordering of transfer shapes it would be necessary to optimize various parameters of the model such as the actual widths and lengths of the receptive fields (in terms of visual angles), and the ratio of fields in one orientation to fields in other orientations. This could be achieved by means of a computer programme and the existing behavioural data are sufficiently extensive to evaluate the goodness of fit which resulted after optimization.

It is almost certain that the type of coding discussed here is only one of several types of coding proceeding in parallel in the visual system. Two simple operations have been suggested which could be performed on information coded in this way: namely, to count the total number of fields fired and to count the ratio of fields fired in one orientation to those fired in others. Obviously many other operations could be and indeed must be performed. Thus the two operations discussed would not detect a difference between mirror image shapes; since such shapes can be discriminated by the octopus (though only with great difficulty), some additional operations are required. It is suggested only that the two operations may be among the commonest performed in the visual system of *Octopus*.

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GEOMAGNETIC AND PALÆOMAGNETIC EVIDENCE OF FOSSIL AXES OF ROTATION OF THE EARTH

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PALÆOMAGNETIC work on Quaternary lava flows from many parts of the world indicates that the direction of the geomagnetic field averages that of an axial dipole over times of the order of 100,000 years. Individual readings of the field as given, for example, by precise measurements of the fossil magnetization of particular lava flows differ from the axial dipole field direction as does the direction of the geomagnetic field at the present epoch. The standard deviation of the distribution of individual flow measurements from a suite

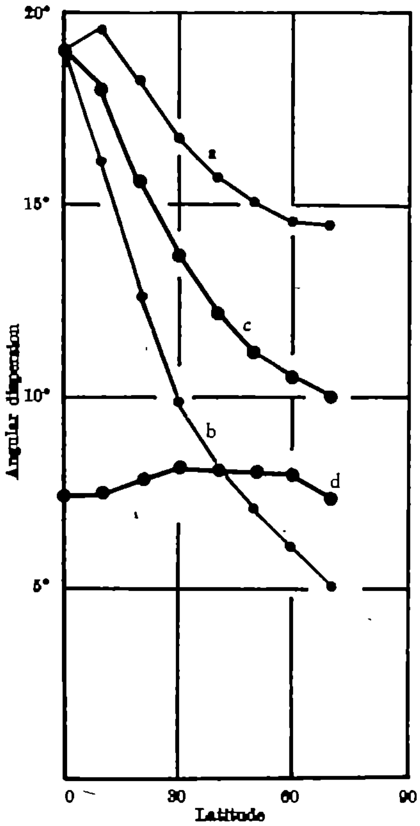

Fig. 1. Angular dispersion of 1945 geomagnetic field around lines of latitude: *a*, of field directions in southern hemisphere; *b*, of field directions in northern hemisphere; *c*, average of *a* and *b*; *d*, of virtual poles for northern and southern latitudes combined

of lava flows gives information about the palæosecular variation, but only a few attempts have been made by workers in palæomagnetism to compute this[1]. If each of N palæomagnetic readings of the field are represented by a unit vector and their resultant is R, an estimate of their standard deviation is given by an angular dispersion $\delta°$ equal to $\cos^{-1} R/N$. It may alternatively be supposed that each palæomagnetic reading is due to a non-axial but geocentric dipole and that the palæosecular variation is due to fluctuations of such dipoles about an axial mean[2]. The poles of such dipoles are conveniently referred to as virtual poles[3]. The palæosecular variation may also be estimated by calculating the angular dispersion of these.

The scatter of geomagnetic field directions at a given place during several tens of thousands of years may be estimated by rotating the present field about the geographic axis, and it has been shown by this means that the angular dispersion around lines of latitude is latitude-dependent and greater in the southern hemisphere than the northern[1] (Fig. 1). In a similar analysis of virtual poles[4] corresponding to geomagnetic field directions around lines of latitude higher dispersion factors are also observed for the southern hemisphere than for the northern, but when data for north and south latitude lines are combined the angular dispersions are independent of latitude (Fig. 1).

It was thought at first[1] that the higher dispersions in the southern hemisphere were due to chance. Of the ten or so regional sources of non-dipole field, more now happen to lie under the southern hemisphere than the northern but the situation might be reversed in time, the time-constant of such a source being about 2,000 years[5]. However, another asymmetry was revealed when the angular dispersion of the group of virtual poles from the Pacific hemisphere was shown to be significantly lower than that for the opposite hemisphere[4]. It has been realized for the past 20 or 30 years that the non-dipole field in the Pacific is lower than elsewhere, and it has been suggested that this might be a permanent feature of the field[7]. Furthermore, palæomagnetic directions for the Hawaiian lava flows are said to exhibit less scatter than usual for rocks of similar age and type from elsewhere[8].

By rotating the present field through 360° as described here, we cannot detect sectorial differences in angular dispersion, but this might be done by rotating it through a smaller angle which should be large enough to contain a sample of the non-dipole field representative of that likely to exist at a particular place with the passage of time, that is, it should contain parts of more than one regional source. 60° has been chosen as a reasonable compromise so as not to smooth out sectorial differences too much. In order to obtain a rather better sample of the local non-dipole field than by simply rotating it through 60°, virtual poles have been formed into groups

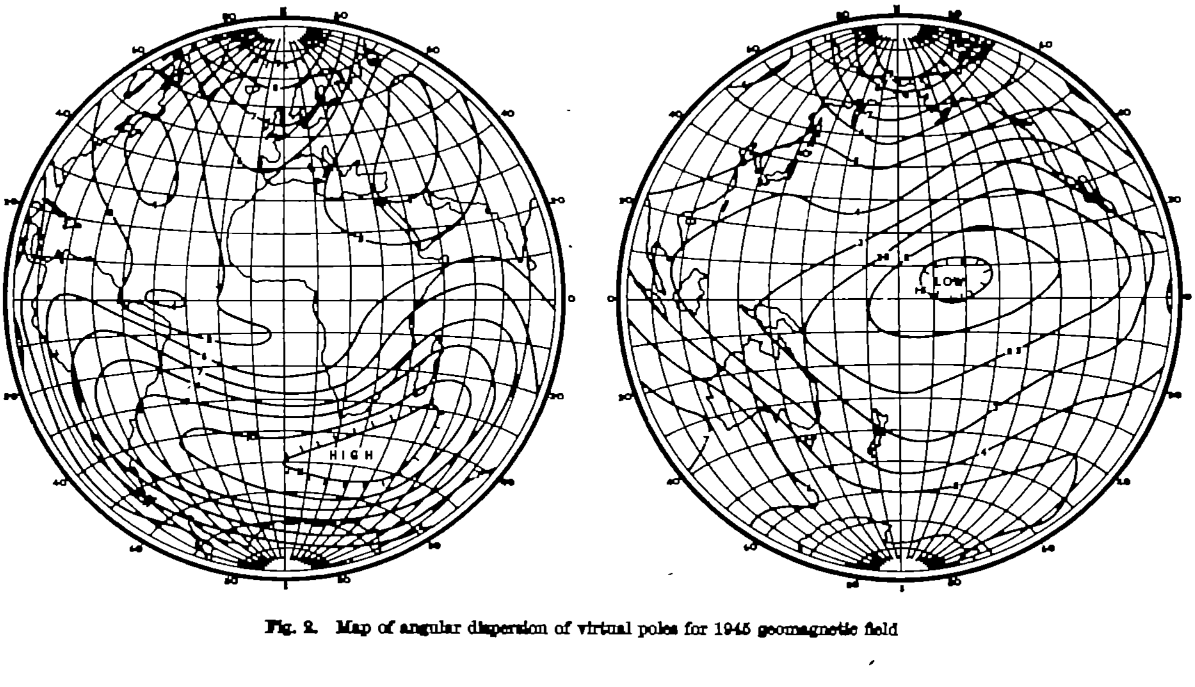

Fig. 2. Map of angular dispersion of virtual poles for 1945 geomagnetic field

lying within small circles of radius 30° described about the place for which the angular dispersion is being estimated. This has been done for all points on a 10° latitude-longitude grid. The angular dispersions so computed vary from less than 1·5° to more than 11° and a contour map is shown in Fig. 2. The map has 3 main features: (1) a region of low dispersion in the Pacific centred at 6° N., 165° W.; (2) a region of high dispersion just to the south of South Africa at about 45° S., 30° E.; (3) a region of high dispersion around the present north pole.

Whether these features are permanent, perhaps related to differences in conductivity in different parts of the mantle, might be revealed by computing the palæomagnetic angular dispersions of virtual poles from mean directions of magnetization of individual Quaternary lava flows within suites of flows from a world-wide distribution of sites. Although a considerable amount of palæomagnetic work has been done on Quaternary lavas, the data have not been presented in terms of mean lava flow directions. More systematic studies with the main object of determining the palæomagnetic angular dispersions due to past geomagnetic fields are necessary.

It is reasonable to suppose that sources of the non-dipole field are equally likely to occur at any longitude near the core surface, the total number existing at any one time being limited by their diameter, which has been estimated at about 50° (ref. 9). To an observer on the surface the effective period of field changes due to these sources is given by dividing the period of westward drift[8], 1,800 years, by the number of non-dipole sources of the same sign occurring round a line of latitude, that is, about 2, giving a period of about 10^3 years. McDonald[10] estimates that the conductivity at the base of the mantle is about 400 ohm^{-1} m^{-1} and that it decreases slowly for the inner 1,500 km and then rapidly with increasing radius. This value is too low to explain a suggested attenuation of the changing non-dipole field under the Pacific. Even allowing for the exponential increase of conductivity with temperature of the semi-conducting mantle rock, it is easy to show that a temperature difference of nearly 1,000° is required to explain why the strength of the regional sources in the Pacific is one-tenth of those near Africa. Runcorn[11] has suggested that a layer of iron as yet unabsorbed by the core might be responsible, and by taking a period of 10^2 years and a conductivity of 10^7 ohm^{-1} m^{-1} for iron at the temperature and pressure near the base of the mantle he calculates that skin depth of this material is about 10 km. For a period of 10^3 years as suggested here, however, the skin depth becomes 30 km and the presence of such a layer of iron might be detectable by seismic methods. The iron must form a layer, for if it were distributed in the mantle material much more would be required to produce a given attenuation; for example, a random distribution of iron spheres of concentration 50 per cent would only increase the conductivity of the mantle by a factor of about 5 (ref. 12).

Starting from the supposition that the Earth accreted cold with iron and stony material rather uniformly distributed, Elsasser[13] has developed a hypothesis which explains how the iron in the mantle might have become asymmetrically distributed. Suppose that the Earth heated up at a fairly uniform rate due to radioactivity from an initial temperature of about 1,000° K. The melting-point would be first reached near the top of any homogeneous layer because the melting-point-pressure gradient for silicates is much steeper than the adiabatic gradient. The actual temperature gradient would not be very different from the latter. Thus the iron would fall towards the centre in the outer layers first of all, and as it fell it would be slowed down owing to an increase of viscosity with depth as the gap between the actual temperature and the melting-point of the silicates decreased. It would thus form a coherent layer which, as the material contained afterwards heated up further owing to continued radioactivity, would become gravitationally unstable, resulting in the formation of quite large drops. These would then fall to the centre forming the proto-core. One of Elsasser's most important conclusions is that horizontal motion of iron must have occurred at certain levels leading to the formation of much larger drops than those corresponding to the original accretion. The time taken to form a proto-core is estimated to have been less than 10^8 years and the time for a drop to fall 10^5 years. As such a drop fell through the almost fluid silicate material the general flow pattern of the latter would at first be nearly streamline, but since the viscosity of the mantle is con-

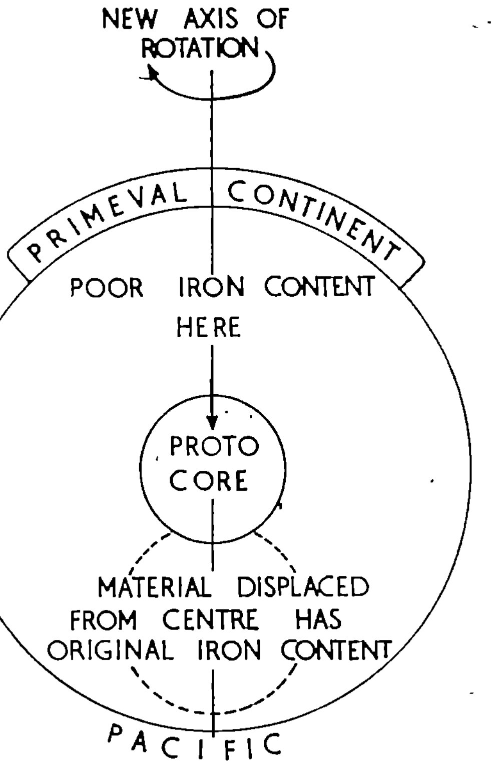

Fig. 3. Relationship between drop axis and asymmetrical distribution of iron in the mantle

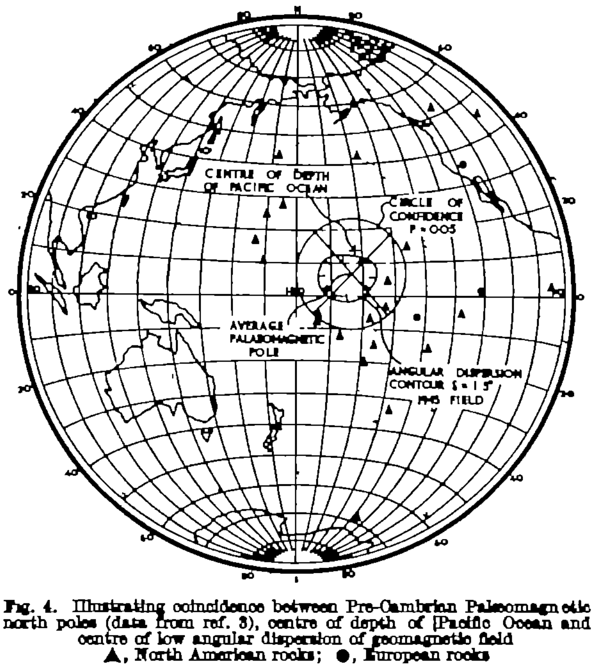
CENTRE OF DEPTH OF PACIFIC OCEAN
CIRCLE OF CONFIDENCE P = 0·05
AVERAGE PALAEOMAGNETIC POLE
ANGULAR DISPERSION CONTOUR S = 1·5° PMS FIELD

Fig. 4. Illustrating coincidence between Pre-Cambrian Palæomagnetic north poles (data from ref. 3), centre of depth of Pacific Ocean and centre of low angular dispersion of geomagnetic field
▲, North American rocks; ●, European rocks

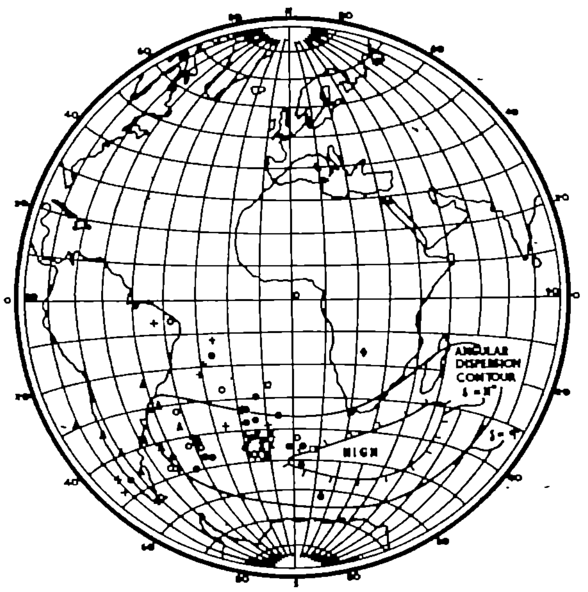

Fig. 5. Permo-Carboniferous Palæomagnetic south poles (data from refs. 3 and 17), and region of high dispersion of geomagnetic field

Continent	Carboniferous	Permian
N. America	△	▲
W. Europe	○	●
U.S.S.R.	×	+
S. America	□	
Africa	◇	

sidered to increase with depth, the inner portion would not have time to flow but would be pushed outward by the falling drop of iron as shown in Fig. 3.

The composition of the mantle would thus become asymmetrical, the material displaced to the antipodal point containing iron, while that above the drop lost most of its iron. The drop axis would then precess about the axis of rotation and spiral towards it. Further differentiation of the iron-free material would occur later, sial rising to the surface forming a primeval continent at one pole, antipodal to an ocean above a mantle region relatively rich in iron. Thus the possible presence of a layer of iron near the base of the mantle under the Pacific could be a relic of this primeval asymmetrical distribution of iron.

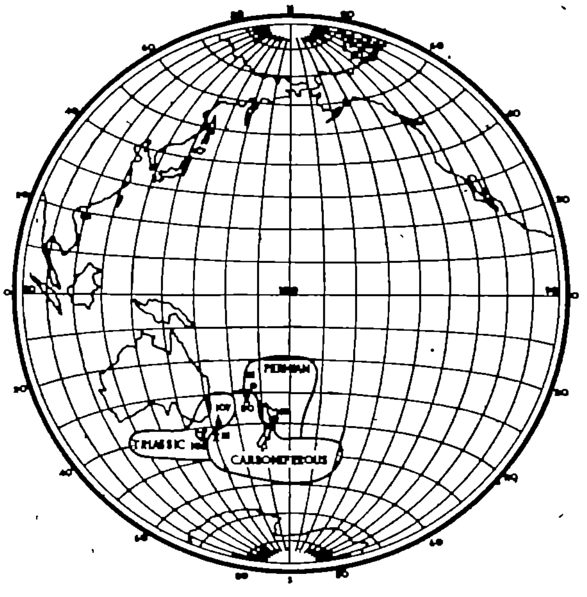

Fig. 6. Palæomagnetic poles determined from Australian rocks

By the end of the Pre-Cambrian, however, this primeval asymmetry might have decreased sufficiently so that the formation and settling of a new drop could topple the Earth relative to its axis of rotation. Although the difference in moment of inertia due to the absorption by the core of a drop, say, of diameter 500 km (that is, about ½ per cent of the volume of the proto-core) is only a few per cent of the difference in moments of inertia $(C - A)$ of the Earth's figure, it is more than enough to cause a movement of the pole because of the plasticity of the Earth[15].

An interpretation of the features shown by the map in Fig. 2 is now attempted using Elsasser's hypothesis. The region of low angular dispersion is close to the centre of depth of the Pacific ocean at 14° N., 161° W., and also to the mean palæomagnetic pole for North American rocks at 8·5° N., 163·5° W. (Fig. 4). The coincidence of the latter two features has previously been noted by Munk and MacDonald[14] and Elsasser[12], and they have suggested they might mark a primeval pole of rotation. The presence of the region of low angular dispersion supports this. In using the palæomagnetic data it is implied that North America has not moved relative to the Pacific basin during geological time.

It is possible that the region of high dispersion under the present north pole is due to the formation of a drop which cleared the mantle of iron beneath that region, hence allowing changes in the non-dipole field to pass through with less attenuation than in other regions.

The Permo-Carboniferous palæomagnetic south poles for west Europe, Siberia and North America lie fairly close to the region of high dispersion near South Africa (Fig. 5). The agreement in position is as good as can be expected: first, because the map (Fig. 2) is only an estimate of the angular dispersion of the geomagnetic field with time and hence of variations of conductivity in the mantle; secondly, because relative movement between the continents and that region could have occurred; and, thirdly, because of inaccuracies (of the order of 10°–15°) in some of the palæomagnetic data. If the position of the Permo-Carboniferous pole of rotation is marked in the mantle and revealed by the region of high dispersion, there is no need to postulate appreciable drift of South America relative to Africa since then to explain the Permo-Carboniferous glaciation. Fig. 5 shows that the few palæomagnetic poles available for rocks of this age from these two continents lie close to the group of palæomagnetic poles from the northern continents. Australia, however, must have suffered a considerable displacement since then because it is now situated a long way away from the 'high'. This suggestion is confirmed by the position relative to Australia of the palæomagnetic poles as shown in Fig. 6. The same applies to India; although there are no Permo-Carboniferous palæomagnetic data for that continent the position of the Cretaceous pole[3] is far from the 'high' and also far from the present south pole. For all other continents the Cretaceous pole lies between the Permian and present poles.

That the third important feature on the map in Fig. 2 lies on the present axis of rotation, strengthens the arguments for the hypothesis that the Pacific 'low' and the South African 'high' also mark ancient rotation axes. The large magnetic anomaly in the Arctic has been recognized for some time, but attempts[16] to explain it have relied on the supposed existence of ferromagnetic material in the crust.

If past axes of rotation are fossilized in the Earth's mantle, the consequences will be of great importance in geophysics, particularly in palæomagnetism, for in our interpretation of the data it will enable us to separate the effects of polar wandering (that is, a toppling of the Earth as a whole relative to the axis of rotation, which remains fixed in space) from those of continental drift. The arguments presented for the existence of such fossil axes of rotation are very speculative. It should be possible, however, as described above, to confirm or refute the ideas presented here by palæomagnetic investigations.

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BEHAVIOUR OF THE GEOMAGNETIC FIELD DURING THE MIOCENE PERIOD IN SOUTH-EASTERN OREGON

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THE vast area and thickness of the lavas of the Columbia River Plateaux (Fig. 1, inset) offer an ideal opportunity to examine the behaviour of the Earth's magnetic field during the Tertiary era. During the summer of 1962 a palæomagnetic survey was made in part of south-eastern Oregon just north of the California and Nevada State boundaries (Fig. 1, inset). Previous work by Campbell and Runcorn[1] in northern Oregon and Washington revealed the existence of approximately equal numbers of normally and reversely magnetized lava flows of Miocene to Pliocene age, and Cox[2] found that normal and reversed Eocene lavas in north-western Oregon have, respectively, unusual north-east and south-west palæomagnetic azimuths.

The geology of the area investigated, which is part of the 'basin and range' province, has been summarized by Baldwin[3]. The region is more than 4,000 ft. in elevation and Steens Mountain rises to nearly 10,000 ft. The main characteristics exhibited are major northerly trending faults, which have produced exposures of up to 4,000 ft. of nearly flat-lying basalt flows, with some tuffs and andesites. A common and widespread petrological type is the Steens Mountain Basalt, which is a coarse-grained porphyritic diktytaxitic basalt[4].

Sedimentary beds with an Upper Miocene vertebrate fauna lap with a very slight unconformity on to the basalts[5]. Six flows at the top of sections *B* and *D* (Fig. 1) are separated from the underlying section by a thin wedge of sediments containing Upper Miocene vertebrates (G. W. Walker, personal communication), and these six flows are not discussed in this communication. In the western part of the area, Peterson[6] has described Lower Miocene silicic pyroclastic rocks. According to Walker[5], comparable rocks occur beneath the Steens type basalt, a few miles west of Lake Abert. Field relationships suggest that all the sections sampled are within these Lower to Upper Miocene age limits.

Seven hundred accurately orientated cores were collected from ten different sections (Fig. 1, *A–J*) ranging in vertical extent from 150 to more than 1,000 ft. The distance between the most widely separated sections is more than 100 miles. Measurement of the remanent magnetization has now been completed on at least one sample from each of 250 flows. Partial demagnetization of representative samples from all sections in alternating fields up to 800 oersteds generally reduced the intensity of magnetization to less than 10 per cent of the original intensity, but did not modify greatly the direction of

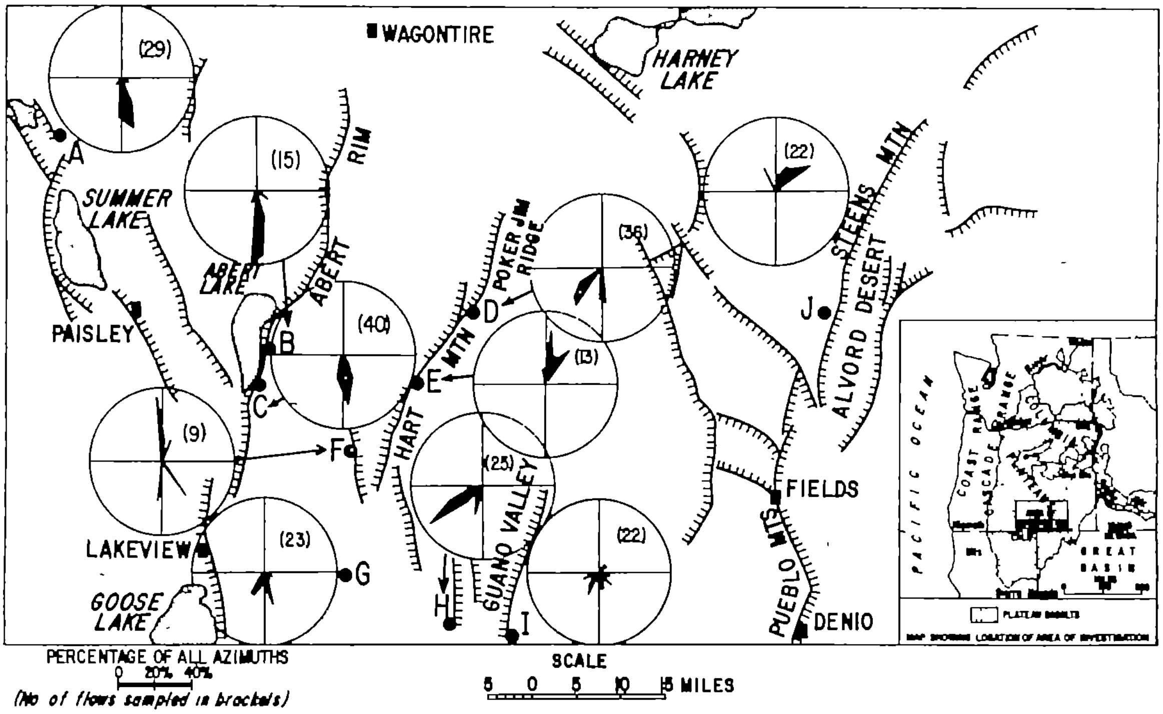

Fig. 1. Map with rose diagrams of palæomagnetic declinations in sections of Miocene lavas in south-eastern Oregon

magnetization. All the samples were subjected to demagnetization in an alternating field of 50 oersteds. At about this field the reversely magnetized specimens reached maximum intensity, indicating a complete removal of an unstable component parallel to the present field direction. A total of five samples with extremely high magnetic intensity, which may represent basalt remagnetized by lightning[7], have been disregarded. Twenty samples taken from baked soils associated with the lavas have magnetic directions in the same sense as those in the overlying lavas, lending evidence to the absence of the self-reversal type of magnetization observed in some lavas[8].

Eighty per cent of the flows possess a reverse magnetization. All the sections, excepting section *I*, show a close grouping of the directions of magnetization. Sections *F* and *I* possess both normally and reversely magnetized flows. The inconsistent directions in section *I* include a definite gradual transition between normally and reversely magnetized flows across a succession of six flows which may represent intermediate directions of the geomagnetic field during the process of reversal. Since this reversal is likely to accompany a decrease in intensity of the main dipole through a minimum to a maximum in an opposite direction, control of the ambient field by non-dipole sources would be expected to predominate close to this time, and hence a more rapid variation of magnetic direction would occur, resulting in a scatter of measured palæomagnetic directions.

The most significant result of the measurements is a systematic variation of the azimuth of the magnetic directions of each section from a north–south direction in the west of the area, to a north-east–south-west direction in the east of the area. These azimuths are plotted as rose diagrams in Fig. 1. Analysis of the directions of magnetization from the 84 flows measured in sections *A*, *B* and *C* would yield a virtual geomagnetic pole slightly west of present geographic north. This determination would contradict that made from sections to the east.

The systematic variation of palæomagnetic declination could be interpreted to be the result of differential tectonic rotation about vertical axes, subsequent to the original creation of parallel magnetic directions in these lavas of restricted relative age. This would be similar to Irving's[9] suggested explanation of Cox's[3] results from north-eastern Oregon. Such an interpretation cannot be accepted in the present investigation, however, when sections in the central part of the area are examined in detail.

It can be seen in Fig. 1 that a bimodal distribution of palæomagnetic azimuth is evident in sections in the centre of the area. Fig. 2 shows the magnetization directions for all flows from sections *D* and *E* in detail. A definite anti-clockwise swing of the palæomagnetic azimuth is observed suddenly in passing down the section from younger to older lavas. This has been observed in section *D*, where the flows are typical Steens Basalt, and are reversely magnetized; and in section *E*, which is composed of alternating coarse pyroclastics and fine olivine basalts, where the flows are normally magnetized. No sign of a significant geological hiatus between the critical flows is visible in the sections. Field relationships indicate that section *E* is older than section *D*. In section *H* (Fig. 3), the ambient magnetic field direction was relatively unchanging during the times when the youngest twelve flows were poured out. Flows 13–16 exhibit palæomagnetic directions which are flatter and more southerly than the younger flows. Then follows an oscillation of directions from southerly to westerly (17); westerly to southerly (18 and 19); southerly to westerly (20–25); and finally back to southerly (26).

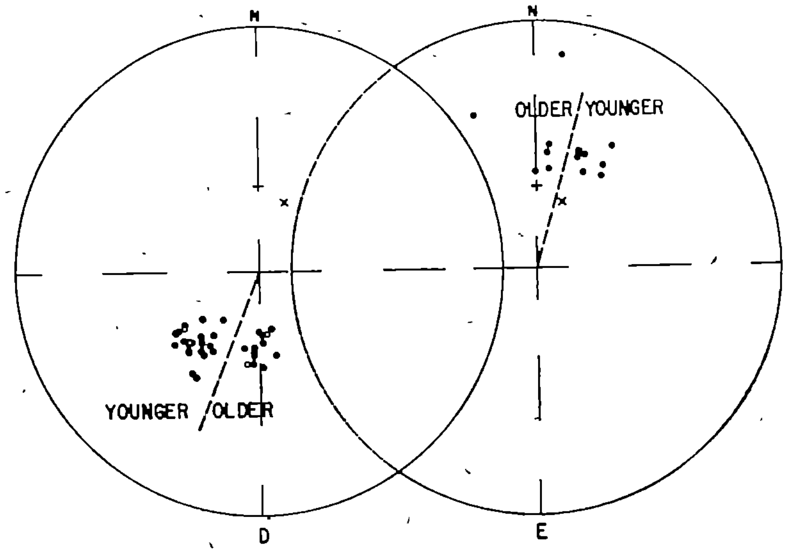

Fig. 2. Details of magnetic directions in sections *D* and *E*. Equal area projection. +, Direction of axially symmetrical dipole; ×, present geomagnetic field direction in south-eastern Oregon; ●, north-seeking polarity downward; ○, north-seeking polarity upward

Explanation of these dual palæomagnetic azimuths within single sections by differential tectonic movement is inconceivable. The cause of the systematic variation of declination across the area, as well as in single sections, is likely to lie in a variation of the ambient magnetic field, superimposed on the relatively small variation in age of the lavas investigated.

Whereas much laboratory work has been devised and carried out in recent years in order to eliminate unstable components of the measured magnetic directions in rocks and thus arrive at a true record of the ancient direction of the magnetic field, the exact meaning of these measured directions is far from certain. The working model of the origin of the Earth's magnetic field accepted for palæomagnetic purposes is that due to Elsasser[10]. Acceptance of this dynamo theory results in the averaging out of

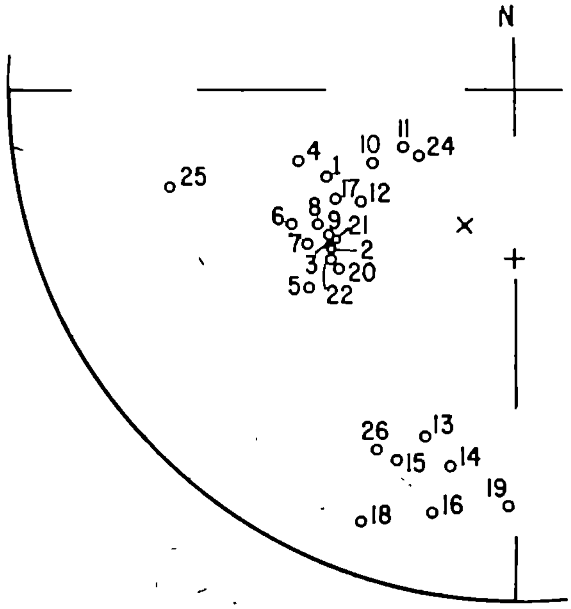

Fig. 3. Detail of change of magnetic directions in section *H*. Equal area projection. Numbers refer to flow, increasing in age with increasing number

○, North seeking polarity upward, +, direction of axially symmetrical south-seeking dipole field in south-eastern Oregon; ×, present south-seeking geomagnetic field direction in south-eastern Oregon both the main dipole and the non-dipole components of the magnetic field to the Earth's rotation axis if measured directions cover a long period of time[11], although it should be noted that movement of the main dipole has not yet been observed directly[12]. Exactly how many lava flows need to be sampled to be able to cancel out non-axial dipole components is a matter of considerable conjecture. Doell and Cox[13] suggest that sections of more than fifty flows in Hawaii may have accumulated in periods of time so short that the non-axial dipole components do not average out to the spin axis, or alternatively, non-axial dipole components persist for times long enough for the accumulation of relatively thick sections. The latter possibility is emphasized in an analysis of the present field by Cox[14], who has shown that the non-dipole component of the geomagnetic field is almost entirely absent from much of the Pacific Ocean.

Bearing in mind, then, that doubt exists about the details of the past behaviour of the geomagnetic field, the following explanations for the observations made of the Miocene lavas in south-eastern Oregon can be suggested:

(1) The lavas were poured out in rapid, but irregular, succession, and the movement of the measured magnetic directions represents movement of the ambient magnetic field in part of an ordinary change of the non-dipole field. The separation of the magnetic directions represents a small pause in the outpouring of the lavas.

(2) Alternatively, the lavas were poured out in regular succession over a fairly long period, and the separation of directions represents a sudden movement of the non-dipole, or possibly the dipole, field.

(3) The movement of the geomagnetic field, whatever its source, appears to have occurred in similar fashion during times when the ambient field was both north-seeking and south-seeking. Furthermore, the movement of the ambient field between two fairly well-defined directions may have been cyclic for at least a limited period.

Although further work on the basalts may reveal additional sets of magnetically normal and reversed zones which can be utilized for assisting in broad stratigraphical correlation, the preliminary indications of finer variations of the Miocene geomagnetic field in Oregon outlined in this article may permit a much more detailed correlation to be carried out.

I thank G. W. Walker (U.S. Geological Survey) for invaluable guidance during the geological work involved; Drs. A. V. Cox and R. R. Doell (U.S. Geological Survey) for generous loan of equipment, for guidance and review of the manuscript; and to Dr. G. A. Thompson (Stanford University) for help and encouragement.

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NEWS and VIEWS

British Association for the Advancement of Science

Dr. N. C. Wright, deputy director-general of the U.N. Food and Agriculture Organization, has been appointed to succeed Sir George Allen as secretary of the British Association for the Advancement of Science as from October 1.

Sir George Allen was appointed secretary of the Association in 1954, having relinquished the vice-chancellorship of the University of Malaya two years previously. For much of his career he has been engaged in medical research and education in Africa and South-east Asia. He was principal of the College of Medicine, Singapore (1929–47), and president of Raffles College, Singapore, during 1932–34 and again during 1947–49, when he became vice-chancellor of the University of Malaya. He is at present chairman of the Council of Queen Elizabeth College (University of London) and vice-chairman of the Sponsors of the University of Kent. Due to his leadership, the Association now provides all the year round, and all over Britain, a service of lectures, junior meetings, and science fairs, which are doing much to interpret science to the public.

Dr. Norman Wright was the first director of the Hannah Dairy Research Institute. He served as chief scientific adviser to the Ministry of Food and then chief scientific adviser (food) to the Ministry of Agriculture, Fisheries and Food from 1947 until 1959, when he became deputy director-general of the Food and Agriculture Organization of the United Nations. He is a past general officer of the British Association and has been a member of its Council since 1950.

Personnel Moves in the Ministry of Aviation

In a written answer to a question in the House of Commons on December 20 regarding the number of civil servants in the Ministry of Aviation over the past ten years who had sought permission to take other posts within two years of retirement or resignation, the Minister of Aviation, Mr. J. Amery, said that applications by ninety-nine officers had been approved They included nine chief scientific officers, seven deputy chief scientific officers, seven senior principal scientific officers, six principal scientific officers, five senior scientific officers, one chief scientist, four senior experimental officers, six experimental officers, five assistant experimental officers, twenty engineers and two chemists.

The Post Office Engineering Research Station

In answering a series of questions in the House of Commons on December 18 regarding the siting of the Post Office Engineering Research Station, the Assistant Postmaster-General, Miss M. Pyke, maintained that working requirements necessitated a location nearer London than the areas of high unemployment. The staff of the Dollis Hill Research Station numbered 1,306, of whom 1,075 were established. The total included 57 scientific officers, 52 assistants (scientific), 92 experimental officers, 166 professional engineering grades, 145 assistant engineers, 76 drawing office staff, 255 technical officers and 205 technicians.

Aid to Economic and Social Progress of Developing Countries

In a written answer in the House of Commons on December 21, the Secretary for Technical Co-operation, Mr. D. Vosper, stated that he recognized the need, which had been emphasized to him by the Overseas Research Council, to sponsor research into problems which impede the economic and social progress of developing countries, both independent and dependent, as part of Britain's technical assistance effort. It was his intention, subject to the approval of Parliament, to provide separately in the Estimates of his Department for the support of research in and for overseas countries, who may welcome this form of aid, and such assistance would be available to any countries with which Britain had technical assistance arrangements. It would not be restricted to dependent territories, as under the terms of the Colonial Development and Welfare Acts. Funds previously available under the present Act for this proposal would be merged in the new provision, and an advance of £950,000 would be made from the Civil Contingencies Fund to cover expenditure between now and the approval of a Supplementary Estimate shortly to be presented to Parliament.

Dragon High-Temperature Reactor Agreement Extended

THE agreement extending the Organization for European Co-operation and Development's *Dragon* high-temperature reactor project to March 31, 1967, has now been approved and signed by all the participants in the project. Under the agreement, the total duration of the project is extended from five to eight years and the original £13·6 million budget is increased to £25 million. The main objective of the extended programme is "to provide the Signatories with information leading to the design of an economic land-based, gas-cooled, carbon-moderated, high-temperature power reactor". The original *Dragon* agreement, concluded between the U.K. Atomic Energy Authority, the Austrian and Swiss Governments, the national atomic energy authorities of Denmark, Norway and Sweden, and the Euratom Commission (representing Belgium, France, Germany, Italy, Luxembourg and the Netherlands), covered a period of five years from April 1, 1959. The project is carrying out a programme of research and development in the field of high-temperature gas-cooled reactors, and is designing and constructing a reactor experiment to obtain practical experience of this type of installation. The *Dragon* reactor experiment is now at an advanced state of construction at the U.K. Atomic Energy Authority's Atomic Energy Establishment at Winfrith, Dorset, where the team has its own technical building and has already carried out extensive research and development. Fuel loading in the reactor is expected at the end of 1963.

Dust-explosion Hazards in Factories

THE danger of dust explosions in industry in general appears to be less widely appreciated than the danger of gas or vapour explosions, although the number of industries in which a dust-explosion hazard may exist has increased rapidly during the present century. This is of some concern to the Committee on Industrial Fires and Explosions of the Department of Scientific and Industrial Research and Fire Offices Committee Fire Research Board, who asked for a review of the literature of dust explosions in factories to be prepared. This has been carried out at the Safety in Mines Research Establishment as part of the programme of the Joint Fire Research Organization (Research Report No. 201: *Dust Explosions in Factories—a Review of the Literature*. By K. C. Brown and Dr. G. J. James. Pp. 67. Sheffield: Ministry of Power, Safety in Mines Research Establishment, 1962. 6*s*. 6*d*.). The review covers methods and conclusions of many investigations of dust explosions, especially in connexion with the hazard in factories, up to June 1959. The subjects considered in the review include: combustion of single particles; forming dust clouds for experimental purposes; possible sources of ignition of dust clouds; studies of the propagation of flame through dust clouds; large-scale experimental work on the venting of explosions and of preventing the spread of flame. There is an excellent summary of precautions that can be taken against dust explosions in factories, suggestions of a number of aspects of the subject that merit early investigation and a list of more than 230 references. This critical review is far from the dry report it might have been. It is an important contribution to the subject of dust-explosion hazards and deserves wide circulation and publicity among those interested in the scientific and the practical industrial aspects of the problem.

Preventing Accidents in the Factory

EVERY year nearly 200,000 serious accidents occur in industry. Employers have legal and moral obligations to prevent accidents, but effective action towards accident prevention also needs co-operation from the man on the shop floor. To help employers in securing such co-operation from their workers, the Royal Society for the Prevention of Accidents has published a new booklet entitled *This is Your Life*, which states in simple, direct language how workers can help to prevent accidents to themselves and their fellow workers (Pp. 16. London: Royal Society for the Prevention of Accidents, 1962). Designed for distribution by employers to their workers, the booklet is written with a light touch and is illustrated with cartoons. It contains much sound advice on such highly practical matters as dressing for the job, safe operation of machines and tools, fire precautions, electrical hazards, personal hygiene, good house-keeping in the factory, first aid, and the avoidance of practical joking. In connexion with the last-named, there is a timely warning about the dangers of playing about with compressed air lines. Falls are a frequent cause of accident, and this danger is stressed in the booklet with appropriate illustrations.

Supplementary Letters of Sir Joseph Banks

IN December 1958 the Trustees of the British Museum (Natural History) published *The Banks Letters*, in which more than 7,000 letters were calendared of Sir Joseph Banks, the English naturalist (1743–1820). A number of further letters have now become available, and of the 139 letters summarized in a recent publication there are four important series, namely: (1) 38 letters from Banks to the antiquary, Francis Douce, in the Bodleian Library; (2) 28 letters from Banks to the second Earl Spencer, which are preserved in the Muniment Room at Althorp, Northamptonshire; (3) 26 letters from Banks to Dawson Turner in the Library of Trinity College, Cambridge; and (4) 16 letters from Banks to Matthew Flinders and his wife, now in the possession of Miss A. Flinders Petrie (*Bulletin of the British Museum (Natural History)*. Historical Series. Vol. 3, No. 2: *Supplementary Letters of Sir Joseph Banks*. Edited by Warren R. Dawson. Pp. 41–70. London: British Museum (Natural History), 1962. 8*s*.). Several letters are included from copies of which the originals are in American libraries; there is also a small series relating to the appointment of a chaplain to Banks while holding the office of High Sheriff of Lincolnshire in 1794, which were originally in the Evidence Room at Revesby Abbey. The remainder are miscellaneous in character, and among them are ten letters which were inadvertently passed over when *The Banks Letters* was in preparation. The publication contains a summary of each of the various groups of letters.

Comparative Efficiency of Indexing Systems

THE *Report on the Testing and Analysis of an Investigation into the Comparative Efficiency of Indexing Systems*—an investigation supported by a grant to Aslib by the National Science Foundation—is essentially for the specialist (Pp. 305. Cranfield: Aslib Cranfield Research Project, College of Aeronautics Library, 1962). After outlining the main test programme of this Aslib Cranfield Research Project, Mr. C. W. Cleverdon gives the results of the main test, the statistical analysis and analysis of failures and some results in testing existing indexing systems. Although he comments briefly on the results of the whole investigation and discusses briefly basic problems in retrieving information, from the point of view of the general reader or scientist this report adds little to the interim report already noted in *Nature* (189, 359; 1961).

Nonconventional Technical Information Systems

THE third of a series of reports on *Nonconventional Technical Information Systems in Current Use*, issued by the National Science Foundation, describes 87 such systems from 73 different organizations, six of whom reported two systems each, one three, and one seven (No. 3. NSF–62–34. Pp. xx + 209. Washington, D.C.: National Science Foundation, 1962). About half the organizations are new to the series, and three systems outside the United States are included. As in previous reports, the information is arranged under three main headings: systems which store references; systems which store data; and systems which produce general search aids. Comments on the compilation are invited and also information on other systems for possible inclusion in future reports.

The U.S. Office of Aerospace Research

Bulletin No. 15, September 1962, *NSF* 62–17, *Scientific Information Activities of Federal Agencies*, issued by the National Science Foundation, the first of four bulletins to deal with the U.S. Air Force, describes the activities of the Office of Aerospace Research, which is responsible for most of the basic research carried out or sponsored by the Air Force. Besides describing the various organizational units, the *Bulletin* includes information on the various fields of research, the progress being made and the types of publication issued.

A New High-sensitivity Colorimeter

A NEW Unicam *SP*. 1300 colorimeter, made by Unicam Instruments, Ltd., Cambridge, has a technical specification which incorporates many of the latest advances in colorimetry. Outstanding features include: (*a*) autofill cell—automatic system of filling and emptying cells, saving time and avoiding spillage (minimum volume of 10-mm glass sample well—2 ml.); (*b*) single lever control for three stages of analysis; (*c*) instant selection of filters by rotation of filter disk. Initially introduced with a series of sixteen clinical methods, the *SP*. 1300 is equally suitable for a very wide range of colorimetric analyses. The instrument will be supplied complete with a multiring binder containing operating instructions and methods of analysis for sixteen separate clinical determinations (other 'method sheets' will be made available to the users within a short space of time). The styling of this new Unicam instrument will fit well into the modern laboratory. It is compact, sturdy and easily transportable.

Ground Reflexion Interference Patterns

THE purpose of the National Bureau of Standards Monograph 38, issued on April 2, is to give the results of a detailed computation of ground reflexion interference patterns in the lower ionosphere at VHF over a spherical earth (*Radiation Patterns in the Lower Ionosphere and Fresnel Zones for Elevated Antennas Over a Spherical Earth*. Pp. iii + 128. Washington, D.C.: Government Printing Office, 1962. 70 cents). The computations cover parallax, tropospheric refraction and defocusing, spherical divergence, and near horizon diffraction. From the patterns, three antenna-siting parameters—the antenna height and elevation angle for placing the maximum of the first lobe at the path midpoint (called the lobe alignment height); the distances from the antenna to the edges and the quarter-wave contour of the first Fresnel zone on the Earth's surface; and information for the determination of effects of obstacles located in the first Fresnel zone—have been computed. The antenna height-gain function calculated from the results has been previously published (*Institute of Radio Engineers Transactions on Communications Systems CS*–8, 14–19; March 1960). The function shows that the optimum height is lower than the lobe alignment height and that a broad range of lower heights is essentially equivalent in gain to the lobe alignment height. The results presented in the monograph refer to an ionospheric scattering layer height of 85 km at frequencies of 30–55 Mc/s, which have been found to be the most useful values. They correspond to computed path lengths of 1,000–2,400 km. Two refractivities representing 'standard refraction' and temperate over-water or tropical 'wet' refraction were used. The monograph contains some hundred graphs, and in the appendixes lobe alignment antenna heights and other data, together with symbols and equations, are tabulated and a mathematical discussion of the lobe shift is given.

Farm Buildings Research

A BIBLIOGRAPHY, *Farm Buildings Research*, was issued by the Agricultural Research Council in 1958, covering publications of the previous three years. The first supplement to Part I of the general bibliography, covering publications of the three years ending in 1961, and also items omitted from the previous issue, has recently been published (Pp. 69. Agricultural Research Council, 1962. 4*s*.). This deals with buildings for pigs and provides a brief annotation for each reference quoted. An author index to the references is included.

Animal Disease in Israel

THE reports of the Veterinary Department in Israel for 1959 and for 1960 were published in Hebrew, but a report in English has now been issued for both years. Israel is inevitably an important area so far as the field of animal diseases is concerned. With its very central situation on certain travel routes between East and West and with some extensive terrain in its vicinity with, perhaps, less well-developed veterinary services, opportunities can occur of certain infective diseases to pass into regions that might be danger areas to other countries. With its strong scientific outlook Israel is a source of strength, in that new introductions of spreading disease are likely to be detected at an early stage, with quick introduction of control measures. In recent years, African horsesickness, as an escape from Africa, has been the cause of great losses as far afield as India—Israel being a stepping-stone in its progress. Bluetongue of sheep has similarly escaped from Africa and spread quite disastrously in recent decades. Similarly, African swine fever, caused by a virus different from that of the well-known swine fever, has invaded European countries with serious results, though, fortunately, active control measures have limited the risk to all but a few countries. Details are given of the activities in Israel for dealing with thirty-five scheduled diseases of mammals and with scheduled poultry diseases—the diseases given special attention in all countries because of particular liability to spread. The Government veterinary field service is handicapped by a serious lack of manpower. Rabies occurred from 1948 onwards in animals, including, as well as domestic animals, jackals, foxes, hyenas, wolves and a mongoose. There were reports of 69,995 persons being bitten, and 11,588 were given anti-rabies treatment.

Natural History of the British Solomon Islands

THE fourth volume of the scientific results of the joint Danish (1951) and British Museum (Natural History) (1953) expeditions to Rennell Island has been published under the general editorship of Torben Wolff (*The Natural History of Rennell Island, British Solomon Islands*. 4: (Invertebrates, Pars; and addition to Vertebrates). Pp. 120 + 18 plates. Copenhagen: Danish Science Press, Ltd. Published by the University, Copenhagen; and the

British Museum (Nat. Hist.), London, 1962. 35 Danish Kr.). Among the articles is one by J. E. Hill describing a little-known fruit-bat from Rennell Island. Diana Bradley has examined additional records of birds from Rennell and Bellona Islands, and Edward O. Wilson the ants of Rennell and Bellona Islands. Other articles include one by Carlton M. Clifford and Daniel Sohenshine on a new bat-infesting *Ornithodoros* (Ixodoiea, Argasidae) of the sub-genus *Reticulinasus* Schulze, 1941, from Rennell Island; J. Gaud on the bird parasites of Rennell Island, *Sarcoptiformes plumicoles* (Analgesoidea); J. P. Harding on *Mungava munda* and four other new species of ostracod crustaceans from the stomachs of fish; T. G. Howarth on *Rhopalocera* of Rennell and Bellona Islands; J. Balfour-Browne on Coleoptera from Rennell and Bellona Islands; R. T. Thompson on Curculionidae from Rennell and Bellona Islands; and Paul E. S. Sjalley on *Pyraloidea* from Rennell and Bellona Islands.

Deer Mouse in California

CALIFORNIA'S deer mouse is equally at home in mountains two miles above sea-level as at, for example, Death Valley, 200 ft. below sea-level. It is not known whether there is a fundamental difference in the physiology of the high-level as compared with the low-level mouse, or whether the differences are a matter of individual acclimatization. The mountain-top mice are heavier than the others, but they have relatively smaller adrenal glands. According to Dr. Raymond J. Hook, of the University of California, they have, as might be expected, larger hearts (*The Sciences*, 2, No. 6; August 15, 1962). Transplanted to lower altitudes, however, the already sluggish mountain-top mice lose 50 per cent of their endurance in the tread-mill test. The low-altitude mice also lose half their endurance when working at 12,000 ft. The decrease in efficiency when the mountain mice are brought to sea-level may be the result of 'oxygen drowning'—a not very well defined malady that is thought to have caused the deaths of some Peruvian Indians who left their homes at the turn of the century to work as servants in sea-level cities. To determine whether the differences in the mice are genetic, or merely the result of acclimatization, Dr. Hook is planning to raise litters at both altitude extremes, then move half of each crop of young deer mice to the opposite regions. His present expectation is that the mice will prove to be an adaptable species, adjusting themselves readily to environmental extremes.

The Institute of Biology

AT the fourteenth annual general meeting of the Institute of Biology, held on January 10, the following officers and members of Council were elected: *President*, Prof. G. E. Blackman; *Vice-Presidents*, W. H. Dowdeswell and Prof. I. A. Preece; *Honorary Secretary*, Dr. L. Broadbent; *Honorary Treasurer*, W. V. Harris; *Council Members*, K. G. Brocklehurst, Dr. J. A. Freeman, Dr. H. C. Gough, Prof. O. V. S. Heath and A. T. Thompson.

The Leopoldina German Academy of Sciences : Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Astronomy*, Prof. A. Unsöld (Kiel); *Chemistry*, Prof. F. Feher (Cologne); *General Biology*, Prof. J. Brachet (Brussels); *Physiological Chemistry*, Prof. J. Heller (Warsaw); *General Pathology and Pathological Anatomy*, Prof. J. Baló (Budapest) and Prof. Ilona Banga-Baló (Budapest); *Ophthalmology*, Prof. G. B. Bietti (Rome) and Prof. A. Bangerter (St. Gallen).

The Iron and Steel Institute

THE following elections to the Council of the Iron and Steel Institute have been announced: *President-elect*, F. B. George (managing director, Consett Iron Company, Ltd.); *Honorary Vice-presidents*, W. C. Bell (managing director, Iron and Steel Development, Stewarts and Lloyds, Ltd.), R. A. Hacking (metallurgical consultant to Richard Thomas and Baldwins, Ltd.), Sir Robert Shone (director-general, National Economic Development Council), C. H. T. Williams (chairman, Park Gate Iron and Steel Company, Ltd.); *Vice-presidents*, W. F. Gilbertson (assistant managing director, (production), Richard Thomas and Baldwins, Ltd.), Lt.-Com. G. W. Wells (chairman, Appleby-Frodingham Steel Company, and managing director (production), the United Steel Companies, Ltd.); *Members of Council*, R. W. Evans (director and general manager, Steel Division, the Steel Company of Wales, Ltd., Abbey Works), G. Foster (managing director (production), Dorman Long (Steel), Ltd., and director, Dorman Long and Company, Ltd.), L. R. P. Pugh (managing director, Guest Keen Iron and Steel Works, Cardiff).

Paul Instrument Grants Fund

THE Paul Instrument Fund Committee has made grants as follows: £1,550 to Dr. S. Evans, senior assistant in polar research, University of Cambridge, for experimental work in the Arctic, in continuation of the grant of £2,736 which was made in 1962 for the development of an instrument for measuring the depth of continental ice sheets by a radar technique; £3,000 to Prof. J. McMichael, professor of medicine in the University of London and director of the Department of Medicine, Postgraduate Medical School of London, for the development of a miniature electromagnetic flowmeter; £2,600 to Mr. J. Rawcliffe, lecturer in electrical engineering, and Prof. C. Adamson, professor of electrical engineering, in the Manchester College of Science and Technology, for the construction of a differential quartz thermometer. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

The Oliver Memorial Award for Work in Blood Transfusion

THE Committee of the Oliver Memorial Fund announces that it will make the fifteenth annual Award of £50 and plaque to any British subject whose original work or service in the field of blood transfusion is considered to be an outstanding contribution to the science, organization or donor service aspect of this subject. The Committee welcomes communications directing its attention to suitable candidates, and, in accordance with the rules, the Award this year will be restricted to members of the medical profession. These must be submitted by March 31, and should be sent to the honorary secretary, Mrs. F. E. Bedborough, 105 New Park Avenue, Palmers Green, London, N.13.

Mass Spectroscopy

A SHORT course of five lectures on "Mass Spectrometry" is to be held at Battersea College of Technology, during January and February. The lectures will be held on Thursday evenings beginning on January 24. The course is under the direction of Mr. G. R. Parlett. The topics to be included are the introduction and theory, medical, organic chemistry and metallurgical applications and instrumentation; speakers will come from both university and industry. Further information and application forms can be obtained from the Secretary (Spectroscopy Courses), Battersea College of Technology, London, S.W.11.

Plant Pathogens in Soil

AN international symposium, "Factors Determining the Behaviour of Plant Pathogens in Soil", has been organized by the National Academy of Sciences–National Research Council Committee on Biological Control of Soil-borne Plant Pathogens. It will be held at the University of California, Berkeley, during April 7–13, 1963. Because the objective of the symposium is primarily to facilitate discussions between active workers in this field, participation will be by invitation. Invitations have now been issued. Those who are actively studying biological control of soil-borne plant pathogens and who would like an invitation can apply to Dr. W. C. Snyder, chairman of the NAS–NRC Committee, Department of Plant Pathology, University of California, Berkeley 4, before March 15, 1963.

University News :

Belfast

THE following appointments to lectureships have been announced: Dr. T. Williams (light electrical engineering); A. H. Garmany Love (medicine); C. W. R. McCreary (mycology and plant pathology).

Birmingham

THE following titles have been conferred: *Professor*, Dr. P. L. Krohn (endocrinology). *Reader*, Dr. R. L. Riddiford (high-energy physics); Dr. Anita Mandl (reproductive physiology); Dr. E. H. Ashton (primate anatomy); Sir Francis Knowles (comparative endocrinology); Dr. J. B. Finean (molecular biology). The following appointments have been announced: *Lecturer*, Dr. Bertha Singer (physiology). *Research Fellow*, R. F. Cheeney (geology); J. A. McCracken (physiology); Dr. H. S. H. Sherratt (medical biochemistry and pharmacology); Dr. E. F. Marshall (experimental neuropharmacology).

Borough Polytechnic

IN view of the fact that the advanced work in the Borough Polytechnic, London, S.E.1, is increasing rapidly session by session, both in volume and complexity, the former Department of Electrical Engineering and Physics is to be divided into two parts—one a separate Department of Physics under the headship of Mr. A. J. L. Collinson, and the other the Department of Electrical Engineering which will continue with Mr. V. Pereira-Mendoza as head. In addition, the former Department of Chemistry and Food Technology has been divided. One portion, known as the Department of Chemistry and Chemical Technology, will function under the headship of Dr. S. Cotson, and the other, designated the Division of Food Science and Technology, will be under the headship of Dr. D. B. Smith. In addition to this reorganization, all work in science and engineering up to and including Ordinary National Certificate Level will be grouped in a General Science and Engineering Section in charge of Mr. L. M. Clark and Mr. C. E. T. Maguire.

The College of Aeronautics

THE following appointments have been announced: J. Murdoch, senior research fellow in the Department of Production and Industrial Administration, to the post of senior lecturer, with effect from January 1, 1962; Dr. P. L. B. Oxley, lecturer at the Manchester College of Science and Technology, to the post of senior lecturer in the Department of Production and Industrial Administration, with effect from July 1, 1962; A. Battersby, senior technologist, British Petroleum, Ltd., to the post of senior organizer in the Work Study School, with effect from September 1, 1962; G. S. Ram, senior scientific officer, National Aeronautical Laboratory, Bangalore, to the post of research fellow in the Department of Aerodynamics, with effect from May 1, 1962; R. J. L. McLaren, engineer, English Electric Co., Ltd., to the post of research fellow in the Department of Aircraft Propulsion, with effect from September 1, 1962.

Leeds

THE following appointments to lectureships have been announced: Dr. M. P. Crosland and J. E. McGuire (history and philosophy of science in the Department of Philosophy); Dr. J. G. Shoesmith (bacteriology); J. W. T. Richards (psychology).

London

THE title of professor emeritus of western asiatic archæology in the University has been conferred on Prof. M. E. L. Mallowan. (Prof. Mallowan was professor of western asiatic archæology at the Institute of Archæology from 1947 until 1962.) The title of professor of molecular biology in the University has been conferred on Dr. M. H. F. Wilkins, in respect of his post in the Department of Biophysics at King's College. Dr. L. B. Strang, senior lecturer at the Institute of Child Health, has been appointed to the readership in pædiatrics tenable at University College Hospital Medical School.

Nottingham

THE title of professor has been conferred on John Edward Adkins, at present reader and head of the Department of Theoretical Mechanics, who is well known for his work on non-linear continuum mechanics with special reference to non-linear elasticity.

Southampton

AN independent Institute of Sound and Vibration Research is to be established within the Faculty of Engineering with effect from October 1. The first director of the Institute will be Prof. E. J. Richards, at present professor of aeronautics and astronautics in the University

Announcements

MR. P. M. HOLLINGSWORTH has been appointed deputy chief engineer of British Insulated Callender's Cables, Ltd. Mr. Hollingsworth joined the former British Insulated Cables Ltd. in 1928 after graduating from the University of Liverpool. In 1938 he went to Johnson and Phillips, Ltd. as manager, Cable Test and Research Department. He rejoined B.I.C.C. in 1954 as chief engineer, Power Cables Division, afterwards becoming assistant chief engineer.

AT the annual general meeting of the Photobiology Group, held at the Institute of Ophthalmology, on October 19, Dr. L. R. Fisher of the National Institute for Research in Dairying, Reading, was elected honorary secretary in succession to Dr. D. Vince. Dr. H. J. A. Dartnall of the Institute of Ophthalmology was elected as chairman to succeed Prof. N. Millott.

THE pharmaceutical business operated by the Distillers Co. (Biochemicals), Ltd. has been transferred to the Eli Lilly Group of Companies as from January 1. The business is being operated by a new company named Dista Products, Ltd.

A SHORT course for graduates on "Flow Processes in Polymeric Systems" will be held at the Bradford Institute of Technology during March 8–9. Lectures to be delivered will include: modern trends in rheology (Prof. K. Weissenberg); rheology of polymer melts (Dr. E. R. Howells); rheology of concentrated polymer solutions (Dr. D. W. Saunders); measurements of flow properties in polymeric systems (Dr. M. F. Culpin); rheology of surface coatings (T. R. Bullett); viscosity–temperature relationships for polymer solutions (Dr. W. R. Moore). Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

THE jubilee meeting of the British Ecological Society will be held at Queen Elizabeth College, Campden Hill Road, London, W.8, during March 28–31, and not March 8–31 as announced in *Nature*, 196, 1271; 1962.

THE tenth international symposium on "Combustion", organized by the British Section of the Combustion Institute, will be held at the University of Cambridge during August 16–21, 1964. Further information can be obtained from Dr. W. G. Parker, Department of Chemistry, College of Advanced Technology, Gosta Green, Birmingham 4.

THE BRITISH COKE RESEARCH ASSOCIATION

THE annual report for 1961 of the British Coke Research Association, Chesterfield, is noteworthy not only for the review of progress in all aspects of the Association's recent activities but for special mention of a number of new ventures which were begun during the year*. Support by the Department of Scientific and Industrial Research had enabled the Association to provide member-organizations with several new services in the field of technical information, the purpose being to further the effect with which the results of research can be applied in industry. The new developments have covered briefly the establishment of a more comprehensive abstracts service, improved technical liaison between the Research Centre and producers and consumers of coke, a field trials officer and a fully equipped mobile laboratory to demonstrate among other activities methods of sampling, analysis and testing of coke, and the assessment of smoke emission from coking plant. The provision of a mobile laboratory for plant trials under industrial conditions is becoming a most valuable instrument of technical advancement in a wide range of practice.

This Association is unique for the fact that it is the only co-operative research organization in the world that is devoted solely to the study of the production of coke and its associated problems. The Research Centre in Chesterfield has accordingly become a focal point for the industry. There is special interest in the fact that the Centre is capable of carrying out modern research on a large scale, as well as the fundamental and applied research that must remain the source of the basic ideas on which progress in many techniques associated with coking plant must depend. The result has been a growing flow of interested visitors from other technical organizations in Britain and overseas.

As to the achievements of the year and the use of the test plant in regard to the improvement in the quality of coke and yield of metallurgical plants, the investigations have been concentrated on the effect of variation of specific operating conditions. Thus the addition of breeze in the charge, pre-drying, the addition of wet or partially dried filter coke, the rate of carbonization and the use of high bulk density. The use of the 10-ton oven in evaluating with an established accuracy the relative merits of such variables defines, in effect, an optimum practice, which could not readily, by other means, be ascertained.

In respect of the fundamental studies of the structure and properties of coke an interesting preliminary investigation has been made of the potentialities of polarized light microscopy for the study of coke structure. The early indications are that the optical method utilized is probably more informative in respect of structural orientations of significance than some X-ray techniques, but that its application to technical cokes may be limited by the sampling difficulties. Investigations of magnetic data collected on a variety of cokes and carbons have given rise to tentative conclusions of significance in respect of the reactivity of coke. Thus the magnetic and probably chemical behaviour of high-temperature coke can be attributed, in part, to the excess or deficiency in the mobile electrons normally associated with the graphitic crystallites in the coke. These are influenced in their turn by the origin and temperature of formation of the coke.

The layman may be interested to learn that the subject of the tailoring of the domestic coke to suit the small boiler used for hot-water heating has been receiving important attention. Conclusions relative to the size, shape and ash content of the coke in this context inevitably have their effect on the character of the coke to be found in the market. Most favourable features appear to lie with the ½–1-in. coke.

Under the sponsorship of the Association are the liquid effluent studies carried out in the Houldsworth School of the University of Leeds. A particularly valuable service has been the biological assessment of coking-plant wastes in those cases where some form of treatment plant is envisaged. The biological treatment of spent liquor from ammonia stills is a relatively easy matter, but certain rogue liquids also produced in a by-product plant can do permanent damage to the activated sludge. These effects have been traced to the hydrogen sulphide and cyanide contents of the waste liquors.

In the Northern Coke Research Laboratory at Newcastle the research objectives included the study of the structure, reactivity and electron-spin resonance properties of carbons prepared from organic polymers. The maximum free-spin concentrations were shown for carbonization temperatures between 500° and 700° C. Two problems were revealed: (1) in the way in which the unpaired electrons were associated with the carbon structure; (2) in the effect of the heat-treatment temperature of the carbonized products on the observations made. Other experimental techniques would have to be considered in the final interpretation of the observations made, which could be of considerable interest from the point of view of reactivity. As the problem is looked at from the angle of the metallurgist in the manufacture of iron and steel it is a relatively simple matter to produce reactivity, but what is more frequently required, and difficult, is to produce unreactivity. To be able to understand the why and the wherefore is to go a long way towards solving the problem. Further, in respect of the domestic needs, the attainment and control of reactivity in respect of coke supplies is a most important matter, since it controls that most important requirement—the rate at which the fuel burns.

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*British Coke Research Association. Eighteenth Annual Report for the year 1961. (Chesterfield: British Coke Research Association, 1962.)

FOREST PRODUCTS IN CHILE

IN the recently created Instituto Forestal* in Santiago, Chile, representatives of all Chilean forest products laboratories joined in a conference on the "Co-ordination of Forest Products Research" during August 30–31, 1962.

The Instituto Forestal commenced its activities in November 1961 as the result of an agreement between the Chilean Government and the United Nations (Special Fund and Food and Agriculture Organization). Under its draft statutes, its object is "to contribute to the promotion, development, and investigation of the forest resources and industries within the territory of this country [Chile]". It comprises four technical sections, namely, forestry, forest industries, marketing, and extension. Research on forest products is being undertaken in collaboration with established forest products departments in the various universities of the country, as the Institute does not have its own laboratory.

The purpose of the conference was to co-ordinate research on forest products in Chile, and to agree on a plan of co-operation, in order to encourage research projects of national importance.

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* Postal address: Instituto Forestal, Casilla 3085, Santiago de Chile.

The research needs of the Chilean wood-using industry were presented by A. Pérez, the Institute's officer-in-charge of forest products research. He directed attention to the urgent necessity for applied research and education in the basic conversion processes such as sawmilling, veneer and plywood, preservation, seasoning, and pulping, as well as for fomenting auxiliary industries such as manufacture of adhesives. The Institute's Food and Agriculture Organization adviser on research, Dr. W. G. Kauman (senior research officer, C.S.I.R.O., Division of Forest Products, Australia; on leave of absence), pointed out that of the three main types of research, that is: (1) short-term problem solving; (2) determination of basic properties; (3) academic research, the second constitutes the most urgent task for the university laboratories, but that the third must be given due importance to ensure a future supply of professional and other academic staff.

Prof. G. Mittak, of the recently formed Austral University of Valdivia, emphasized the importance his laboratory is attaching to wood anatomy, in particular identification, and to drying of difficult *Nothofagus* species. For the Catholic University of Santiago, H. Ayarza presented a comparison of the mechanical strength of *Pinus radiata* determined on small clear specimens and on commercial sizes, and work on various timber joints and connectors. Research on timber in the Institute for Testing Materials in the University of Chile, Santiago, as outlined by H. Albala, is mainly concerned with mechanical strength of Chilean timbers. Due attention is being given to statistical design of the experiments, a factor which has been largely neglected in much of the past work in Chile. Nineteen species have so far been investigated. E. Cuevas presented work on timber physics and preservation in the Forest Engineering School of the University of Chile. Interesting results have been obtained on the frequency distribution of density in fifteen species, and correlations of other properties with density are being examined. E. Bluhm, of the University of Concepción, stated that his laboratory is concentrating on wood chemistry and pulp and paper, and reported a laboratory project on pulping by the nitric acid process. In the Technical University Federico Santa María of Valparaíso, forest products work so far has dealt with pulping, minor forest products and utilization of lignin. Prof. R. Ripa reported on an investigation of production of detergents from sulphonated lignin derivatives. Prof. J. Hirschmann, of the same University, gave details of a new professional course for timber and plastics engineers, now in its third year.

The participants agreed on a plan for co-ordinated research on forest products proposed by Dr. W. G. Kauman, of the Instituto Forestal, and resolved to work in close collaboration to counteract the difficulties created by the dispersion of facilities.

GAMMA-RAY STERILIZATION OF MEDICAL UTENSILS

THE world's first privately owned cobalt-60 irradiation plant designed specifically for medical purposes began continuous operation on November 10 at Johnson's Ethical Plastics Ltd., Slough. It is to be used to sterilize disposable, or single use, plastic hypodermic syringes. The only other plants which resemble it are the United Kingdom Atomic Energy Authority's experimental unit at Wantage, Berks., and a privately owned installation in Australia used for sterilizing goat hair. The plant at Slough was designed and constructed by H. S. Marsh Ltd., Reading, in collaboration with the United Kingdom Atomic Energy Authority.

The radioactive source which emits the sterilizing γ-rays consists of 120 cobalt rods which have become radioactive by treatment in a nuclear reactor at Calder Hall. The rods have a half-life of five years. The capacity is 50,000 curies, but provision has been made to measure the number of rods to a maximum of 720 with a possible rise to half-a-million curies. The cell in which the cobalt is loaded is a concrete block weighing some 700 tons, and occupying most of the space of the building of 2,500 sq. ft., which houses the plant.

The main object of the disposable or single-use syringes is to reduce or avoid the incidence of cross-infection. Gamma irradiation provides the safest and surest way of assuring sterility. The syringes are made of polystyrene, the grummets of surgical rubber and the needles of stainless steel. It is claimed that it is more economical to use disposable syringes than to wash and clean glass syringes, clean and sharpen needles, and sterilize them by orthodox methods. The present production and sales of disposable syringes is more than 12 million per annum.

At the inauguration luncheon held on November 9 in London the guest of honour was Sir Roger Makins, chairman of the United Kingdom Atomic Energy Authority, and it was announced that to mark the occasion of the opening of the cobalt-60 plant, the Medical College of St. Bartholomew's Hospital, London, had accepted a grant of £2,400 spread over three years made by Johnson's Ethical Plastics Ltd. The money is to be used by Prof. J. Rotblat in the Department of Physics to employ a radio-biologist as assistant in his radiation research.

RESPONSE OF AN ASTATIC MAGNETOMETER TO A CYLINDRICAL ROCK SPECIMEN

By J. L. ROY

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SPECIMENS of weakly magnetized rocks are often cut in cylindrical form for measurement with an astatic magnetometer. It will be shown here that the response of a two-magnet astatic system depends on the relative dimensions of the specimen, that is, on the ratio of height to radius. A knowledge of the nature of this response variation is important if, as is usually the case with weakly magnetized rocks, the largest possible deflexion for a given intensity of magnetization is required. The standard practice has been to use rock specimens in the form of thin disks. It is concluded that the height of cylindrical specimens should be comparable with their diameter.

The design theory and description of an astatic magnetometer can be found elsewhere[1-3]. In brief, the intensity and direction of magnetization of a specimen of magnetic moment p are measured by placing the centre of the specimen at a distance z below the axis of the lower magnet (of the magnetometer) and at a distance $x < z$ from the plane of magnetization of the magnetometer.

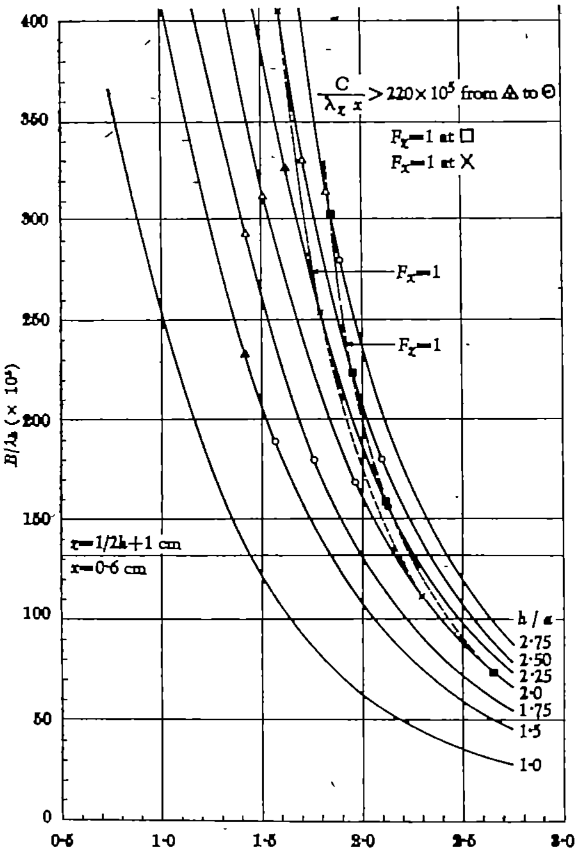

Fig. 1. Variation of B/λ_h with $z/a = v$ for given ratios $h/a = u$ calculated from equation (10)

It can be shown that, if the distance between the two magnets of the system is much larger than z, the vertical component p_z of the magnetic moment of the specimen is given by:

$$p_z = CH_1z^4/3x \quad (1)$$

H_1 is the field required at the lower magnet to produce a deflexion of 1 mm at a scale placed at a fixed distance from the instrument and is given in oersteds per mm deflexion. C is the linear deflexion in mm measured at the scale.

The horizontal component p_x perpendicular to the plane of magnetization of the magnetometer is given by:

$$p_x = B_x H_1z^3 \quad (2)$$

The horizontal component p_y parallel to the plane of magnetization is obtained by rotating the specimen through 90° in azimuth and is given by:

$$p_y = B_y H_1z^3 \quad (3)$$

and the total horizontal component $p_h = (p_x^2 + p_y^2)^{1/2}$ of the magnetic moment can be written:

$$p_h = (B_x^2 + B_y^2)^{1/2} H_1z^3 = BH_1z^3 \quad (4)$$

In the calculations of these expressions, it has been assumed that the dimensions of the specimen were small compared with the distance z. Very often, however, it is necessary, owing to the weak magnetization, to cut large specimens with dimensions comparable to z. Papapetrou, quoted in Blackett[1], has calculated the horizontal fields produced outside and near the vertical axis of a circular cylinder of radius a, height h and uniformly magnetized. The expressions obtained show that, if the geometrical centre of the specimen is placed at the same distance $(x^2 + z^2)^{1/2}$, with its axis vertical, the foregoing expressions can be used provided that equation (1) is divided by the correcting factor F_z and equations (2) to (4) are divided by $F_x(= F_y = F_h)$ where:

$$F_z = \frac{1}{6U}\left[\frac{1}{\{(1-U)^2+V^2\}^{3/2}} - \frac{1}{\{(1+U)^2+V^2\}^{3/2}}\right] \quad (5)$$

$$F_x = \frac{1}{2UV^2}\left[\frac{1-U}{\{(1-U)^2+V^2\}^{1/2}} - \frac{1+U}{\{(1+U)^2+V^2\}^{1/2}}\right] \quad (6)$$

$$U = h/2z \text{ and } V = a/z$$

Size and ratio of height to radius of specimen. If we define λ_h and λ_z as the horizontal and vertical intensities of magnetization of the specimen, we have (using the previous expressions):

$$B/\lambda_h = \pi a^2hF_x/H_1z^3 \quad (7)$$

$$C/\lambda_z = 3\pi xa^2hF_z/H_1z^4 \quad (8)$$

In general, these ratios should be made as large as possible so that large deflexions can be obtained from weak intensities of magnetization and so that the effects of artificial disturbances affecting the magnetometer, such as vibrations and drift, can be minimized.

We shall, then, investigate the manner in which these ratios depend on the dimensions of a cylindrical specimen.

If K is the vertical distance from the centre of the lower magnet to the upper surface of the specimen, then:

$$z = (1/2).h + K \quad (9)$$

The response ratios B/λ_h and C/λ_z can be expressed in terms of the quantities $u = h/a$, $v = z/a$ and K. Using these variables, equations (7) and (8) are written:

$$B/\lambda_h = \pi uF_x/H_1v^3 \quad (10)$$

$$C/\lambda_z x = 3\pi u\{v - (1/2).u\}F_z/H_1Kv^4 \quad (11)$$

In Figs. 1 and 2, B/λ_h and $C/\lambda_z x$ are plotted against v for fixed values of u, assuming $H_1 = 4{\cdot}7 \times 10^{-5}$ oe/mm defl. and $K = 1$ cm. For values of K other than 1 cm, equation (11) shows that the ordinates in Fig. 2 would have to be divided by K. For values of H_1 other than $4{\cdot}7 \times 10^{-5}$ oe/mm defl., the ordinates of both figures would have to be multiplied by $4{\cdot}7 \times 10^{-5}/H_1$.

Figs. 1 and 2 show that while B/λ_h increases indefinitely as v decreases, C/λ_z, for a given x, goes through a maximum as v is varied. Thus, while it is desirable to have both these ratios large, care must be taken to choose values of u and v which do not increase B/λ_h unduly at the expense of C/λ_z.

In Fig. 2, a line has been drawn at $C/\lambda_z x = 220 \times 10^5$ in order to form a comparison between values of B/λ_h and C/λ_z. The values of h/a and z/a for which $C/\lambda_z x = 220 \times 10^5$ are carried over to Fig. 1, where the corresponding values of B/λ_h are shown. If x is taken to be 0·6 cm (this value can be considered as the maximum permissible

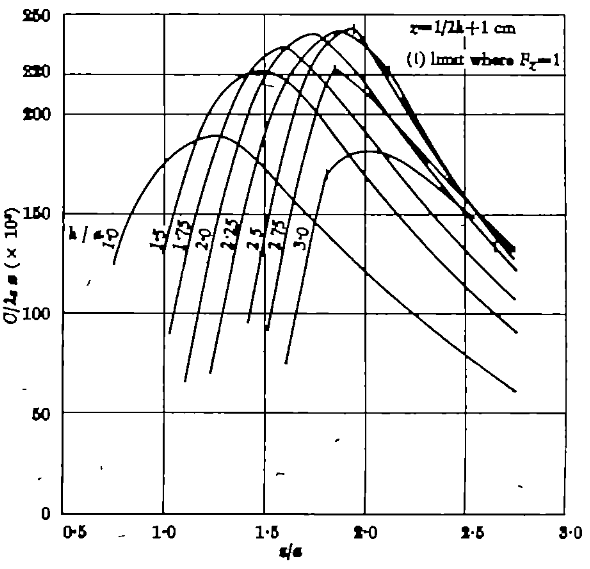

Fig. 2. Variation of $C/\lambda_z x$ with $z/a = v$ for given ratios $h/a = u$ calculated from equation (11)

value of x since the calculations are made under the assumption that x is much smaller than z which, in general, is of the order of a few centimetres), then $C/\lambda_z = 132 \times 10^5$ and a line is drawn in Fig. 1 showing this maximum value.

It is clear that, for any combination of u and v giving $C/\lambda_z = 132 \times 10^5$, we have:

$$B/\lambda_h > C/\lambda_z \qquad (12)$$

when $K = 1$ cm and $x = 0{\cdot}6$ cm. Furthermore, an increase of K to any value greater than 1 cm or a decrease of x to any value less than 0·6 cm will decrease C/λ_z proportionately (11). Although a reduction of K to a value less than 1 cm would increase C/λ_z, a damping disk beneath the lower magnet usually prevents a much closer approach of the specimen. Thus, the foregoing inequality holds for all practical values of K and x. It is concluded, therefore, that u and v should, in general, be chosen so that C/λ_z is close to its maximum possible value. Fig. 2 indicates that this condition can be fulfilled if:

$$1{\cdot}5 \leqslant h/a \leqslant 2{\cdot}75 \qquad (13)$$

and the appropriate value of z/a is chosen.

An exception to this rule might occur with a specimen for which B/λ_h and hence B is very small. In this case, it might be desirable to increase B/λ_h at the expense of C/λ_z in order that both B and C be large enough for accurate measurement. This could be done by increasing u and/or decreasing v. It should be noted, however, that the absolute dimensions of a specimen are given in terms of u, v and K by:

$$\begin{aligned} h &= 2uK/(2v - u) \\ a &= 2K/(2v - u) \\ z &= 2vK/(2v - u) \end{aligned} \qquad (14)$$

These relations show that h and a increase indefinitely as u approaches $2v$. Thus the process of increasing u or decreasing v cannot be carried beyond the point at which practical dimensions of the specimen are exceeded. Besides requiring large coring equipment and magnetic washing apparatus, large specimens might also be difficult to collect in the field.

It has been noted that optimum values of $u = h/a$ are, in general, such that the height is approximately equal to the diameter. It is clear from both figures that the use of thin disks leads to reduced response to both horizontal and vertical components of magnetization in the specimen.

Elimination of the correcting factors: F_z and F_x. Since F_z is required in equation (8) because the dimensions of the specimen are comparable to z, it is clear that F_z will tend towards unity if for a given ratio h/a z/a is made large enough.

The value of $v = z/a$ required to make F_z approximately equal to unity ($F_z > 0{\cdot}995$) has been calculated from equation (5) (for the various fixed values of $u = h/a$ considered here) and is shown in Fig. 2 by a short vertical stroke drawn across the curve of h/a used in the calculation. Any combination of h/a and z/a lying to the right of the curve which could be drawn through these strokes gives a value of F_z equal to unity. We see, then, that F_z will be equal to unity and, at the same time, $C/\lambda_z x$ will be larger than 220×10^5 if h/a is chosen so that:

$$2{\cdot}25 \leqslant h/a \leqslant 2{\cdot}75 \qquad (15)$$

with appropriate ratio z/a.

Similar calculations have been made for F_x and the results are shown in Fig. 1. Any combination of h/a and z/a lying to the right of the dashed line X give a value of F_x equal to unity. Now, carrying the combinations of h/a and z/a giving 'limit where $F_z = 1$' from Fig. 2 to Fig. 1, we find that, whenever h/a and z/a are chosen so that $F_z = 1$, then F_x is automatically equal to unity, since the curve of $F_z = 1$ lies to the right of the curve of $F_x = 1$.

We conclude, therefore, that it is preferable to cut a specimen according to the dimensions given by equation (15) since, besides obtaining the largest deflexions B and C, we also eliminate both correcting factors F_z and F_x.

In palæomagnetic work, the distance K is determined by the degree of non-uniformity of magnetization of the specimen and should be chosen large enough so that the error due to this inhomogeneity is negligible.

I thank Dr. E. R. Niblett for help with preparation of this manuscript.

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[1] Blackett, P. M. S., *Phil. Trans. Roy. Soc.*, A, 245, 303 (1952).

[2] Collinson, D. W., and Creer, K. M., *Methods and Techniques in Geophysics*, 1, 168 (Interscience Publishers, London, 1960).

[3] Roy, J. L., *Publ. Dominion Obs.* (In the press).

CLASSIFICATION OF CALDERAS: 'KRAKATOAN' AND 'GLENCOE' TYPES

By Dr. G. J. H. McCALL
University of Western Australia, Perth

TWO short communications by Yokoyama and Tajima[1] and Yokoyama[2] have recorded the recognition of low and high Bouguer anomalies, centred on 'Krakatoan' and 'Glencoe' type subsidence calderas respectively. These two communications from Japan were of considerable interest to me, because I have examined a group of similar but smaller calderas in the central rift valley of Kenya.

I initially studied the 35 square mile subsidence caldera of Menengai[3,4]; striking similarities to Santorin and Aso[5] led me, at that time, to invoke a 'Krakatoan' mechanism. Later, however, a detailed study of the caldera was made (McCall, "Geology of the Nakuru–Thomson's Falls–Lake Hannington Area", awaiting publication, Geological Survey, Kenya) and the smaller (3 sq. miles) Kilombe caldera to the west of Menengai was briefly examined (McCall, unpublished results); the calderas of Suswa volcano to the south were also examined (McCall, unpublished results, investigation in progress in collaboration with C. M. Bristow) and these recent investigations together with a visit to Norway, where the Baerum cauldron, near Oslo[6,7], came to my notice, have led me to revise my earlier interpretation of the Menengai caldera, an interpretation which appears to have been too simple. Menengai closely matches the Baerum cauldron in shape, position and orientation in a meridional graben, in size, in the presence of narrow magmatic screens along the marginal fractures (vestiges of a ring feeder of trachyte-obsidian and pumice have now come to light in the south-east and southern margins of the caldera, following on the recognition of a much more completely preserved ring-feeder in the outer caldera of Suswa), and in the local repetition of the bounding fracture (evidenced in the case of Baerum by the repetition of the narrow syenitic ring-dykes, and in the case of Menengai by step-faulting in the caldera walls). It seems certain that Menengai represents the surface expression of a subsidence cauldron such as Baerum, the much lesser degree of erosion experienced by the Plio-Pleistocene structure of Menengai allowing for the preservation of the surface-levels, completely removed by erosion in the case of the Permian structure of Baerum. Menengai is then, primarily, a 'Glencoe' type subsidence caldera[8].

What then of the very real 'Krakatoan' characteristics of Menengai, the strong resemblance to Santorin and Aso, both in the form of the caldera and the characteristics of the associated eruptions ? Can it be that the 'Glencoe' mechanism is more commonly associated with calderas of subsidence than Williams in his well-known definitive treatment of the caldera problem[5] allows. I feel that this is so, that the 'Glencoe' mechanism is the primary mechanism involved in the majority of subsidence calderas, and that the 'Krakatoan' mechanism is in the nature of a special case, a complication of the 'Glencoe' mechanism, operating where volatiles are particularly abundant within the magma chamber at the time of subsidence. Complete gradation is believed to be possible between calderas showing only the effects associated with ring-fracture subsidence and the famous catastrophic 'Krakatoan' calderas. Menengai is supposed to exemplify a more weakly 'Krakatoan' sub-type, the explosive eruptions of pumice, which initiated the caldera, being nowhere nearly as strongly represented as in the case of Krakatoa, Santorin and Aso[6]. The outer caldera of Suswa, though larger than Menengai (43 sq. miles), shows less evidence of initiating pumice eruptions, Kilombe shows little or none at all, and the inner caldera of Suswa shows none. In spite of these dissimilarities, all these calderas are believed to have stemmed from the same primary process: the fundamental process and the secondary variations imposed on it are evisaged by me as outlined here.

(1) Main sequence of eruption from a broad shield volcano: up-doming by the agency of magmatic distension is a likely adjunct; the typical products of this main phase of eruption are lava flows, showing weathered surfaces indicative of periods of quiescence, punctuating the sequence of eruptions.

(2) The eruption, as a whole, wanes and the superstructure, probably up-arched by the main eruptive upsurge, collapses; two possible causes of this collapse may be entertained: a purely gravitational collapse and a deflation in which magma plays an active part. In the first case, the superstructure founders due to withdrawal of support. Rapid emptying of the magma chamber is usually invoked to explain this; but another factor may well be the increase in volatile content held dissolved in the residual magma, causing a decrease in viscosity. The watery end-stage magma so produced would provide a poor cushion for the superstructure, while the previous more viscous magma would sustain it. The alternative to the purely gravitational hypothesis, suggested by Oftedahl in the case of Baerum cauldron[7], invokes recession of the magma within the chamber, a cylindrical block following the magma downwards, that is being drawn down by the receding magma. Whatever the motivating force, the initiating movement downwards will open up steeply inclined fractures. These will be of essentially concentric pattern, but some complication by a limited degree of control exerted by other tectonic lines of weakness—often the intersecting fracture lines which control the position of the volcanic focus in the first place—will, perhaps, be reflected in rectilinear patterns in the caldera walls and lateral sector grabens.

(3) This initial movement downwards will set off a subsidiary reaction; the opening of the superstructure causes a sudden pressure release and the volatile-rich residual magma suffers ebullition, surging up ring-fracture conduits. If this magma is heavily charged with volatiles, pumiceous material will be erupted in vast quantities, in a series of rapid and explosive eruptions from vents arranged roughly on the trace of the ring fractures at the surface. In the case of magma with somewhat less volatile content ebullition will be weaker, and little or no ring-fracture eruption will ensue. It has been found, in the case of the Kenya calderas, that the magma composition varies only slightly throughout the eruptive sequence, and that the silica-saturated lavas, such as trachytes, are associated with evidence of more violent ebullition in this phase, having apparently more inherent volatile content, while undersaturated lavas, such as phonolites, are associated with evidence of less violent ebullition.

(4) The descent of the foundering block, which includes the superstructure and the solidified upper part of the pluton, will be stayed by violent ebullition, the gas upsurge sustaining it without further subsidence for perhaps hours or days, but, once the secondary ebullitive phase has waned, the cylinder will continue on its downwards path. This picture seems compatible with the fact that the pumice erupted in the ebullitive phase of eruption is typically truncated by the caldera fracture. In the case of certain calderas, associated with weak ebullition, the relationships observed may be different, since the pressure of ebullition is insufficient to stay the subsiding cylinder. If pumiceous products of ebullition do reach the surface, in this case they will be erupted on to the already lowered floor of the caldera. The small amount of pumice-tuff infilling the very bottom of Kilombe caldera (*op. cit.*) may well represent such a case. Sometimes only a steam-blast will reach the surface.

(5) New cycles of eruption may occur after cauldron subsidence; usually the area of activity is smaller than in the first cycle, suggesting a restricted reactivation within the original pluton. After the main upsurge of this phase of renewed eruption has waned, a second cauldron subsidence may ensue. This has occurred in the case of Suswa (*op. cit.*). However, because of the fracturing, which the superstructure will have undergone in the first episode of subsidence, it is likely that the pressure seal will never be as efficient as before, so eruptions are not likely to be so explosive in this second subsidence cycle as in the first—unless differentiation leading to more volatile-rich magma has occurred. In the case of Suswa no explosive activity heralded the formation of the second caldera.

(6) In the case of a few subsidence structures of this type, the cylinder may fail to resume its movement downwards after the ebullitive phase: this would produce an embryonic caldera, an annular pattern of vents. This pattern seems to occur within the Valles caldera, New Mexico[8], and is also seen in the internal crater progression of Clavius on the Moon.

Perhaps the most vexed question involved in the explanation of subsidence calderas is the volume deficit. Williams's calculations[5,9,10] of the volume of pumice emitted during the formation of Crater Lake, Aso, Santorin, Coseguina, and Hakone calderas led him to conclude that the volume of pumice invariably showed a deficit against the volume of the caldera formed. Magma movement at deep levels, involving dyke injection, was suggested as the agency responsible for the extra volume unaccounted for. Williams's conclusions regarding calderas[5,9,10] provide the basis of any study in this field and his unbiased assessment of the evidence has never been challenged. However, I suggest the possibility that no deep-seated migration of magma, other than injection into ring-fracture conduits on ebullition, is necessary to account for the volume deficit. Updoming, caused by magmatic distension, is known to accompany volcanic eruption (for example, ref. 11) and some relaxation and withdrawal of the magma-level without bodily migration at the end of the eruptive sequence seems acceptable. Shrinkage on crystallization, loss of volatiles, and a tendency for eruptions to continue under the influence of secondary triggering mechanisms, such as pressure release, long after the primary upsurge (whatever its nature) has waned, may all contribute to a process of simple deflation, which may, as has been suggested, be the primary agency involved in cauldron subsidence. No computations as carried out by Williams (*op. cit.*) have yet been attempted for the Kenya calderas, but observations suggest that the deficit is proportionally greater in the case of these weakly 'Krakatoan' calderas than in the catastrophic type-calderas cited by Williams.

Besides the volume deficit, there is a second anomaly apparent in the equation of subsidence calderas and cauldrons. Inwards-dipping feeder-dykes have been recognized, marginal to the calderas of Crater Lake, Oregon[9], Suswa and Menengai. In the case of Suswa the dip can be seen to steepen downwards. A study of the literature, however, reveals that ring-dykes associated with cauldrons show steep outwards dips or are vertically inclined. Rare exceptions have been attributed to later movements. If the ring feeders are the surface reflexion of ring-dykes, why this reversal of dip? Belousov[12] has shown experimentally that at the surface or near to a surface a subsiding cylinder in clay produces ring fractures exactly as seen in the Kenya calderas, inclined inwards and steepening downwards. Anderson[13,14] calculated mathematically that such a downward movement should produce steep-outward inclined fractures, which is the normal cauldron pattern. The probability would seem to be that Anderson's pattern is valid under strong containing pressure, but where the rock/air interface above the structure affects the picture, Belousov's pattern develops. In three dimensions, then, ring-dykes at deep levels should steepen and flare out into reversed cone form.

The structure envisaged seems important since it involves vertical movement producing both normal and reversed fault displacements, and suggests a higher tensional zone and a lower compression zone. Calderas are, in my view, annular analogues of the rift valleys in which predominantly vertical movements are involved (the predominant type in Kenya) and the same processes of magmatic distension, eruption, deflation and subsidence (the latter probably involving dragging downwards) seem to have produced both types of structures. If the fracture pattern suggested, combining Anderson's and Belousov's models, is the true one, then the extension of this concept to the linear volcano–tectonic rift structures may solve some of the conflicts of evidence which have caused so much controversy.

With regards to the geophysical evidence from Japan, I believe that the types distinguished as 'Krakatoan' and 'Glencoe' overlap, and that the 'Glencoe' mechanism is the primary mechanism, not simply of calderas classified as 'Glencoe' type, but of 'Krakatoan' calderas as well. I have carried out a gravimetric survey over the broad shield volcano of Menengai (report awaiting publication, Nakuru–Thomson's Falls–Lake Hannington area) and have found that a series of high values for the Bouguer anomaly are grouped around the caldera, the typical example of a 'Glencoe' type caldera on which weak 'Krakatoan' characteristics have been superimposed. This anomaly, superimposed on the low values which here characterize the Rift Valley as a whole, seems related to the ridge volcano of Menengai, and not to be direct reflexions of the caldera.

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URANIUM AND THORIUM ABUNDANCES IN POSSIBLE UPPER MANTLE MATERIALS

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It is of considerable geochemical importance that accurate determinations should be made of the uranium and thorium abundances in possible upper mantle rock types since quite severe restrictions can be placed on the absolute concentrations of radioactive elements existing in the Earth's upper mantle. For example, Birch[1] has shown that the present rate of loss of heat through the Earth's crust is equal, within a factor of two, to the rate of production of heat if the Earth as a whole was composed of material with the uranium and thorium contents of the chondritic meteorites. Assuming an overall chondritic composition for the Earth, MacDonald[2] has further calculated that the material in the Earth's upper mantle above a depth of 600–700 km should have an overall uranium content of about 0·05 p.p.m. and (assuming a thorium/uranium ratio of 3·5) a thorium content of about 0·175 p.p.m.

Many workers have suggested that rocks of ultra-basic composition (for example, peridotites, garnet peridotites or dunites) are the predominant materials in the Earth's upper mantle. Modern, accurate analyses of uranium and thorium abundances in ultra-basic rocks are very few and only three different rocks have been examined so far[4,5], and even within this small sample there is not always good agreement between different analytical methods. However, what little data there are available (Table 1) suggest that the uranium and thorium abundances measured in ultra-basic rocks are about 50 times less than MacDonald has calculated for upper mantle materials.

Following an earlier suggestion by Goldschmidt, Lovering[3] has proposed that a considerable portion of the Earth's upper mantle is basic in composition but composed of pyrope-garnet and omphacitic pyroxene assemblages characteristic of eclogitic rocks. The only data available on uranium and thorium abundances in eclogites are those of Holmes and Paneth[6] published in 1936. Their results (Table 1) would suggest that the uranium and thorium abundances in the eclogitic inclusions from kimberlite pipes in South Africa are very much higher than would be consistent with their occurrence in the upper mantle. However, the methods then used for the determination of the uranium and thorium have been superseded by more precise methods (for example, neutron activation, isotope dilution, etc.), and it became obvious that a study should be made of uranium and thorium abundances in eclogites using one of these techniques. Neutron activation analysis was selected as the most appropriate technique for the determination of the relatively low levels predicted for eclogite rocks from the mantle and a method was devised to simultaneously determine uranium and thorium in the sample after neutron irradiation in the *Hifar* reactor of the Australian Atomic Energy Commission. The details of the analytical method will appear elsewhere[7]; but it can be shown that individual analyses for both uranium and thorium have relative deviations of about 2·5 per cent. Good general agreement has been found with the most reliable methods of analysis used for the determination of uranium and thorium in the Standard Granite *G*-1 and the Standard Diabase *W*-1.

Eclogitic rocks occur in two main environments on the Earth's surface. There are the pipe eclogites which occur along with ultra-basic and granulitic rocks as inclusions in kimberlitic and basic igneous pipes in various parts of the world. Some of the pipe eclogites contain

Table 1. PREVIOUS DETERMINATIONS OF URANIUM AND THORIUM IN ECLOGITIC AND ULTRA-BASIC ROCKS

		Uranium			Thorium		
Rock type	Locality	p.p.m.	Method	Reference	p.p.m.	Method	Reference
Eclogites	Inclusions in kimberlite pipes, South Africa	0·66 1·26 1·47	Radium method	3	0·50 1·30 2·10	"Thorium-X" method	3
Dunite	Twin Sisters, Washington	0·016	Isotope dilution	4	0·06	Isotope dilution	4
		0·001	Neutron activation	5	—	—	—
Peridotite	Inclusion in basalt, San Bernardino, Cal.	0·007	Isotope dilution	4	—	—	—
Peridotite	Inclusion in basalt, Hualalai, Hawaii	0·005	Isotope dilution	4	—	—	—

Table 2. NEUTRON ACTIVATION DETERMINATION OF URANIUM AND THORIUM IN PIPE ECLOGITES AND CRUSTAL ECLOGITES

Eclogite type	Rock	Number	Locality	Uranium p.p.m.		Thorium p.p.m.		Th/U
Pipe Eclogites	Eclogite	*R*11	Delegate, N.S.W., Australia	0·053 0·051	0·052	0·19 0·18	0·18	3·6
	Hornblende eclogite	*R*117	Delegate, N.S.W., Australia	0·074 0·072	0·073	0·30 0·28	0·29	3·9
	Olivine eclogite	*R*394	Delegate, N.S.W., Australia	0·044 0·042	0·043	0·15 0·15	0·15	3·5
	Eclogite	*R*269	Roberts Victor Mine, Orange Free State, South Africa	0·041 0·041	0·041	0·24 0·24	0·24	5·8
	Olivine eclogite	*R*419	Salt Lake Cone, Oahu, Hawaii	0·043 0·041	0·042	0·10 0·10	0·10	2·4
Crustal Eclogites	Eclogite	*R*388	Junction School, Healsburg Quadrangle, California	0·18 0·21	0·20	0·43 0·43	0·43	2·2
	Eclogite	*R*391	Silberbach-Hof, Münchberg gneiss massif, Germany	0·25 0·23 0·25	0·24	0·58 0·58 0·64	0·60	2·5
	Eclogite	*R*371	Hof Bavarra, Germany	0·053 0·064	0·059	0·27 0·30	0·28	4·8
	Olivine eclogite	*R*372	River Tessin, Southern Switzerland	0·026 0·025	0·025	0·009 0·020	0·015	0·6
	Bronzite pyropite ("eclogite")	*R*375	Sittampundi Complex, Madras, India	0·024 0·013	0·018	0·011 0·024	0 018	1·0

diamond as a primary phase and have certainly formed at depths of at least 130 km in the mantle[8]. There is strong evidence that these eclogites represent material brought directly from the upper mantle of the Earth during the emplacement of the pipes.

The other class of eclogites we have are called the crustal eclogites. These eclogites occur as lenses or discontinuous masses often (but not always) associated with peridotite and garnet peridotite masses in orogenic zones. It is possible that these eclogites have also been brought directly from the mantle during the orogenic episode which mobilized them, but the evidence for a direct mantle origin is very much less compelling than that of the pipe eclogites. In the present work samples of both crustal and pipe eclogites have been studied to give as complete a picture as possible of the uranium and thorium abundances in eclogitic rocks.

Pipe eclogites. Igneous pipes containing eclogite inclusions are known from many parts of the Earth's surface. Most of these pipes are filled with a matrix rock of kimberlitic composition in which fragments of eclogite and other rock types are included (for example, South Africa, Siberia, etc.). An eclogite (*R*269) inclusion from the Roberts Victor Mine, Orange Free State, South Africa, made available by Dr. F. A. Venter of the South African Geological Survey, has been analysed in the present work. More recently pipes filled with massive and brecciated alkali–olivine basalts and containing a suite of eclogitic and other inclusions virtually identical with those in the kimberlitic pipes have been found in eastern Australia. The Australian pipe containing the least-altered inclusions occurs near Delegate in southern New South Wales and three eclogites with slightly different mineralogical composition have been analysed from this pipe (for example, *R*117 hornblende eclogite, *R*11 eclogite, *R*394 olivine eclogite).

The eclogitic samples described so far occur in pipes which penetrate the continental crust and presumably these eclogites represent material from the continental upper mantle. It is very important to examine pipe eclogites in oceanic areas to see whether there are any significant differences between eclogites occurring in continental and oceanic upper mantle regions. Oceanic eclogites have been described as inclusions in palagonite tuff, Salt Lake Cone, Oahu, Hawaii by Winchell[9] and samples of an olivine eclogite (*R*419) from this locality were made available by Dr. G. A. Macdonald.

Crustal eclogites. Four eclogites from crustal environments have also been analysed. Eclogite *R*388 from Junction School, Healsburg Quadrangle, California, is a relatively unaltered sample of the Californian eclogites which are associated with ultra-basic rocks and glaucophane schists[10] and was made available by Dr. Iris Borg. Eclogite *R*391 (Silberbach-Hof, Fichtelgebirge) is a sample of the eclogite occurring as pods in gneisses of the German Münchberg gneiss massif[11] and was collected by Dr. R. R. Marshall. Eclogite *R*371 comes from a similar geological environment at Hof Bavarra, Germany. The olivine eclogite *R*372 occurs with ordinary eclogites and peridotites in lenses within amphibolite facies rocks of the River Tessin region of southern Switzerland. Both specimens *R*371 and *R*372 were made available by R. O. Chalmers from the collection of the Australian Museum. Other rocks described as eclogite facies rocks by Subramaniam[12] occur with meta-anorthosites in an apparently layered sequence within the Peninsular gneisses near Sittampundi, Madras. One of these rocks, classified as a 'bronzite pyropite', *R*375 has also been analysed in the present work.

The results of the analyses of these rocks are shown in Table 2, and the following conclusions can be drawn from these observations.

(1) Eclogites from the continental upper mantle have very similar uranium (range 0·04–0·07 p.p.m.) and thorium (range 0·15–0·29 p.p.m.) abundances with mean values of 0·052 p.p.m. uranium and 0·22 p.p.m. thorium. These values are at least an order of magnitude higher than those reported for ultra-basic rocks (Table 1) and something like an order of magnitude less than the average values usually quoted for basic igneous rocks[14]. The levels of uranium and thorium found in these eclogites agree very closely with the estimates made by MacDonald[1] for possible uranium and thorium abundances in upper mantle materials.

(2) The thorium/uranium ratios for the eclogites from the continental upper mantle range from 3·5 to 5·8 with a mean value of 4·2. This is very close to the value of 4·1 which Cannon *et al.*[15] have calculated for the upper mantle on the assumption that lead ore deposits have been derived from the Earth's upper mantle.

(3) The one eclogite analysed which may well represent part of the oceanic upper mantle has a uranium content identical with continental eclogites; but the thorium content is somewhat lower, so that the thorium/uranium

ratio (2·4) is considerably lower than the ratios in the continental eclogites. However, as only one oceanic eclogite has been analysed up to the present, it is not known whether there is in fact a real difference between continental and oceanic eclogites.

(4) The crustal eclogites measured had uranium contents between 0·018 p.p.m. and 0·24 p.p.m., thorium contents between 0·015 p.p.m. and 0·60 p.p.m. and thorium/uranium ratios between 0·6 and 4·8. Only one crustal eclogite (*R*371 from Hof Bavarra) had a uranium and thorium content, and a thorium/uranium ratio within the range of the continental pipe eclogites. The others show very different abundance-levels for uranium and thorium as well as very different thorium/uranium ratios from those of the pipe eclogites. However, it is not possible to conclude from the present work whether or not the majority of crustal eclogites do in fact represent portions of the upper mantle intruded into the crust during orogenic episodes. It may well be that their present uranium and thorium abundances reflect drastic modifications of normal upper mantle abundances of these elements after the particular eclogites became modified by geochemical processes within the upper crust.

(5) Marshall[11] has examined the isotopic composition of the lead extracted from the same sample of the Silberbach-Hof eclogite which was studied in our work and concluded that in order to account for the lead developed during 4·5 × 10^9 years and observed by him to-day in this sample, there should be 0·10 p.p.m. uranium and 0·41 p.p.m. thorium present. The actual amounts present in this sample (Table 2) are considerably higher than Marshall's estimates, and would suggest that the Silberbach-Hof eclogite has not remained a closed system with regard to uranium, thorium or lead over geological time.

The work is at present being extended to include granitic, amphibolitic and granulitic inclusions in the igneous pipes described previously on the assumption that these rock types probably represent samples of the deep continental crust intruded by the pipes. It is hoped that in this way a more complete picture may be built up of uranium and thorium distributions within the continental crust. Other possible upper mantle materials (such as garnet peridotites and peridotites) occurring in the pipes are also being studied.

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LARGE RING STRUCTURES IN A SOUTH AUSTRALIAN PRECAMBRIAN VOLCANIC COMPLEX

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IN 1961 I noted an arcuate pattern on the outcrop of the Gawler Range porphyry 50 miles west-south-west of Port Augusta, South Australia. The Gawler Range porphyry is a loose term given to a vast (12,000 sq. miles) outcrop covering northern Eyre Peninsula. The predominance of red-weathered felspar porphyry suggested a persistently similar rock-type, comparable rocks being known in scattered outcrops 100 miles north of the railway. Lack of known mineralization within this outcrop led to neglect since its discovery by H. Y. L. Brown in 1885.

The age of the Gawler Range porphyry was uncertain. It was compared by R. Lockhart Jack[1,2] with the richly copper-bearing Moonta porphyry of Yorke Peninsula about 100 miles to the south-east. This rock intrudes Precambrian gneisses usually described as Archaean. Lockhart Jack suggested that the Gawler Range porphyry was an effusive equivalent of the Moonta porphyry.

I suggested[3] that the copper-bearing Wooltana volcanics of the northern Flinders Ranges (which are haematite-rich trachytes underlying unconformably the Sturtian glacials), the Moonta Porphyry and the Gawler Range porphyry were of common genesis and of much the same age. Other volcanics occurring in scattered outcrops between Wooltana and Port Augusta (for example, Brunnschweiler[4]) proved to be of Wooltana type. These are often either diapirically placed[5] or occur along major lineaments.

A field check of the arc noted in the Gawler Range porphyry showed that it had parallel banding and that devitrified obsidian was common. Rocks almost indistinguishable from typical Wooltana-type trachytes were found only 15 miles east of the main porphyry outcrop and recently H. W. Fander and I found similar rocks well within the main outcrop.

Eroded vents with diameters of about one mile were revealed near the arc. Numerous curved lineaments were detected, some concentric with the vents, among innumerable straight lineaments forming part of a superbly displayed rectilinear joint pattern. This pattern is ascribed to torsional stress associated with block-faulting.

An assembly of 40 to one inch to one mile mosaics, covering the whole of the main outcrop revealed very large circular structures. In the area south-west of the southern end of Lake Gairdner, and which forms the south-western part of the main outcrop, a valley with a constant width of about half a mile extends along a semicircle, having a diameter of about 35 miles. This is centred approximately on Yardea head station (that is, sheep station). Diametric to this valley (which is obviously quite distinct from innumerable straight valleys of variable width, most of which are probably widened joints) a perfect graben four miles wide extends from near Yardea for twenty miles eastward. The graben meets the semicircle on the south-westernmost inlet of Lake Gairdner, and this point is itself the centre of a complex of elliptical shape, with a larger diameter of 12 miles.

Field examination of the large semicircular structure showed no major change in rock-type across the valley, which like most valleys in the Gawler Ranges is flat-bottomed and entirely covered by alluvium, except at Paney out-station, 17 miles south-west of Yardea, after which the feature has been named. At Paney low hills between higher plateaux are formed of flow-banded porphyritic rhyolites much paler in colour than most of

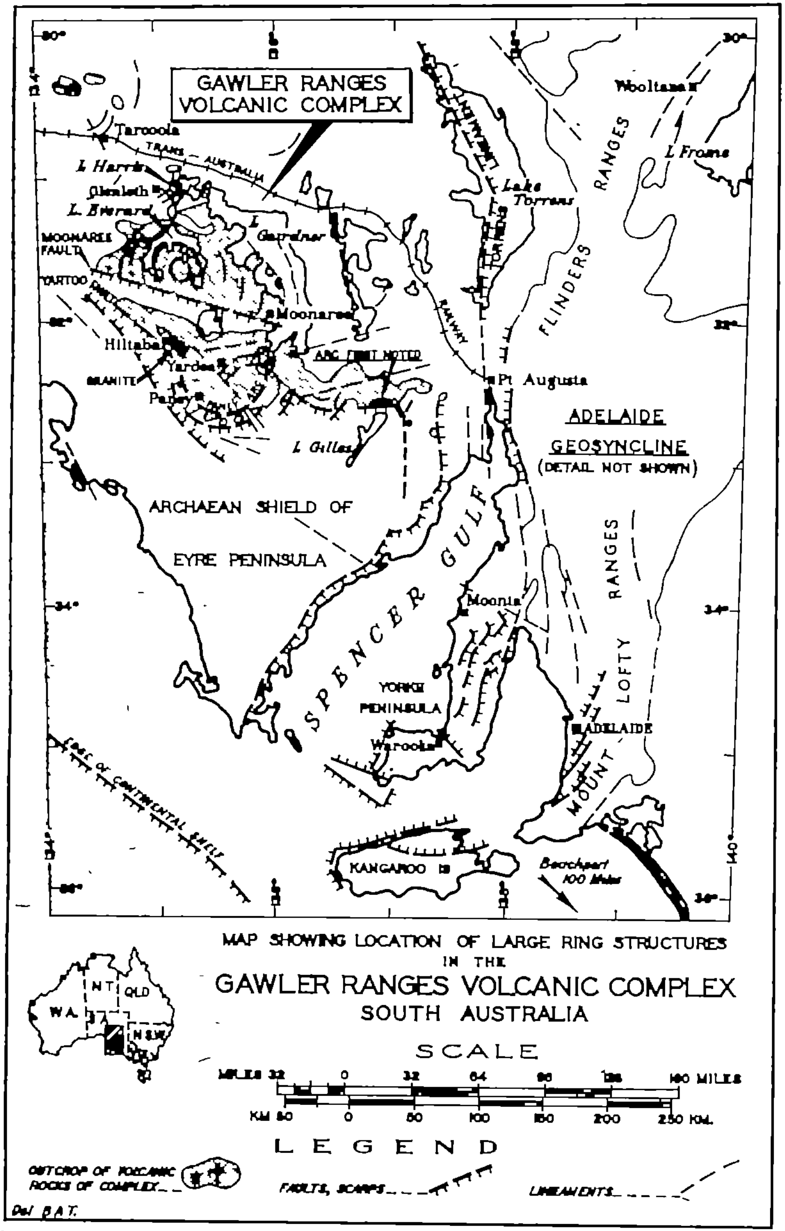

Fig. 1

the porphyry, and showing extremely tortuous, swirling textures, confined to a narrow zone about a quarter of a mile wide along the line of the semicircle. The rocks to the north and south are different, and flow-banding is not common.

A major east-north-easterly lineament extends through the Gawler Ranges for 100 miles and is almost tangential to the Arc. Along this lineament intense brecciation and chemical alteration is common and uncharacteristic rocks occur. The area within the Paney Arc appears, so far as can be determined without distant oblique air views, to have a topographic expression which is saucer-like.

Radial to the Paney Arc (and parallel to the Yartoo Fault) is a body of granite which occupies a graben-like area near Hiltaba H.S. This has a longer dimension of 15 miles, aligned west-north-west. It is a vuggy fluorite-bearing granite quite distinct in type from others in northern Eyre Peninsula. As it intrudes the porphyry to the south of it with a sharp vertical contact it may be a large radial dyke.

Numerous smaller ring structures exist, much of the southern boundary of the outcrop being formed by their intersection; some are faults which have moved in Quaternary time. Evidence for other large ring structures is accumulating. On a recent field visit during which ring patterns south of Lake Everard were checked it was clear that these are concentric with the southern shores of that lake, which had an extraordinary shape. This area too has a saucer-like topography. In so far as the westernmost limbs of the lake are also concentric, it seems almost certain that ring structures of at least 55 miles in diameter exist. The relationship of these to sympathetically curved lineaments to the east between Lake Everard and Lake Gairdner is as yet obscure, but structures of 70 miles in diameter may exist. If certain lineaments noticed in the bed of Lake Gairdner are related, this figure would have to be increased to 100 miles.

Similar arcs entirely control the west shores of Lake Harris farther north (on most maps these lakes are all inaccurately delineated) and with radial lineaments form sector-like bays. Others include andesite dykes in the adjacent Glenloth goldfield, the bedrock outcrops of which appear to form part of an annulus. In the Tarcoola district andesite dykes known to be gold-bearing are radial intrusions into sediments which are apparently down-faulted within an annulus. Copper minerals occur at Tarcoola in fine-grained variants of Gawler Range type volcanic rocks.

Numerous long, straight lineaments occur. Most are north-westerly, west-north-westerly, or east-north-easterly in direction. The most obvious is the Moonaree Fault, which extends for more than 80 miles west-north-westward from the west shore of Lake Gairdner.

Of 400 rock specimens being examined, H. W. Fander has already (personal communication) noted many welded tuffs. Age determinations by the strontium–rubidium method are in progress (by Dr. W. Compston) at the Australian National University.

The deeper implications of the large circular structures and their associated straight lineaments are still under study. One obvious comparison is with lunar structures. Jeffreys[4] states that the terrestrial structures most like lunar 'craters' are the ring-dykes first noted by Clough, Maufe and Bailey in the Tertiary volcanic complexes of the Scottish Highlands. Jeffreys noted that the largest known ring-dykes were very much smaller in diameter than the average lunar crater, but did not regard this as important. While it has not yet been possible to obtain a three-dimensional picture of the large circular structures in the Gawler Ranges Volcanic Complex, I provisionally regard the Paney Arc as having been the edge of a cauldron subsidence. The retention of original topography (as is suggested by the saucer-like topography) in rocks of such age is very unlikely but it seems possible that a rejuvenation of the major structural controls may have assisted the development of what can perhaps be described as 'palimpsest topography'.

The centre of the Paney Arc lies on the projection of a line joining the epicentres of South Australia's largest earthquakes, namely, September 19, 1902, Warooka, Yorke Peninsula, and May 10, 1897, April 8, 1948, Beachport. The centre is about as far from Warooka as Warooka is from Beachport. This line is parallel to the edge of the continental shelf off Eyre Peninsula.

The economic implications are important. The Tarcoola and Glenloth goldfields undoubtedly owe their mineralization to the volcanics. The association with copper is significant. Magmatic copper occurs in the Wooltana rocks, and gold is known. (The widespread copper mineralization of the associated Adelaide System sediments has long been regarded by me as sedimentary-exhalative in type.) 'Porphyry copper' is therefore thought to be a likely prospect in at least the north-west of the Complex. It may be significant that the only tin localities in South Australia are at and near Glenloth. Minor gold occurrences within the Complex are known. Most interesting of all is the possible connexion with the exceedingly rich hæmatite bodies of the Middleback Ranges, all of which lie in a narrow north–south belt regarded as a major structural lineament parallel to one of the most fundamental in Australia, the Torrens lineament separating the West Australian shield from the Adelaide geosyncline. Iron Monarch is the richest body and is the nearest to the porphyry outcrop. Miles *et al.*[7] believed the Gawler Range porphyry to be much younger than the ore bodies and therefore to have played no part as a magmatic source. The question should be reconsidered. It may be very significant that a large aeromagnetic anomaly lies under the northern end of Lake Gilles, due west of Iron Monarch, and at a point central to the Arc first noted; that the rocks of the Complex are invariably rich in hæmatite; and that 'Gawler Range porphyry' was recorded under the Iron Monarch orebody at about 650 ft. below surface in one drill-hole. It is also curious that a line passing through the Pancy Arc centre, and through several other centres, and parallel to the Moonaree fault also passes through the Iron Monarch orebody.

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HIGH-TEMPERATURE PHASE EQUILIBRIA IN THE SYSTEM $2CaO.SiO_2 — 3CaO.P_2O_5 — CaO$

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A DETAILED examination has been made of phase equilibria in the system $2CaO.SiO_2 - 3CaO.P_2O_5 - CaO$ characterized by liquidus temperatures in the region of 2,000° C, and by the presence of high-temperature phases which cannot be preserved by rapid cooling. This system is of importance to processes in the cement, steel and fertilizer industries, and has been much investigated, but earlier work suffered from lack of suitable experimental methods. The investigation benefited from recent elucidation of the binary system $2CaO.SiO_2 - 3CaO.P_2O_5$ (ref. 1) and of the melting behaviour of tricalcium silicate[2], and use was made of special techniques of high-temperature microscopy[1–3] capable of giving direct insight into crystal growth and crystallization sequence at temperatures up to 2,450° C.

The liquidus surface, established by these methods, which is given in Fig. 1 with abbreviated liquidus data, derives from direct distinction on the microscope stage of the phases present in the melt. This surface includes a primary crystallization field, extending to 13 per cent phosphorus pentoxide, for tricalcium silicate, the most important cementing component of Portland cement. This field was not detected by earlier investigators[4]. There are two reaction points. The first of these R_1, a ternary eutectic point between CaO, $4CaO.P_2O_5$ solid solution and $2CaO.SiO_2/3CaO.P_2O_5$ solid solution, has the composition 60·75 per cent CaO, 4·75 per cent SiO_2 and 34·5 per cent P_2O_5 (by weight) and its liquidus temperature is 1,636° C. The simultaneous presence of the three phases at the liquidus at this point was established experimentally. The tie lines shown in Fig. 1 were established at a temperature of 1,500° C.

The crystallization products compatible at 1,500° C which are shown in Fig. 1 by the compatibility triangles are based on extensive examination by X-ray and chemical analysis, and by microscopy, of selected compositions in the system after prolonged heat-treatment at 1,500°. These compositions were made from reagents at least 99·9 per cent pure. A phosphate and lime modified tricalcium silicate (C_3S'') of the approximate composition 74 per cent CaO, 25·5 per cent SiO_2, 0·5 per cent P_2O_5 (by weight) exists along the join $C_3S'' -$ PSS′ and there is solid solution between C_3S and C_3S''. The locus of the joins $C_3S - C_3S''$ and $C_3S'' -$ PSS′ was established by 'free lime' analysis of selected ternary compositions in which lime is one of the three stable crystallization products at 1,500° C. Subtraction of the 'free lime' gave the corresponding two-phase compositions on these joins. Similar treatment revealed tetracalcium phosphate altered by silica and lime (C_4P') of approximate limiting composition 60·1 per cent CaO, 3·8 per cent SiO_2, 36·1 per cent P_2O_5 (by weight) existing along the join $C_4P' -$ PSS″, there being a region of solid solution between C_4P and C_4P'. X-ray analysis of compositions in various parts of the system revealed good agreement with the phase diagram of the system $2CaO.SiO_2 - 3CaO:P_2O_5$, due to Nurse, Welch and Gutt[1], the products of sub-solidus reactions obtained at 1,500° being those predicted by the phase diagram due to these authors with the following exception. Their diagram indicates no unmixing of the continuous solid solution series of $2CaO.SiO_2$ and $3CaO.P_2O_5$ above 1,500° and would predict therefore that the joins from C_3S'' and C_4P' would be directed to PSS (75·9 per cent $2CaO.SiO_2$, 24·1 per cent $3CaO.P_2O_5$ (by weight)) the composition of maximum liquidus in the binary system and not to the compositions PSS′ (86·9 per cent $2CaO.SiO_2$, 13·1 per cent $3CaO.P_2O_5$ (by weight)) and PSS″ (62·4 per cent $2CaO.SiO_2$, 37·6 per cent $3CaO.P_2O_5$ (by weight)). The work reported here indicates that some such unmixing does occur above 1,500° into two structurally very similar solid solutions the composition of which depends on the temperature. It is suggested that PSS′ and PSS″ mark the compositions of these solid solutions at 1,500° C.

The composition PSS″ lies in the small region of the system $2CaO.SiO_2 - 3CaO.P_2O_5$ in which $\bar{\alpha}3CaO.P_2O_5 \equiv \alpha 2CaO.SiO_2$, the primary phase throughout this system[1,5], can be preserved to room temperature. The uniqueness of the PSS″ composition is supported by the sharpness of the $\bar{\alpha}3CaO.P_2O_5$ X-ray diffraction pattern that it gives. This is sharper than is given by other compositions in the small region in which $\bar{\alpha}3CaO.P_2O_5$ can thus be preserved. The same argument cannot be advanced with regard to PSS′, which inverts on cooling to the α' form of the solid solution rich in $2CaO.SiO_2$.

Evidence in support of the significance attached to the composition PSS′ was obtained by reheating at 1,650° C the compositions which led to the join $C_3S'' -$ PSS′ valid at 1,500° C, and repeating 'free lime' analysis. This

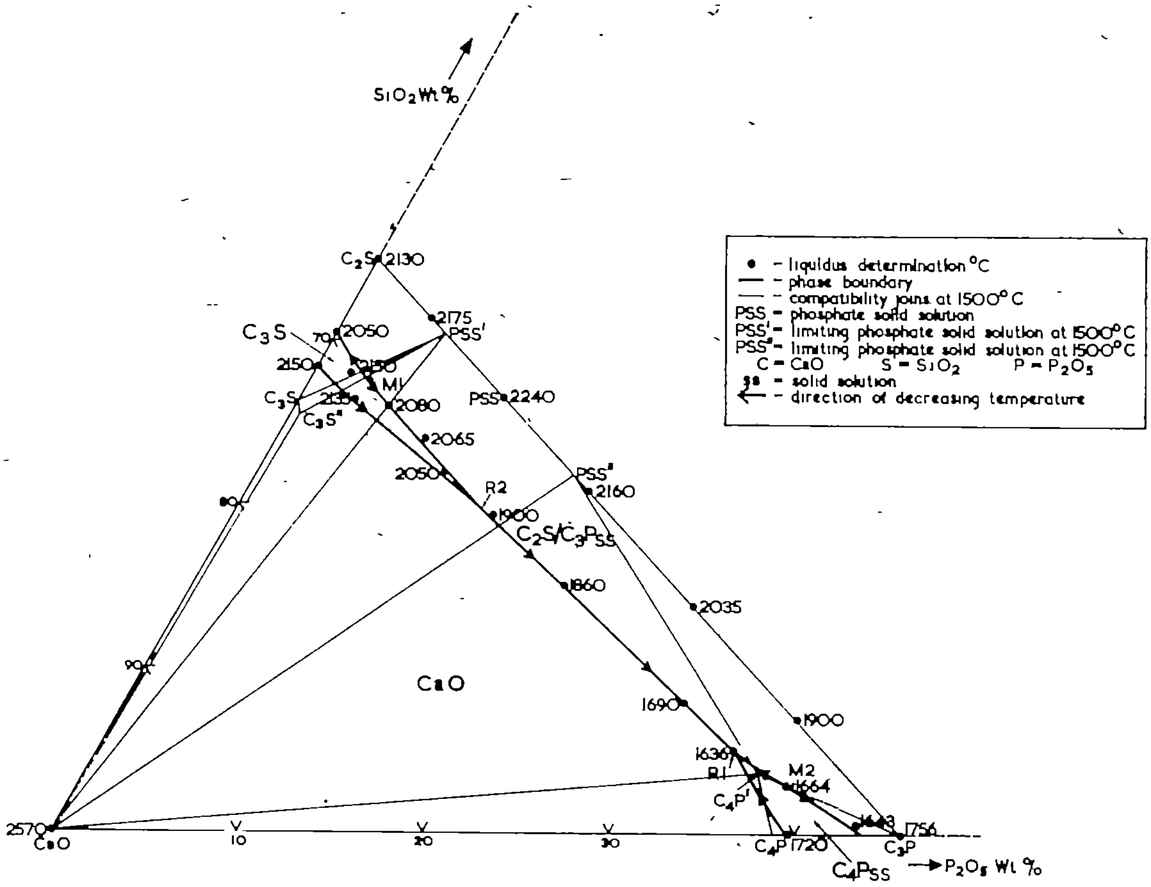

Fig. 1. Phase diagram of the system C_2S-C_3P-CaO

led to a displacement of the join from C_3S' towards a point on the $2CaO.SiO_2 - 3CaO.P_2O_5$ join richer in $3CaO.P_2O_5$ than the composition PSS′.

The liquidus maximum M_1 on the $3CaO.SiO_2$ – $2CaO.SiO_2/3CaO.P_2O_5$ solid solution boundary is in accordance with the Alkemade theorem, even assuming some displacement of PSS′ toward $3CaO.P_2O_5$ as the temperature rises.

The second reaction point R_2 between $3CaO.SiO_2$, CaO and the $2CaO.SiO_2/3CaO.P_2O_5$ solid solution occurs at the composition 67·2 per cent CaO, 19·8 per cent SiO_2, 13 per cent P_2O_5 (by weight) and its liquidus is 1,970° C. This is a 'rising reaction point'. The temperature along two of the boundaries meeting here falls towards this point and along the third the temperature rises towards it.

The liquidus maximum M_2 which may be displaced somewhat by the movement of PSS″ towards $2CaO.SiO_2$ with rising temperature on the $4CaO.P_2O_5 - 2CaO.SiO_2/3CaO.P_2O_5$ solid solution boundary is also required by the Alkemade theorem.

The incongruent melting of $3CaO.SiO_2$ in the system $CaO-SiO_2$ may occur at a temperature some 70°–80° C higher than reported by Welch and Gutt[3], but in all other respects their findings were confirmed.

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MECHANISM OF THE ACTION OF NITRIC OXIDE ON PARAFFIN PYROLYSES

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THE effect of nitric oxide on paraffin pyrolyses has been the subject of numerous reviews[1,2]. Briefly, the addition of nitric oxide in small amounts causes a reduction in the initial rate of decomposition of the paraffin. As the pyrolysis proceeds, however, the reaction accelerates to a maximum rate before the rate falls off towards completion. Thus, the pressure-time curve for the inhibited reaction is sigmoid in form, in contrast to the normal non-inflected curve observed for the uninhibited pyrolysis. Because of this, inhibited rates are usually defined by measurement of the initial slopes of the pressure-time curves. This procedure is sometimes complicated by the existence of either initial periods of no (or, on occasion, of negative) pressure change[3] or initial 'bursts'[4] of somewhat variable extent and duration. It is not clear whether these are genuine or are in some way due to the experimental technique, but in general they are discounted when obtaining the inhibited rate from the initial slopes. For example, spurious initial pressure decreases can be due to solution of material in stop-cock lubricant, while initial bursts can be produced by incomplete mixing (due to the practice of mixing gases in the reaction vessel) or by the presence of traces of oxygen in the reactants. It is often difficult to tell from the descriptions what precautions were taken against these difficulties. An alternative method that has been used to eliminate the effect of these 'disturbances' is to take the inhibited rate as the average rate of pressure rise between two fixed percentage pressure rises[4]. Compared with the initial rate, the subsequent stages of this self-accelerating reaction have received little attention. If increasing quantities of nitric oxide are added to the paraffin the initial rate of pyrolysis passes through a region of minimum rate in which the rate is virtually independent of the 'inhibitor' concentration, it then increases, eventually for large amounts of nitric oxide varying linearly with the concentration of inhibitor—in what is called the induced reaction. The minimum rate reached is sometimes called the residual or limiting rate. For a large number of paraffins and indeed some other substances (for example, aldehydes and ethers) it has been claimed that this limiting rate is the same under given conditions irrespective of whether nitric oxide or olefines are used to inhibit the reaction.

n-Pentane[5] is an example that is relevant to the following discussion. This equality, however, is known to be destroyed by changes of reaction temperature in the cases of 2-methyl pentane and *n*-pentane[5], by changes of the reaction vessel surface in the case of *n*-heptane[6], and by changes of reactant pressure in the case of propaldehyde[7]. The equality is only approximate; for example, in the cases of iso-butane and iso-pentane the rates differ by some 15–20 per cent, which is well outside experimental error[8]. It is well established that the olefines themselves decompose by radical mechanisms, often at appreciable rates at the temperature at which they are used as inhibitors; for example, propylene and isobutene have been used[8] to inhibit the pyrolyses of isobutane, isopentane, and *n*-pentane, at 560° C, although these olefines have been found to decompose at this temperature at rates comparable with those of the paraffin pyrolyses[9]. We think that the equality of limiting rates has not been established as exact and general. The explanation of this equality, therefore, is not a necessary requirement of any mechanism postulated for the reaction. Nevertheless, there is an overall similarity in the effects of nitric oxide and of olefines in paraffin pyrolyses that almost certainly reflects a similarity of mechanism.

Several attempts have been made to explain the observed characteristics of the effect of nitric oxide on paraffin pyrolyses in terms of detailed mechanisms[4,10–12]. In our opinion these have paid insufficient attention to the fate of the products of the reactions between the alkyl radicals and nitric oxide. The fate of these products is of the greatest significance to a clear understanding of 'inhibition'. These products are known to include oximes[13]. The pyrolyses of formaldoxime and acetaldoxime have been investigated[14,15], and found to occur very rapidly at temperatures well below those normally used for paraffin pyrolyses. The decompositions of these oximes have also been shown to be greatly accelerated by very small quantities of nitric oxide[14,15]. In the case of formaldoxime at about 400° C a violent explosion occurred when nitric oxide was added[14]. In the inhibited paraffin pyrolyses, where excess paraffin is present, this explosion might well exist incipiently. The mechanism postulated to account for the pyrolysis of acetaldoxime has been shown to give rise to the possibility of chain branching occurring in systems containing ethyl radicals and nitric oxide[15]. The reactions involved are:

$$NO + C_2H_5^{\cdot} = C_2H_5NO \rightarrow CH_3CHNOH$$

$$NO + CH_3CHNOH = (CH_3CHN)^{\cdot} + HNO_2$$

$$(CH_3CHN)^{\cdot} = CH_3^{\cdot} + HCN$$

$$HNO_2 = OH^{\cdot} + NO$$

It is suggested here that the self-accelerating nature of paraffin pyrolyses inhibited by nitric oxide can be explained by the occurrence of such a chain branching mechanism in a degenerate form. The initial 'inhibited rate', as defined by previous workers, would then correspond to a situation where the branching has not yet become significant. On this basis we suggest a simple scheme here which gives a good account of the observed characteristics of this initial rate. In the absence of more direct evidence of the fate of the products of the reactions between alkyl radicals and nitric oxide within an actual inhibited pyrolysis it is evident that any detailed mechanism proposed for these inhibited pyrolyses must to some extent be speculative. Nevertheless, the scheme which is proposed here takes account of the facts recorded above and is suggested as an alternative possibility to previous schemes: it is based on the concept of a unified mechanism for all phases of the reaction.

The three main stages in the effect of the addition of nitric oxide to decomposing paraffins, that is, inhibition, residual reaction, and induced reaction, have often been treated separately as virtually independent reactions, the overall rate of pyrolysis being obtained simply by adding together these three components in proportions appropriate to the various stages of the reaction. This approach has recently been used by Blackmore and Hinshelwood[4] to explain the three phases of the nitric oxide effect in terms of a detailed mechanism and to evaluate the relevant rate parameters involved by comparison with experimental results. Their approach postulates a true molecular decomposition which predominates in the region of maximum inhibition, and an attack by nitric oxide on the paraffin which predominates in the region of the induced reaction[1]. These authors emphasize that, since the inhibitable part of the reaction rate ($k_0 - k_\infty$), in their notation, is not very much smaller than the uninhibited reaction rate (k_0), either value may be used as the rate of the chain reaction for the purposes of the arithmetical calculation without producing very large changes in the values of the derived rate parameters. This purely numerical result does not alter the fact that the postulate of a molecular reaction is essential to their interpretation of the inhibition curve.

Thus, the fact that back extrapolation of the linear induced reaction curve to zero nitric oxide concentration gives a finite rate of pyrolysis, together with the observation that the consumption of nitric oxide relative to that of paraffin decreases markedly from near unity in the induced region to some small unknown value in the region of maximum inhibition, has been taken to indicate the existence of the true molecular component. This argument is only valid subject to the assumption of independence of the various reactions stated above. This independence is *a priori* very unlikely, particularly so for radical chain reactions. Further, the continuity observed in certain product ratios throughout the inhibition by propylene militates against separate concurrent reactions in this case[16]. A single scheme embracing the whole range of reaction would be more satisfactory, but has been discounted by Blackmore and Hinshelwood[4] on the grounds of the varying kinetics observed in the different regions. Such a single scheme which satisfies these kinetics, however, is presented here.

In the simplified scheme for a paraffin pyrolysis written below, the symbols have the following significance: P = paraffin, R_L = 'large' radical (for example, C_2H_5, C_3H_7, etc.), R_S = 'small' radical (for example, CH_3, H), M = inactive product, Ol = olefinic product.

$$P = R_L + R_S \tag{1}$$

$$R_S + P = M + R_L \tag{2}$$

$$R_L = Ol + R_S \tag{3}$$

$$R_L = \text{end-products} \tag{4}$$

The termination step (4) is written as first order for simplicity; its kinetics are not important when the extent of inhibition is large. If we represent the oximes isomeric with RNO by *Ox*, then in the presence of nitric oxide the following reactions occur:

$$R_L + \mathrm{NO} = \mathrm{RNO} = Ox \qquad (5)$$

The oximes formed in this manner will undergo both a rapid pyrolysis and a nitric oxide catalysed pyrolysis. As outlined above this latter reaction cannot lead to significant branching in the initial stages of the reaction, so we may write:

$$Ox + \mathrm{NO} = R_s + \text{products} \qquad (6).$$

For example (6) could be:

$$\mathrm{CH_3CHNOH + NO = CH_3^{\cdot} + HNO_2 + HCN}$$

If, for simplicity, we assume that the uncatalysed decomposition of *Ox* yields only inactive products we may include:

$$Ox = \text{products} \qquad (7)$$

Should this decomposition, in fact, involve radicals which sensitize the paraffin pyrolysis[16], then it may be shown that this will not influence the overall kinetics of the reaction, but will lead only to some alteration in the coefficients of [NO] in the rate expression given below. The reactions (1)–(7) written above constitute a mechanism for the pyrolysis of a paraffin in the presence of nitric oxide which has been much simplified by the grouping together of many reactions and the exclusion of many others. In spite of this it may still be expected to represent the basic pattern of the true mechanism.

Applying a steady-state treatment to R_L, R_s, and *Ox* (*Ox* is very unstable at the temperatures normally used, for example, at 800° K, $(k_7/k_1) > 10^5$ for $R_L = \mathrm{C_2H_5^{\cdot}}$) leads to the following result for the rate of paraffin destruction in the 'steady' initial state:

$$\frac{1}{[P]}\,\frac{\mathrm{d}[P]}{\mathrm{d}t} = 2k_1\left\{1 + \frac{(k_2k_7/k_5k_6) + (k_3/k_5)[\mathrm{NO}] + [\mathrm{NO}]^2}{(k_4k_7/k_5k_6) + [(k_7/k_6) + (k_4/k_5)][\mathrm{NO}]}\right\}$$

This result predicts the observed shape of the 'inhibition curve', that is, a very rapid drop in rate for small additions of NO, to a minimum rate which is not zero, followed at larger concentrations of NO by an 'induced' reaction which is first order in both [NO] and [*P*] and which does not extrapolate back to zero but to a finite rate, equal to $2k_1\{1 + (k_3/k_5)/[(k_7/k_6) + (k_4/k_5)]\}$, at zero [NO]. This extrapolated first order rate constant being a complex function of several rate parameters would not be expected to show the normal properties of a single unimolecular constant. The unusual variations of order and activation energy with pressure observed by Hinshelwood *et al.* for this extrapolated rate constant which conflict with the predictions of present theories for true molecular reactions would be explainable on this basis.

As an example, we may take the case of 100 mm Hg of *n*-pentane at 540° C studied by Blackmore and Hinshelwood[4]. The curves in Fig. 1 are calculated from the foregoing expression using $k_1 = 1{\cdot}78 \times 10^{-5}(s^{-1})$, $k_2/k_4 = 17{\cdot}6$, $k_3/k_5 = 2{\cdot}18 \times 10^{-6}$ (mole c.c.$^{-1}$), $k_6/k_7 = 0{\cdot}587 \times 10^7$ (c.c. mole^{-1}). This value of k_1 compares favourably with that evaluated for *n*-butane by several workers, for example, Trotman-Dickenson[17] gives $10^{17} \exp(-80{,}000/RT) = 3 \times 10^{-5}(s^{-1})$ at 540° C, while Quinn[18] gives $10^{17{\cdot}6} \exp(-86{,}300/RT)$, that is, $0{\cdot}4 \times 10^{-5}$ (s^{-1}) at 540° C. The ratio k_2/k_4 is the uninhibited chain length and the value of 17·6 is therefore well within the accepted range. If k_3 is taken to be $10^{11} \exp(-23{,}000/RT)$ as given by Bywater and Steacie[19] for the corresponding decomposition of the butyl radical, this is 6×10^4 (s^{-1}) at 540° C. This, together with the above value for k_3/k_5, gives $k_5 = 3 \times 10^{10}$ (c.c. mole$^{-1}s^{-1}$). This value is only slightly less than that measured for $\mathrm{CH_3^{\cdot}}$ + NO, that is, 10^{11} (c.c. mole$^{-1}s^{-1}$), and this is not unreasonable in view of the larger radicals involved in reaction (5). Further, if k_5 is in fact slightly greater than that for butyl radicals this will increase the estimate of k_3. The value of k_6/k_7 for the case $R_L = \mathrm{C_2H_5^{\cdot}}$ may be independently estimated from the known acceleration of acetaldoxime pyrolysis by NO at 500° K. This gives $k_6/k_7 = 4 \times 10^7$ (c.c. mole^{-1}) at 500° K. The slightly lower value taken here could correspond to E_7, being greater than E_6 by some 5 or 6 kcal or to larger radicals being involved. Hence it may be concluded that the rate parameters assumed here are all in quite reasonable agreement with established values.

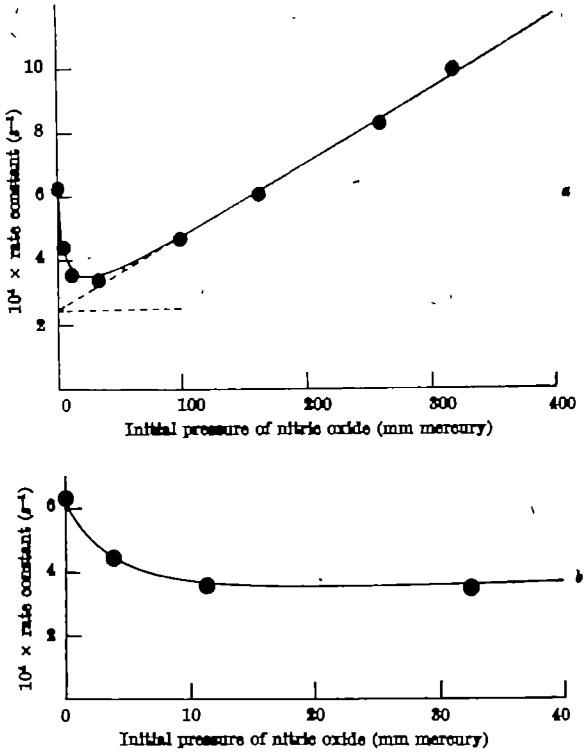

Fig. 1. Comparison of a calculated inhibition curve with the experimental points of Blackmore and Hinshelwood (ref. 4) for 100 mm mercury of *n*-pentane at 540° C

The experimental points in Figs. 1*a* and *b* were obtained from the graph of Blackmore and Hinshelwood[4]. Fig. 1*b* shows the first part of Fig. 1*a* on a larger scale to illustrate the similarity of the calculated curve to the 'flat' minimum normally associated with inhibition curves. The experimental points on the latter are rather rough, being taken from the larger graph. Unfortunately, the curve cannot be compared with the inhibition data obtained in this region by Stubbs and Hinshelwood[8] for 100 mm mercury of *n*-pentane at 530° C since their results as presented are not comparable with those of Blackmore and Hinshelwood to which the curve was fitted. In the former work the rate was reduced by a factor of seven at the minimum, while in the latter investigation only a factor of two reduction was obtained. This divergence between results as variously recorded strengthens the case against the use of inhibition curves in the manner adopted by these latter workers. The close similarity between these calculated curves and those observed experimentally is very striking. Clearly, by suitable choice of the rate parameters the curves could be made to fit the data for any other particular case, and furthermore the values so obtained would be well within the accepted limits for these rate constants.

As further examples, Table 1 gives values of the rate parameters obtained by fitting the foregoing expression to the graphs of Blackmore and Hinshelwood[4] for the three isomeric pentanes at 540° C. The values are somewhat approximate, owing to the error involved in matching the curve to a small scale graph.

The trends in both k_1 and k_2/k_4 with increasing degree of branching of the paraffin chain are the same as those deduced by Blackmore and Hinshelwood[4] and are therefore capable of the same explanations. The trends in k_3/k_5

Table 1. PARAMETERS FOR THE THREE PENTANES AT 540° C

	n-Pentane	Isopentane	Neopentane
k_1 (s^{-1})	$1·8 \times 10^{-3}$	$2·4 \times 10^{-3}$	$3·0 \times 10^{-3}$
k_3/k_4	17·6	12·6	1·8
k_3/k_6 (mole c.c.$^{-1}$)	$2·2 \times 10^{-6}$	$1·8 \times 10^{-6}$	$0·4 \times 10^{-6}$
k_6/k_7 (c.c. mole^{-1})	$5·4 \times 10^{6}$	$8·4 \times 10^{6}$	$0·5 \times 10^{6}$

and k_6/k_7 are also regular. Taking the quotient of the second and third lines gives k_6/k_4 to vary in the ratios 8 to 7 to 4·5 from normal through iso- to neo-pentane. This, therefore, shows a regular trend of decreasing rates of addition of nitric oxide to the more branched (and therefore sterically hindered) radicals. This is in contrast to the results of Blackmore and Hinshelwood for k_6/k_4 which do not show a regular variation, though they agree in that this ratio is smaller for neopentane than for normal or isopentane. The marked decrease in k_6/k_7 for neo-pentane seen in Table 1 is perhaps understandable, as in this case some of the nitroso compounds present are unable to isomerize to the oxime without undergoing structural isomerization of the carbon skeleton. Since this process is likely to be slow these tertiary nitroso compounds may suffer alternative fates. This would increase the rate of termination of chains by NO compared with propagation, and hence decrease the effective value of k_6/k_7.

The present approach, therefore, agrees with that of Blackmore and Hinshelwood in producing regular trends in rate parameters. Moreover, it has been shown to lead to more reasonable absolute values, particularly for k_6. The value of k_1 for *n*-pentane given above ($1·78 \times 10^{-3}$ s^{-1}) agrees very well with that of Blackmore and Hinshelwood, that is, $1·7 \times 10^{-3}$ s^{-1}, but the discrepancy increases going through iso- to neo-pentane. The value given by these workers for this latter case, that is, $> 1·9 \times 10^{-4}$ s^{-1}, cannot easily be accepted, however, since it exceeds their experimentally observed value for the overall rate of pyrolysis in the absence of NO.

The ratio of the rates of consumption of paraffin and of nitric oxide is given by this scheme as:

$$\frac{\dfrac{d[\mathrm{NO}]}{dt}}{\dfrac{d[P]}{dt}} = \frac{\left(\dfrac{k_7}{k_6}\right)[\mathrm{NO}] + [\mathrm{NO}]^2}{\left(\dfrac{k_7}{k_6}\right)\left(\dfrac{k_4}{k_5} + \dfrac{k_3}{k_5}\right) + \left(\dfrac{k_7}{k_6} + \dfrac{k_4}{k_5} + \dfrac{k_3}{k_5}\right)[\mathrm{NO}] + [\mathrm{NO}]^2}$$

Hence, as observed experimentally, this ratio tends to unity at large concentrations of NO. During the inhibition, however, at small concentrations of NO this result predicts a very small consumption of NO compared with paraffin. For example, taking the values for *n*-pentane in Table 1, we obtain for this ratio where the induced reaction is negligible or corrected for:

$$\frac{d[\mathrm{NO}]/dt}{d[P]/dt} \simeq \frac{(k_6/k_3)[\mathrm{NO}]}{1 + (k_6/k_7)[\mathrm{NO}]} = 6 \times 10^{-2} \text{ for NO at 25 mm mercury pressure}$$

Thus a single simple radical chain scheme can be constructed using only known reactions to explain the whole range of the inhibition curves from true inhibition, through a 'flat' minimum rate region, to a linear 'induced reaction' region. It is therefore not necessary to consider these regions as separate and completely independent reactions, and the back extrapolation of the induced reaction to a finite intercept at zero nitric oxide concentration cannot be regarded as evidence for a true molecular component in the reaction. Nor can the low rate of consumption of nitric oxide in the region of minimum rate be so regarded.

Doubtless, numerous other similar schemes can be devised which will produce a rate expression similar to that derived here. For example, the following likely reactions could be added to those used here:

$$R_L + \mathrm{NO} = \mathrm{Ol} + \mathrm{HNO}$$
$$\mathrm{HNO} = \mathrm{NO} + \mathrm{H}$$

This is equivalent to a catalysis of reaction (3) by NO, so that k_3 is replaced by (k_3 + k'_3[NO]). This leaves the form of the rate expression unchanged, only the coefficients of [NO] and $[\mathrm{NO}]^2$ in the numerator being altered. Again, suitable choice of the rate parameters could reproduce the experimental curves.

The mechanism proposed here may be an oversimplification of what actually occurs in the reaction, but it serves to show the importance of a clear knowledge of the role of oximes, their decomposition and reaction with nitric oxide, in these inhibitions.

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ANTIGENICITY OF SYNTHETIC POLYPEPTIDES WITH KNOWN AMINO-ACID SEQUENCE

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ELUCIDATION of the precise structural requirements for antigenicity constitutes a critical problem of modern immunochemistry. Despite concerted efforts in this area, many aspects of this intriguing question still remain unsolved. A rather recent approach to this problem involves use of a variety of synthetic polyamino-acids of the following types: (1) linear polymers of single amino-acids; (2) linear copolymers of two or more amino-acids in varying ratios; (3) branched multi-chain copolymers; (4) polypeptidyl proteins in which new polypeptides and possibly some unsubstituted amino-acids have been coupled to reactive amino groups of a native protein. Utilization of these model compounds has yielded information on the influence of various structural features on antigenicity and the antigen–antibody interaction[1]. But lack of precise knowledge of the sequential

Table 1. STRUCTURE AND ACTH ACTIVITIES OF SYNTHETIC PEPTIDES*

Peptides†	Ref.	Structure	ACTH activity (units/mg)
P-13	3	$\text{Ac.ser.tyr.ser.met.}\overset{\substack{\mathrm{NH_2}\\ \bullet}}{\text{glu}}\text{.his.phe.arg.try.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.pro.val.(NH}_2\text{)}$	Inactive
P-16	4	$\text{Ac.ser.tyr.ser.met.}\overset{\substack{\mathrm{NH_2}\\ \bullet}}{\text{glu}}\text{ his.phe.arg.try.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.pro.val.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.(NH}_2\text{)}$	Inactive
P-20	5	$\text{Ac.ser.tyr.ser.met.}\overset{\substack{\mathrm{NH_2}\\ \bullet}}{\text{glu}}\text{.his.phe.arg.try.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.pro.val.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.arg.arg.pro.val.(NH}_2\text{)}$	Inactive
P-23	6	$\text{Ac.ser.tyr.ser.met.}\overset{\substack{\mathrm{NH_2}\\ \bullet}}{\text{glu}}\text{.his.phe.arg.try.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.pro.val.gly.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.arg.arg.pro.val.}\overset{\substack{\mathrm{F}\\ \bullet}}{\text{lys}}\text{.val.tyr.(NH}_2\text{)}$	Inactive
F-23	6	H.ser.tyr.ser.met.glu.his.phe.arg.try.gly.lys.pro.val.gly.lys.lys.arg.arg.pro.val.lys.val.tyr.(OH)	116 ± 21

* Ac = acetyl; F = N^{ϵ}-formyl.

† In each designation, the number refers to the number of amino-acid residues in the peptide, while the letters *P* and *F* refer to the protected and free peptides, respectively.

arrangement of the amino-acid residues in these polymers, as well as their heterogeneity, restricts their usefulness for this purpose. In an effort to circumvent these difficulties, we undertook an investigation of the possible antigenicity of synthetic homogeneous polypeptides possessing a defined sequential arrangement of their amino-acid residues. Polypeptides satisfying these criteria have become available to us as an outgrowth of extensive studies in this laboratory on the chemistry of peptides related to the amino-acid sequence of the corticotropins[2]. Unexpectedly, it was found that certain of these peptides were markedly antigenic.

Male albino guinea pigs of the Hartley strain (350–450 g) were housed in individual cages with wide-mesh screen bottoms and fed a laboratory chow diet (Purina guinea pig chow, Ralston Purina Co., St. Louis). Structures and ACTH activities of synthetic peptides utilized in these investigations are shown in Table 1. Solutions of peptides in isotonic saline were emulsified with an equal volume of Freund's complete adjuvant and 0·1 ml. of the resulting emulsion injected into each of the four foot pads. Unless indicated otherwise, a total of 200 μg of the peptide administered in this fashion constituted the primary immunizing injection. In a few instances, emulsification was accomplished with an incomplete Freund's adjuvant lacking *Mycobacteria*. For booster injections, the peptides were always emulsified in complete Freund's adjuvant and administered intraperitoneally. Skin reactions were observed following intradermal injection of 10 μg of the peptide in 0·1 ml. of isotonic saline. Area and intensity of erythema, together with changes in skin thickness, served as criteria for estimation of degree of reaction[7]. Reactions of skin were characteristic of the delayed hypersensitivity type with no response at 4 h and maximum reaction at 24 h following challenge. No skin reactivity to any of the peptides was observed in non-immunized animals. Reactions at 24 h were classified as negative when the diameter of erythema was < 5 mm and change in skin thickness < 0·25 mm; strongly positive (+++) reactions exhibited a diameter of erythema > 20 mm and a change in skin thickness > 1·0 mm. Good correlation existed between degree of erythema and skin thickness changes. Anaphylactic reactivity was determined by injecting 2 mg of the peptide in 0·2–0·4 ml. of isotonic saline via a vein of the penis or intracardially. Serum agglutinins were estimated by the hæmagglutination procedure of Boyden[8] and Stavitsky[9]. For sensitization of the sheep erythrocytes, 5 ml. of a 2·5 per cent suspension of tanned erythrocytes in isotonic saline were added to 20 ml. of buffered saline (pH 6·4) and the resulting suspension coated with 5 mg of the respective peptide dissolved in 5 ml. of isotonic saline. Sensitivity of splenic mononuclear cells was determined with a previously described procedure[7]. In this method, migration of splenic cells from a sensitized animal is inhibited *in vitro* through contact with the respective allergen. Similar cells from non-immunized animals remain unaffected by this treatment.

Studies of the antigenicity of various peptides as measured by production of (*a*) skin hypersensitivity (Table 2), (*b*) anaphylactic sensitivity (Table 3), (*c*) circulating agglutinins (Table 4), and (*d*) *in vitro* sensitivity of splenic cells revealed the following salient features. Peptide *F*-23 did not effect an immunological response in any of the foregoing systems. Furthermore, this peptide exhibited no cross-reactivity with *P*-23, *P*-20, or *P*-16 in the skin hypersensitivity or *in vitro* sensitivity tests. In contrast, both *P*-23 and *P*-20 were antigenic. Peptide *P*-23 elicited an immunological response in all four systems while the antigenicity of *P*-20 was evidenced in the two reactions studied, that is, skin hypersensitivity and *in vitro* sensitivity of splenic cells. In these two systems, *P*-23 and *P*-20 were capable of cross-reacting with each other. Animals immunized with either *P*-23 or *P*-20 cross reacted with *P*-16 and *P*-13 in the skin tests. Peptide *P*-16 was less potent than either *P*-23 or *P*-20 in its ability to induce delayed hypersensitivity reactions of skin and was incapable of sensitizing splenic cells.

In this study we have utilized a series of peptides corresponding to N-terminal sequences of ACTH. With the exception of *F*-23, which possesses essentially the full biological activity of ACTH, these peptides are practically devoid of adrenocorticotropic activity (Table 1). It is of

Table 2. DELAYED HYPERSENSITIVITY REACTIONS IN SKIN*

Peptides† utilized for		Skin reactions							
		7 days‡				14 days‡			
Immunization	Skin challenge	0	+	++	+++	0	+	++	+++
F-23	*F*-23	4				4	1		
	P-23	3				1			
	P-20	2							
	P-16	2							
F-23§	*F*-23	6				4			
P-23	*F*-23	4					1		
	P-23		5					10	3
	P-20	1	2				3	1	
	P-16		1	1			1	1	
	P-13						2	1	
P-23§	*P*-23	3	3				3		
P-20	*F*-23	3				3			
	P-23	1	1	1		1		2	
	P-20	2	4				1	2	
	P-16	1	2				1	1	
	P-13						3		
P-20§	*P*-20	4	2						
P-16	*F*-23	3				3			
	P-23	2	1						
	P-20	2	1			2	1		
	P-16	3	2			2	2		
	P-13					2	1		
P-16§	*P*-16	5	1			5	1		

* Values indicate number of individual animals exhibiting the designated reactions 24 h following skin challenge.

† See Table 1 for structures of peptides.

‡ Days after immunizing injection.

§ Emulsified with incomplete Freund's adjuvant.

Table 3. ANAPHYLACTIC REACTIONS

Peptides utilized for Sensitization	Challenge	Primary sensitizing injection (μg)	Challenge after primary sensitizing injection (days)	Reaction*
F-23	*F*-23	200	15	0/3
F-23†	*F*-23	100	23	0/2
P-23‡	*P*-23	200	15	1/2
P-23	*P*-23	200	15	4/5 (1)
	P-23	100	15	2/3
	P-23	200	23	3/3 (1)
P-23†	*P*-23	100	23	3/6 (2)
P-23§	*P*-23	200	37	2/2 (1)

*No. of animals exhibiting symptoms of anaphylaxis/No. of animals tested. Symptoms include respiratory distress, lowered body temperature and convulsions. No. in parenthesis indicates No. of animals, showing symptoms, which died within a few minutes after challenge.

† Booster injection of 100 μg 16 days after primary sensitizing injection.

‡ Emulsified with incomplete Freund's adjuvant.

§ Booster injections of 100 μg 23 and 30 days after the primary sensitizing injection.

Table 4. AGGLUTININ FORMATION*

Immunizing peptide	Antibody determinations (days)†	Erythrocytes coated with *P*-23		*P*-20	
F-23	21	0			0
		0			0
P-23	21	0	160	0	40
		80	320	0	160
		80			
P-23‡	37	10,240	2,560		
		10,240	320		
		10,240			

* Reciprocal peak titres of individual animals are presented.

† Days after primary immunizing injection.

‡ Booster injections of 100 and 400 μg 23 and 30 days after the primary injection, respectively.

considerable interest that protection of the *N*-terminus by an acetyl group and formylation of the lysine ε-amino groups eliminates almost completely the ACTH activity. The adrenocorticotropic potency of the unprotected eicosapeptide is essentially the same as that of the unprotected tricosapeptide (*F*-23) (ref. 5). In contrast to their protected analogues *P*-16 and *P*-13, the corresponding unprotected *N*-terminal hexadecapeptide and tridecapeptide also possess ACTH activity albeit of a low order of magnitude (< 0·1 U/mg). The observation that antigenic potencies of *P*-23 and *P*-20 exceed that of *P*-16 emphasizes the correlation between antigenicity of the protected peptides and adrenocorticotropic activities of their unprotected analogues and suggests that the antigenic and corticotropic determinants may be related chemically.

The demonstration that the synthetic tricosapeptide (*F*-23) of established structure[4] which corresponds to the arrangement of the *N*-terminal 23 amino-acid residues of pig corticotropin possesses essentially the full adrenocorticotropic potency of the natural hormone strongly supports the proposed structure for this section of the molecule. Since this portion of ACTH has been shown to be identical in all species investigated, it is likely the *N*-terminal sequence of guinea pig ACTH. The non-antigenicity of *F*-23 suggests that the antigenic determinants of the ACTH molecule reside in this *N*-terminal sequence. Failure of *F*-23 to serve as an antigen could also be interpreted on the basis that this biologically active compound becomes bound to naturally occurring receptor molecules and thus fails to reach sites of antibody synthesis. On the other hand, the non-attached, biologically inactive protected peptides would be free to exert antigenic activity.

Impressive progress in peptide synthesis and purification has provided almost unlimited possibilities for structural variations and has served to identify structural features essential for a variety of biological functions. Our demonstration of the antigenicity of relatively low molecular weight synthetic peptides has provided a new tool for studies on relation of structure to antigenicity and the antigen–antibody interaction.

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OBSERVATIONS BY BIOCHEMICAL ANALYSIS AND AUTORADIOGRAPHY ON LABELLED DEOXYRIBONUCLEIC ACID IN THE NORMAL AND REGENERATING LIVER OF MICE

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IT is generally assumed that the deoxyribonucleic acid (DNA) of the cell nucleus is a very stable substance. Much evidence has been collected which indicates that once DNA has been synthesized, it remains in the cell until the cell dies. This is a basic assumption for much of the fundamental work which is being done by means of labelled precursors of DNA, and subsequent identification of labelled DNA in autoradiographs[1,2]. In recent years it has been suggested that DNA also has metabolic characteristics[3], but this view has been contested. The present communication reports data which indicate that DNA, as characterized by conventional biochemical methods, displays different physico-chemical properties under different physiological conditions.

The observations which led to the present series of experiments were made during a series of experiments designed to study the possible biological effect of a radioactive label incorporated into DNA in long-term experiments with mice. Tritiated thymidine was used as labelled precursor. Thymidine is considered as a precursor specific of DNA, and the activity demonstrated in a tissue after administration of labelled thymidine as an indication of the amount of labelled DNA present.

We recorded the total activity in several organs of mice at varying times after injection of tritiated thymidine. In normal mice we observed a decrease in the total activity

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Table 1

I	II Liver (μc. × 10⁻⁴ per mg total activity)	III Per cent activity in DNA	IV Activity in acid-soluble fraction per cent of DNA	V Average liver wt., per cent of body-wt.	VI Average body-wt.	VII Kidney (μc. × 10⁻⁴ per mg)	VIII Liver per cent labelled cells
Partial hepatectomy							
1 day after injection	11·3 (*7*) (8·9–12·9)	92 (4)	8 (3)	4·2	22·7	3·8 (*5*) (3·6–4·1)	4·0 (*7*)
8 days	11·8 (*4*) (10·2–12·6)	98 (4)	2·3 (2)	4·6	24·4	2·6 (*4*) (1·7–3·3)	5·0 (*4*)
30 days	9·7 (*4*) (7·2–13·8)	97 (4)	2·2 (2)	4·4	26·1	1·4 (*5*) (0·9–1·9)	2·0 (*5*)
116 days	8·9 (*5*) (7–9·8)	96 (5)	1·4 (2)	4·3	27·8	1·2 (*5*) (0·9–1·5)	3·2 (*6*)
Controls							
1 day after injection	7·2 (*7*) (6·2–8·7)	81 (4)	18 (3)	4·6	25·6	4·3 (*5*) (3·0–4·9)	0·4 (*7*)
8 days	7·9 (*4*) (6·4–9·4)	94 (4)	8 (2)	4·2	26	2·8 (*4*) (2·1–4·0)	0·28 (*4*)
30 days	3·7 (*4*) (3·2–4·7)	87 (4)	18 (2)	4·7	26·3	1·6 (*5*)	0·23 (*4*)
116 days	1·9 (*5*) (1·6–2·1)	78 (5)	17 (2)	4·3	28·1	1·1 (*5*) (0·9–1·3)	0·17 (*4*)

Results of analyses for radioactivity, and per cent labelled cells. The single figure in italics in parentheses of columns II, VII and VIII indicates the number of mice. The figures in parentheses of columns III and IV indicate the number of analyses performed. Owing to the small numbers the variations have not been characterized by statistical symbols, but the highest and the lowest values recorded have been given in parentheses under the mean figures of columns II and VII

of the liver very similar to the results published by Gerber *et al.*[4], who measured the specific activity in the DNA isolated from the liver of normal rats after injection of tritiated thymidine. From their data it is seen that the activity of the DNA of the liver was reduced to one-half in about 15 days. On the other hand, Hecht and Potter[5], and Fresco and Bendich[6] found that the specific activity of the DNA in the liver remained essentially constant for a period of up to 4 months, after injection of orotic acid-6-^{14}C, adenine-1,3-^{15}N, and glycine-2-^{14}C, respectively. These latter experiments were, however, performed with rats that had been subjected to partial hepatectomy shortly before being injected with the labelling substance.

An experiment was therefore set up to compare the activity of labelled DNA in the liver of partially hepatectomized and normal mice, following injection of tritiated thymidine. The activities in the whole organ, and in the isolated DNA fraction, were determined quantitatively, and supplemented by the study of autoradiographs of sections of the organs.

Forty-four male mice of the *WL* Oslo strain were used (85–88 days old, and weighing about 25 g). By means of a thermocauter, about 25 per cent of the liver was removed in 22 mice, under ether anaesthesia. After 42–44 h 20 μc. tritium-labelled thymidine in 0·25 ml. sterile saline was injected intraperitoneally (Radiochemical Centre, Amersham, Great Britain, specific activity 4·5 c./m.mole). The same amount was simultaneously injected, in an identical manner, from the same solution, into each of 22 normal mice from the same batch, which were to serve as controls. The mice were killed by neck luxation 1 day, 8 days, 30 days, and 116 days after injection. The liver was removed and rapidly frozen, and kept below − 20° C until the radiochemical analyses were performed. For these analyses the livers from each sampling were pooled. A small part of the livers was not frozen, but fixed in 4 per cent neutralized formalin solution for autoradiography. Material from the kidneys was secured in the same way, to serve as a cross-check between the two groups.

The pooled liver samples were homogenized in a Potter–Elvehjem homogenizer. Aliquots of the 10 per cent homogenates were subjected to direct assay of the radioactivity. The remaining parts of the homogenates were used for separation of the nucleic acids. The nucleic acids were isolated as described by Schneider and Potter[7], and DNA was separated from ribonucleic acid (RNA) by alkaline digestion[8].

The concentration of DNA was determined quantitatively by the specific colorimetric method of Ceriotti[9]. Tritium was measured by dissolution of the sample in hyamine and liquid scintillation counting[8]. Radioactivity was determined in DNA, RNA, and in the acid-soluble fraction. No tritium was detected in the separated RNA. The acid-soluble fractions contained small amounts of tritium.

For autoradiography the fixed material was dehydrated by means of ethanol, transferred through xylene, embedded in paraffin wax, and sections were cut at 6μ thickness. The sections were deparaffinized in xylene, carried through ethanol to water, and mounted with Kodak *AR* 10 stripping film[10]. They were exposed for 30 days at + 4° C before being developed (the 8 day series were exposed for 60 days).

The solutions of tritiated thymidine were stored for four months at about − 22° C, when they were subjected to paper chromatography. 91–94 per cent of the activity were recovered in the thymidine fraction, confirming the purity of the preparation.

The results of the analyses have been corrected arbitrarily to a body-weight of the mice of 20 g, by multiplying the activities by a factor = body-wt. (g)/20 g assuming that changes in organ weight were proportional to those in body-weight. No corrections were made for the autoradiographic counts. The amount of liver tissue removed was not corrected for, since the weight of the liver one day after injection differed less than one standard deviation from the arithmetic mean of all the controls (4·5 ± 0·45 per cent of body-weight). The removal of about 25 per cent of the liver tissue had at this time, about 3 days post operation, been compensated for by an increase in the liver weight to near normal values. The results are given in Tables 1 and 2.

Table 2

	Total activity ratio hepatectomy/control		Labelling index ratio hepatectomy/control	
	Liver	Kidney	Liver	Kidney
1 day	1·6	0·88	10	2·1
8 days	1·5	0·93	18	1·4
30 days	2·6	0·88	9	0·5
116 days	4·7	1·09	19	1·6

The figures of the total activity and the numbers of labelled cells in the partially hepatectomized mice have been divided by the corresponding figures of the control mice, to show the relation between the results of the test and the control series.

Six main points may be noted from the Tables.

(1) The total activity of the liver on the first day was only 1·6 times higher in the partially hepatectomized mice than in the control mice. In a previous experiment we found that the activity in the liver of normal mice killed 2–3 h after injection was 1·6 times higher than the activity in the livers sampled 24 h after injection. For the sake of argument we therefore consider it safe to state that the initial incorporation of radioactivity was similar in the two groups.

The total activity of the partially hepatectomized group remained at nearly the same level during the four months of the experiments, whereas that of the control group underwent a substantial decrease, in accordance with the results obtained by the authors quoted here[4–6].

(2) In the partially hepatectomized group the activity was almost completely accounted for by the DNA fraction. Although the main part of the activity was also

found in the DNA fraction in the control group, the deficit was consistently bigger.

(3) The activity in the acid-soluble fraction was low in the partially hepatectomized group, but consistently higher in the control group.

(4) The labelling index of the liver in the partially hepatectomized group was one order of magnitude above that of the control group; the number of silver grains in the film above the labelled nuclei was also distinctly higher in the partially hepatectomized group than in the control group. It should be noted that the autoradiographs of the two groups were exposed and processed together.

(5) From published data a substantial increase in the rate of DNA synthesis was to be expected in the regenerating liver at the time of injection, as is apparently borne out by comparing the autoradiographs. The high index of labelled nuclei observed in the partially hepatectomized series is in agreement with results published by Lesher *et al.*[11]. It is therefore striking that the incorporation of activity in the normal liver was of the same magnitude as that of the regenerating liver.

(6) From Table 1 it is seen that the total activity of the kidneys was not significantly different in the partially hepatectomized group and the control group.

From Table 2 it is seen that the labelling index observed in the kidneys was also very similar in the two groups, taking into account the considerable variability of the results to be expected from quantitative autoradiographic analysis with tritium used as a label.

Point 6 is taken as supporting evidence that the differences observed in the liver in the two series by two different techniques are real.

The gradual decrease in activity of DNA of the control series, and the low degree of labelling of the same observed in relation to the activity recorded, may be explained in several different ways: (*a*) by cell turn-over; (*b*) by metabolic DNA turn-over; (*c*) by heterogeneity of DNA; (*d*) by labelled DNA precursors.

(*a*) Assuming that the label is located only in DNA, a higher turn-over of cells in the normal liver than in the regenerating liver might account for the decrease in the control series. This in itself does not seem very likely. Also, it does not fit well with the fact that mitoses are so rarely seen in the liver and that it is scarcely compatible with a cell turn-over of the order of one month or less[4], though this possibility cannot be completely discarded. It does not explain the autoradiographic results.

(*b*) A metabolic turn-over of DNA in the control series is conceivable, though a difference in DNA turn-over would have to be assumed in cells of a normal and a regenerating liver. The reduced labelling in the control series is not explained in this way.

(*c*) The differences observed may be due to different physico-chemical properties of DNA synthesized in normal and in regenerating liver and it may be an expression of heterogeneity of DNA[12]. This could account for the different percentage of activity found in the DNA fraction of the two series (2), and the differences in the acid-soluble fractions (3). It could also account for the different degree of labelling observed in the autoradiographs, but not so easily for the decreasing activity of the control series.

(*d*) The same arguments as under (*c*) apply to labelling of DNA precursors. These would also seem more likely than DNA having a metabolic turn-over.

Taking into account the observation mentioned under point (6), we favour the opinion that the substance in the DNA fraction of the analysis is heterogeneous and may perhaps include DNA precursors besides what may be termed true, genetically stable DNA. It also seems likely that the labelled thymidine is utilized differently in normal and in regenerating liver.

The results described have implications for the theoretical background behind the use of labelled thymidine as an indicator of DNA synthesis. Assuming that DNA is metabolic stable, thymidine cannot be regarded as a precursor for the stable DNA only: and vice versa, if one assumes that thymidine is either promptly incorporated into DNA only or degraded, it is scarcely reconcilable with DNA as a metabolic stable substance.

Most experience with labelled thymidine relates, however, to rapidly dividing cells, such as in bone marrow, intestinal epithelium, and tissue culture, as also is the case in the regenerating liver. It is possible that thymidine under such conditions is very specific as a DNA precursor, and that it is utilized only or mainly for stable DNA, as seems likely from the available experimental evidence.

But the results presented in this communication indicate that the substance(s) into which the labelled thymidine is incorporated may display different physico-chemical properties under different physiological conditions, and that the label is not always to be found in the metabolic stable DNA.

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MYELOCYTE-METAMYELOCYTE TRANSITION IN THE BONE MARROW OF THE DOG

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THE granulocytes constitute a steady-state renewal system, cells being born in the bone marrow and lost in the periphery. Although the general chronology of granulocyte development is known[1,2], the dimension of time alone reveals only a part of the over-all picture. It is necessary to determine the flow of cells to understand the population relationships. This article is concerned with the flow of cells from the proliferating to the non-proliferating compartments in the bone marrow of the dog as revealed by autoradiographic analysis after labelling with tritiated thymidine. There are several transitions during granulocytopoiesis, that between myelocyte and metamyelocyte being particularly important, since the former is the last of the dividing granulocytes and the latter the first of the non-dividing granulocytes in the developmental sequence.

Two eight-month-old beagles were injected intravenously with tritiated thymidine (0·3 mc./kg body-weight; specific activity 360 mc./m.mole). The experimental procedures of bone marrow sampling and autoradiography have been described elsewhere[3]. It is generally agreed that tritiated thymidine is incorporated initially only in those cells that are synthesizing deoxyribonucleic acid (DNA) in preparation for mitosis (*S* period). Since granulocytes eventually reach a stage of maturation in which mitosis does not occur, some cell types, for example, metamyelocyte, will become labelled only with the passage of time. Labelled metamyelocytes appear within 3–4 h after injection of tritiated thymidine; the percentage of labelled cells then increases linearly for a few hours to a plateau followed by a second rise after 14 h. The results are given in Fig. 1.

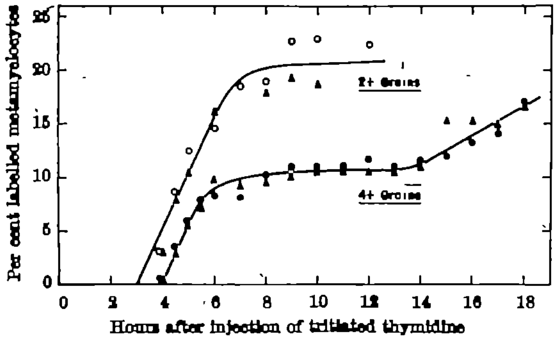

Fig. 1. Initial pattern of labelled metamyelocytes after injection of tritiated thymidine. (Each point is derived from enumeration of 1,000 metamyelocytes; triangles, dog *A34C*; circles, dog *A34E*)

It will be noted that the initial rate of increase of labelled metamyelocytes seems to be the same whether the autoradiographic analysis includes cells with 2 grains or more (2+) or only cells with 4 grains or more (4+). The linear increase extends over about 4 h with 2+ cells and 2 h with 4+ cells; it begins earlier and persists longer in the former instance. These results suggest that the more lightly labelled cells may be derived mainly from myelocytes which were near the beginning or end of their DNA synthetic period, while the more heavily labelled cells may be derived from a cohort near the middle of the *S* period. If this is so, the rate of uptake of thymidine must increase to a maximum around the middle of the *S* period and then decrease. The period of linear increase of 2+ metamyelocytes approximates 4·5–5-h duration of the myelocyte *S* period[4]; the agreement would perhaps be even closer if the progeny of myelocytes with less than 4 grains, which would appear as metamyelocyte false negatives, were included. The close correspondence of the duration of the *S* period and of the initial increase in labelled metamyelocytes indicates that there is little variation in the transitions from myelocyte DNA synthesis to myelocyte mitosis to metamyelocyte. A second rise in labelled metamyelocytes occurs some 10 h after the first increase. The rate of increase is less at this time, possibly because of the variation in the period preceding DNA synthesis, and also because of further dilution of the label by mitosis with resulting increase in false negatives. The 10-h interval may be a reflexion of the minimum duration of the myelocyte generation cycle.

Since the initial rate of entry of metamyelocytes is about 5 per cent per hour (Fig. 1), the turn-over or transit time would be 20 h. Labelled band cells were not seen even at 18 h after injection of tritiated thymidine, which means that the minimal transit time must exceed 15 h. It is noteworthy that a 20-h transit time was also obtained in earlier work by comparison of the time of appearance of label in metamyelocyte and band cell[1]. The initial rate of appearance of labelled metamyelocytes was considerably less in the latter instance, probably because insufficient thymidine was given to label all the myelocytes in synthesis of DNA. Since in the earlier investigation only about half the myelocyte mitoses were labelled during the first several hours after injection of tritiated thymidine, labelled and unlabelled cells must have entered the metamyelocyte compartment at the same time.

The magnitude of the metamyelocyte influx should correspond to the myelocyte efflux, assuming that few cells die or otherwise escape from the proliferating compartments. The initial labelling index of cells capable of mitosis and the duration of the *S* period provide a rather precise estimate of the birth or proliferation rate. This estimate will not be influenced by the possibility that: (1) all cells of a given type may not divide even though all may be capable of division; (2) the morphological boundaries of a given cell type may not include a complete generation cycle, that is, from the end of one mitosis to another. Some 37 per cent of myelocytes are labelled (2+ grains) a half-hour after injection of tritiated thymidine which, with an *S* period of 5 h, corresponds to a birth rate of about 7·5 per cent per hour. Since the metamyelocyte turnover is 5 per cent per hour, and the relative distribution of myelocytes to metamyelocytes in the marrow smear is 1 to 0·75, this means that for every 7·5 myelocytes that are born, only 3·75 myelocytes are transformed to metamyelocytes.

Cells are also entering the myelocyte compartment, and hence the myelocyte efflux must be greater than the myelocyte proliferation rate in the steady state with constant population. If we assume that all myeloblasts and promyelocytes eventually become myelocytes, the myelocyte efflux will be given by the sum of the proliferation rates of myeloblast, promyelocyte and myelocyte plus the presumed stem cell efflux (myeloblast influx). The latter is an indeterminate quantity at present, but it is probably very small relative to the succeeding fluxes. 40 per cent of myeloblasts, promyelocytes and myelocytes as a group are labelled at a half-hour after injection of tritiated thymidine; thus, the combined birth-rate with a 5-h *S* period is 8 per cent per hour. The proportional distribution of this group of cells to metamyelocytes is 1 to 0·6 and on this basis the relative efflux from the proliferating compartments would be 8 and the metamyelocyte influx 3. Whether we consider only the myelocyte proliferation rate or the approximate total myelocyte efflux, the results indicate a substantial 'over-production' or 'ineffective' granulocytopoiesis. A similar conclusion can be reached independently by comparing the expected yield after mitotic doubling of myelocytes labelled initially with 4 grains or more to the number of metamyelocytes with 2 grains or more. We can infer from the *S* period and the metamyelocyte appearance curve that all labelled myelocytes will have completed mitosis by 8 h after thymidine injection.

If only a part of the granulocyte production flows into the metamyelocyte compartment, it should be possible to account for the excess cells. Apropos of this, there is an increase in the percentage of labelled myelocytes during the first several hours after injection of tritiated thymidine. It is difficult to estimate the increase as long as some labelled cells are still undergoing mitosis. However, when myelocytes with 8+ grains at 0·5 h (21 per cent positive) are related to myelocytes with 4+ grains at 8 h after injection of tritiated thymidine (30 per cent positive), there is a 40 per cent increase. A similar increase does not occur in myeloblasts and promyelocytes. Thus, there is an appreciable 'sink' in the myelocyte compartment for accumulation of labelled cells. It follows that there must be a substantial number of myelocytes that do not participate in mitosis, but rather that are in a transitional phase prior to their loss in some way other than maturation to metamyelocyte. The transition from myelocyte to metamyelocyte requires about 1 h (3-h appearance time less 2 h for post-S period and mitosis); however, this could not be a factor in the accumulation of labelled myelocytes 8 h after thymidine administration. A prolonged myelocyte–metamyelocyte transition, postulated in our earlier experiments[1], would be inconsistent with the present finding that the initial rate of entry of labelled cells into the metamyelocyte compartment is sufficient to account for its turn-over. It is of interest that there is no evidence of metamyelocyte attrition; labelled metamyelocytes can be accounted for afterwards as labelled band cells[1].

Others have alluded to the possibility of some 'ineffective' erythrocytopoiesis[5,6] and the fact that 'ineffective production' occurs in the case of the granulocytes is perhaps not too surprising. The myelocyte overproduction cannot be attributed to possible toxicity from incorporated tritium[3] or to possible stimulation by thymidine[7]. If either of these factors were of consequence, this should be apparent in the rate of appearance of labelled metamyelocytes; the metamyelocyte turn-over is consistent with data obtained in other ways and after other doses of tritiated thymidine. It should be pointed out that this work was performed on young dogs and, conceivably, the degree of overproduction could vary with age. We are inclined to think that the so-called 'ineffective' granulocytopoiesis represents an important facet of granulocyte regulation. Flexibility of the granulocyte system could be brought about by adjustment of the balance of 'effective' and 'ineffective' production. The population relationships and their regulation will be discussed in greater detail elsewhere[8].

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A PRESUMED OBSTACLE TO MAMMALIAN POLYPLOIDIZATION

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THE sex of *Drosophila* is determined by ratios between autosome sets and X's. The Y lacks sex determining genes. From this Muller[1] inferred that the sex chromosomes might be an obstacle to polyploidization in animals by virtue of the difficulty in maintaining the foregoing ratio.

Contrary to its inertness in *Drosophila*, the mammalian Y chromosome has a strong male-determining effect[2–4]. Still, the ratio of autosomal sets to X chromosomes ($A : X$) might complicate polyploidization due to the presence of sex chromatin or Barr bodies in female somatic interphase cells.

The transformation alternately of one paternal or one maternal X of the female embryo cells into the sex chromatin[5–7] is initiated during early embryogenesis by anaphase chromatid tension[8,9] due to pseudo-chiasma formation on one of the X's.

Most presumably, the X or the part of it which constitutes the sex chromatin is genetically inactivated[5–7]. This implies that the female zygote and a few subsequent cell generations have two active X's, but the somatic cells of later cell generations, only one. Thus, the somatic ratio of diploids would approach $2A : 1X$ in both sexes.

The present problem mainly concerns the resulting gene balance in the soma. In females this balance is the result of the embryonic X inactivation. Consequently, the theories trying to explain how many X's get inactivated should not take endopolyploid somatic cells as models. Such polyploid cells might have originated at a later stage and would represent the statically balanced situation of their predecessors. The events recorded under II (Table 1) are in agreement with all the balance theories proposed[9–12].

The resulting somatic $A : X$ ratio of polyploids perhaps would be $nA : 1X$ because in diploids supernumerary X's are transformed into sex chromatin. However, it is not definite that only one X of the polyploid would be active. Possibly, the gene ratio of the zygote and the succeeding few cell generations before the soma is formed determines whether the inactivation process starts or not. One could expect that the events always start at or below the level $A : X = 1$ and stop at $A : X = 2$, because these levels are characteristic of normal diploids. The formation of sex chromatin within presumably haploid $1A : 1X$ embryos[9] is in agreement with the supposition about the starting level. The haploids, however, do not tell us anything about a final ratio.

There are several instances of polyploid embryos reported from experimental mammals[13,14] but the lack of information concerning their sex chromosome conditions leaves the imaginable courses of X chromosome inactivation only to conjecture.

Table 1. ANTICIPATED X-ACTIVITY IN ZYGOTE, GERMLINE, SOMA BEFORE X CHROMOSOME INACTIVATION AND IN SOMATIC CELLS FROM EMBRYOS AT VARIOUS PLOIDY LEVELS

Diploidy of one of the gametes (restitution nuclei at one of the two meiotic divisions) or doubled zygotes is considered. (Normal ratios and verified numbers of sex chromatin bodies in italics)

Zygote All X's active	Germline, and soma before X inactivation All X's active		Soma after X inactivation (I) Inactivation of X's approaching the ratio at which all but one X's are inactivated			(II) Inactivation of X's approaching the normal diploid ratio $2A : 1X$		
Chromosome complement	$A : X$	Sex chromatin bodies	Genotype X, active (X), inactive	$A : X$	Sex chromatin bodies	Genotype X, active (X), inactive	$A : X$	Sex chromatin bodies
$A+X$	1 : 1	0	$A+(X)$	1 : 0	*1**	$A+(X)$	1 : 0	*1**
$2A+2X$	*1 : 1*	*0*	$2A+X+(X)$	*2 : 1*	*1*	$2A+X+(X)$	*2 : 1*	*1*
$2A+X+Y$	*2 : 1*	*0*	$2A+X+Y$	*2 : 1*	*0*	$2A+X+Y$	*2 : 1*	*0*
$3A+3X$	*1 : 1*	0	$3A+X+2(X)$	3 : 1	2	$3A+X+2(X)$ or $3A+2X+(X)$	3 : 1 or 1·5 : 1	2 or 1
$3A+2X+Y$	1·5 : 1	0	$3A+X+(X)+Y$	3 : 1	*1*†	$3A+X+(X)+Y$ or $3A+2X+Y$	3 : 1 or 1·5 : 1	*1*† or *0*‡
$3A+X+2Y$	3 : 1	0	$3A+X+2Y$	3 : 1	0	$3A+X+2Y$	3 : 1	0
$4A+4X$	*1 : 1*	0	$4A+X+3(X)$	4 : 1	3	$4A+2X+2(X)$	*2 : 1*	2
$4A+2X+2Y$	*2 : 1*	0	$4A+X+(X)+2Y$	4 : 1	1	$4A+2X+2Y$	*2 : 1*	0

* (ref. 9) † (ref. 20) ‡ (ref. 11)

To my knowledge there are three reports of human beings containing a majority of triploid cells in which the sex chromosome constitution were analysed[15-17]. In each case the triploid cells all had the constitution $3A : XXY$. In two cases the embryos were spontaneously aborted at an early stage[16,17] and in one case, where an abnormal young male patient was examined, he was found to be a mosaic composed of triploid and diploid cells[11]. This human triploid–diploid mosaic is another example of what has been found in a male rabbit[18] and pig[19]. It is at present possible to understand the principles for the origin of triploid–diploid mosaics in mammals[14], and the evidence of their post-embryonic existence is accumulating. However, newly arisen pure polyploid mammals have not so far been found as adults.

The events connected with the X chromosome activity in the zygotes, and at the subsequent differentiation of somatic cells from the germline, are summarized in Table 1.

Within a triploid–diploid mosaic there would be a selection on the somatic level against triploid cells, because they are unbalanced. One of the possible sources of this unbalanced condition may be the relation between active X and autosomal genes. The lack of a sex chromatin body[11] or the presence of one[20] has been reported in these cells. This would imply that either two or one genetically active X functions respectively. From this follows that the two expected $A : X$ ratios in the $3A : XXY$ triploid cells, somewhat deviating from the normal 2 : 1 ratio, are realized. Possibly, cells with one and two active X's have a different survival value. It would contribute to a further understanding of the problem to know the viability of a human triploid–diploid mosaic, in which the triploid cells display one sex chromatin body instead of none.

It seems very probable that the diverging relationships between active sex chromosomal and autosomal genes, which would result in the somatic cells of pure triploids, cause the serious disturbances and lethality which seems to be their characteristic[14].

Female and male tetraploids would be normally balanced both in their germlines and their somatic cells, provided the inactivation of X's occurs according to the second possibility scheduled in Table 1, which is the most probable event. However, if all but one of the X's are inactivated in accordance with the first possibility scheduled, the balance in the somatic cells of tetraploids would be very abnormal. Of course, the tetraploids may have too many chromosomes to function very well even if their $A : X$ ratio is in accordance with the one in diploids, both in germline and soma.

If the present assumptions are correct, they would imply that occasional tetraploids of both sexes resulting from zygote doublings in mammals have a fair chance to survive. At least there should not be any obstacles due to $A : X$ imbalance either in the germline cells at very early embryonic life or later on in the soma. The mammalian pure triploids, however, would have a minimum chance of survival because of a necessarily abnormal chromosome balance in the early embryonic cells of males and in the soma of both sexes.

Thus, the newly obtained knowledge about 'dormant' X chromosomes would explain why mammalian triploids have not been able to participate in the establishment of tetraploidy.

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ELASTASE PRODUCTION BY MICRO-ORGANISMS

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BECAUSE of the possible implication of pancreatic elastase in the aetiology of vascular diseases[1,2], it was felt that elastase derived from sources other than the pancreas might provide interesting comparative characteristics. Thus, in conjunction with a study of pancreatic elastase and its serum inhibitor, under way in this laboratory, our attention was turned to the microbial flora of soil. Soil is a source known to provide micro-organisms capable of breaking down all natural substrates. This article describes some of the characteristics of an elastolytic enzyme isolated from a fungus obtained from soil. Also, the possible implication of this elastase-producing fungus in the etiology of temporal arteritis is discussed.

Elastin, prepared according to the method described by Gilfillan *et al.*[3], was used in the assay system. An agar medium containing only elastin, agar and inorganic salts was prepared as shown in Table 1. About 1 g of soil obtained from a flower bed in the hospital grounds was dispersed in 99 ml. of sterile distilled water. A 0·1-ml. aliquot was spread over the entire surface of elastin agar plates which were then incubated at room temperature (22°) and at 37° for a 3–5 day period. Isolates which showed clearing of the elastin were picked and restreaked on elastin agar medium for pure culture clones.

A 3–5-day culture of one of the organisms grown in Fildes tryptic digest broth (BBL), afterwards identified as *Streptomyces flaveolus*, was used for the preparation

Table 1. COMPOSITION OF ELASTIN MEDIUM*

Components	Amount of components in stock solution	Volume of stock solutions for 100 ml. of complete medium
Inorganic salts *A*	25 g	
K_2HPO_4		
KH_2PO_4	25 g	
Distilled water	250 ml.	0·5 ml.
Inorganic salts *B*		
$MgSO_4.7H_2O$	10 g	
$NaCl$, $FeSO_4.7H_2O$	0.5 g of each	
$MnSO_4.4H_2O$		
Distilled water	250 ml.	0·5 ml.
Elastin*	10 g	
Distilled water	100 ml.	10 ml.
Distilled water		87·5 ml.
Agar		1·5 g

* Water–sodium hydroxide elastin is used. It is autoclaved for 10 min at 10 lb. pressure. The resulting pH is 7·0.

of crude elastase. The mycelial mat was removed by filtering through 3–4 pads of cheese-cloth. Extracellular elastase was purified by fractionation with ammonium sulphate. Ammonium sulphate was added to 60 per cent saturation and precipitation was allowed to proceed overnight at 2°–5°. The resulting precipitate was collected after centrifugation at 12,000*g* for 45 min. It was then suspended in carbonate buffer *p*H 8·4 and dialysed against this buffer overnight before assay.

The crude intracellular enzyme was prepared by grinding the mycelial mat with alumina (80–200 mesh, Fisher Scientific Co.). About 2 vol. of carbonate buffer, *p*H 8·4, was added to the extract and allowed to stand in the cold for 1 h. The alumina was removed by centrifugation at 2,000*g* for 15 min, and the supernatant was clarified further by centrifugation at 20,000*g* for 60 min. The resulting clear supernatant was collected and served as a source of crude intracellular enzyme. Both intra- and extra-cellular enzyme was assayed by the plate technique, as described by Sbarra *et al.*[4,5], and nepholometrically, as described by Gilfillan *et al.*[6]. The crude intracellular enzyme was used in all our studies. The extracellular enzyme was found active both on plates and nephelometrically. These results will be reported later.

Normal human serum was obtained from healthy personnel of the laboratory and tested for inhibitor as previously described[3]. A number of mice and guinea pigs, weighing approximately 20 g and 350 g respectively, were injected intraperitoneally with a 5-day culture of *Streptomyces flaveolus*. The animals were killed at intervals, from day 1 to day 150, after challenge, and histological sections of liver, kidney, blood vessels, spleen, intestine, skin and lungs were prepared. Two sets of slides were made, one was stained with hæmatoxylin and eosin, and the other with periodic acid–Schiff stain.

Five different organisms were isolated from soil which have the ability to hydrolyse elastin. Two of these have been placed in the genus *Streptomyces*; one has been completely characterized and classified according to *Bergey's Manual of Determinative Bacteriology*[7], as *Streptomyces flaveolus*, but the other has not yet been assigned a species designation. A yellow pigmented organism, capable of digesting elastin and belonging to the genus *Flavobacterium* was also isolated. In addition, a mould and a Gram-positive spore-forming rod were also observed to digest elastin and subsequently identified as *Aspergillus niger* and a *Bacillus* sp. respectively.

The elastolytic activity of *Streptomyces flaveolus* on agar containing elastin is shown in Fig. 1.

The results obtained when the crude intracellular enzyme was allowed to hydrolyse elastin, in the absence and presence of serum, are shown in Fig. 2. It can be seen in Fig. 3 that the optimum elastolytic activity occurs at 30°. Table 2 shows that the enzyme is completely inactivated by heating for 30 min at 54°. Such treatment effectively inhibits hydrolytic activity but subsequent incubation at 37°, in the presence of the substrate, restores activity so that at 24 h it is equally as active as untreated enzyme. Reactivation does not occur following treatment at 100° for 15 min.

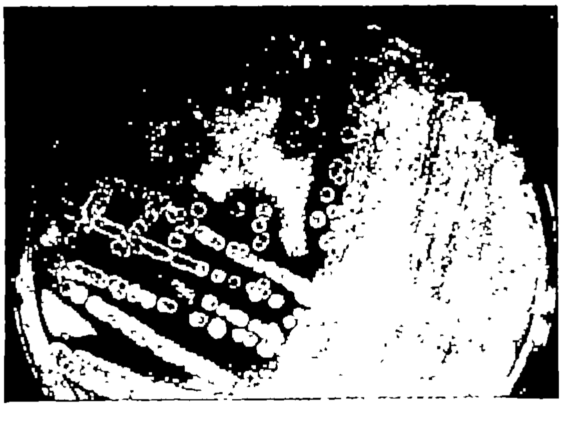

Fig. 1. Elastin agar plate inoculated with *Streptomyces flaveolus*. Afterwards incubated at room temperature (22° C) for 72 h. Clearing around colonies due to liberation of elastase

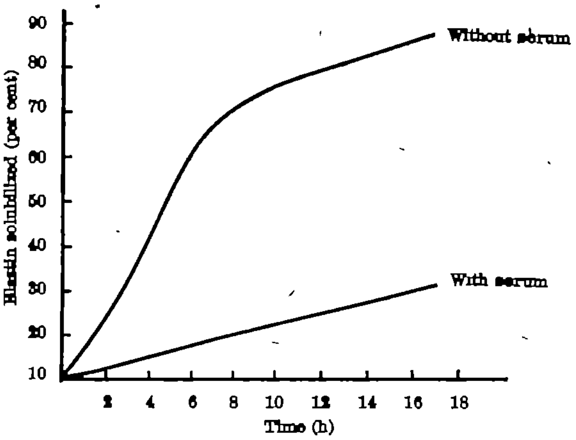

Fig. 2. Effect of *Streptomyces flaveolus* elastase on elastin in the absence and presence of serum. The reaction was followed nephelometrically in a Bausch and Lomb Spectronic 20 colorimeter at 550 mμ. The reaction mixture consisted of 0·5 ml. of elastase (0·9 mg protein), 24 mg elastin was made to 4·0 ml. with carbonate–hydrochloric acid buffer *p*H 8·4, ionic strength 0 05. All reactants were warmed to 37° and enzyme was added at zero time

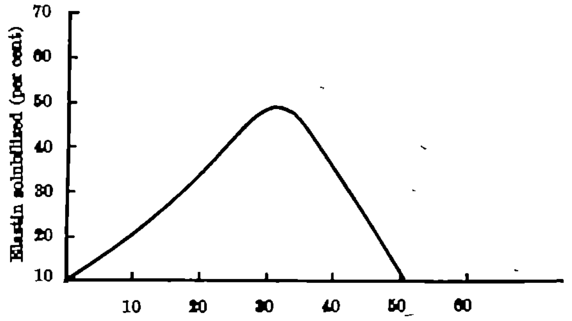

Fig. 3. Effect of temperature on solubilization of elastin by *Streptomyces flaveolus* elastase. Substrate-disappearance was measured at half-maximum velocity in a system containing 0·9 mg enzyme protein

The only pathological changes seen so far have been the appearance of granulomas in the liver and lungs of both mice and guinea pigs that had previously been injected with a 5-day culture of *Streptomyces flaveolus* twice weekly for one month. Histological sections of liver and lungs of autopsied guinea pigs showed the presence of acidophilic homogeneous spherules and elongated structures which gave a positive reaction to the periodic acid–Schiff stain. These structures are tentatively considered as spores or mycelial fragments. Animal tissues from uninfected animals did not reveal these structures.

The finding that different micro-organisms have the ability to produce elastase is not new, but the significance of such findings has received little, if any, attention. Horton and Magath[8,9], in an investigation concerned with

Table 2. REACTIVATION OF HEAT-TREATED *Streptomyces flaveolus* ELASTASE*

Treatment	% Elastin hydrolysed at 37° † Incubation time 3 h	 24 h
None	50	70
54° 30 min	0	67
100° 15 min	0	0

* See Fig. 2 for composition of reaction mixture. Elastase concentration, 1·8 mg. protein.

† Enzyme exposed to respective temperatures and immediately assayed at 37°.

the ætiology of temporal arteritis, were able to isolate an 'Actinomyces' from resected temporal arteries in two out of seven temporal arteritis patients studied. After limited bacteriological studies they concluded that these organisms were laboratory contaminants, and thus were not associated with ætiology. The organism involved could have been a *Streptomyces*, a common soil organism, according to current classification. Inasmuch as five of the seven patients examined were farmers, the organisms could have gained entry through the mouth. It is also possible that dental procedures might permit entrance of an otherwise non-pathogenic organism. The fact that *Streptomyces* produces elastase and since mycelial fragments were observed at biopsy may indicate a primary or secondary association of these organisms in the elastic tissue destruction (modification) observed in this disease. Further, these observations suggest that epidemiological studies in this disease may prove fruitful.

In limited animal studies, so far, we have been able to detect granulomas in the livers of mice and guinea pigs injected with *Streptomyces flaveolus*. It has also been possible to stain mycelial-like fragments in the liver of guinea pigs and to recover, on occasion, organisms from the livers of injected mice. No other pathological changes have been detected so far. The significance of the liver and lung granulomas is, as yet, unknown. The possibility that elastase-producing micro-organisms may be associated with the ætiology of vascular diseases is intriguing and is at present under investigation.

The fungal enzyme, like the pancreatic elastase, is inhibited by serum. Mandl and Cohen[11] have reported a bacterial elastase produced by *Flavobacterium elastolyticum*, that is not inhibited by serum. This bacterial enzyme differs from that described since it was found to have optimal activity between 50° and 55°. The fungal enzyme has a temperature optimum of 30°. Thus, it appears that these two enzymes are different in at least two respects. On the other hand, the fungal enzyme appears similar to the pancreatic elastase in respect to serum inhibition and temperature optimum. The significance of these findings is unknown.

The reactivation of the enzyme by incubation at 37° is of interest. Perhaps the enzyme chain is inactivated at 54° by a folding phenomenon, and subsequent incubation at 37° results in unfolding with a concomitant restoration of activity. Investigations are under way to further purify the enzyme.

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BIOCHEMICAL ANALYSES OF THE CARCASS IN LETHAL STAPHYLOCOCCAL INFECTIONS OF MICE

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In previous investigations[1] we found that mice killed by injections of virulent staphylococci show a high blood concentration of transaminase, alkaline phosphatase and inorganic phosphorus associated with a low sodium. There was some indication also of less-definite decreases in the blood levels of sugar, carbon dioxide and cholesterol. It appeared necessary to extend the work to see if the alterations of the blood were coincidental or an essential part of the mechanism of death. In order to avoid conclusions from biochemical changes restricted to the blood stream, we have studied the total biochemistry of the animal body.

The hæmolytic, coagulase-positive Smith strain of *Staphylococcus aureus*, the properties of which have been published previously[2,3], was used. The mice used were Carworth Farms (New City, New York) white, Swiss males. The animals weighed 15–18 g and were given food and water *ad libitum* before examination. Mice were inoculated with a heavy dose (0·1 c.c. = 10^9 organisms) intraperitoneally, as we have shown that the dose of organisms (above a certain minimum quantity) and route of infection were not important in the production of death in mice from staphylococcal infections[3]. Homogenates of whole mice (less skin, tail and feet—which did not blend[4] and gut to avoid Gram-negative organism contamination) were made by adding the carcass to 50 ml. of chilled distilled water and grinding for ½ min at slow speed and 2½ min at high speed[3,4].

The carcass homogenates made at death were transferred to the biochemistry laboratory and the determinations were made by standard methods. Sodium fluoride (10 mg/c.c.) was added to prevent enzymatic degradation of the glucose before analysis.

Biochemical values for normal and infected Carworth Farms white mouse carcasses are given in Table 1. A comparison of the results of the normal and intraperitoneally infected mice show statistically significant differences ($P \leq 0{\cdot}05$) in sugar, total protein, cholesterol, inorganic phosphorus and transaminase (Table 1).

Significant and comparable chemical changes in glucose, inorganic phosphorus, transaminase and cholesterol are found in the blood and carcass. The blood proteins show a decrease parallel to the carcass proteins but the extent of the change is not significant[1]. If it is assumed that inflammation destroys the semipermeable membranes of the body leading to an adynamic equilibrium of intra- and extra-vascular substances, then the changes in alkaline phosphatase in the blood are easily explained. Possibly a similar mechanism explains the discrepancies in the carbon dioxide values. Further study is needed to correlate the sodium loss in the carcass with the sodium loss in the blood.

Two additional strains of *S. aureus* were used for challenging the same strain of mice. Analysis of the biochemical results detailed in Table 1 showed no discrepancies from the conclusions reached with the Smith strain.

Limited studies of carcass biochemical values have been completed for other routes of inoculation (Table 2). Good correlation was observed in all values except inorganic

Table 1. Carcass Biochemical Values for Carworth Farms White Mice

	No organisms		Live organisms				Heat-killed organisms	
	Normal	±*S.D.*	*S. aureus*	± *S.D.*	*S. albus*	± *S.D.*	*S. aureus*	± *S.D.*
Sugar (mg %)	58·6	13·2	18·8*	9·9	6·5*	4·0	12·8*	3·4
Urea nitrogen (mg %)	6·2	0·8	7·4	3·6	6·2	0·4	4·6*	1·9
Creatinine (mg %)	0·39	0 17	1·13	1 90	0·2*	0	0 23*	0·08
Uric acid (mg %)	3·06	1·48	1·5	1·7	1·75*	0·36	1·43*	0·47
CO_2 (m.equiv.)	1·84	0·81	2·87	1·91	0 62*	0·53	0·88*	0·37
Chloride (m.equiv.)	4·7	1·6	5·6	1·2	5·18	0·90	7·17*	1·61
Potassium (m.equiv.)	10·0	5 9	17·5	14·2	8·42	2·48	14·5	2·3
Sodium (m.equiv.)	29·1	15·8	23·5	7·2	15·2	4·7	11·08*	4·07
Total protein (g %)	1 73	0·48	1·07*	0·64	0 96*	0·27	1·4	0·3
Cholesterol (mg %)	31·1	11·2	10·8*	10·5	18·7*	7·1	14 2 *	12·2
Inorganic phosphorus (mg %)	9·0	6·9	21·2*	0·8	10·8	3·1	17·43*	6·41
Alkaline phosphatase (S.U.)†	53·7	11 6	40·3	19·0	31·5*	12·4	37·2*	8·5
Acid phosphatase (S.U.)	13·1	9·2	6·96	1·91	14·1	4·7	1·71*	0·24
Transaminase (S.F.U.)‡	1,937	1,503	2,583*	1,166	4,007*	951	3,436*	607

* Significant by the *t* test ($P \leq 0·05$.)

† S.U., Sigma units. ‡ S.F.U., Sigma–Frankel Units

Because of the turbidity of the homogenate calcium, bilirubin, cephalin flocculation, thymol turbidity, zinc turbidity and protein partition estimations were not made.

Table 2. Biochemical Values at Death

		Route of inoculation†				
Determinations	Control animals	Intraperitoneal (0 1 c.c.)	Intravenous (0·1 c.c.)	Intracranial (0·05 c.c.)	Intramuscular (0·5 c.c.)	Subcutaneous (0·3 c.c.)
Glucose	58 6	5·60	4·76	7·03	4·82	6·70
Total protein	1·73	1·07	8·3	0·9	1·1	1·0
Cholesterol	31·1	10·8	6	15	12·3	16·0
Inorg. phosphorus	9·0	21·2	18·0*	16·8*	19·3	11·6*
Transaminase	1,937	2,583	1,160*	1,440	3,093	2,340

* Significantly different than the intraperitoneal route ($P \leq 0·05$)

† Titre $= 10^{10}$ organisms/c.c.

phosphorus when the results were compared with those obtained following intraperitoneal infection.

Additional significance can be given to these chemical findings by showing a steady trend from the time of infection to the time of death. Animals have been studied at hourly intervals after intraperitoneal challenge with *S. aureus* Smith and a significant trend was shown (Fig. 1) for glucose.

To differentiate between the consumption of glucose by *S. aureus* and consumption by the mouse host, an *in vitro* investigation was made. Mice were injected with *S. aureus* in the usual manner and immediately killed and the carcasses were ground. After an aliquot was taken for glucose determination, the homogenates were placed in the 37° C incubator. Glucose determinations were made every hour for 6 h and the results were compared with those obtained from the *in vivo* study (Fig. 2). It is evident that microbial consumption of glucose does not explain the loss of glucose from the carcass at death from staphylococcal infection. Normal uninfected carcasses placed in the incubator and sampled at intervals did not change significantly in glucose content.

Other studies in our laboratory have shown that animals infected with the Smith strain of *S. aureus* ($10^9 = 0·1$ c.c. intraperitoneally) can regularly be cured by 400 mgm/kg of penicillin *G* if the drug is given subcutaneously 1 h after infection[8].

Hourly glucose determinations were made to see if glucose-levels of staphylococcal infected mice would return to normal after treatment with penicillin. It may be seen in Fig. 3 that although there is an initial fall in glucose, a gradual rise is evident although not as marked as we had expected.

Similar challenge with heat-killed (80° C for 30 min) Smith strain of *S. aureus* given intraperitoneally revealed, in addition to the changes found with live virulent *S.*

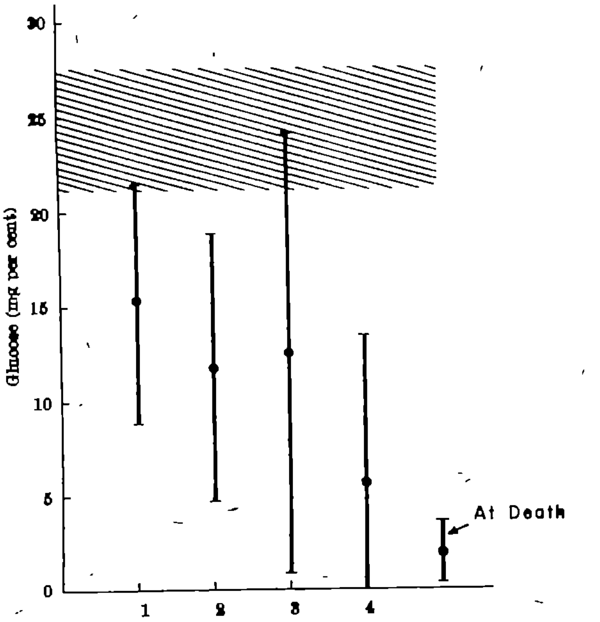

Fig. 1. Disappearance of glucose from mouse carcass at stated intervals after infection. Shading, normal mean − 1·96 *S.D.*; vertical lines, mean ± 1·96 *S.D.*

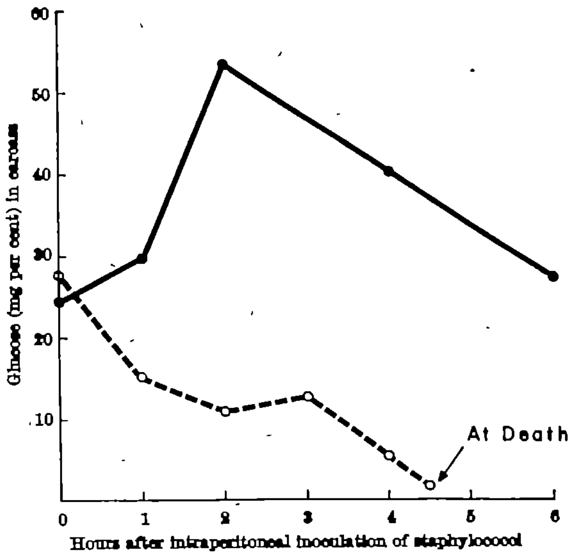

Fig. 2. Glucose present in carcasses of animals at intervals after intraperitoneal infection if animals are killed immediately and stored at 37° C compared with glucose in carcasses of animals killed at stated intervals after infection. Staphylococci $\log_{10}$. ●, *in vitro* 11·40 inoculated, 9·70 recovered; ○, *in vivo* 10 04 inoculated, 10 65 recovered

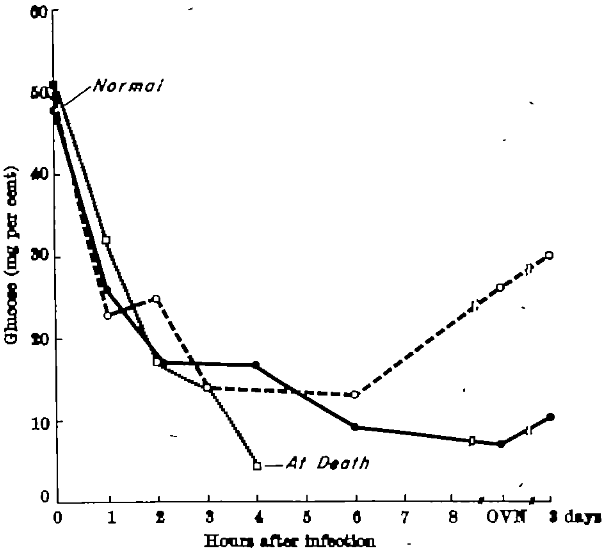

Fig. 3. Effect of penicillin treatment on the carcass glucose of mice infected with staphylococci compared with control values. □, Staphylococci only; ●, staphylococci and penicillin; ○, penicillin only

aureus, significant differences in the values for urea nitrogen, creatinine, uric acid, carbon dioxide, chloride sodium, alkaline phosphatase and acid phosphatase when compared with the values for healthy animals (Table 1).

Three live virulent human strains of *S. albus* (*S. epidermidis*—coagulase negative) obtained from patients (cases 6, 7, and 9, ref. 5) were inoculated in large doses (0·1 c.c. = 10^{10} intraperitoneally in mice. The biochemical values for each of the strains were virtually identical so that all the values were analysed as a group and compared with controls. The findings were similar to those with live *S. aureus* organisms except that creatinine, uric acid and carbon dioxide values were significantly decreased compared with the controls (Table 1). Inorganic phosphorus was not significantly elevated.

The excessive biochemical changes with heated *S. aureus* and *S. albus* may be caused by degradation products released by heat or by the destruction of *S. albus* by host phagocytes.

Because of the reproducible fall in glucose values, sodium lactate should, in theory at least, be beneficial to the infected animal by providing, as pyruvate, an available source of glucose replacement or as blood lactic acid a potential source of liver glycogen.

It has been observed by Berry *et al.*[8] that certain endotoxins will block the conversion of blood glucose to liver glycogen, thus forcing the conversion to muscle glycogen instead. Therefore, the only pathway to liver glycogen is by way of blood lactate. If a severe energy requirement is pressed on an organism without simultaneous increase in respiration, this requirement is largely met by enhanced glycolysis by way of blood lactate.

Preliminary evidence from biochemical findings in patients dying from staphylococcal disease indicates carbohydrate derangement and protein depletion[9].

We have shown previously that glucose given orally was deleterious to fasting mice infected intravenously with staphylococci[10]. This effect could only be detected during the initial phase of infection in fasted animals and in mice that survived infection; those receiving glucose during the fasting period seemed better able to destroy staphylococci than those receiving only saline as drinking fluid.

Studies to be detailed elsewhere[7] show that treatment with glucose, 1/6 M sodium lactate or inorganic phosphorus will significantly lengthen the survival time of infected mice whereas mouse plasma proteins and transaminase are deleterious. Cholesterol treatment was ineffective.

We conclude that the severe derangements of carbohydrate metabolism in mice dying from severe staphylococcal infections are part of the death mechanism as survival time in infected animals is significantly increased by appropriate replacement.

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PASSIVE AGGLUTINATION TESTS

Bismuth Tannate Test

THE passive hæmagglutination test described by Boyden[1] as well as variations of this method find widespread application as sensitive procedures for the detection of antibodies. Collodion and latex particles have been used instead of the tannic acid-treated red blood cells. However, laborious techniques and unsatisfactory antigen binding limited the use of these particles.

In this communication the use of bismuth tannate particles for the passive agglutination test is described.

Bismuth tannate is a brownish yellowish powder containing 40 per cent bismuth monoxide, insoluble in saline solutions. The bismuth tannate particles are of varying size and before use the larger particles are removed. This is performed by preparing a suspension of 2 g of bismuth tannate in 10 ml. of a 0·85 per cent sodium chloride solution. This suspension is then homogenized for 15 min in a standard homogenizer at high speed. It is next transferred to a test tube, left undisturbed for 30 sec before decantation into another test tube. The larger particles of bismuth tannate remaining in the first tube are discarded. The suspension is centrifuged for 3 min at 2,000 r.p.m. This stock suspension may be kept in the refrigerator for many weeks. For use 0·3 ml. of the sediment are added to 9·7 ml. of saline.

Equal volumes (10 ml.) of the bismuth tannate suspension are added to an antigen solution in saline and kept at room temperature for 30 min with repeated shakings. The sensitized bismuth tannate particles are then washed three times in 15 ml. of saline to remove the unfixed antigen. After the third washing 10 ml. of saline are added to the antigen-coated bismuth tannate particles.

The antiserum dilutions in volumes of 0·2 ml. (in tubes 14 mm × 80 mm) are performed with a dilution of 1·5 per cent of normal rabbit serum in saline. To each tube one drop of antigen-coated bismuth tannate particles is added. The tubes are shaken vigorously for 3 min and placed in a refrigerator for 2 h. After 2 h the tubes are again vigorously shaken and left at room temperature for at least 4 h before reading results. A second reading is performed after overnight incubation of the tubes in the racks without any shaking. Special care must be taken in order not to disturb the suspension in the tubes. It was found practicable to place the tubes in clear plastic racks on a glass surface with a mirror underneath at an angle of 45°. In such a way the sedimentation pattern could be observed in the mirror without any handling of the tubes.

As the pattern of the bismuth tannate sedimentation is different from that of the tannic acid-treated erythrocyte passive hæmagglutination, it was found practicable to run a parallel control of every experiment by adding the antigen-coated bismuth tannate particles to dilutions of a normal serum. This precaution is unnecessary after gaining some experience when one tube of the 1·5 per cent normal rabbit serum in saline with the antigen-coated bismuth tannate particles serves as control.

The negative controls and the negative higher dilutions of the antiserum showed a regular sedimentation of the bismuth tannate particles either in the form of a ring with or without a central point or as brownish coating of the whole bottom of the tube. The positive results are recognized by the sedimentation of the bismuth particles in an irregular thick denticular ring form, as a coarse brownish bottom or merely as a ring with a more pronounced design. These patterns depend on the antigen–antibody system used and on the concentration of these components. Therefore, when working with an unknown system, positivity must be appreciated only by comparing every tube with its negative control. The test was performed with different antigen–antibody systems; it did not require any buffers, and gave very satisfactory results also with the inhibition technique. The inhibiting antigen was added to the antiserum 30 min before the addition of the bismuth tannate antigen-coated particles. The coating of the bismuth tannate particles with non-protein antigens was unsuccessful. Some systems gave better results with the bismuth tannate particles; others, however, were superior when the tannic acid hæmagglutination test was utilized. The most probable explanation for these discrepancies are differences of antigen coating in the two techniques. Bismuth tannate may be prepared by allowing an aqueous solution of $Bi(NO_3)_3 . 5 H_2O$, slightly acidified by HNO_3, to react with an alcalic solution of tannic acid. The yellowish precipitate obtained at room temperature was washed five times with large amounts of saline and thereafter dried. The residue after drying was thoroughly grounded and the powder obtained used as indicated above.

This test was developed in connexion with an investigation of immunological aspects of corneal transplantation and supported by grant *NB* 02018–04 from the U.S. National Institutes of Health.

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Barium Sulphate Test

COLLODION[1], bentonite[2], tanned red blood cells[3], latex[4] and bismuth tannate (preceding communication) have been used for the detection of circulating antibodies in passive agglutination tests. These particles serve as vehicles for the antigen which is in solution or a suspension of very minute particles.

In the presence of the corresponding antibody the antigen-coated particles are agglutinated, precipitated or sedimented in an altered pattern.

As the pattern of sedimentation may easily be disturbed by very slight shaking, it is convenient to place the tubes in clear plastic racks on a glass surface with a mirror underneath at an angle of 45°. In this way results are read through the mirror without handling the tubes.

This communication describes the use of barium sulphate for a passive agglutination test. The barium sulphate passive agglutination method provides an additional, very simple, rapid and sensitive method for the detection of circulating antibodies.

Equal volumes of 3 per cent barium sulphate suspension in saline and antigen are mixed and shaken. After 2-min contact at room temperature, the sensitized barium sulphate particles are washed three times in saline. The packed sediment obtained is resuspended in normal rabbit serum 10 per cent in saline so as to regain the original 3 per cent concentration.

One drop (0·05 ml.) of the sensitized barium sulphate suspension is added to each tube (14 mm × 80 mm) containing 0·2 ml. of the antiserum dilutions. The dilutions are performed in normal rabbit serum 5–10 per cent in saline. For every antigen–antibody system the optimal concentration of normal rabbit serum within a range of about 5–10 per cent must be determined, in order to get a completely negative control. The tubes are shaken vigorously and placed at room temperature. The pattern of the sedimentation of the barium sulphate particles is examined after 30 and after 60 min. For another reading the tubes may be thoroughly shaken and the pattern of sedimentation is again obtained.

As control, one drop of antigen-coated barium sulphate suspension is added to 0·2 ml. of the diluent. A negative result is indicated by sedimentation in a form of a regular ring (Fig. 1, right tube). The strong positive tubes show sedimentation in the form of denticular curved lines (Fig. 1, five left tubes) and the intermediate positive tubes show no well-defined distribution.

This test has certain advantages over other passive agglutination methods. It does not require early washings or pre-treatment with tannic acid. The incubation periods are also very short. The interaction between the barium sulphate and the antigen is very rapid and so is the sedimentation of the sensitized particles. Results may be read after 60–90 min from the beginning of the test. The simplicity and the fact that no buffers or expensive materials are needed make it cheap and easy to perform even in small laboratories.

The barium sulphate particles are stable; the connexion between them and the antigen is firm. The coated particles may be preserved for at least 14 days before their addition to the antiserum. The sedimentation patterns, both the positive and the negative results, may also be preserved for days without changes.

No absorptions of the antiserum or of the normal rabbit serum are needed as the barium sulphate particles are antigenically inert. This method may therefore replace the tannic acid hæmagglutination test in cases where the antiserum contains anti-red blood cell antibodies.

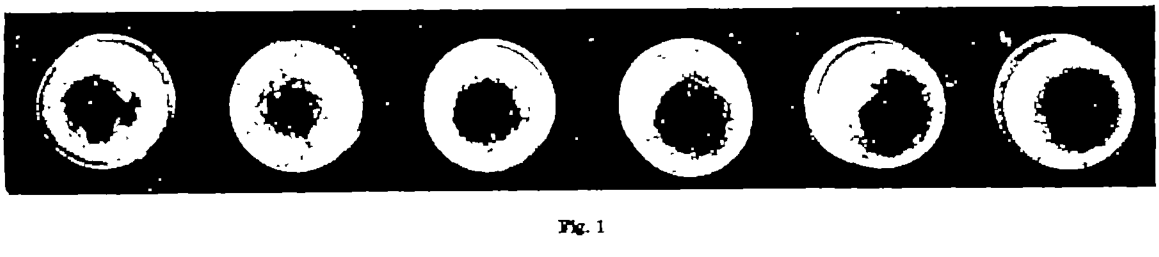

Fig. 1

The sensitivity of the passive barium sulphate agglutination test is greater than that of agar-gel diffusion, precipitation or direct agglutination techniques. In some antigen–antibody systems it is, however, somewhat less sensitive than the tannic acid hæmagglutination test, while in other systems the sensitivity is even greater than that of the tannic acid hæmagglutination test.

The passive barium sulphate agglutination test was performed with the following antigens and their corresponding antibodies: (1) whole human serum—rabbit anti-human serum; (2) human γ-globulin—rabbit anti-γ-globulin serum; (3) chorionic gonadotropin—rabbit anti-chorionic gonadotropin serum; (4) human thrombocyte extract—rabbit and monkey anti-human thrombocyte serum; (5) human leucocyte extract—rabbit and monkey anti-human leucocyte serum; (6) snake venom (*Vipera*)—horse anti-snake venom serum.

In all these systems no false positive results were encountered.

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BLOOD COAGULATION IN ARTERIOSCLEROTIC RATS

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MALE and female rats exposed to the stress of repeated breedings spontaneously develop arteriosclerosis[1-3]. Microscopically, the arterial lesions demonstrate marked alterations in mucopolysaccharide ground-substance and connective tissue elements (Fig. 1). Duguid[4] and others[5] have emphasized the participation of abnormal coagulability of blood and thrombosis in the pathogenesis of arteriosclerosis. With this in mind, an investigation of the coagulability of blood in these arteriosclerotic rats was begun.

Virgins and discard breeder rats of approximately nine months of age were obtained from a commercial breeder (Sprague–Dawley). A total of 300 breeders were weighed, bled and then autopsied. At autopsy, the aortæ were examined for gross evidence of arteriosclerosis. All blood samples were taken by cardiac puncture under the lightest possible anæsthesia with ether. Blood was drawn into 5 ml. siliconized syringes (G.E. dry film *SC*–87). Needles were coated with 'Monocote *E*' (Armour). The whole blood clotting time was determined by the Lee–White method[6]. Prothrombin time was determined by the one-stage method of Quick[7], using Bacto thromboplastin. The foregoing procedure was also carried out in saline-diluted plasma (12·5 per cent). Dilution of excess of clotting factors present in blood makes possible the detection of more subtle quantitative changes. Recalcification time was utilized to demonstrate the presence of a circulating anti-coagulant in the blood of breeder rats. Anti-coagulant activity can be demonstrated by mixing plasma from the breeder rats with plasma from virgin rats which show a normal clotting time. The plasma was prepared from oxalated blood by centrifugation at 1,750 r.p.m. for 5 min. Samples containing 0·1 ml. of plasma were placed in clean 13 × 100 mm test tubes and incubated for 3 min at 37°. Since the recalcification time becomes shortened with prolonged incubation, it is important that the incubation period be standardized. Calcium chloride, 0·2 ml. (0·01 M), was added to the plasma. The tubes were tilted at 30-sec, 1-min, and at 20-sec intervals thereafter. The recalcification time was taken as the interval from the addition of the calcium chloride to the formation of a solid clot. To examine the effects of breeder plasma on the recalcification time of virgin rat plasma, 0·05 ml. quantities of breeder and virgin plasma were mixed and the changes in recalcification times were measured.

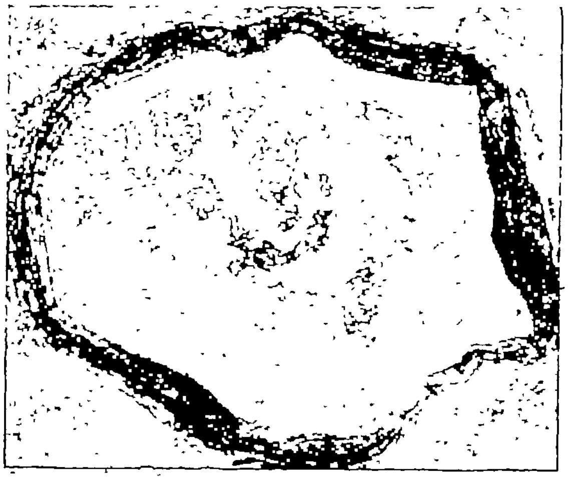

Fig. 1. Thoracic aorta of a repeatedly bred female Sprague–Dawley rat with severe arteriosclerosis. The dark black material in the photograph shows the heavy concentration of acid mucopolysaccharides in the disrupted ground substance of the arterial wall. (Hale stain, × c. 44)

At autopsy, the entire aorta (arch to iliac portion) was removed, dissected free of extraneous tissue including the adventitia, and blotted dry between filter paper. 1-g samples of aorta were collected from male and female breeders and from male and female virgin rats. The samples were homogenized in a Ten Broeck homogenizer and extracted with 25 ml. of distilled water. The samples were then exposed to ultrasonic vibration in a Raytheon sonic oscillator for 30 min at 1·25 amp and at maximum 'frying' noise to break up the complex of bound mucopolysaccharides. Next, the samples were boiled for 1 min to destroy the protein constituents, diluted to the original volume (25 ml.) and centrifuged for 5 min at 2,500 r.p.m. The supernatants were then lyophilized, dissolved in 3 ml. of distilled water, and frozen for storage. To facilitate the accurate measurement of the effects of aortic extracts on the clotting mechanism, the optical density was determined during the formation of a fibrin clot at 450 mμ (Coleman Junior spectrophotometer). A calcium chloride solution, 0·6 ml. of 0·01 M concentration, was added to a mixture containing 0·1 ml. plasma, 0·1 ml. extract, and 0·1 ml. distilled water, and placed in microcuvettes (10 mm int. diam.) which were fitted with microspace cuvette adapters. The time was noted, and readings taken at 10-sec intervals following the onset of the galvanometer deflexion. The changes in optical density were plotted against time. The intercept between two straight lines, one drawn through the initial plateau and the other drawn through that portion of the curve which shows a linear relationship between the rate of change of optical density and time, was taken as the end point. Control determinations were made in which 0·1 ml. of saline was substituted for the extract sample.

First, a comparison was made of the whole-blood clotting times of male and female non-atherosclerotic virgins and arteriosclerotic breeders. The results indicated that there was prolonged clotting in breeder rats when compared with virgins.

Next, the prothrombin times were investigated to learn if, in this factor, a similar relationship existed between virgin and breeder rats. The results indicated that there was no significant difference between the prothrombin times of virgin and breeder rats. Therefore, these results suggested that the active factor or factors producing the prolongation of clotting mentioned above might be among the variables which are not measured by Quick's one-stage prothrombin test. Such variables might be alterations in the concentration of factors V and VII, and heparin in quantities sufficient to interfere with prothrombin conversion or with the conversion of fibrinogen to fibrin by thrombin[6].

The next question was whether the prolongation of clotting could be due to the presence of a 'circulating anti-coagulant'. Six samples of breeder blood with prolonged clotting were individually added to six separate samples of virgin blood with normal clotting. An average of 54 per cent prolongation of the clotting time of virgin blood was demonstrated on addition of the breeder blood. Blood taken from virgin rats was added to other virgin blood as a control. No such prolongation was observed. This crude estimation of coagulability indicated that an unknown substance, present in the circulation of breeder animals but not in virgin controls, would retard the clotting of plasma. Further experiments were designed to delineate the nature and activity of this substance (or substances).

Breeder rats show a high concentration of mucopolysaccharides in the walls (Fig. 1) of their major arteries[3]. We became interested in the possibility that the intramural aortic mucopolysaccharides found in these arteriosclerotic rats may have anti-coagulant activity and might account for the prolonged clotting. Aortæ from male and female, virgin and breeder rats, were extracted as previously outlined. Blood samples freshly collected from virgin female rats were used as substrate. The effect of aortic extracts on the spectrophotometrically recorded clotting times was tested utilizing a pool of virgin rat plasma as a uniform substrate. The results are given in Table 1. The aortic extracts, each compared with the saline control, uniformly exhibited marked anti-coagulant activity. The average value of anti-coagulant activity of the virgin females was somewhat greater than that of the virgin males. The breeders exhibited the most profound anti-coagulant effect in extracts of their aortæ, that is, an average of 80 per cent.

Table 1. ANTI-COAGULANT ACTIVITY OF AORTIC EXTRACTS* ON PLASMA SUBSTRATE AS MEASURED BY THE TIME REQUIRED FOR THE FORMATION OF A CLOT

Source of aorta extracts (equivalent to 1 g fresh tissue)	Average of seven separate trials
Saline (control)	163†
Virgin males (no arteriosclerosis)	191
Breeder males (minimal arteriosclerosis)	231
Virgin females (no arteriosclerosis)	229
Breeder females (minimal to severe arteriosclerosis)	292

* To collect 1 g of virgin aorta required killing an average of 12 rats; for 1 g of breeder aorta, 7 rats.

† Time of clotting (sec) as measured by the spectrophotometric procedure.

In summation, the arteriosclerosis which develops in rats by repeated breedings is characterized by heavy accumulations of mucopolysaccharides (Fig. 1). The mucopolysaccharides, heparin and chondroitin sulphate B, are active anti-coagulants, and are found in the arterial wall[5]. If these aortic constituents were discharged into the blood stream, they presumably could materially alter the coagulability of the blood. Thromboses, although present in these animals, occur infrequently. The growth of atheromata as the result of the deposition of fibrin on intimal surfaces with subsequent endothelial overgrowth and eventual incorporation of the clot, as suggested by Duguid[4], does not appear to be applicable to these animals. It is interesting that prolonged clotting instead of accelerated clotting was found in these arteriosclerotic breeder rats. The initial observation that breeder plasma when added to virgin plasma produced prolonged clotting (54 per cent increase) has suggested the possibility that a circulating anti-coagulant is operative. Gore and Larky[7] and Dyrbye and Kirk[8] and Kirk[9] have demonstrated that animal and human aortic tissue contains an extractable anti-coagulating material which is mucopolysaccharide in nature. A search was made for a similar factor in these animals. Because of the difficulty of separating mucopolysaccharides from protein and other complexes, we resorted to exposure of the aortæ to ultrasonic oscillation. It was further necessary to boil these extracts to unmask the anti-coagulating activity. Tests of the unboiled extracts showed acceleration of the clotting process rather than prolongation, most likely due to the presence of tissue thromboplastic components and related substances.

All of the aortic extracts prepared in the foregoing manner demonstrated anti-coagulant activity. Statistically, analysis of variance of these results in Table 1 indicates that the physiological activity of the unknown factor is highly significant ($P = 0{\cdot}005$). The highest anti-coagulating activity was found in female breeder rats. It is suggestive that these are the animals which also showed the greatest degree of gross arteriosclerosis and the greatest concentration of mucopolysaccharides (Fig. 1). Extracts of aorta from the male breeders exhibited less anti-coagulant effect and showed less mucopolysaccharide histologically.

Our findings are similar to those of Gore and Larky[7] in that we have found that the aorta contains an anti-coagulant in its wall. In addition, our results show that anti-coagulating activity is actually more pronounced in animals with arteriosclerotic aortæ. The prolonged clotting of blood found in these arteriosclerotic rats *in vivo* coincides with the demonstration of an anti-coagulating factor in the *in vitro* tests. The present consensus is that the normal aorta contains an anti-coagulant which maintains the normal fluidity of blood and prevents the formation of mural thrombi[4,5]. It has been suggested that during the development of atherosclerosis the nature of the mucopolysaccharides of aortic ground substance is altered resulting in the loss of this protective mechanism, that is, prevention of thrombus formation. Our experience with atherosclerotic rats indicates that the latter mechanism does not apply in this experimental situation. On the contrary, atherosclerotic breeder rats show increased anti-coagulating activity with increasing concentration of aortic mucopolysaccharides which is proportional to the degree of advancement of atherosclerosis.

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MATERNAL BEHAVIOUR IN THE RABBIT: EFFECTS OF ENVIRONMENTAL VARIATION

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MAMMALIAN reproduction, in its broadest essence, starts with mating behaviour and terminates when the young are weaned. Between these two events is a complex chain of genetic, biochemical, physiological, environmental and behavioural phenomena all of which are critical for the survival of the young[1]. Recent work has shown that disruption of this chain at different points can have deleterious effects on mammalian reproduction. Parkes and Bruce[2] have reviewed the evidence showing that implantation is blocked in the mouse if the female is exposed to the odour of a strange or alien male during the first four days of pregnancy. Eleftheriou *et al.*[3] have obtained the same results with the deermouse; in addition, they found that changes in the physical environment also resulted in a significant decrease in implantation. Calhoun[4] has reported that pregnant rats living under conditions of maximal population density frequently failed to carry their young to term; in addition, many of the litters which were successfully delivered died before weaning. In all these species, manipulation of dimensions of the external environment significantly affected reproductive physiology and maternal behaviour. The purpose of this article is to extend this generality to another species—the rabbit.

As part of a programme investigating determinants of maternal behaviour in the rabbit, a special experimental maternity cage was built. This cage permits quantification of various aspects of maternal behaviour including nest-building, number of entries into the nest-box, and general activity outside the nest-box. Dutch-belted female rabbits, born in the laboratory, were maintained in stock-room cages after weaning under uniform conditions until sexual maturity. They were then removed, mated and placed individually into standard colony cages or into the experimental maternity cages.

The standard colony cage consisted of a battery of four cages with an area approximately twice that of the stock-room cages. The cages were of wire construction and contained a nesting box 11 in. × 11 in. × 22 in. The experimental cages were made of wood with a wire top and bottom. These cages had an area approximately four times as great as the stock-room cages. A nesting box (10 in. × 7 in. deep × 20½ in.) was at one end of the cage. Figs. 1–3 illustrate the cages.

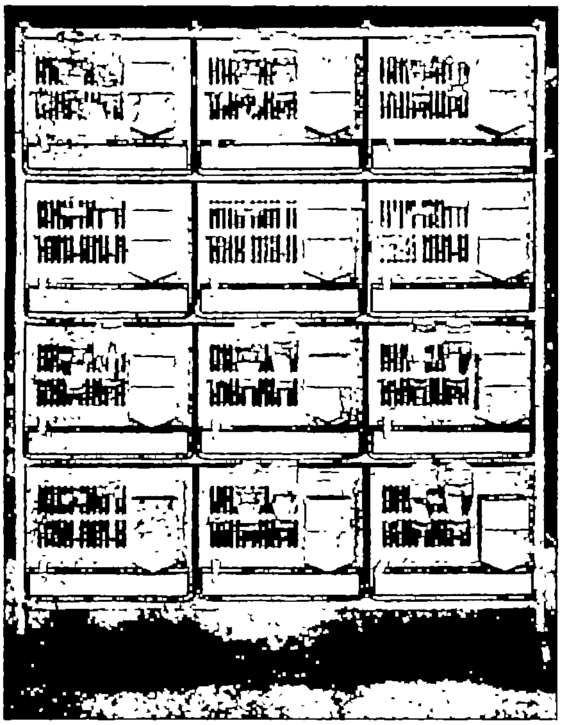

Fig. 1. Twelve-unit stock-room cage in which animals were housed from weaning to sexual maturity, when they were removed, mated and placed either in the four-unit standard colony cage (Fig. 2) or in the experimental maternity cage (Fig. 3). The respective sizes of the individual cages were 13·5 in. × 18 in. × 24 in.; 36 in. × 16 in. × 13·5 in.; 24·5 in. × 21·5 in. × 76·5 in.

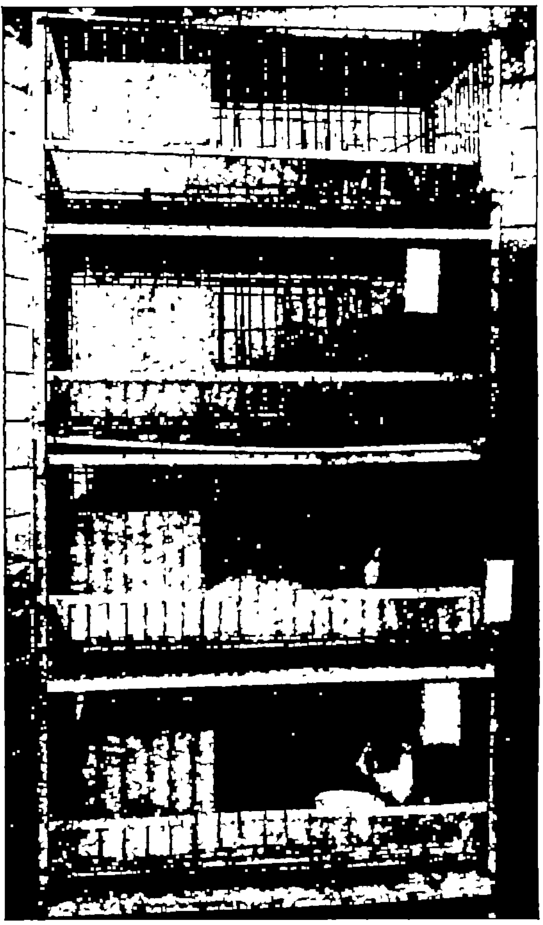

Fig. 2. Standard colony cage

Fig. 3. Experimental maternity cage

The control animals ($N = 100$) and experimental animals ($N = 27$) were provided with nesting material and nest-boxes as soon as they were placed into their respective cages. The controls were given access to straw for nesting material while the experimental rabbits were provided with 'Excelsior' (this was used as nesting material because it could be made into 'wads' which were easily counted). After being placed in the maternal environments the animals were minimally disturbed. All animals were kept under standard conditions of food and water (supplied *ad libitum*), light (13 h light per 24 h), and temperature (70°–72° F).

The following results were recorded: (1) Length of gestation; the day of mating was defined as day one of gestation. (2) Number of females building a maternal nest, that is, a nest of straw or 'Excelsior' into which the female had incorporated hair plucked from her body[5]. (3) Incidence of scattering, that is, the birth of one or more young outside the nest-box. (4) Incidence of cannibalism, that is, eating part or all of one or more young. (5) The percentage of young which survived through weaning.

Table 1 summarizes the findings. The mean length of gestation did not differ significantly between the two groups. All other differences are significant well beyond the 0·01 level as determined by χ^2 or exact probability tests. The rabbits which delivered in the experimental cages were deficient in maternal nest-building and exhibited a greater incidence of scattering and cannibalization. In addition, 87·3 per cent of the young born of control mothers survived until weaning but only 25·9 per cent of the young born to the experimental mothers survived.

Table 1. SUMMARY OF STATISTICS ON MATERNAL BEHAVIOUR IN THE RABBIT

Variable	Control ($N = 100$)	Experimental ($N = 27$)
$\bar{X}$ Length of gestation	31·8	31·8
Per cent built maternal nest	98·0	48·2
Per cent scattered young	6·0	66·7
Per cent cannibalized young	3·0	22·2
Per cent young surviving Through weaning	($N = 552$) 87·3	($N = 143$) 25·9

The experimental maternity cages are approximately four times as large as the stock-room cages and differ from the stock-room cages in terms of material, pattern, shape, amount of light admitted, etc. For example, the experimental maternity cages are much longer and made of wood, while the standard colony cage is smaller and much more comparable with the stock-room cages. This suggests that placing the animals into a markedly different environment after mating is enough to interfere with normal maternal behaviour. These results, in combination with other findings[2–4], strongly suggest that the external environment, rather than merely having a supportive role, has a dynamic effect in guiding the course of mammalian reproduction. There are other data suggesting that these effects may have long-term consequences for the species since manipulation of maternal factors have been shown to affect the offspring's behaviour[6–8]. Harlow's[9] findings are particularly pertinent here. He has shown that monkeys raised from birth by mother surrogates exhibit maladaptive mating behaviour and, in the few instances when pregnancies have occurred, defective maternal behaviour.

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ACTIVATION PRODUCT AND THE FIBRINOLYTIC SYSTEM

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ACTIVATION product was first prepared by Waaler[1] by the 'Celite' contact activation of factor XII (Hageman factor) and its subsequent interaction with factor XI (plasma thromboplastin antecedent). As prepared, it is free from significant contamination by other clotting factors: its addition to blood in a siliconed or plastic container leads to a considerable shortening of the clotting time. Interest in a possible connexion between the initial phase of *in vivo* clotting and the activation of fibrinolytic mechanisms led to an investigation of the fibrinolytic properties of activation product.

Activation product was prepared by the method of Waaler. Preparations were stored at − 30° C for up to 4 weeks before use. Techniques used for identification of components of the fibrinolytic system were the fibrin plate method[2,3] using both unheated and heated fibrin, and the standard clot method[4]. Plasminogen was activated by urokinase or streptokinase. Proactivator activity was estimated by the capacity of the test material to bring about lysis on an unheated bovine fibrin plate in the presence of streptokinase. Acceleration of clotting activity was assessed by the power of the activation product to shorten the recalcification time in a plastic tube of citrated plasma, collected and prepared without contact with an activating surface.

Activation product alone produced no lysis on unheated or heated fibrin plates (Table 1), and its incorporation in a standard clot prepared from human fibrinogen and bovine thrombin did not shorten the clot lysis time (Table 2). It was therefore concluded that activation product contains no free plasmin or plasminogen activator.

Activation product treated with urokinase or streptokinase, both potent activators of human plasminogen, failed to produce lysis on a heated fibrin plate (Table 1). This

Table 1. FIBRIN PLATE TESTS TO SHOW PRESENCE OR ABSENCE OF PLASMINOGEN, PLASMINOGEN ACTIVATORS AND 'PROACTIVATOR' IN ACTIVATION PRODUCT

Test mixture	Area of lysis in mm² Unheated plates	Area of lysis in mm² Heated plates
Activation product + saline	0	0
SK* + saline	0	0
Human serum + saline	0	0
Activation product + SK	80	0
Human serum + SK	117	85

0·02 ml. amounts of test mixture placed on fibrin plates prepared from bovine fibrinogen and thrombin. Lysed area measured after 18–20 h at 37° C.
*Streptokinase—'Streptodornase', 'Varidase'. Lederle Laboratories.

Table 2. STANDARD CLOT TESTS FOR PLASMINOGEN, PLASMINOGEN ACTIVATOR AND PLASMIN IN ACTIVATION PRODUCT

Test mixture (ml.)			Standard clot		
Activation product	Saline	SK 10 units/ml.	Fibrinogen* 500 mg/100 ml.	Thrombin† 5 units/ml.	Lysis time (min.)
—	0·7	0·1	0·2	0·1	60
0·7	—	0·1	0·2	0·1	25
0·7	0·1	—	0·2	0·1	68

* Human fibrinogen, Kabi, Stockholm.
† 'Bovine Thrombin Topical', Parke Davis and Co.

appeared to indicate that plasminogen was absent, but as the fibrin plate method is known to be insensitive to small degrees of fibrinolytic activity, this result was not accepted as conclusive. A more sensitive standard clot preparation, containing activation product and urokinase or streptokinase, lysed in a shorter time than a control clot (Table 2), indicating the presence of some plasminogen. This was confirmed by estimation of the esterase activity of the same mixture. (By courtesy of Dr. A. J. Johnson, New York University College of Medicine, New York.) Attempts to measure the amount of plasminogen in several preparations of activation product always indicated less than five units to be present, one unit in this instance being defined as the amount of plasminogen which, when activated, would form the amount of plasmin necessary to lyse a 1·1 ml. volume standard clot in 10 min. By this standard, normal human plasma contains 5,000–7,000 units of plasminogen per ml.[4].

Streptokinase (SK) alone will not activate bovine plasminogen[5], but if a small amount of human plasma or serum is mixed with the streptokinase, activation of bovine plasminogen is marked. This fact is explained by assuming that the human reagent provides a 'proactivator' which reacts with streptokinase to form plasminogen activator[6]. Proactivator has not been isolated and may or may not be part of the human plasminogen molecule[7]. It was found that the addition of activation product to streptokinase on an unheated bovine fibrin plate produced a considerable degree of lysis but, as has already been mentioned, lysis did not occur with the same mixture on a heated plate (Table 1). This finding is interpreted as indicating that activation product possesses proactivator activity. This proactivator activity is stable at room temperature and is still present in activation product diluted to 1 : 32. It is not destroyed by heating at 54° C for 4 h or at 80° C for 30 min. A trace of activity was found in samples which had been heated at 80° C for 4 h (Table 3).

Parallel experiments to measure clotting acceleration, proactivator activity and plasminogen activity in samples of activation product incubated at different temperatures, showed proactivator and plasminogen to be independent of the clotting activity of activation product. The latter was destroyed completely by heating at 54° C for 10 min (Table 4), while plasminogen and proactivator remained unaffected after 4 h at this temperature (Tables 3 and 5). This independence of clotting activity and fibrinolytic activity was confirmed by tests on activation product prepared from the plasma of patients deficient in factor XI or factor XII. The plasminogen and proactivator content of these preparations equalled that of activation product from different samples of normal plasma.

Table 3. PROACTIVATOR ACTIVITY OF ACTIVATION PRODUCT AFTER INCUBATION AT VARIOUS TEMPERATURES

Incubation time	Area of lysis in mm² after incubation at: 0° C	56° C	80° C
0	120	120	120
10 min	110	100	36
20 min	121	100	24
30 min	96	94	12
1 h	121	124	trace
2 h	120	110	trace
4 h	110	94	trace

Samples of activation product were incubated at temperatures shown. Sub-samples were taken at intervals, mixed with SK and tested on unheated bovine fibrin plates* as in Table 1.
* No lysis occurred on heated fibrin plates.

Table 4. THE CLOTTING ACCELERATION ACTIVITY OF ACTIVATION PRODUCT AFTER INCUBATION AT VARIOUS TEMPERATURES

Non-activated human plasma (ml.)	Type of tube	Added reagent (0·2 ml.)	Recalcification time (sec)
0·2	Plastic*	Saline	587
0·2	Glass	Saline	175
0·2	Plastic	Activation product	150
0·2	Plastic	Activation product after 4 h at 0° C	162
0·2	Plastic	Activation product after 10 min at 56° C	600
0·2	Plastic	Activation product after 10 min at 80° C	561

Samples of activation product were incubated at temperatures shown. The ability of sub-samples to shorten the recalcification time of unactivated plasma was tested at intervals.
* Polystyrene.

Table 5. PLASMINOGEN CONTENT OF ACTIVATION PRODUCT AFTER INCUBATION AT VARIOUS TEMPERATURES

Incubation time	Lysis time (min) after incubation at: 0°	56°	80°	Control (no activation product)
0	30	30	30	75
10 min	29	33	77	77
20 min	30	33	76	74
30 min	32	38	79	76
1 h	30	35	76	79
2 h	30	37	76	69
4 h	30	38	80	73

The lysis time of a standard clot preparation* containing activation product and SK.
* Standard clot preparation as for Table 2.

Plasminogen, measured by the standard clot method, was destroyed by heating at 80° C for 10 min, but proactivator activity remained measurable after 30 min at this temperature and a trace was still present even after 4 h. The fact that proactivator activity could be demonstrated in the absence of definable plasminogen may indicate proactivator to be a separate entity, or may merely reflect the insensitivity of the test for plasminogen.

The experiments described fail to provide information about possible links between the initiation of clotting and fibrinolysis *in vivo*. But it has been shown that activation product prepared from human plasma, although said to be free from contamination with other clotting proteins, does contain certain components of the fibrinolytic system. Free plasmin and plasminogen activator were not present, but small amounts of plasminogen were demonstrable and all batches prepared possessed proactivator activity to a considerable degree. This activity was independent of the ability of activation product to shorten the recalcification time of unactivated plasma. No clear-cut distinction between plasminogen and proactivator activity was obtained; slight variation in heat stability could be due to difference in sensitivity of the methods used.

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A 'NEW' VARIANT OF HÆMOGLOBIN A_2 AND ITS SEGREGATION IN A FAMILY WITH HÆMOGLOBIN S

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DURING the investigation of the family (Fig. 1) of a patient with sickle cell anæmia, a 'new' minor hæmoglobin component was encountered. This hitherto undetected hæmoglobin appeared to be a variant of hæmoglobin A_2. Since it was first observed in a subject from the Flatbush area of New York City, it has been tentatively designated hæmoglobin Flatbush (abbreviated to Hb. Flat.)[1]. This article deals with some of the characteristics of Hb. Flat., and with family studies which provided data concerning the linkage of Hb. Flat. with Hb. S.

Haemoglobin Flatbush. During electrophoresis at *p*H 8·8 on starch gels in borate buffer[2], Hb. Flat. migrated in approximately the same position as did Hb. S, and was estimated to account for 1 or 2 per cent of the total pigment (Fig. 2). Hb. Flat. was also detected on electrophoresis on starch granules at *p*H 8·6 (ref. 3), but a long period of electrophoresis (40 h) was required to achieve separations which were adequate for quantification. This new variant was not demonstrated by electrophoresis on agar gels at *p*H 6·2 (ref. 4), or on paper at *p*H 8·6. Preparations of Hb. Flat. purified by electrophoresis on starch granules exhibited slightly greater anodic mobility on starch-gel electrophoresis than did Hb. S; but we have thus far been unable to demonstrate directly the presence of Hb. Flat., in the presence of Hb. S. Hæmolysates containing Hb. Flat. did not contain increased amounts of alkali-resistant hæmoglobin, and a sample of Hb. Flat. eluted from an electrophoretic separation on starch granules had approximately the same resistance to denaturation by alkali as did Hb. A.

In each of 9 carriers of Hb. Flat., hæmoglobin A_2, the minor basic component described by Kunkel *et al.*[5] was notably decreased (Table 1). In these 9 subjects, Hb. A_2 values, ranging from 0·8 to 1·1 per cent, were approximately half the values obtained in normal subjects. In 8 family members whose hæmoglobin appeared entirely normal, hæmoglobin A_2 values of 1·8–3·2 per cent were within the normal range in our laboratory. The method of direct quantification of Hb. Flat. by elution after a 40-h electrophoretic separation on starch granules was established late in the investigation and was applied to only 3 hæmolysates. Even in these prolonged electrophoretic separations, it was at times difficult to separate Hb. Flat. completely from Hb. A. Hb. Flat. apparently accounted for 1–2 per cent of the total hæmoglobin in carriers who had Hb. A_2, Hb. Flat., and Hb. A. In 3 individuals with sickle cell trait (Fig. 1, II, 5; II, 8; and II, 16), Hb. Flat. could not be demonstrated directly, but its presence was inferred from the decreased amounts of Hb. A_2 observed on starch gels, and from the family studies.

Ceppellini *et al.*[6] described the first recognized variant of Hb. A_2, which is designated Hb. B_2. Hæmoglobin B_2 was readily detected and quantified since it migrated to the cathode on electrophoresis at *p*H 8·6. In Hb. B_2 carriers, Hb. A_2 and Hb. B_2 each accounted for about 1 per cent of the hæmoglobin. Thus the sum of Hb. A_2 and Hb. B_2 was approximately equal to the usual A_2 value of 2 per cent. Further evidence of allelism of Hbs. A_2 who noted the absence of Hb. A_2 in an individual whose and B_2 may be inferred from the studies of Horton *et al.*[7]. Hb. B_2 amounted to 2·1 per cent. Although this individual was probably homozygous for Hb. B_2, family studies could not be carried out. The results of fingerprinting studies of Hb. B_2 (ref. 6) have indicated that it differs from Hb. A_2 in a single peptide of the δ-chain.

A comparable situation was observed in the heterozygotes for Hb. Flat. While incomplete separation from Hb. A probably resulted in erroneously elevated values for Hb. Flat., the normal amount of Hb. A_2 was clearly reduced by about a half, and the sum of Hb. A_2 and Hb. Flat. was 2·5 per cent of the total pigment. Hæmoglobin A_2 is a tetramer of two kinds of polypeptide chains ($\alpha_2^A\delta_2^{A_2}$). Since the α-chain of hæmoglobin A_2 is apparently identical with the α-chain of hæmoglobin A ($\alpha_2^A\beta_2^A$), any alteration in the α-chain would be expected to result in another major hæmoglobin component as well as a second minor component. Only 3 hæmoglobins, A_2, Flat. and A,

Table 1. HÆMOGLOBIN ANALYSES OF CLASSES OF SUBJECTS IN KINDRED 48

	Electrophoresis on starch gels (ref. 2) Inspection					Per cent alkali-resistant (average)	Hb. A_2 was not quantified when Hb. S was present. Electrophoresis on starch granules (ref. 3) per cent of component (average)				
Class	No. of subjects	A_2	S	Flat.	A		No. of subjects	A_2	S	Flat.	A
Normal	8	Normal	–	–	+	1·2	8	2·3			97·7
Normal Hb. plus Flatbush	9	Dec.	–	+	+	1·0	9	1·0			99·0*
							3†	0·7		1·7‡	97·6
Sickle cell trait	14	Normal	+	–	+	1·3	9		44		56
Sickle cell anæmia	2	Normal	+	–	–	12·4					
Sickle cell trait plus Flatbush	3	Dec.	+*	(–)	+	1·1	3		46*		54

* These components were assumed to include Hb. Flat.
† Electrophoretic separation for 40 h.
‡ Value may represent slight contamination with Hb. A.

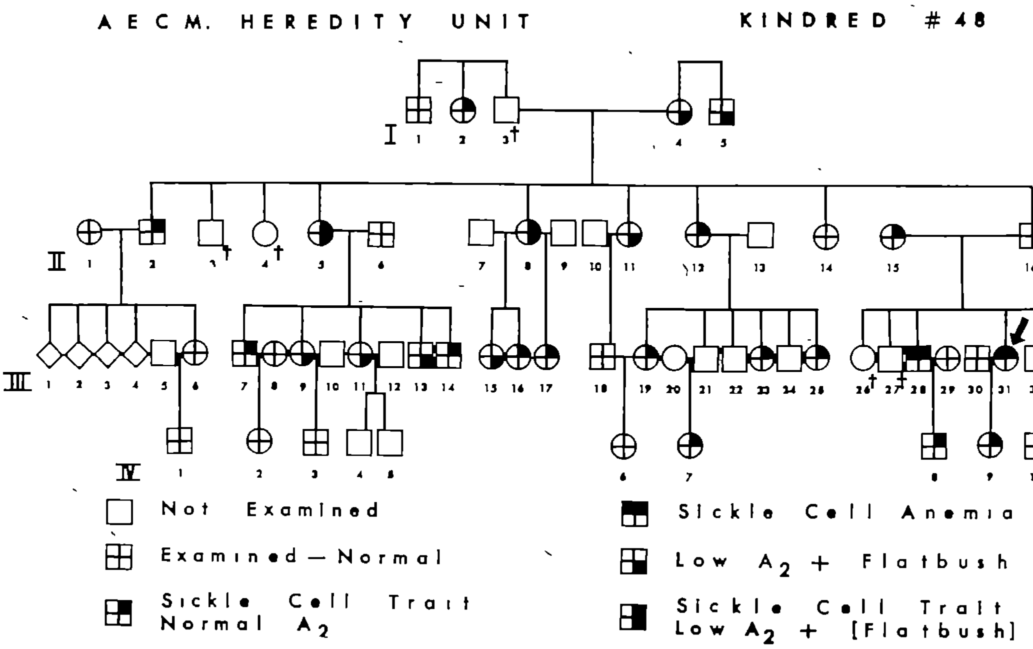

Fig. 1. Kindred segregating for hæmoglobin Flatbush and hæmoglobin S. No information is available concerning individuals represented by diamond symbol. *Subject IV, 11 was examined at 8 months of age: he had decreased Hb. A_2 and small amounts of a component with the mobility of Hb. Flat. However, since alkali-resistant hæmoglobin was present, final interpretation has not been made

were observed in Hb. Flat. carriers. The evidence therefore suggested that Hb. Flat. contained an alteration in the δ-chain, and its formula might consequently be written $\alpha_2^A\delta_2^{\text{Flat}}$.

Further evidence that Hb. Flat. contained an altered δ-chain was obtained by recombination experiments utilizing hæmoglobin $I_{\text{Burlington}}$ (ref. 7). As in the case of hæmoglobin A_2 (ref. 8), recombination was observed only after long periods of acidification of the mixture of hæmoglobin I and Hb. Flat. After acidification of a mixture of these hæmoglobins (as cyanmethæmoglobin) for 96 h, two new species of hæmoglobin of mobility intermediate between Hb. I and Hb. Flat. were observed:

$$\begin{array}{cccccccccccc}
\alpha_2^I\beta_2^A & + & \alpha_2^A\delta_2^{\text{Flat}} & \rightarrow & \alpha_2^I\beta_2^A & + & \alpha_2^I\delta_2^{\text{Flat}} & + & \alpha_2^A\beta_2^A & + & \alpha_2^A\delta_2^{\text{Flat}} \\
\text{Hb. I} & & \text{Hb. Flat.} & & \text{Hb. I.} & & \text{double abnormal Hb.} & & \text{Hb. A} & & \text{Hb. Flat.}
\end{array}$$

There were several differences between Hb. Flat. and the previously described minor components of approximately the same electrophoretic mobility. Hæmoglobin Lepore[9], hæmoglobin Pylos[10], and hæmoglobin 'G' (ref. 11), have about the same electrophoretic mobility as did Hb. Flat., but Hbs. Lepore, Pylos, and 'G' occurred in larger amounts (6–12 per cent). In the heterozygous state, these three previously described hæmoglobins have been associated with the hæmatological findings of thalassæmia minor. The simultaneous presence of Hb. Lepore (or Pylos) and high A_2 thalassæmia led to a marked interaction, resulting in an illness clinically resembling thalassæmia major[9,10]. In contrast, none of the individuals with Hb. Flat. had any hæmatological abnormalities and the simultaneous presence of Hb. Flat. and Hb. S did not result in any evident interaction. Currently available evidence concerning the structure of Hb. Lepore is conflicting[12,13], suggesting that Hbs. Lepore from different sources may have differences in structure.

Family studies. The family (Fig. 1) in which Hb. Flat. was detected was of Puerto Rican ancestry. Blood groups, including ABO, MN, Rh and Kell, were determined on all available members of the family. No evidence for non-paternity was found.

In generation I, the mother (I, 4) and her brother (I, 5) were Hb. Flat. carriers. In generation II, four hæmoglobin phenotypes were detected: AA – A_2A_2, AA – Flat. A_2, AS – A_2A_2, AS – Flat. A_2. The assumption that the father (I, 3), who was dead, must therefore have had sickle-cell trait was strengthened by the finding of sickle-cell trait in his sister (I, 2). In generation II, three individuals (II, 5; II, 8; II, 16) had decreased Hb. A_2 and sickle-cell trait. As noted here, the presence of Hb. Flat. was inferred from the decreased levels of Hb. A_2. These three individuals had 12 children; 7 of the children inherited the Hb. S and the remaining 5 the Hb. Flat. from the doubly heterozygous parent. (The genetic contribution of the father (II, 16) to his children (III, 28; III, 31; III, 33) was clearly defined even though the mother (II, 15) had sickle-cell trait; two of the children inherited Hb. S from each parent and had normal levels of Hb. A_2, the third child had the hæmoglobin phenotype AA – Flat. A_2.) None of these 12 children in generation III had entirely normal hæmoglobin or Hb. S with decreased A_2. The results from the first three generations indicated that, in this kindred, the β-chain locus responsible

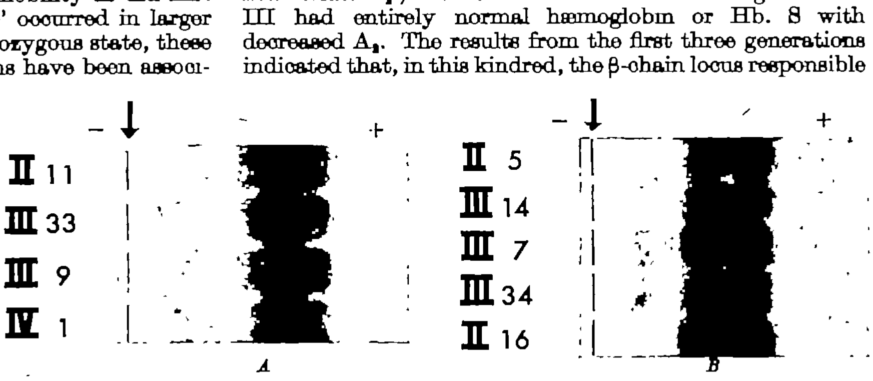

Fig. 2. Benzidine stained vertical starch-gel electrophoretic analyses (ref. 2) in borate buffer pH 8·8 of hæmoglobin (concentration 3 g/100 ml.)

A, components (cathode to anode): hæmoglobins A_2, Flat., A. In hæmolysate of II, 11, III, 33, and III, 9, the presence of Hb. Flat. and decreased Hb. A_2 was noted. Hæmoglobin of IV, 1 appeared normal

B, components (cathode to anode): hæmoglobins A_2, S and A. In hæmolysates of II, 5 and II, 16, Hb. A_2 was decreased, but Hb. Flat. was not detected in the presence of Hb. S. The hæmoglobin patterns of III, 14, III, 7, and III, 34 indicated sickle cell trait with normal Hb. A_2

for the synthesis of Hb. S, and the δ-chain locus responsible for the synthesis of Hb. Flat. were in the repulsion phase in the three doubly heterozygous individuals in generation II. The hæmoglobin phenotypes of the 12 critical offspring in generation III are consistent with linkage of the β- and δ-polypeptide chain genetic loci.

Evidence for the linkage of the δ-chain locus with high A_2 thalassæmia was provided in the kindred studied by Huisman *et al.*[14], in which these two loci appeared to be in the coupling phase in 3 individuals with thalassæmia minor and Hb. B_2. Of the 14 children of the doubly heterozygous individuals in their study, 11 had both Hb. B_2 and thalassæmia and 3 were normal. Since the loci for high A_2 thalassæmia and for the β-polypeptide chains appear to be linked[15], the family studied by Huisman *et al.*[14] provides evidence for linkage of the δ- and β-chain loci. Direct evidence concerning δ–β-linkage had been obtained earlier by Ceppellini[5], who studied one family in which the father was heterozygous for both Hb. S and Hb. B_2 and the mother had normal hæmoglobin. Of their 6 children, 4 had sickle cell trait and the others had hæmoglobin B_2. The family studied by Horton *et al.*[6] yielded less information concerning linkage of the β- and δ-loci: while the father was doubly heterozygous for hæmoglobins B_2 and S, the mother also had Hb. B_2.

The homozygous state for Hb. Flat., in which hæmoglobin A_2 would be expected to be completely absent, has not been encountered. Such a finding would provide definite evidence concerning the allelic relationship of Hb. Flat. and Hb. A_2. However, when the evidence provided by the present kindred with Hb. Flat. is considered together with the accumulated data on Hb. B_2, it seems very likely that Hb. Flat. represents another allele of Hb. A_2 with an alteration of the δ-polypeptide chain.

The family studied by Ceppellini[5] and the present kindred together include eighteen offspring of individuals who were heterozygous for both β- and δ-polypeptide chain variants. The two hæmoglobin phenotypes which are consistent with linkage of the β- and δ-chain genetic loci were observed in all of the eighteen children. The other two possible phenotypes were not encountered. Statistical analysis of these results ($P = < 0·005$ from χ^2 test) appears to establish linkage of the genetic loci responsible for β- and δ-polypeptide chain synthesis.

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We thank Dr. R. Gross, who assisted in obtaining blood samples from the members of the family in Puerto Rico.

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STATISTICAL VARIATION FOUND AT POCK AND PLAQUE COUNTING OF VACCINIA VIRUS

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IN *Nature* of March 31, 1962[1], the opinion of some authors is quoted according to which the count variance in the method of counting pocks in the chick chorio-allantois is so great that no valid estimate of the over-all coefficient of variation is possible. The quotation, although verbatim for the rest, is incomplete, lacking the supplement "—though others disagree—" used in the original text[2] as a qualification to these authors mentioned but not named.

The conclusions drawn in the review are strongly biased in favour of indirect particle counting, represented above all by the rabbit scarification method with the improvements given by Kolb *et al.*[3], but still based on measuring the percentage area covered either by confluent eruptions or individual lesions. The doubtfulness of adding up confluent reactive areas, which may conceal uncontrollable lots of infective units, together with single pocks is obvious. This fundamental disadvantage is similarly common to all titration devices establishing relations between dilutions and percentage effects; it may, however, be moderated by correct statistical handling as usual with the so-called ID_{50} methods. Compared with direct counting all indirect methods are highly debited with loss of information and, therefore, not economic, as Cooper[4] has recently pointed out on the occasion of discussing the relationship of plaque-forming units to end-point dilution units.

For these reasons as well as with regard to our own experience with direct pock and plaque counting of vaccinia virus we should like to join those "others disagreeing". Investigations with chorioallantoic membrane pock counting[5–9], cited by Kolb[3] as a warning, failed to explain the high count variance inherent to this technique. We feel that this may be due, at least partially, to the fact that most workers in the field of pock counting neglected to use Fisher's index of dispersion for small samples from a Poisson distribution, first inaugurated by him in connexion with plating counts of soil bacteria[10] and afterwards conveyed into his famous text-book[11]. The only exception seems to be the study of Reid *et al.*[12] on fowl pox virus titration which, favoured by the additional circumstance of low sample means, fulfilled the conditions of Poisson expectation. The habit of estimating the counting accuracy by comparing the counts of several dilutions made from the same suspension, adopted in the early stages of the method[13] and thoroughly preserved, may be of great secondary help but cannot

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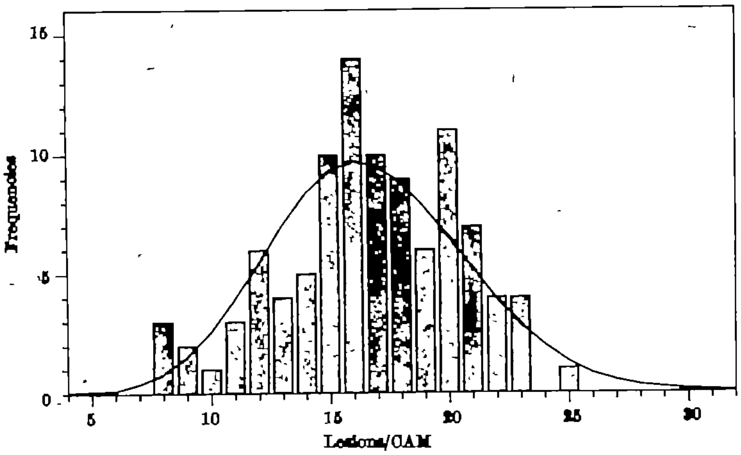

Fig. 1. Distribution of pock counts on 100 chorioallantoic membranes (CAM) inoculated with a 10^{-3} diluted suspension of vaccinia virus (lot 8/2 from bovine embryonic muscle t. c.). The line connecting frequencies of the Poisson series pertinent to the sample mean of 16·71 (± 0·41) designates expectation. Goodness of fit is ascertained by a χ^2 of 10·26 (12 d.f.) resulting in a probability higher than 0·6, which is exceptional for chorioallantoic membrane samples even of this size

replace examinations of the variance in the counts gained of one single dilution.

The index of dispersion is calculated by dividing the sum of squared deviates from the sample mean by the mean itself (1). Thus a sum of squared normal deviates is obtained, generally known as a χ^2, provided that the variance of the underlying distribution be

$$\chi^2 = \frac{\Sigma(x - \bar{x})^2}{\bar{x}} \qquad (1)$$

numerically equal to the mean, which is in fact one of the criteria natural to the Poisson distribution, here the hypothesis under test. Note (2) that the index of dispersion, replacing the sampling variance v for subsequent considerations, is proportional to this statistic (the sum

$$\chi^2 = \frac{(n - 1).v}{\bar{x}} \qquad (2)$$

of squares divided by the number of degrees of freedom) since the mean $\bar{x}$ and the number n of observations in the sample can be regarded as constants for the actual case. For the use of these formulæ in the way of approximating the Poisson distribution to a normal one with its variance equal to the mean, a condition as to the order of magnitude has to be satisfied, the product $n\bar{x}$ being supposed to be larger than about 30, which is the case with our data as it certainly will be in similar investigations.

In the experiments leading to this article the chorioallantoic membrane pock-forming units were counted by the method of Westwood *et al.*[9], slightly modified; for counting plaque-forming units a tissue culture of bovine embryonic muscle with hæmadsorption plaque reading[14] was used.

Having obtained (Fig. 1) sufficient support for the actual occurrence of the presumed distribution type from experiments with enlarged samples (100 embryonated eggs or 200 tissue culture tubes respectively, inoculated with one virus suspension at a time) we applied the χ^2 index to samples of routine size (5–8 chorioallantoic membranes, 5–10 tissue culture tubes). The lot of values gained by computing each sum of squares following one of the usual formulæ and dividing it by the corresponding mean turned out to be useful in several ways:

(1) Each sample under consideration can be tested for homogeneity by referring to the 1 or 5 per cent significance points of a tabulated χ^2 distribution; in cases of significant departure from the Poisson hypothesis the mean cannot be regarded as reliable, so the counting may have to be repeated, the reason for the development of a variation greater than explicable by chance should be found out, etc. Modifications of the established significance levels are practicable in special cases which will be referred to.

(2) A more comprehensive evaluation of the indexes is at hand if χ^2's and proper degrees of freedom are added up. Samples of different size can thus be put under test together, and arrangements following certain technical characteristics of the samples, for example, the dimensions of means, will supply particulars about experimental deficiencies.

(3) Arraying the indexes into a frequency distribution for comparison with the theoretical expectation leads to a most sensitive and versatile test. Classes are readily deduced from the probability divisions of a χ^2 table, their number and ranges depending on the number of indexes to be filed up. Departure of the observed distribution in the direction of high probabilities designates a degree of particle dispersion better than predictable by chance alone, while the other direction stands for an over-all variation greater than expected, if not for influences depressing the sample means, for example, overcrowding. For measuring the extent to which the variance is affected by such a shift of distribution (in order to rectify *S.E.* by an allowance fitted to the actual discrepancy between the ideally and partially fulfilled Poisson conditions) Fisher's own example may be followed, set up in connexion with the first display of the dispersion index under similar experimental circumstances[10]: if a distribution of indexes deviates significantly from expectation, a factor is easily determined indicating the amount of change in the variance by means of modifying the χ^2's of the distribution class limits so as to fit the actually found frequencies.

Parts taken from experimental data that will be published elsewhere in full detail[15] are given in Tables 1–4 as an illustration of the statistical treatments briefly enumerated here. Over-all variances differing widely between the two counting methods in question appear to be the most striking result, following from adding the sample indexes just as well as from the comparison of their distributions (Tables 1 and 3). The chorioallantoic membrane data so far demonstrated are all derived from counts of tissue culture vaccines; the findings are, however, quite the same with equally treated calf lymphs. Since further technical circumstances (sample size, suspension diluting procedure, etc.) also turned out to be of no fundamental influence, the overcrowding of pocks, already accused or at least taken under suspicion of seriously interfering with the chorioallantoic membrane titration[5–7], had again to be considered; it is, in fact, responsible to a remarkable extent for the high pock counting variability, as is shown by the d values in Table 2 increasing with the sample means. (The concomitant rise of the sample numbers per class is, of course, incidental, as provable by adding, for example, the samples of the second and third row to obtain from the resulting 26 indexes a d value of 2·92 which exactly fits the column ranking.) The rise of single pocks out of more than one infective unit each, depressing the mean and thereby increasing the χ^2 index, is altogether not surprising if the invariable smallness of the receptive area is kept in mind; this area is sometimes not even fully utilized, concluded from local accumulations and sparsenesses of chorioallantoic membrane lesions occasionally observed in spite of meticulous care taken with the inoculation, whereas the area available for tissue culture counting depends entirely on the experimenter's choice and is free to observation through the proceedings. No support for any overcrowding effect on plaque counts is obtained if tissue culture data are arranged in the way of Table 2; the d values, most

Table 1. ADDITION OF χ^2'S. COMPARISON OF POCK AND PLAQUE COUNTING METHODS

Counts per sample	No. of samples	*df* (degrees of freedom)	χ^2	*d*
	pock-forming units (CAM counting)			
5	100	400	642·5	7·6
6	100	500	923·2	11·4
7	27	162	379·1	7·5
8	34	238	404·7	6·6
		1,300	2,349·5	17 6
	plaque-forming units (tissue culture counting)			
5	100	400	320·3	−3·0
10	100	900	857·9	−1 0
		1,300	1,178·2	−2 4

$d = \sqrt{2\chi^2} - \sqrt{2df - 1}$

with one-sided significance limits of $d = 1\ 64$ for $P = 0\ 05$
$= 2·33$ $= 0·01$
$= 3\ 09$ $= 0·001$

The negative sign of *d* designates a variance lower than predicted by chance in the tissue culture counts.

Chorioallantoic membrane results differ from expectation with high significance. Note the differences in the χ^2 values for the two methods (equal sums of *df*).

Table 2. INFLUENCE OF SAMPLE MEAN LEVELS ON THE VARIANCE IN POCK COUNTING

100 samples, each comprising 6 chorioallantoic membranes. Distinct overcrowding effect shown by increasing *d* values

Sample mean	No. of samples	*df*	χ^2	*d*	*P*
up to 5	5	25	39·15	1·85	about 0·05
5 to 10	10	50	76·02	2·38	,, 0 01
10 to 15	16	80	127·45	3·36	,, 0·001
15 to 20	17	85	147·39	4 17	lower than 0 001
20 to 25	23	115	240·63	6·88	
higher than 25	29	145	292,36	7·18	
		500	923·20	11·38	

Table 3. FREQUENCY DISTRIBUTION OF χ^2
Comparison of pock and plaque counting

P	Expected	Frequencies of χ^2 observed in groups of 100 samples: Pock counts (CAM) 5 CAM per sample	Pock counts (CAM) 6 CAM per sample	Plaque counts (bovine embryonic muscle t.c.) 5 t.c. tubes per sample	
	f_{exp}			f_{obs}	$\frac{(f_{obs} - f_{exp})^2}{f_{exp}}$
	1	—	—	1	0
0·99					
	4	2	—	8	4
0 95					
	5	5	1	7	0·8
0·90					
	10	—	6	12	0·4
0·80					
	10	7	7	15	2·5
0·70					
	20	16	14	22	0·2
0·50					
	20	18	7	19	0·05
0·30					
	10	5	11	5	2 5
0 20					
	10	17	15	7	0·9
0 10					
	5	9	9	1	3·2
0·05					
	4	9	18	2	1
0·01					
	1	12	12	1	0

$\chi^2 = 15·55$
P (for 11 *df*)
higher than 0·1

of them negative in sign, do not show any tendency whatever.

The slight but already significant tendency of the indexes derived from tissue culture samples towards a probability higher than predicted, meaning better particle dispersion than chance alone would yield, might be explained by mutual repulsion of the particles during sedimentation; if the presence of the tissue culture fluid were really responsible for bringing about this scatter effect, it would intimate an additional technical advantage over the chorioallantoic membrane from which small volumes of liquid are almost instantaneously resorbed. At last, the fact may be recalled that embryonated eggs will always represent a whole population of hosts, subject to biological variation in many respects; so the assumption of a variable susceptibility to virus infection in comparison with the homogeneous tissue culture seems to be reasonable.

The chorioallantoic membrane remains, however, a valuable counting recipient, easy at hand and inexpensive, therefore not even dispensable for those laboratories familiar with tissue culture work. So we are glad to have found a way of expressing the increase of the variance in case the Poisson variability is not fully achieved. Table 4 shows χ^2 table values modified by multiplication as to fit a distribution derived from 100 samples of 6 chorioallantoic membranes; the allowance of 60 per cent designated by the factor adjusted to 1·6 supplies a measure applicable to the over-all variance of the group. If one of these 100 samples has produced a mean of, say, 24 pocks, its *S.E.* can now be stated as being ± 2·53 instead of ± 2, the latter calculated assuming unrestricted Poisson conditions (variance equal to mean, *S.E.* expressed by the square root from the ratio of variance to number of counts). The example illustrates the fact that the *S.E.* for each sample in this group has to be raised by about 25 per cent (strictly 26·5 per cent, the square root of 1·6 being 1·265). The greatest necessary allowance for the variance in a group of samples amounted to 80 per cent; thus a raise of the *S.E.* by a third will include the highest possible risk of additional variation involved with the chorioallantoic membrane technique.

Table 4. FREQUENCY DISTRIBUTION OF χ^2 ARRANGED IN QUINTILES AND MODIFIED BY AN ALLOWANCE OF 60 PER CENT FOR THE VARIANCE
100 samples, each comprising 6 chorioallantoic membranes (cf. second observation values column in Table 3)

χ^2 (table values)	*P*	f_{exp}	f_{obs}	χ^2 (values multiplied by 1 6)	$f_{obs\ 1\ 6}$	$\frac{(f_{obs\ 1·6} - f_{exp})^2}{f_{exp}}$
		20	54		23	0·45
7 289	0·2			11·662		
		20	16		24	0 8
5·132	0 4			8·211		
		20	8		18	0·2
3·655	0 6			5·848		
		20	15		13	2 45
2·343	0 8			3·749		
		20	7		22	0·2

$\chi^2 = 4\ 1$
P (for 4 *df*) about 0 4

Precautions for practical work can be deduced from the foregoing. Significance limits for evaluating individual sample indexes properly modified and samples of still significant variability eliminated, the standard error of the means will not exceed 15 per cent (or 20 per cent as an extreme). Analogous calculations are practicable with confidence limits, significance testing for differences of sample means, etc., all based on the adjusted variances. The bearing of this for more differentiated laboratory work with vaccinia virus, for example, neutralization testing of sera, need not be emphasized.

The general conclusion from these findings points clearly to the validity of the Poisson distribution for pock as well as plaque counting of vaccinia virus. Restrictions as to the variability of chorioallantoic membrane pock counts can be explained by technical circumstances and compensated statistically to uphold the reliability of sample means. This ought to dispel doubts about the suitability of an efficient and economic method.

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LETTERS TO THE EDITOR

ASTRONOMY

A Typical Leonid Meteor Spectrum

CONSIDERABLE efforts have been made by many workers[1] to obtain meteor spectra. From the earlier observations it has been established that all meteor spectra from each shower are remarkably similar to each other, having the same general features within the limitations of photographic resolution and emulsion sensitivity[2].

This communication describes the first spectrum taken from India, at Waltair. The meteor appeared at 2 h 47 min I.S.T. on the night of November 15–16, 1961. Its visual magnitude has been estimated as brighter than – 2. It appeared green and had a terminal burst at which the wake remained visible for more than a minute. From the plot of its path on a star map it was found to proceed from the Leonid radiant. The spectrum of this meteor has been photographed on an Ilford *HP*3 film (*ASA* No. 400) using a conventional meteor spectrograph with the rotating shutter arrangement. The Agfa 35 mm camera used for this purpose carried before its $f/2$ lens a Bausch and Lomb plane replica transmission grating, with 200 lines per mm and blazed for 5000 Å. From the durations of the segments produced by the rotating shutter, the velocity of the meteor has roughly been estimated at 71 km/sec, which is in close agreement with the mean velocity for the Leonid shower.

The spectrum of the meteor is reproduced in Fig. 1. Five segments of the trail could easily be identified in the photograph. The brightness of the trail has gradually increased from the first to the last segment. The sudden increase in the brightness of the last segment corresponds to the terminal flare. The maximum intensity is found to occur in the yellow region in the first three segments, whereas it has shifted to the violet in the last segment, showing an increased excitation towards the end of the trail. This feature has already been noticed by Millman[2]. A weak secondary spectrum having a few lines has been recorded in the gap between the first and second segments. This is supposed to be produced by the material which broke off from the main mass and trailed behind it[3]. The spectrum of the persistent train has also been recorded in between the 4th and 5th segments. The analysis of this wake spectrum will be presented in another communication.

Table 1. WAVE-LENGTHS AND IDENTIFICATIONS OF LINES IN THE SECONDARY SPECTRUM BETWEEN THE FIRST AND SECOND SEGMENTS

Wave-lengths	Multiplets identified
3605 1	FeI (23)
3885·7	FeI (4), FeI (20)
3933·1	CaII (1), FeI (4), FeI (20), SiI (3)
5174·3	MgI (2), CaI (23), FeII (42), FeI (1)
5328·6	FeI (15), FeII (48), FeII (49).
5576·4	OI (3F)
5892·9	NaI (1)

Table 2. MULTIPLETS OF ELEMENTS IDENTIFIED IN THE PRIMARY SPECTRUM IN THE FOURTH AND FIFTH SEGMENTS

FeI	(1)	FeI	(21)	FeII	(27)	CaI	(3)	SiI	(3)
	(2)		(41)		(28)		(4)		(9)
	(3)		(42)		(37)		(20)	SiII	(1)
	(4)		(43)		(38)		(23)		(2)
	(5)		(152)		(42)	CaII	(1)		(3)
	(13)		(168)		(48)	MgI	(1)		(5)
	(15)		(318)		(49)		(2)	NaI	(1)
	(16)		(350)		(74)		(3)		(5)
	(20)		(1087)	CaI	(2)	MgII	(4)		

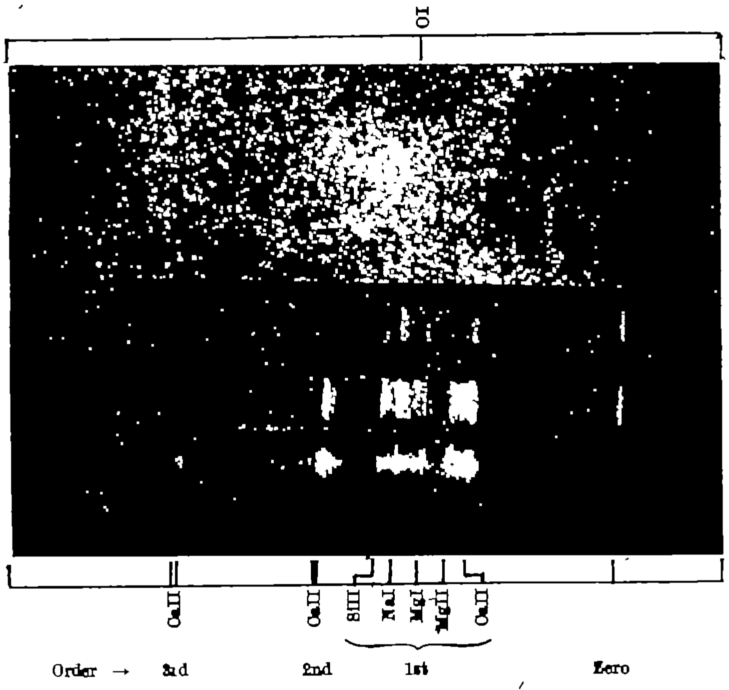

Fig. 1. Spectrum of the Leonid meteor observed at 02h 47m on the night of November 15–16, 1961. (As the secondary spectrum HI (1) and NI (20) lines are very faint, they have not clearly shown up in the reproduction. However, they could easily be identified in the negative)

Seven lines have been measured in the secondary spectrum, and their wave-lengths are presented in Table 1 along with their identifications. Five of them are found in the secondary spectrum of the Perseid meteor obtained by Millman[3]. A continuous line extending from above the first to the third segments has been identified as the oxygen green line. This line has been observed in 17 meteor spectra taken earlier[4] out of which only one corresponds to a Leonid meteor, and most of the others also correspond to high-velocity meteors. The present observation is consistent with the general feature that the oxygen green line occurs at heights greater than that of the maximum light in the meteor spectrum. The HI (1) (6568·1 Å) and the NI (20) (6656·3 Å) lines have also been identified in the first segment. The presence of the hydrogen, nitrogen and oxygen gases seems to lend support to Whipple's comet model[5], and also to Kuiper's suggestion that the water of crystallization of certain compounds may possibly be present in cometary meteoroids[6].

About 35 lines have been measured in the primary spectrum, which is clearly resolved in the fourth and fifth segments. The various possible multiplets, identified in this spectrum, are listed in Table 2. There the multiplet numbers correspond to those used in Dr. C. E. Moore's multiplet table[7]. These multiplets have all been identified in Perseid meteor spectra[3,4]. All the multiplets observed in the Leonid meteor spectrum of Millman[4] have been found in the present list excepting FeI (38).

Eight Leonid meteor spectra obtained by Millman[3] have been classified under type *Y* spectra, having the H and K lines of ionized calcium as the most prominent feature. The present spectrum also exhibits the same feature, confirming that the Leonid group is composed mainly of stone meteors. The presence of the strong lines of CaII, MgII and SiII in this spectrum supports Millman's suggestion[8] that the excitation may depend on the geocentric velocity.

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RADIO ASTRONOMY

Radio-Transit Telescopes steerable in Azimuth

WHEN considering radio-transit telescopes, one ordinarily speaks in terms of instruments steerable in altitude. However, transit telescopes steered in azimuth have potentialities which should be evaluated. Bracewell[1] and Drake[2] have made suggestions for azimuth steerability. Both their designs utilize offset foci and the concept of a segmented reflecting surface.

The instrument proposed here would comprise a paraboloidal dish with fixed altitude supported by an infrastructure that is free to move in azimuth. Railway tracks may provide the azimuth mount; the 250-ft. Mark I reflector at Jodrell Bank is an example of this design[3]. The rotation of the whole structure through the desired angle accomplishes the steering. The structure behaves like a transit instrument for all sources at the declination which corresponds to the fixed altitude and adjustable azimuth. As usual with transit instruments, no guiding or scanning is possible.

The disadvantage of the instrument, compared with the altitude-steerable transit types which are designed to give horizon to horizon coverage, is that only a limited band of the celestial sphere between two declinations is accessible to observations. The altitude of the paraboloid determines the width of the band, and in general any source that lies within this band can be observed twice every 24 sidereal hours. To achieve complete sky coverage a number of such transit instruments at varying geographical latitudes would have to be erected, the number being determined by the altitude. If the altitude is too small, observations will be made too near the horizon and the height of the instrument may be too great if the diameter is large. If, on the other hand, the altitude is too great, there will be small sky coverage which will necessitate a larger number of instruments. Thus, for example, two azimuth-steerable transit telescopes each with an altitude $\alpha = 45° + \varepsilon$ ($\varepsilon \sim 1°$), with a diameter of D ft. and a maximum height $h \sim D \cos \alpha$ ft. situated at geographical latitudes 45° N. and S., would give complete sky coverage plus overlap at the equator with a half-power beam width of 112 λ/D min of arc at wave-length λ cm.

The number-diameter-altitude-cost-capability relationship of such a design could well be evaluated because of the following desirable features. (1) Greater resolution is achieved by spreading a greater projected surface area of the dish over a larger ground area. The rigidity of the Earth is therefore used to advantage. (2) The elimination of the degree of freedom in altitude simplifies construction problems. (3) The angle between the direction of gravity and any element both of the reflecting surface and the focus mounts will be the same for all positions of the telescope, and thus the structural members will not be subjected to varying loads during steering. (4) The instrument can be designed with a focus tower built directly upon the ground. This possibility would free the reflecting surface and its supporting infra-structure from the static loads that would occur if the feed were supported conventionally by a tripod mount, and greater accuracy of the reflecting surface might therefore be possible. In this case the structure must be designed to rotate about an axis coincident with the focus tower and on circular tracks with the base of the focus tower at centre.

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GEOPHYSICS

Susceptibility Anisotropy Measurements on some Silurian Siltstones

ANISOTROPY of magnetic susceptibility, in certain sediments, is probably due to the preferred orientation of elongated magnetic grains. Rees[1] has shown that for varved silt redeposited in the laboratory the direction of the axis of maximum susceptibility is controlled initially by the geomagnetic field and in the final stages of deposition by water current and 'compaction' effects analogous to those which have been shown to affect the direction of remanent magnetization. This work reported here was undertaken to determine whether natural sediments also have an anisotropy which can be related to the direction of palæocurrent flow indicated by geological observations.

Orientated samples were collected from siltstone beds in the Kerry, south-west Clun Forest and Bishop's Castle areas of the Welsh borderland. The siltstone beds are of Lower Ludlovian age, and it has been suggested by Cummins[2] that they were deposited by turbidity current flows. Evidence of the direction of these currents is provided, at many sites, by sedimentary structures and other forms of lineation occurring at the base of, and within, the siltstone beds. Fourteen samples were collected from such sites, and 45 orientated cylindrical specimens have been obtained from these by coring.

The siltstones have a median diameter of 30–60 μm and are composed of between 30 and 40 per cent of clastic quartz in a matrix of finely divided sericite and/or calcite. Opaque minerals including magnetite form about 5 per cent of the rock.

For each specimen the directions of the principal axes of susceptibility were determined using a simple torsion magnetometer[3]. These directions have been plotted on a stereographic net. The maximum and intermediate axes of susceptibility lie nearly in the bedding plane, the minimum axes thus being perpendicular to it. A mean direction of the maximum susceptibility axis was estimated for each sample, from measurements on 3 or more specimens. These mean directions were plotted (Fig. 1) with respect to magnetic north, and were seen to have a considerable scatter. As an alternative, the mean directions were plotted with respect to the known current directions, determined from macroscopic indications, for the individual sampling sites (Fig. 2) and this considerably reduced the scatter. The directions of the mean maximum susceptibility axes appear to be aligned in the current direction and dip into this direction at an angle of about 10°.

Forces acting at the time of deposition control the orientation of the grains. On touch-down on the bed the grains tend to take up a position so as to offer least resistance to the flow. Rusnak[4] showed, from studies on sand

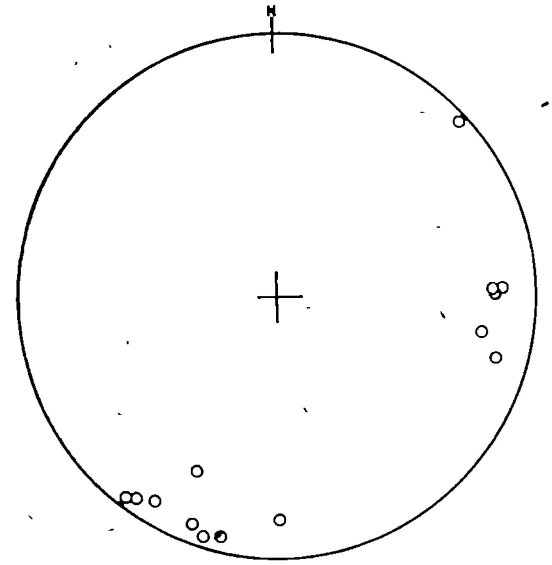

Fig. 1. Mean maximum susceptibility axes plotted with respect to magnetic North. All points lower hemisphere

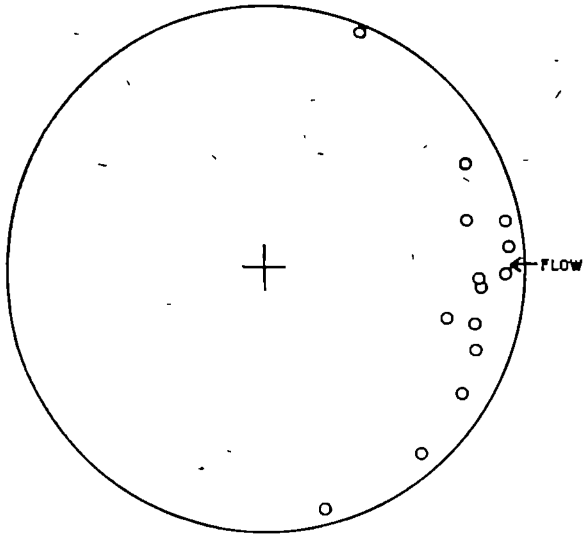

Fig. 2. Mean maximum susceptibility axes plotted with respect to known current direction. All points lower hemisphere

grains, that this is achieved when the grains are lying with their axes of elongation in the direction of flow. However, the magnetic field acting at the time of deposition might also be expected to produce some preferred orientation of the grains, but it is thought that the deposition of the siltstones occurred under high fluid stress conditions, where the effect of the magnetic forces in producing grain alignment was negligible compared with the effect of the hydrodynamic forces. Thus the present observations agree with Rusnak's findings. Furthermore, the dip of the axes into the upcurrent direction is of the same order as that described by Rusnak for sand of 100 µm diameter.

These results suggest that measurements of the directions of the axes of maximum susceptibility of rocks of the type described may, in certain cases, prove a reliable method for estimating current directions and be a valuable supplement to other palaeocurrent data. Earlier studies of grain orientation in fine-grained sediments, for example those of Dapples and Rominger[5], and Curray[6], have involved measurement of the projections of the grains in two dimensions. The present method enables the magnetic grain orientation to be determined in three dimensions and thus gives a clearer indication of the magnetic fabric of the sediment, which for these siltstones is thought to be the same as that exhibited by the non-magnetic grains.

The collection of the siltstone samples was made possible by the guidance in the field of R. J. Bailey, and I am indebted to him for permission to draw on unpublished data concerning the current directions in the area. I also thank the Department of Scientific and Industrial Research for providing a maintenance grant.

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GEOLOGY

Multiple Solid Solution on Sulphides in Sphalerite

The mineral sphalerite is well known as a ready solvent, at elevated temperatures, for a number of other sulphides. On cooling, such a homogeneous solid solution unmixes, producing exsolution bodies of the solute aligned, ideally, along the structure planes of the sphalerite host.

Experimental work has established certain maximum solubilities while chemical analyses of minerals indicate varying degrees of solid solution under natural conditions. Sphalerite from St. Agnes, Cornwall, for example, contains as much as 26·2 per cent iron[1]. A number of solid solutions with sphalerite as solvent are known or are inferred from unmixing textures. The most common solutes are chalcopyrite, pyrrhotite and tetrahedrite. Others have been recorded but are rare. Unmixing textures involving more than one sulphide exsolved from a given sphalerite host are occasionally observed, for example, chalcopyrite and pyrrhotite sometimes with additional exsolved cubanite and/or valleriite.

A recent mineragraphic study[2] of a suite of polished sections from a small deposit of silver-rich mixed sulphide ore from Rockvale, near Armidale, New South Wales, has revealed a hitherto unexpected diversity of minerals exsolved from solid solution in sphalerite. The trends of unmixing are shown in Fig. 1.

The Rockvale ore is extremely complex with twenty-five different minerals in intimate intergrowth. The principal constituents are arsenopyrite, galena, argentian tetrahedrite, sphalerite, chalcopyrite, stannite, pyrite and composite grains of several ruby–silver minerals with

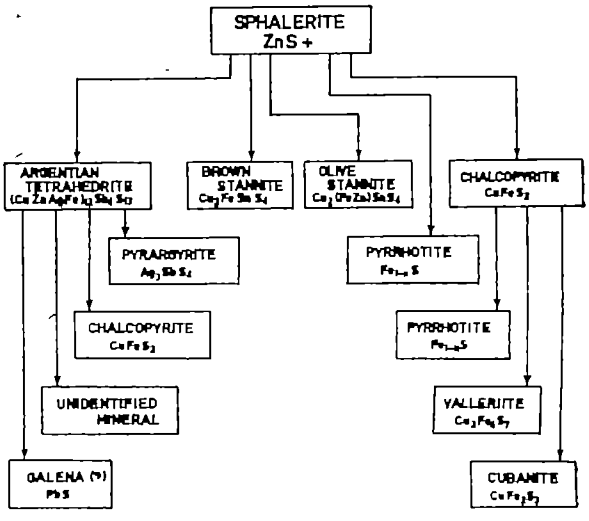

Fig. 1. Trends in the multiple unmixing of a solid solution of eight mineral phases of sphalerite

pyrargyrite predominating. Other constituents are accessory. Each of the principal constituents was deposited directly from the ore fluids and some of them also figure in exsolution processes in sphalerite. A number of them, in addition to sphalerite, also contain exsolution products. These include galenobismutite and traces of pyrargyrite in the (100) planes of galena, sphalerite in the basal planes of stannite, chalcopyrite and an unidentified grey-white mineral as interstitially exsolved solutes in argentian tetrahedrite.

The first mineral to unmix from solid solution in the Rockvale sphalerite was argentian tetrahedrite, which occurs as small rods in the (100) and the (111) planes of the host. A second-stage unmixing (Ramdohr's "*unterentmischen*") of the exsolved tetrahedrite yielded extremely minute wisps of chalcopyrite, the unknown grey-white mineral and small amounts of pyrargyrite. The possible exsolution status of occasional lamellæ of galena, orientated in the structure planes of the sphalerite and sometimes co-linear with tetrahedrite lamellæ, is questionable.

Further unmixing from the sphalerite produced lace-like aggregates of two varieties of stannite—an olive-coloured stannite and a brown stannite. These may correspond to zinc-rich and zinc-poor stannites, respectively.

Minute lamellæ of pyrrhotite precipitated in the (111) planes of the sphalerite occur in places.

Vigorous diffusion during unmixing of chalcopyrite is evident with the development of sphenoidal crystals aligned in the (111) directions of the sphalerite. Some of these exsolved crystals are monomineralic; the majority, however, have also undergone second-stage unmixing yielding, within the chalcopyrite, irregular areas and (111) precipitations of pyrrhotite exhibiting widmanstetten-like texture. Other chalcopyrite crystals show either cubanite and/or valleriite precipitated in the (111) planes of the chalcopyrite.

This unique unmixing of an initially homogeneous sphalerite has yielded no fewer than eight distinct mineral phases one of which (stannite) consists of two variations. So profuse was the unmixing that single grains of sphalerite only 0·5 mm diameter contain up to six or seven of these exsolved phases.

Omitting galena, the crystal structure of which would inhibit solid solution in sphalerite but which nevertheless has an exsolution appearance, an extended sphalerite composition (Zn, Fe, Cu, Sn, Sb, Ag)S is manifest for the homogeneous material prior to unmixing. The five non-essential metals (as sulphides) were assessed at some 10–15 per cent of the total.

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METEOROLOGY

A Lunar Influence on Ice Nucleus Concentrations

RECENT papers by Bradley, Woodbury and Brier[1] and by Adderley and Bowen[2] have shown a remarkable relation between the phase of the Moon and precipitation.

The former showed that using the dates of heavy precipitation for each month recorded at the 1,544 United States weather stations which have operated continuously during the period 1900–49, there was a marked tendency for extreme precipitation near the middle of the first and third weeks of the lunar synodic month. The second paper showed a very similar result for the rainfall at 50 New Zealand stations in the same period, and the authors suggested that the Moon might perhaps modulate the amount of meteor dust collected by the Earth.

If this were so, and Bowen's theory that meteor dust reaches the lower atmosphere about 30 days after its arrival in the upper atmosphere were correct, then the ice nucleus concentrations at the ground should show corresponding maxima. It is scarcely conceivable that nuclei of local or terrestrial origin would be influenced by the Moon to a significant degree.

For several years ice nucleus concentrations have been measured in Australia, New Zealand and South Africa[3,4] during the summer months. These data have now been examined for a lunar effect in the following way: adopting Bradley, Woodbury and Brier's convention, the angular difference on apparent longitudes of the Sun and Moon at Greenwich noon was expressed as hundredths of the synodic month of 29·53 days, new Moon being 0·00 and full Moon 0·50. The temperature at which most observations were made in a particular sequence of ice nucleus measurements was then chosen, and the mean concentrations at that temperature for each day was entered against the appropriate 'synodic decimal'. Moving averages over 0·10 lunar months were taken (roughly equivalent to a 3-day average), as in the rainfall data, giving the curves shown in Fig. 1. In order to compare curves satisfactorily

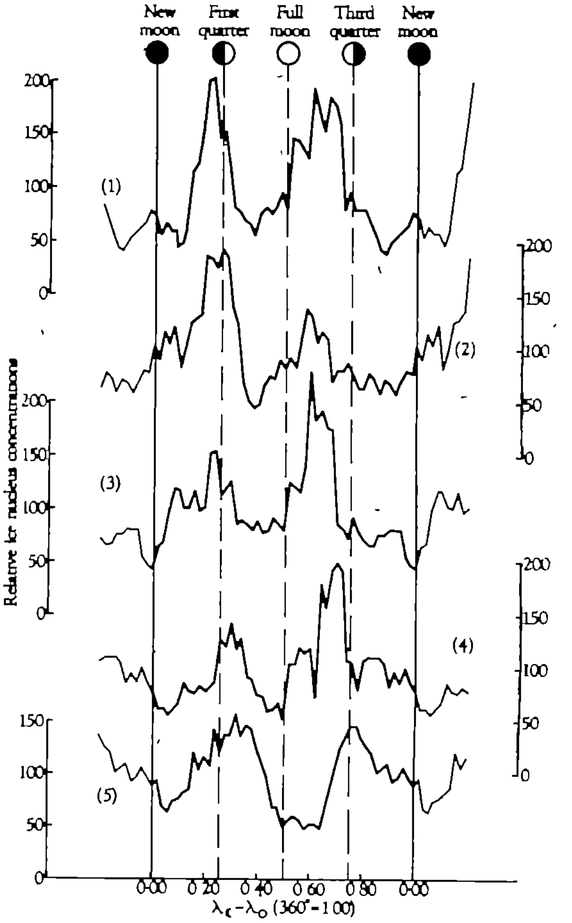

Fig. 1 Relation between summer ice nucleus concentrations in the southern hemisphere and lunar phase

Curve	Location	Time	Instrument
(1)	41° S., 175° E., Wellington, N.Z.	Oct. 1957–Mar. 1958	10 *l* Expansion chamber
(2)	34° S., 151° E., Sydney, Australia	Nov. 1958–Jan. 1959	Continuous automatic cold chamber
(3)	34° S., 151° E., Sydney, Australia	Oct. 1956–March 1957	10 *l* Mixing chamber
(4)	26° S., 153° E., Caloundra, Australia	Nov. 1956–Feb. 1957	10 *l* Mixing chamber
	25° S., 114° E., Carnarvon, Australia	Nov. 1955–Feb. 1956	1 *l* Mixing chamber
(5)	23° S., 14° E., Swakopmund, South-West Africa	Nov. 1956–Jan. 1957	3·5 *l* Expansion chamber
		Dec. 1957–Jan. 1958	10 *l* Expansion chamber

the resulting concentrations were expressed as percentages of the mean for the lunar month.

The result is as surprising as the rainfall discoveries. In every case, there are two distinct maxima in the lunar month, about first and third quarters of the Moon, and the total range is three or four to one, even with the smoothing used. There appears to be a slight shift with latitude, the peaks occurring later nearer the equator (the curves 1–5 are arranged in order of decreasing latitude).

It is apparent, therefore, that, in addition to the effect on rainfall, the Moon has a large effect on the freezing nucleus count. It is well known that the effect of the Moon on other meteorological variables like barometric pressure, surface temperature, is extremely small; the implication is strong, therefore, that the effect on the freezing nucleus count does not originate in the lower atmosphere. However, a possible mechanism is that the Moon in some way controls the incoming meteor rate and so affects the number of freezing nuclei falling into the lower atmosphere. This possibility will be examined in a forthcoming paper.

In the meantime it is interesting to compare the summer rainfall of a continental climate (on which ice nuclei are likely to exert their greatest influence) with those in Fig. 1. In Fig. 2 the daily rainfall of Alice Springs (lat. 24° S., long. 134° E.) during 1873–1952 has been analysed in the same way as the ice nucleus data.

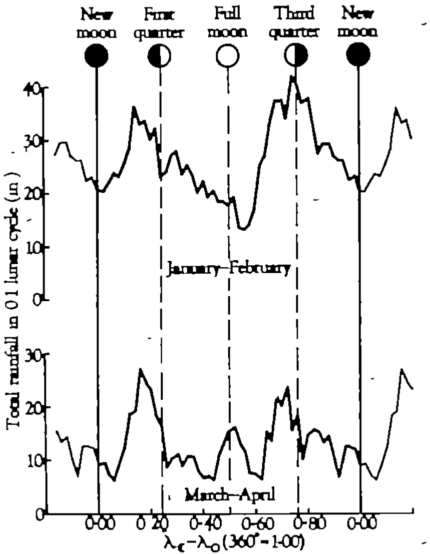

Fig. 2. Relation between total daily rainfall at Alice Springs and lunar phase

The variations are remarkably similar to those of Fig. 1 (and those of the earlier papers) and show that the effect is present not only in the heaviest rainfalls but also in the total daily rainfall at a suitable location. The extreme range of three or four to one in the rainfall is the same as in the ice nuclei, suggesting that on the average there is a progressive increase in rainfall with increase in the ice nucleus count.

I thank the U.S. Weather Bureau for its tables of decimal ephemeris of Sun and Moon which were used for the figures, and the Director of the New Zealand Meteorological Service for the Wellington ice nucleus measurements which have not been published previously in detail.

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PHYSICS

Re-entrant Motion in Special Relativity

Phipps[1] has shown that for a disk rotating with edge velocity v, the 'round trip' velocity $v_{rt} = v(1-v^2/c^2)^{-1/2}$ where v_{rt} = circumference of disk/period of revolution. The following argument derives a similar relationship between observed velocity and 'round trip' velocity for linear 'to-and-fro' motion.

Consider two rods AB and CD, each of rest length x, which slide along each other. Initially (as viewed from C) the rods are end to end, B touching C. A and C (also B and D) approach each other at relative velocity v. When an observer at C sees A reach C, the relative motion of the rods is reversed. C measures the time from B leaving C to B returning to C. Because of the relativistic contraction of length, C initially observes A at a point P at rest relative to C and distant $x(1-v^2/c^2)^{1/2}$ from C. (This is equivalent to stating that C observes the length of AB to be $x(1-v^2/c^2)^{1/2}$.) As AB is moving with observed velocity v, the time observed by C for A to reach C is $(x/v)(1-v^2/c^2)^{1/2}$. This is also the time C observes for the return of A to P. When C observes A at P, he also observes B back at C. Hence C measures the time of B's journey as $(2x/v)(1-v^2/c^2)^{1/2}$. Now an observer initially coincident with B and C, and in whose frame of reference AB and CD have equal and opposite velocities, will observe A reach C and B reach D simultaneously. Hence if A reaches C, B must reach D. But in C's frame of reference $CD = x$ and the distance travelled by B is $2x$. It follows that C measures the 'round trip' velocity of B to be $v_{rt} = v(1-v^2/c^2)^{-1/2}$.

It is of interest that the time observed by C for the round trip equals the time observed by C on a clock mounted on B (ref. 2). The clock paradox thus does not exist.

I thank Dr. T. E. Phipps for his comments, and for his encouragement to publish this work.

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Pulsed Gaseous Maser

Optical maser oscillation has been obtained during the recombination period following a pulsed radiofrequency or d.c. discharge in helium mixed with either neon or carbon monoxide at wave-lengths of 1·153μ and 1·069μ, respectively. No oscillation occurs during the 10 μsec excitation pulse although in pure neon a period of apparent optical gain and low-power oscillation at about 1·15μ is observed during the pulse.

The usual near-confocal mirror[1] system is used containing a quartz discharge tube 1 m long and 16 mm or 2 cm internal diameter having end windows at the Brewster angle. The tube has been excited either by a high-power S-band magnetron or by d.c. pulses of about 1·5-kV, 20 amp, 10 μsec fed in through tungsten electrodes sealed in side arms of the quartz tube. Pulse repetition rates of up to 1 kc/s have been used for the d.c. excitation.

With the helium/neon mixture the optimum gas pressures appear to be about 1 mm and 70μ mercury respectively, when the oscillation reaches its maximum power of more than 1 W at 30–80 μsec after the end of the excitation pulse and the oscillation pulse then has a half-power width of 40–50 μsec. With S-band excitation it is also possible to obtain powers of about 0·5 W at 1·153μ wave-length when the helium pressure is raised to more than 8 mm. The half-power width of the oscillation pulse is then about 30 μsec and the pulse occurs immediately after the excitation pulse.

The optimum conditions for helium mixed with carbon monoxide involve gas pressures of 2 mm and 8μ mercury respectively. Peak powers of 0·7 W at 1·069μ wavelength are produced about 30 μsec after the excitation pulse and the half-power pulse width is about 40 μsec.

The unusually high powers obtained are thought to be due to the ease with which a high density of 2^3S metastable helium atoms is formed during the recombination of a well ionized helium plasma[2]. Moreover, the high metastable density is obtained without a correspondingly high electron density. These conditions are particularly favourable for producing a population inversion in neon by resonant exchange processes[3].

With carbon monoxide instead of neon, the energy required to dissociate the molecule into a ground state oxygen atom and a carbon atom excited to the $3p^3D$ level is 0·06 eV less than the excitation energy of the metastable helium 2^3S, and a nearly resonant condition for this process is established. The observed wave-length of 1·069μ agrees well with the permitted radiative transition in carbon from the $3p^3D$ state to the $3s^3P^\circ$ state.

The $CI3p^1P$ level being just below the $CI3p^3D$ is also suitable for selective population by this dissociative mechanism. Oscillation originating from this level has been observed by L. E. S. Mathias at these laboratories[4] in a continuously excited helium/carbon monoxide mixture. The lower level is $3s^1P^\circ$ and the wave-length 1·454μ.

This, together with the fact that neither of these upper levels is optically connected to the ground state, is indicative that these oscillations are produced by the dissociative mechanism proposed above, and not by direct electron excitation.

We are indebted to R. S. A. Thorn for performing much of the experimental work. Acknowledgment is made to the Admiralty for permission to publish.

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Growth of Positive Channels in Non-uniform Fields during Impulse Breakdown

THE investigation reported by Menemenlis[1] under the same title as a communication to *Nature* of June 27, 1962, has been continued at the laboratory so that some more concrete results concerning the growth of positive channels can now be communicated.

The channels investigated were produced in an 18-cm non-uniform field air gap by applying 1/50 μsec chopped impulse voltages of a crest value of the full wave lying between that causing without chopping 100 per cent breakdown and that exceeding this value by 20 per cent.

The gap consisted of a small sphere, 5–15 mm in diameter, fixed at one end of a 75-ohm coaxial cable and a circular disk 30 cm in diameter, directly connected to the negative high-voltage terminal of the front capacitor of a single stage 200-kV impulse generator whose positive pole was earthed. The coaxial cable was earthed through a 68 + 7 ohm (75 ohm) attenuator. The voltage drop of the predischarge current (that is, of the first corona discharge and of the chopped channel) across the 7 ohm part of the attenuator was applied on the upper beam pre-amplifier of a double beam, Model 555, Teletronix oscillograph to obtain faithful recordings of the time variation of this predischarge current. In addition, a mixed divider, consisting of a 1,000 ohms resistor in series with a 0·5 μH inductance, was connected in parallel to the 7 ohms part of the attenuator. The resistance end of this divider was connected with the upper point of the 7 ohms resistance of the attenuator while its inductance end was earthed. The voltage drop in the inductance, which is practically proportional to the current slope, was applied on the lower beam pre-amplifier of the oscillograph to register the time variation of di/dt.

By correlating both the faithful current records and the current slope records with corresponding photographs of the channels taken in the way described in the aforementioned communication, the following results regarding the propagation mechanism of the positive channels could be obtained.

The average velocity of the channels at various stages of their propagation was found to vary between 2 and 15 cm/μsec, this being in accordance with the results obtained during previous investigations.

The propagation of the channel is effected in steps. At the end of each step a corona burst discharge having several streamers paves the formation of the following step. The current impulses of these successive corona discharges are weaker than that of the first corona pip, which is responsible for the formation of the numerous stems on the positive electrode; but they are comparable to the current corresponding to each stem.

Under the experimental conditions given here, the channels' steps have a length varying between 0·5 and 1·5 cm. Longer steps were rarely observed. They can then reach lengths up to 4 cm.

As a rule, the corona streamers preceding each successive step become thinner and their number increases as the channel proceeds towards the negative disk.

The time-intervals between successive corona discharges vary from 0·05 to 0·6 μsec. The smaller the time interval, the shorter the length of the step and the smaller the magnitude of the electric charge corresponding to the corona current impulse.

The front of the initial corona current impulse lasts some 10^{-2} μcoul. The successive corona impulses occurring during the progress of the channel are less steep; their front duration can reach values up to 0·2 μsec.

The average current through the gap, as it is known, is exponentially increasing during the development of the channel. The current jumps caused by each of the successive coronas vary between 0·5 and 1·5 amp and are superposed to the average current.

The steepness of the current impulses due to the successive corona discharges is of the order of 10 amp/μsec.

The electric charge that corresponds to each of the successive corona impulses shows a considerable variation; the values obtained are from the order of 0·005 to that of 0·1 μsec. They are, however, comparable with those corresponding to a single stem produced on the anode surface by the initial corona pip.

Four characteristic types of channels could be distinguished among several hundreds of photographs.

(1) Single channels presenting practically no branching; (2) single channels split up on their way to the cathode into two strong channels; (3) multibranched channels. In one case, six developing branches out of a common trunk have been recorded; (4) twin channels, that is, two separate channels of comparable length and brightness starting from different points of the sphere.

As it has been already pointed out[1], the application of the correlation method to study the channel development is mainly feasible only in the case of single, practically unbranched, channels.

An important question arises now concerning the physical phenomenon that is associated with the stepped propagation of the positive channel during the impulse breakdown in non-uniform fields. The experience of a great number of experiments conducted so far shows that this propagation can reasonably be interpreted as follows:

It has already been mentioned that the gaps investigated were directly connected to the front capacitor (4,140 pF) of the impulse generator. Oscillograms of the gap voltage taken during the growth of the channel showed no appreciable differences when compared, up to the chopping time, with the no-load impulse voltage of the generator. This is due to the fact that the charge drawn from the capacitor during the channel propagation is negligible compared with the charge stored in it, and therefore the voltage across the gap is practically independent of the channel current. On the other hand, voltage drops were avoided by the absence of any series resistance in the gap circuit. Hence, it is reasonable to conclude that the stepped propagation of the channel should be attributed to the discharge phenomena occurring during its growth and not to any voltage drops or fluctuation caused by the circuitry. The presence of a series resistance would, of course, affect considerably the time constants and the magnitude of the phenomena involved.

Breakdown occurs when the channel reaches the negative electrode and bridges the gap. This is possible when the crest value of the impulse voltage is, at least, as high as the minimum impulse breakdown voltage of the gap. Under this voltage, and either when the impulse voltage approaches its crest or during the first microseconds of its tail, the first corona discharge occurs that produces a stem able to form the starting point of a channel. The meaning of this is that a notable progress of a channel towards the cathode is then possible, when the first corona occurs under a voltage value comparable with the minimum impulse breakdown voltage of the gap.

Calculations of the field strength in front of the positive electrode of gaps with very pronounced non-uniform fields, as those investigated, subjected to the minimum breakdown voltage, led to values of 70 kV/cm or more, that is, to values that are by far higher than the dielectric resistance of the atmospheric air, which is roughly 30 kV/cm. Hence, the first corona discharge, which is followed by a stem able to lead to the formation of a channel advancing in the gap, must jump from a point of the positive electrode where the field strength has at least the above calculated value. Corona discharges can also occur, of course, under lower field strengths; they have been observed during this investigation even by field strengths on the positive electrode of about 40 kV/cm, but it is believed they are also possible by field strengths slightly exceeding 30 kV/cm. However, these weaker corona discharges are not able to pave the formation of a stem sufficiently long and hot for the further evolution of a channel.

It is well known that the channel starts from a point of the stem situated near its end. It has been observed during this investigation that the first channel step occurs after a corona discharge has jumped from this point; a strong streamer of this corona discharge is then transformed to this first channel step. The next step is produced in the same way: a corona jumps from the end of the first channel step and one of its streamers is transformed to the second channel step. This process is repeated many times until the channel reaches the negative disk or, eventually, until it stops earlier.

It has been deduced from many oscillograms taken during this investigation that, under gap voltages able to cause a notable progress of a channel, both the current intensities and the electric charges involved during the corona discharges of the successive steps are comparable with the corresponding magnitudes of the first corona streamers that start from various points of the positive electrode and become the basis of the stems. Hence, it might be suggested that all corona discharges leading to the formation of the channel steps are roughly as strong as that creating the stem which, in a way, is the first channel step and, therefore, that they jump from points of the stem or of the channel steps where the field strength reaches a value comparable to that producing on the positive electrode the first corona burst, that is, 70 kV/cm or more.

As stated here, weaker corona discharges have also been recorded. They were starting from points of lower field strength but they were not able to lead to a notable progress of a channel. At the limit, even a slight excess of the critical values of 30 kV/cm, of the field strength on the stem or on the channel steps surface should be able to produce a small discharge. This discharge, however, would cease to advance as soon as the field strength at its boundary reaches the minimum value required for ionization, that is, 30 kV/cm. The consequence should be that the field strength on or near the surface of the stems or of the channel steps would never reach with weak coronas the value of about 70 kV/cm which is indispensable, as mentioned here, for a further channel propagation. Therefore, it seems reasonable to suggest that a phenomenon should take place that causes a sudden jump of the field strength on the streamers forming the stems or on the channel steps during their creation from a value of 30 kV/cm to a value of the order of 70 kV/cm. Such a phenomenon could be a sudden 'constriction' of that part of the corona streamers that have been sufficiently heated by the electronic current flowing through them to the positive electrode, so that the high-temperature plasmas of the stems or the channel steps are formed. As a matter of fact, numerous constriction cases could be noticed after a careful examination of a great number of photographs of chopped channels. When they are taken under good focusing and sensitivity condition, they show very distinguishingly bright thin channels surrounded by a quasi-cylindrical space showing a much weaker brightness, which, obviously, was previously occupied by a preceding corona streamer. Hence, it might be concluded that such streamers have been constricted to form the thin channels. In many photographs, constricted sections of channels are followed by corona streamers which are partly ready to be constricted. A conical space connecting the two stages shows a gradually decreasing brightness from the narrow channel to the wider streamer.

The constriction causes a sudden decrease of the diameter of the cylindrical discharge space of a corona streamer from the order of a few millimetres to that of a few tenths of a millimetre, that is, the diameter of the stems or the channel steps. This sudden reduction of the diameter, as well as the abrupt decrease of the gradient along the discharge space, cause the necessary sudden increase of the field strength around them so that it reaches at a point near their end the level required of about 70 kV/cm.

Hence, during the progress of the channel conditions are repeatedly established for the production of corona discharges that are strong enough to create the next channel steps by means of new constrictions.

Such constrictions phenomena, known also under the name of 'glow to arc transitions'[2,3], are believed to occur in gases under low pressures or pressures of the order of 1 atm. Bruce[4] has suggested that a constriction occurs during the formation of the leader-stroke darts in lightning discharges. Constriction is supposed to occur during the formation of positive channels along the traces of the first streamers of positive gliding discharges[3]. The phenomena involved during the transitions are not very well known[2] but some conclusions can be drawn from the experimental data available: (1) the time of the constriction can be as short as 0·01 μsec; (2) the electric conductivity of the discharge path increases very much during its constriction; (3) the voltage gradient along the discharge path may fall from values of the order of 1,000 V/cm, able to conserve high-pressure glow discharges, to values of an arc discharge which are of the order of 50 V/cm. Such differences in the gradient values of the corona streamers and of the channel paths are believed to exist by many authors.

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CHEMISTRY

Polarographic Behaviour of the Hexa-amminechromium(III) Ion: Role of Hydrogen Ions

Hexa-amminechromium(III) ion in acetate buffer solutions is reduced at the dropping mercury electrode, and gives two polarographic waves. The first wave, which is well defined, is considered to be a one-electron reduction of Cr(III) to Cr(II)[1]. Its half-wave potential at 25° C was found to be $-0{\cdot}97_5$ V *versus* saturated calomel electrode in 0·1 M acetate buffer solution which contained 0·9 M potassium chloride and 0·005 per cent gelatin (Fig. 1, curve *a*). The limiting current of this wave was diffusion-controlled and proportional to the concentration of the complex ion up to 5 mM. Both the half-wave potential and the diffusion current remained constant when the *p*H was varied from 4·5 to 5·45. No change was observed when potassium chloride was replaced by ammonium chloride at the constant ionic strength. The plot of log $i/(i_d - i)$ *versus* E, where i means the average current, i_d, the average diffusion current, and E, the potential of the dropping mercury electrode, gave a straight line with a reciprocal slope of 64 mV, whereas the examinations with Kalousek's circuit and alternating current polarograph showed that this wave has nearly irreversible character.

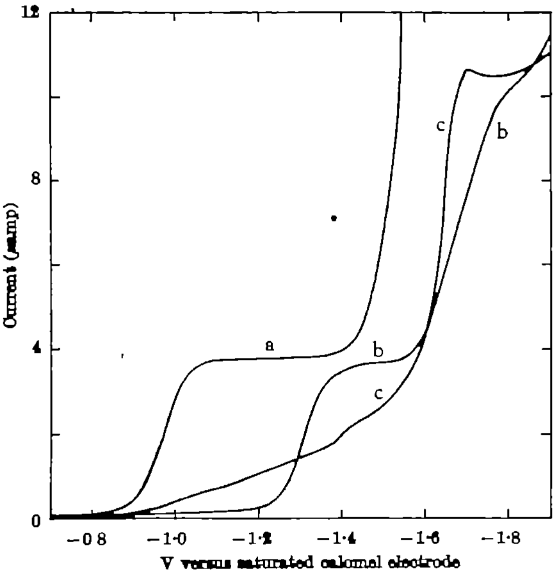

Fig. 1. Polarograms of 1 mM $[Cr(NH_3)_6]^{3+}$ at 25° C in 0 1 M acetate buffer + 0 9 M potassium chloride *p*H 4 6 (*a*); 1 M potassium chloride + 1·56 mM disodium dihydrogen ethylenediamine tetraacetate (*b*); 1 M potassium chloride (*c*). Gelatin was added by 0·005 per cent in all cases

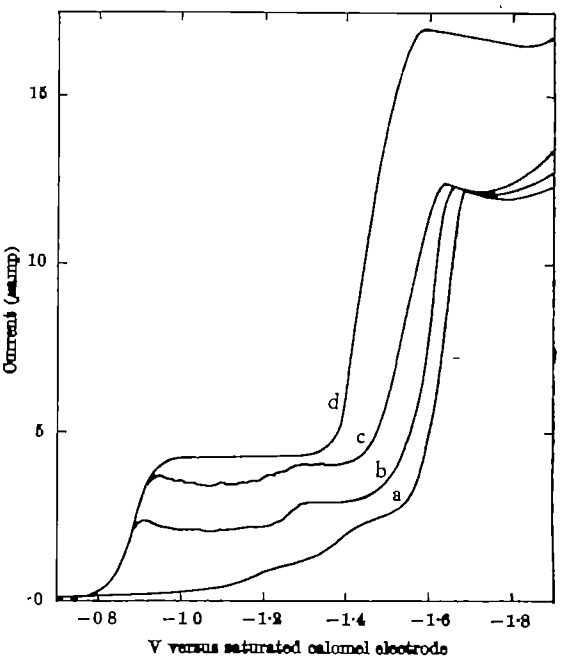

Fig. 2. Effect of hydrochloric acid on the reduction of 1 mM $[Cr(NH_3)_6]^{3+}$ in 0 1 M potassium chloride + 0·005 per cent gelatin + *x* mM hydrochloric acid. *x* = 0 (*a*), 1 0 (*b*), 1 5 (*c*), 2·0 (*d*). In this case current was measured at maximum of current oscillation

In neutral salt solutions, no well-defined one-electron reduction wave was observed (Fig. 1, curve *c*), as has been reported by Maki, Shimura and Tsuchida[2]. The value of the diffusion current at −1·7 V corresponds to a three-electron reduction, that is, Cr(III)→Cr(O). On the addition of hydrochloric acid, the wave at −0·89 V began to appear (Fig. 2). The current at −1·7 V, on the other hand, remained constant until the acid concentration reached a certain critical value; the wave due to hydrogen ion discharge appeared only when this critical concentration was exceeded. This phenomenon is an example of the 'interfering electrode reactions' described by Kolthoff and Lingane[3]. In the present case, hydrogen ions diffusing up to the electrode are neutralized by the ammonia molecules which are liberated by the reduction of hexa-amminechromium(III) ion; reduction of one complex ion will produce six ammonia molecules. The ratio of the critical acid concentration to the concentration of $[Cr(NH_3)_6]^{3+}$ was found to be 1·66, which is in agreement with the value calculated by the equation:

$$(C_{H^+}/C_{Cr(NH_3)_6^{3+}})_{crit.} = 6(D_{Cr(NH_3)_6^{3+}}/D_{H^+})^{1/2}$$

where C is the concentration and D is the diffusion coefficient of the respective species. The wave at −0·89 V attained its full development just when the acid concentration reached the critical value. There was no further essential change in the shape of the developed wave when the acid was added in excess. Hydrogen ions seem to play an essential part in the reduction of hexa-amminechromium(III) ion to the divalent state. However, they cannot be involved in any chemical reaction prior to the electron transfer, because the half-wave potential of the one-electron reduction wave was independent of the hydrogen ion concentration of the solution. They rather act as a neutralizing agent of the ammonia molecules which are liberated from the divalent chromium species. When hexa-amminechromium(III) ion is reduced in a neutral non-buffered solution, the liberated ammonia will make the solution layer near the electrode alkaline, and will cause divalent chromium species to precipitate as an insoluble film on the surface of the electrode. In 0·1 M potassium chloride solution, the current in the potential region between −0·6 and −1·3 V was almost independent of the concentration of the complex ion, and the currents at −1·1 and −1·2 V were linearly proportional to the height of the mercury reservoir. The addition of gelatin increased the current between −1·0 and −1·35 V. These observations support the foregoing conclusion. The fact that the electrode is covered with the insoluble film was confirmed by the observation of the current-time curves during the period of a mercury drop.

It is, therefore, expected that any reagent which can keep divalent chromium from precipitating will make the one-electron reduction wave appear. The polarogram of 1 mM $[Cr(NH_3)_6]Cl_3$ in a neutral salt solution (1 M potassium chloride) containing 1·56 mM of disodium dihydrogen ethylenediamine tetraacetate and 0·005 per cent gelatin (Fig. 1, curve *b*) showed that this is the case.

That the well-defined one-electron reduction wave does not appear in neutral salt solutions though it appears in acid media is a general character of those chromium(III) complexes that have one or more basic ligands, unless the corresponding divalent complexes are stable enough to be maintained in solution. In fact, the following complex ions gave the one-electron reduction wave in acetate buffer solutions (with 0·005 per cent gelatin added), and did not in neutral salt solutions: $[Cr(H_2O)(NH_3)_5]^{3+}$, $[Cr(en)_3]^{3+}$, *trans*-$[Cr(NCS)_2(en)_2]^+$, *trans*-$[Cr(NCS)_2(pn)_2]^+$, $[Cr(ox)(en)_2]^+$, and *trans*-$[Cr(NCS)_4(NH_3)_2]^-$, where en, pn and ox represent ethylenediamine, propylenediamine and oxalate, respectively. On the other hand, a well-defined one-electron reduction wave of Cr(III)→Cr(II) is observed in potassium chloride or sodium chloride solutions for *tris* (dipyridyl)chromium(III)[4], hexathiocyanatochromate (III), and ethylenediamine tetraacetatochromate(III) ions.

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Molecular Addition Compounds of Polycyclic Derivatives of Cinnoline

POLYCYCLIC derivatives of cinnoline are known to form molecular addition compounds with picric acid[1]. Contrary to the report by Slack and Slack[2], I find that benzo[*c*]cinnoline, in common with its methyl derivatives[3], forms a mono-picrate. My compound (found, C, 52·9; H, 2·8; N, 16·8; $C_{18}H_{11}N_5O_7$ requires C, 52·9; H, 2·7; N, 17·1 per cent) has the same melting point as that reported by Slack and Slack, who based their formula solely on a nitrogen content of 17·8 per cent.

The polycyclic derivatives of cinnoline also form addition compounds with trinitrofluorenone in ethanol. These were found to be 1 : 1 addition compounds except in the case of benzo[*f*]naphtho[2,1-*c*]cinnoline, which formed a 1 : 2 compound.

Orchin[4] has suggested that the melting points of molecular addition compounds of the aromatic hydrocarbons, relative to those of the hydrocarbon itself, can be taken as a measure of the effectiveness of the intramolecular forces between the two molecules. From Table 1 it can be seen that the addition compounds of the polycyclic cinnolines melt at temperatures close to or above the melting point of the cinnoline except in the case of benzo[*f*]naphtho[2,1-*c*]cinnoline. This cinnoline is non-planar, as a result of intramolecular overcrowding; and it is evident that this non-planarity reduces the effectiveness of the intramolecular forces responsible for compound formation. The non-planarity of the molecule may also be responsible for the formation of a 1 : 2 compound with trinitrofluorenone, since the cinnoline would present two planes for attachment of molecules of trinitrofluorenone.

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Table 1

Cinnoline derivative		Picrate	Trinitrofluorenone addition compound						
				Found			Calc.		
	m.p.	m.p.	m.p.	C	H	N	C	H	N
Benzo[*c*]	156°	192°	162°	60·5	2·5	14·0	60·5	2·4	14·2
Dibenzo[*c,h*]	192	216	205	63·8	2·8	12·8	63·9	3·0	12·8
Dibenzo[*c,f*]	157	188	182	63·6	2·9	13·0	63·9	3·0	12·8
Benzo[*h*]naphtho[1,2-*c*]	266	245	235	66·7	2·7	11·6	66·7	2·7	11·8
Benzo[*f*]naphtho[1,2-*c*]	185	226	—	—	—	—	—	—	—
Benzo[*f*]naphtho[2,1-*c*]	270	182	148	61·7	2·6	12·9	60·9	2·2	12·7

Solvation of the Proton in Humic Acids

Tuck and Diamond[1] have described the primary solvation of the proton in the solvent extraction of strong acids by basic organic solvents from dilute acids. Their work gives evidence that the hydronium ion, H_3O^+, is a trihydrated hydronium ion, $H_3O(H_2O)_3^+$, in dilute acids. Others have given evidence from X-ray[2], nuclear magnetic resonance[3], infra-red[4], and Raman[5] studies for the existence of the hydronium ion, H_3O^+, in the solid state.

In this communication, we wish to report the apparent existence of trihydrated hydronium ion in humic acids in the solid state.

Preparation of humic acids. North Dakota lignite (supplied by the Bariod Division, National Lead Co., Houston, Texas) was extracted with 2 N sodium hydroxide[6] at room temperature until the extract was light brown or clear. The aqueous sodium humate solution was centrifuged, decanted and treated with hydrochloric acid until a *p*H of 3 was reached. At this point, the humic acids were quantitatively precipitated. After separating the humic acids by centrifugation they were washed with distilled water and dried at 105° for 12 h.

The analyses of these acids are shown in Table 1.

Table 1

	Ultimate analysis					
	%H	% C	% N	% O	(diff.) % S	% Moisture
Lignite	4·20	45·40	0·82	29·04	2·06	12·23
Humic acids	4·14	59·56	0·70	32·4	1·14	3·56
Decarboxylated and dehydrated humic acids	3·62	61·71	0·72	31·3	1·35	1·37

Decarboxylation method. 79 g of humic acids were supported on glass wool in a 38 × 280 mm electrically heated glass column[7]. Two thermometers were inserted into the column to measure the temperature of the humic acids at two depths. Glass wool was placed on the top of the humic acids to prevent the loss of fine particles from the column.

A variable voltage regulator was used to maintain the temperature of the column at 150°. Anhydrous carbon dioxide-free nitrogen was introduced into the column at a rate of 14 l./h. Carbon dioxide and water were quantitatively determined in the exit gases from the column by absorption in U-tubes packed with ascarite and anhydrous calcium sulphate.

The moles of evolved carbon dioxide and water over 65 h of heat treatment at 150° are shown in Fig. 1.

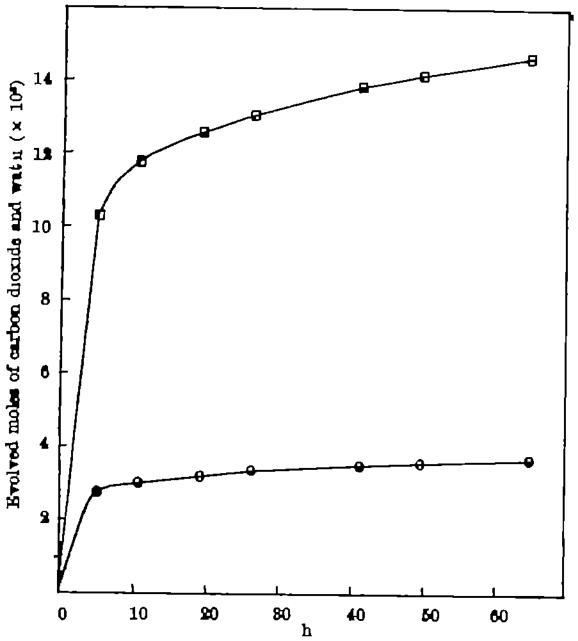
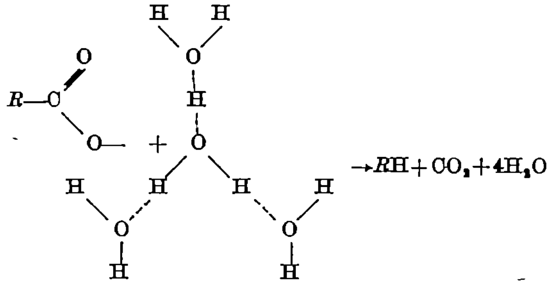


Fig. 1. Effect of heating humic acids at 150° C and 14 l. of nitrogen per h. Moles of carbon dioxide (○) and water (□) versus time (h)

The mole ratio of evolved water to carbon dioxide was about 4. The rate of decarboxylation and dehydration decreased with time. The analyses of the humic acids after 65 h of heat treatment are given in Table 1.

The combined water of humic acids appears to be associated with the carboxyl groups as evidenced by the almost constant ratio of water to carbon dioxide (4 : 1) found in the evolved gases on heat treatment at 150°. The strong co-ordinate solvation in the solid state is postulated, and is very similar to that found by Tuck[1] in his work on extracts from aqueous solutions into organic solvents of strong acids. The primary solvation of the hydronium ion[1] and decarboxylation and dehydration of humic acids are indicated as follows (where *R* is the remainder of the humic acid molecule):



These results appear contrary to those expected. Humic acids, being weak acids, should be in a non-ionized molecular form. Also, one might expect that in the solid state carboxylic acids would be un-ionized.

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BIOPHYSICS

Effect of Oleic Acid on the Mobility of Human Red Cells, and its Effect on Human Serum Protein Fractions

THE higher fatty acids (C-18 acids such as oleic, ricinoleic, arachidonic, etc.) exist both in the adenocarcinomas of the *C3H* mouse[1] and in at least some human tumours[2]. They also occur in normal mouse and human tissues[3], where they are combined, somewhat like the fatty acids in tumours, with lipid and protein inhibitors about which little is known. This communication is concerned with the question whether oleic acid (taken as an example of the higher fatty acids) affects the electrical mobility of human red cells (*A*, below), and whether it interacts with human serum protein fractions.

(*A*) *Method.* One volume (about 3 ml.) of M/50 oleic acid in ethanol is added to 2 volumes of Dole buffer at *p*H 8·6, and is dialysed for 18 h or more at 20° C against large volumes of the same buffer. A suspension of washed human red cells is diluted with the buffer so as to have a volume concentration of 0·08. To 10 ml. of the buffer is added 0·1 ml. of this suspension. When drawn up into the vertical electrophoresis cell[4], such a suspension provides about 20 red cells per field. It is important to avoid working with much more concentrated suspensions, which provide an example of a phenomenon in which the mobility is partly determined, not by single cells, but by groups. To 10 ml. of this suspension is added 0·1–0·3 ml. of the dialysed oleic acid sol, and the mobility of the cells is determined before and after the addition. Larger quantities of sol are lytic. Alternatively the red cells can be suspended in serum or in serum to which the oleic acid sol has been added.

(1) The mobility of washed human red cells in Dole buffer at *p*H 8·6 is remarkably constant at 1·3 μ/sec/V/cm. The addition of 0·15 ml. of oleic acid sol to 10 ml. of a suspension of washed human red cells reduces the mobility to 0·98 μ/sec/V/cm, or by 25 per cent.

(2) If the red cells are suspended in serum which has been dialysed against Dole buffer at *p*H 8·6, the mobility is a little less than 1·3 μ/sec/V/cm, because the viscosity of serum is slightly greater than that of the buffer. Addition of 0·1 ml. of oleic acid sol to 10 ml. of a red cell suspension in serum reduces the mobility by 8 per cent and the addition of 0·2 ml. of the sol reduces the mobility by 15 per cent, that is, the effect of the oleic acid in reducing the mobility is less than when the cells are suspended in buffer alone.

(*B*) *Method.* One part of M/25 oleic acid in ethanol is added to 2 parts of Dole buffer at *p*H 8·6 and then dialysed against a large volume of the buffer for 18 h or more. Because of a volume change, the final concentration of oleic acid is M/100. Serum is dialysed against Dole buffer at the same *p*H for the same time.

Equal parts of the dialysed serum and the dialysed oleic acid are mixed and allowed to stand for 2 h at 22° C. The electrophoretic pattern of this mixture is obtained with the Antweiler micro-Tiselius apparatus at 18° C, and is compared with the pattern obtained with dialysed serum plus one volume of the buffer. A typical result is shown in Table 1, which shows the quantities of the different serum fractions as judged by the areas under their spikes.

The two important points are that the presence of the oleic acid produces an apparent reduction in the quantity of β-globulin, and a corresponding increase in the quantity of albumin plus α_1-globulin. This suggests that the addition of the oleic acid sol confers the mobility of the

Table 1

	$A + \alpha_1$	α_2	β	γ	Total
Serum	126	14	20	17	177
Oleic acid M/100 plus serum	138	11	10	20	179
Differences	12	−3	−10	3	2

albumin plus α_1-globulin fraction on some of the β-globulin fraction. Sometimes a small new spike appears in advance of the albumin spike, as if there were a new fraction with a mobility greater than that of albumin. By using a larger quantity of oleic acid (M/70), the effect on the β-globulin spike is still greater, so that virtually no β-globulin can be demonstrated by this method.

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Density and Structure of Alpha-Keratin

A MODEL of the two-phase structure of fibrous α-keratin has been evolved, starting with the α–β fold of Astbury *et al.*[1] and thence via the concept of α-helices to the coiled-coil models of Pauling and Corey[2] and of Crick[3], and at present to the detailed schemes of packing recently proposed by Fraser, MacRae and Rogers[4] and by Johnson and Sikorski[5]. The successive stages of this development have resulted primarily from attempts to give a more accurate explanation of the observed X-ray diffraction patterns. In addition, however, each advance has been judged by its success in explaining the 'high' densities found for keratin, and all have been found wanting because they led to lower crystalline densities than the values actually measured (definitive values given by Fraser and MacRae[6] range from 1·283 g/c.c. for cow horn to 1·335 g/c.c. for echidna quill tip, at 25° C). This reasoning appears to rest on a false analogy with polymers, involving the assumption that the density of the crystalline phase is necessarily greater than that of the non-crystalline phase. Because this assumption is still influencing current thought on keratin ultrastructure it seems worth while to point out three deficiencies in the polymer analogy.

(1) For many polymers, the density in the crystalline phase does indeed exceed that in the amorphous phase (whether liquid or glassy). These are largely non-polar materials where the intermolecular forces are relatively small and the increase in order associated with crystallization is synonymous with denser packing of the molecules. However, when the intermolecular forces are stronger, as in polar materials and, particularly, in hydrogen-bonded systems, the disordered structure may be more dense than the crystalline state. An outstanding example of this is shown by the 10 per cent increase in density when ice melts. Of more immediate relevance is the work of Bradbury[7], who has recently measured density increases of the order of 1 per cent associated with the helix→random coil transition in synthetic polypeptides in solution.

(2) The polymer situation relates to a material composed essentially of a single molecular species. There is now ample evidence that keratin contains at least two distinct molecular fractions with differing amino-acid compositions, as shown by the separation into keratoses or other high- and low-sulphur fractions[8,9]. Although the direct relation of these components to the crystalline and non-crystalline phases in keratin is still controversial, it is reasonable to presume that the two phases will also differ in composition and therefore in density. Johnson and Sikorski[5] have recently noted that α-keratose contains "a greater proportion of the bulky amino-acid residues" and suggest that this argues against the general belief that the α- and γ-fractions are derived from crystal and from matrix respectively, because of the "greater regularity and better packing" in the crystal. This is an important observation; but the corollary is fallacious because "better packing" is not in this case necessarily identifiable with more dense packing.

(3) The high cystine content of keratin is probably derived more from the matrix than from the crystalline microfibrils and the presence of the additional disulphide cross-links implies a considerable relative densification of this phase.

To relate these points to a specific polymer, consider a strip of unvulcanized natural rubber having a density of 0·91 g/c.c. in the amorphous, unstrained state. If this strip is stretched to 700 per cent extension at 0° C, its density increases[10] by approximately 3 per cent. The crystalline density, using Bunn's unit cell[11], is 1·00 g/c.c. so that about 30 per cent by volume of the rubber is crystalline.

If the matrix alone were now to be cross-linked, its density could be increased to a value equal to or even greater than that of the crystallites. In the simplest case where the cross-links are direct —C–C— bridges a volume contraction of 124 Å³ per cross-link has been found[12] which is somewhat larger than the average volume per amino-acid residue in keratin. As there are about nine residues per cystine cross-link in the whole wool fibre, density increases in the matrix of at least 10 per cent are thus conceivable on the basis of cross-linking alone.

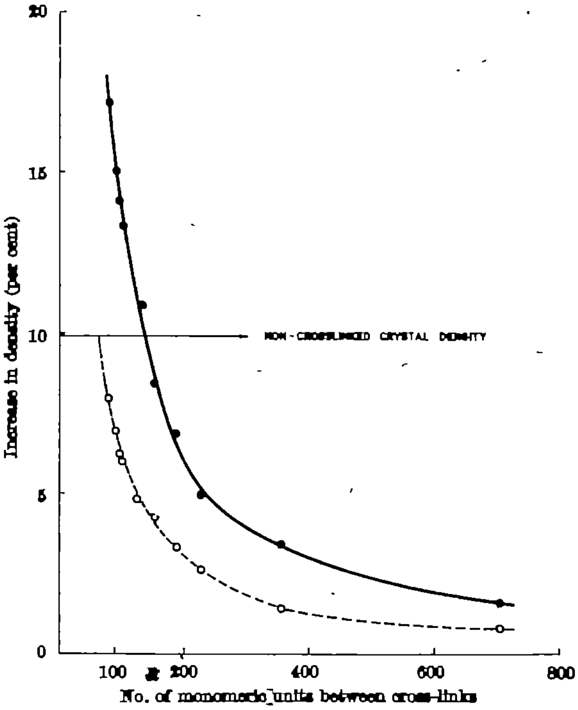

Fig. 1

An even more striking increase in density is observed when the rubber is cross-linked by heating it with sulphur. Fig. 1 shows the variation in density with degree of cross-linking, using the data of Martin and Mandelkern[13]; the degree of cross-linking is represented by n, the number of monomeric units between cross-links, this being estimated by reference to the work of Moore, Mullins and Swift[14]. It can be seen how the density increases rapidly as n decreases towards the levels appropriate to keratin. In particular, the crystalline density is reached at a value of approximately 140 monomers between cross links. As this comparison may be exaggerated by the high density of the sulphur atom, the dotted curve has been drawn in to show the corresponding increase in steric density, that is, the fractional volume occupied by the atoms assuming normal van der Waals radii and covalent bond-lengths. Even on this basis, the crystal density is reached at the comparatively low level of one cross-link per 66 monomers. It should be observed that in this system there is extensive intra-molecular combination of sulphur with the main hydrocarbon chains quite apart from the intermolecular cross-linking[14]. Thus this cross-linked polymer can be denser than its crystalline phase on the grounds of both of the foregoing points (2) and (3).

The foregoing discussion emphasizes the possibility that in keratin the matrix may be denser than the crystallites. It may be noted that Haly and Swanepoel[15] found it necessary to postulate that the matrix in dry wool is the denser component in order to account for the form of their observations of birefringence at different water contents.

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BIOCHEMISTRY

Preparation of [^{131}I]-3-Monoiodotyrosine and [^{131}I]-3,5-Diiodotyrosine of Very High Specific Activity

IN the course of work on the extraction of iodinated tyrosines from human plasma and their subsequent estimation, it was necessary to determine the losses which might occur during the series of manipulations. For this purpose we decided to add quantities of [^{131}I]-3-monoiodotyrosine ([^{131}I]-MIT) and [^{131}I]-3,5-diiodotyrosine ([^{131}I]-DIT) small enough to be below the lower limits of estimation by our method but having a sufficiently high [^{131}I]-activity to be counted easily in a well-type scintillation counter.

The highest published specific activities of these compounds we have found in the literature were those prepared by Lemmon, Tarpey and Scott[1] using the method of Harington[2] and Harington and Pitt-Rivers[3] adapted to micro-scale preparations. The specific activities reported are 498 μc./μmole for [^{131}I]-MIT, and 996 μc./μmole for [^{131}I]-DIT. In these preparations the specific activity was of necessity reduced by the addition of carrier iodine-127. The chloramine *T* radioiodination procedure used by Hunter and Greenwood[4] for human growth hormone was therefore adapted to prepare the [^{131}I]-iodotyrosines so far as possible 'carrier free'.

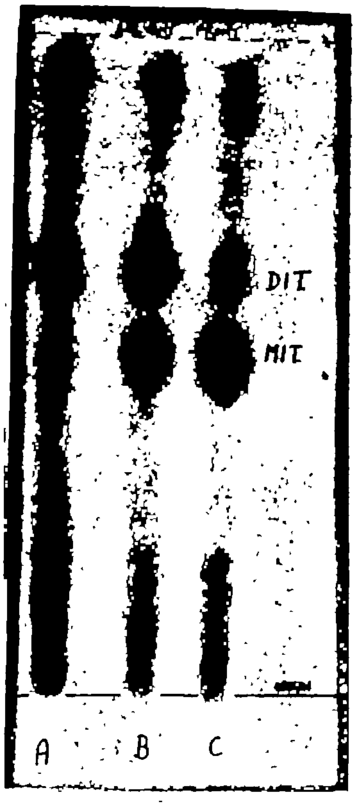

Fig. 1. Autoradiogram of chromatograms of preparations of [^{131}I]-MIT and [^{131}I]-DIT on Whatman No. 1 paper; ascending chromatography in *n*-butanol/2 N acetic acid (1 : 1; equilibrated at room temperature). Run 16 h

All solutions were prepared with de-ionized water, and all the glassware used was, after cleaning, finally rinsed with de-ionized water. However, minute traces of iodine (even a small fraction of a μg), which might be difficult to exclude from glassware and solvents, can multiply many times the actual amount of iodine contained in the 'carrier free' [^{131}I]-iodine solution used in any single preparation. A typical preparation of [^{131}I]-MIT proceeded as follows:

[^{131}I]-iodide in dilute (N/50) sodium hydroxide (2 mc.; 10–20 μl.; carrier and thiosulphate free from Radiochemical Centre, Amersham, Code No. IBS. 3) was added to a solution of L-tyrosine (California Biochem., Res., Corp., *A* grade; 1 μg in 25 μl. borate buffer *p*H 8). A freshly prepared solution of chloramine *T* (B.D.H.; 50 μg in 25 μl. of borate buffer *p*H 8) was then added, the tube stoppered and gently swirled. After 1–2 min, a solution of

Table 1. SUMMARY OF IODINATIONS ILLUSTRATED IN FIG. 1

	L-Tyrosine (μg)	'Carrier free' ^{131}I (mc.)	Per cent total ^{131}I activity appearing on chromatography as	Maximum theoretical yields (μg)	Minimal specific activities mc./μg	c./μmole
A	0·05	2·33	MIT trace DIT − 31	MIT——— DIT − 0·1198	MIT——— DIT − 6·06	MIT——— DIT − 2·62
B	0·10	1·67	MIT − 31 DIT − 38	MIT − 0·1061 DIT − 0·0897	MIT − 4·97 DIT − 7·07	MIT − 1·53 DIT − 3·07
C	1·0	1·75	MIT − 62 DIT − 11	MIT − 1·558 DIT − 0·1948	MIT − 0·696 DIT − 1·012	MIT − 0·214 DIT − 0·438

Aliquots of iodine-131 taken for iodinations were measured with an ionization chamber (type 1383 *A*, General Radiological Ltd., London, with amplifier d.c. type 1388 *B*, Avo, Ltd., London), in which there had been made a previous determination of the total activity of the sample of iodine-131; the absolute value of this sample was given by the Radiochemical Centre, Amersham. An error of about 10 per cent in the measurements was indicated by the difference between the total activity and the sum of the activities of the aliquots.

freshly prepared sodium metabisulphite (B.D.H.; 1·2 mg/ml. in borate buffer pH 8; 100 μl.) was added. When required for ease of subsequent manipulation, the volume of the mixture after reaction was increased by the addition of 0·1 N ammonia in 10 per cent isopropanol.

An aliquot of the reaction mixture was subjected to ascending chromatography in n-butanol/2 N acetic acid (1 : 1), and the dried paper strip was scanned in an argon flow chromatogram counter (Baird and Tatlock Co., Ltd.) to give the percentage of the total activity incorporated into [^{131}I]-MIT. Assuming that all the L-tyrosine had been iodinated, a minimum specific activity could then be calculated. This way of calculation obviates the necessity of assuming a definite abundance of iodine-131 isotopes in the 'carrier free' iodide used in the preparation. These estimates are conservative, and the actual specific activity may be higher.

Fig. 1 gives the autoradiogram of three of our preparations, and Table 1 the relevant data.

For subsequent tracer work the spots were cut out, eluted with 0·1 N ammonia in 10 per cent isopropanol, and stored in a deep freeze. The identity of the material was checked by re-chromatography and autoradiography, running markers alongside the eluted material, which were revealed by spraying with ninhydrin. Chromatograms and autoradiograms of the eluted material mixed with various markers were also used for the identification of the spots.

Work is in progress on the stability under various conditions of these very highly active preparations, and also on the extension of the method to the radioiodination of various thyronines.

These results are part of an investigation on the occurrence of iodotyrosines in human plasma; we thank the Medical Research Council for a grant in support of this work.

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Effect of Acetaldehyde on the Resorcinol Test for Fructose

The Seliwanoff colour reaction of ketohexoses with resorcinol and hydrochloric acid is the basis of several quantitative procedures for the determination of fructose in biological materials in which the absorbance is measured at 470 mμ[1], 480 mμ[2], 490 mμ[3], 510 mμ[4], 520 mμ[5], and 540 mμ[6]. Roe reported that the addition of ethanol[5,7] or acetic acid[5] to the reaction mixture has the effect of increasing the absorbance of the coloured product at 520 mμ. The cause of the effect produced by these solvents is obscure. Roe suggested that the dehydrating conditions of the reaction were improved by introducing ethanol[7] and that the hydrolytic properties of the reagent were improved by the addition of acetic acid[5]. While some workers have noted that the quality of the solvents affected the absorbance at 480 mμ[2,8] and the absorption spectrum[8], the fact that impurities in these solvents may be largely responsible in some cases for the effect attributed to the solvents themselves seems to have passed unnoticed. In this communication we wish to direct attention to the effect produced by acetaldehyde, an impurity in commercial acetic acid[10] and ethanol[11], on the colour formation in the resorcinol test.

The resorcinol test which was developed by one of us[12] to determine 3,6-anhydrogalactose in marine algal polysaccharides was modified to examine the effect of adding acetic acid, ethanol and acetaldehyde on the visible absorption spectrum of the coloured product. The reagent was prepared by adding 100 ml. of 12 M hydrochloric acid to 10 ml. of $1{\cdot}2 \times 10^{-2}$ M resorcinol in aqueous solution. The reagent (10 ml.) was put into a test-tube containing 1 ml. of $1{\cdot}8 \times 10^{-4}$ M fructose in aqueous solution and 1 ml. of the additive being tested. The tube was covered with a glass marble and the reaction mixture was heated for 10 min at 80° C.

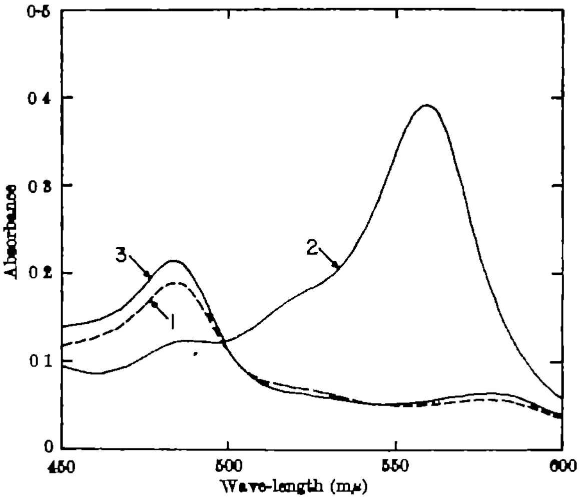

Fig. 1. Absorption spectra of coloured products formed in resorcinol test using as additives (see text for details of procedure): 1, water; 2, commercial acetic acid; 3, purified acetic acid. Spectra determined with a Beckman *DK-2* spectrophotometer using a 10-mm light path

Fig. 1 shows absorption spectra obtained using, as additives, water (curve 1), commercial acetic acid (curve 2), and the same commercial acetic acid after purification by fractional distillation (curve 3). Thus purification of the acetic acid resulted in shifting the wave-length of maximum absorption (λ_{max}) from 555 mμ to 480 mμ and decreasing the absorbance at λ_{max} from 0·4 to 0·2. The similarity between the absorption spectra obtained using water (curve 1) and purified acetic acid (curve 3) should be noted. Spectra similar to those described for acetic acid were obtained with absolute ethanol. From these results we conclude that purified acetic acid and ethanol have a negligible effect on the Seliwanoff reaction carried out according to our procedure.

A consideration of known impurities present in commercial acetic acid and ethanol led us to study the effect of acetaldehyde on the colour reaction. When purified acetic acid (curve 3) containing 0·06 μmole of acetaldehyde per ml. was used, the absorption spectrum was identical to that obtained with commercial acetic acid (curve 2). It was shown that the shift in λ_{max} is independent of the presence of organic solvents in the reaction mixture since it was also obtained when 1 ml. of an aqueous solution containing 0·06 μmole of acetaldehyde was used in the foregoing procedure. Further work was carried out with 1,1-diethoxyethane rather than acetaldehyde since the former compound is less volatile and more stable at room temperature, and is readily hydrolysed to acetaldehyde with a mineral acid catalyst. Its effect on the colour reaction was indistinguishable from that of acetaldehyde. The absorbance at 555 mμ was dependent on the concentration of 1,1-diethoxyethane in the reaction mixture, maximum absorbance being obtained when equimolar amounts of 1,1-diethoxyethane and fructose were present. Beer's law was obeyed unless the molar ratio of 1,1-diethoxyethane to fructose was less than one.

The complete details on factors affecting the production of colour in the resorcinol test for the determination of fructose and 3,6-anhydrogalactose will be presented in forthcoming publications. In view of our results, and since a large number of colour reactions used for detecting and determining carbohydrates is based on the condensation of phenols with products formed from sugars by the action of acids[13], it may be expected that the absorption spectra of other colour reaction products would be affected by aldehyde impurities in organic solvent.

The absorbance at 555 $m\mu$ in the resorcinol test for fructose is not only affected by acetaldehyde but also by other *n*-aliphatic aldehydes. This is the basis for a sensitive procedure for determining *n*-aliphatic aldehydes which will be published shortly.

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Lipase and Glycerokinase Activities in the Adipose Tissue of Obese–Hyperglycæmic Mice

IT has previously been reported that free fatty acid mobilization from the adipose tissue of hereditary obese–hyperglycæmic mice after incubation with epinephrine or fat-mobilizing substance[1] or prolonged fast is far smaller than is seen with tissue from non-obese litter-mates[2,3]. It has also been shown that lipogenesis from acetate in adipose tissue from this animal proceeds at 10–15 times the normal rate[4]. The diminished free fatty release and the increased lipogenesis from acetate occurring in spite of a decrease in glucose uptake[5] and utilization[2,3] suggested to us that this syndrome might entail two abnormalities, possibly interrelated: an impaired lipolytic activity directly related to the epinephrine-sensitive lipolytic system in adipose tissue described by Rizack[6]; and an ability of this tissue to utilize 'non-active' glycerol in the esterification process possibly through a specific 'glycerokinase'.

Accordingly, lipase activity was determined in the epididymal fat from eight obese–hyperglycæmic mice and nine non-obese litter-mates obtained from the Jackson Memorial Laboratory in Bar Harbor, Maine. The animals were approximately four months old and fed Ralston Purina rat chow *ad libitum* until the time of death. Rizack's method for the estimation of lipase activity[6] was used with the exception that the total tissue homogenate (after removal of the fat cake) was used for the determination of enzyme activity. In the second series of experiments, six obese–hyperglycæmic mice and five non-obese litter-mates (all male) about 4 months old were used. Epididymal fat pads were incubated in Krebs-bicarbonate buffer. Glycerol-1,3-^{14}C was obtained from New England Nuclear Corporation and was added to the medium at a level of 2 μc. per flask (containing 3 ml. medium). Unlabelled glycerol was added to bring the total concentration of glycerol to 3 mM/l. In order to provide optimal conditions for tissue fatty acid synthesis, 5 mM glucose and 30 mM acetate were also added to the medium. Acetate was added as sodium salt in substitution for sodium chloride[4]. Incubation of the adipose tissue was carried out as described by Winegrad and Renold[7]. Tissue glyceride-glycerol was isolated according to Cahill *et al.*[8], oxidized with periodic acid, buffered and dimedone reagent was added to precipitate the formaldehyde derived from the α-carbons of glycerol as formaldimedone. The formaldimedone was washed, purified by recrystallization from acetone, washed twice, plancheted, counted and calculated[9].

Activity of the lipolytic enzyme system which appears to be responsible for the release of free fatty acids is significantly decreased in the adipose tissue from obese–hyperglycæmic mice (10·50 ± 0·92 μmoles free fatty acids/mg tissue nitrogen/1 h incubation) as compared with that of non-obese litter-mates (14·29 ± 1·69, $P < 0·02$).

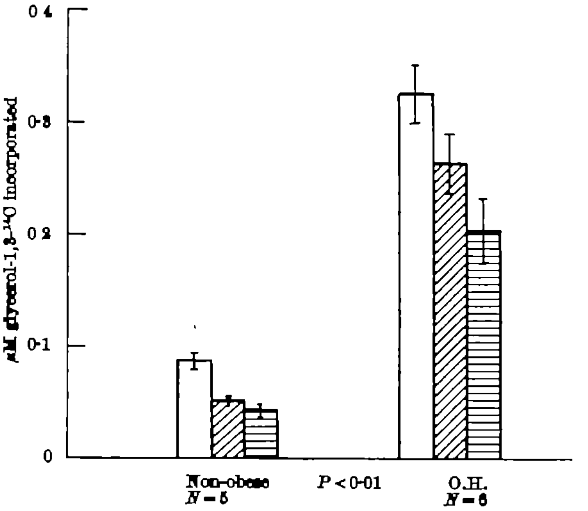

Fig. 1. Incorporation of glycerol-1,3-^{14}C into adipose tissue glyceride-glycerol of obese–hyperglycæmic mice (O.H.) and non-obese litter-mates. Results are expressed in micromoles labelled glycerol incorporated per mg tissue nitrogen per 3 h of incubation, mean ± *S.E.* It is readily seen that incorporation by adipose tissue from obese mice with this syndrome proceeds at 4–5 times the rate observed in tissue from non-obese litter-mates. White, control; oblique hatching, insulin 0·1 U/ml.; horizontal hatching, epinephrine 10^{-4} M

Incorporation of glycerol-1,3-^{14}C into glyceride-glycerol in adipose tissue of obese–hyperglycæmic and non-obese mice is shown in Fig. 1. When no hormone was added to the incubation medium, the incorporation of labelled glycerol into glyceride-glycerol by the adipose tissue from the obese–hyperglycæmic mice was three and a half times greater than that by the adipose tissue from the non-obese mice (0·33 ± 0·026 μmole/mg tissue nitrogen/3 h incubation in the obese–hyperglycæmic mice, 0·089 ± 0·007 in the non-obese mice). On addition of insulin or epinephrine to the incubation medium, the ratio of the rate of incorporation of glycerol-1,3-^{14}C in the adipose tissue from the obese–hyperglycæmic mice to that in tissue from the non-obese mice rose to 5 (0·27 ± 0·026 μmole for tissue from obese mice, 0·05 ± 0·004 μmole for tissue from non-obese mice in the presence of insulin; 0·20 ± 0·029 and 0·045 ± 0·003 μmole for adipose tissue from the obese–hyperglycæmic and non-obese mice, respectively, in the presence of epinephrine).

These results strongly suggest the existence of a glycerokinase in the adipose tissue of mice with the hereditary obese–hyperglycæmic syndrome, even though it is recognized that part of the apparent increase in labelled glycerol incorporated could be due to decreased dilution with the lesser amount of glycerol synthesized from glucose in this syndrome. Experiments now in progress should make it possible to rule out definitely this complication.

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Proton Magnetic Resonance Investigation of Enzyme–Coenzyme Complexes

THE unique potentialities of high resolution nuclear magnetic resonance as a method for obtaining detailed information on molecular structure and molecular interactions in solution are well recognized[1,2]. However, the applicability of the method to the examination of interactions in biological systems has been hampered by its low sensitivity, requiring the use of comparatively concentrated solutions (~ 0·05 M or higher).

We have now applied a technique which has allowed us to perform a series of crude but informative experiments on the binding of diphosphopyridine nucleotide (DPN) to the enzyme yeast alcohol dehydrogenase (ADH) in stoichiometric proportions. Our procedure has been to couple the output of a Varian model 4300*B* high-resolution spectrometer operating at 60 Mc/s to a 400 channel digital average response computer, the Mnemotron model 400 computer of average transients. The sweep of the computer was triggered through a Tektronix model 535 *A* oscilloscope from a marker placed in the sample tube. By an appropriate choice of delay times, calculated from the known chemical shifts of the marker and the DPN peaks, it was synchronized with the desired linear portion of the field sweep, with excellent reproducibility on successive sweeps. The sweep rates found adequate in the present work were of the order of 2·5 mG/sec and the total field change in the course of a single computer sweep was approximately 25–30 mG, corresponding to a spectral region of 100–120 c/s. At this rate the spectrum was converted into digital form using 4 channels per c/s. Since the DPN peaks have a width at half-height of the order of 3 c/s, the resulting resolution was as good as that obtained on a conventional spectrum at higher DPN concentrations. Approximately 500 sweeps were required to obtain a well-defined spectrum of a dilute solution of DPN.

Figs. 1 *a* and *b* show the nuclear magnetic spectrum of 0·1 M DPN in deuterium oxide, *p*H 7·2, and the average response nuclear magnetic resonance spectrum of 0·002 M DPN in deuterium oxide, *p*H 7·2 (on an expanded scale). The assignment of peaks in the aromatic region follows from the spin–spin coupling pattern and is consistent with that given for other pyridine and purine derivatives[3-5]. A comparison of line widths to the coupling constants on the two spectra indicates that errors which could be expected to arise from sweep broadening and random drifts between successive sweeps are of the order of 10–20 per cent of the line widths.

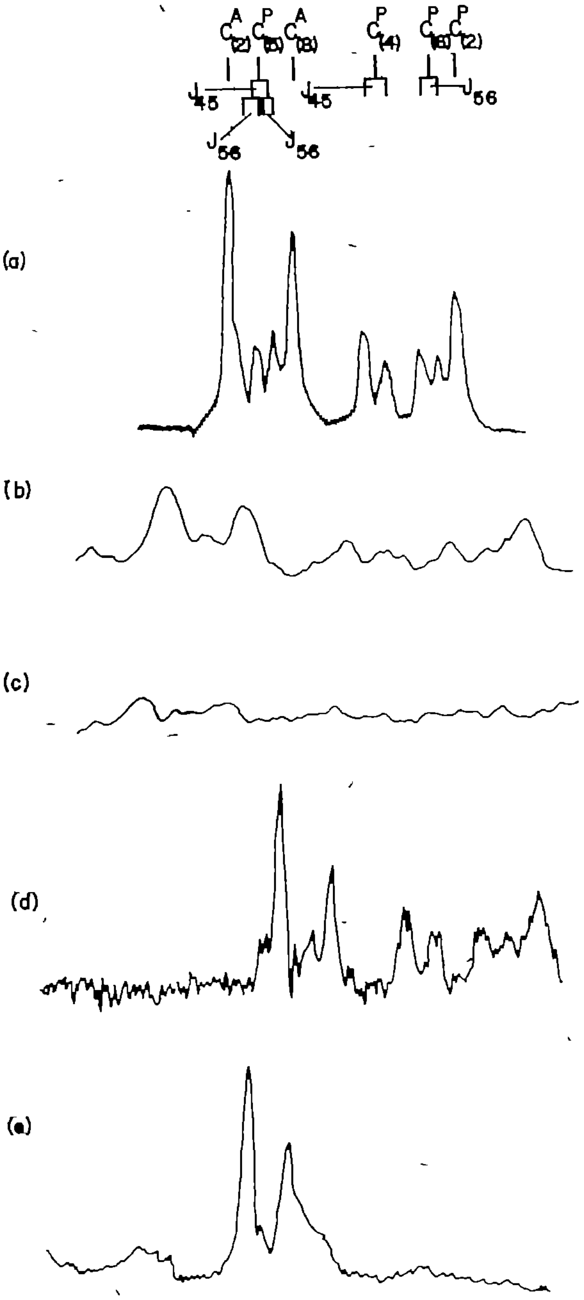

Fig. 1

The spectrum of 0·002 M β-DPN in the presence of 0·0005 M ADH, *p*H 7·2 (calculated on the basis of a molecular weight of 148,000 and a stoichiometric ratio for binding of 4 DPN molecules/molecule of ADH[6]) is shown in Fig. 1 *c*. The two adenine peaks can still be reproducibly detected, while the pyridine region of the spectrum gives random patterns on repeated scans. This would not have been expected in the case of a simple non-specific broadening, since the ratio of intensities of the C_2H of pyridine to the C_2H of adenine should have remained approximately 1 : 1 as it is in the case of the controls[1,2]. The same observation can be made more clearly on a solution containing a slight excess of DPN, as is shown in Figs. 1 *d* and *e*, which represent spectra of a control solution of 0·005 M β-DPN and the spectrum of 0·005 M β-DPN in the presence of 0·0005 M ADH. Whether this disappearance of the pyridine portion of the nuclear magnetic resonance spectrum represents a shift, selective broadening, or a combination of both, cannot yet be settled with certainty.

A scan of other regions of the average response nuclear magnetic resonance spectrum has thus far failed to reveal either these peaks, or the characteristic pyridine peaks of DPNH[7,8]. We therefore tentatively favour selective broadening (with or without an accompanying shift) as the more likely explanation. The possibility of the broadening being due to a difference in pH between the control and the enzyme-containing samples was carefully eliminated, since a pH dependent preferential broadening of the pyridine peaks has been observed above pH 8·5 and attributed to an exchange between the uncharged and the cationic form of the ring[9]. The possibility of a reduction of DPN to DPNH cannot be entirely excluded, but is made unlikely both by the absence of a substrate and by the failure to find the $H_{(2)}$ and the $H_{(4)}$ peaks of the dihydropyridine ring.

The fact that the spectral changes in the pyridine region are observable even when DPN is present in excess (10 molecules of DPN/molecule of ADH) suggests either binding of additional molecules through the pyridine ring or an exchange between the free and the bound species. Data available thus far do not allow a distinction between the two possibilities.

A precise interpretation of the present findings in terms of molecular events must therefore be deferred until the completion of a more detailed study at present in progress. However, regardless of the ultimate specific explanation, the observations made so far provide direct physical evidence for the involvement of the pyridine ring in the interaction between DPN and the enzyme, and provide a basis for defining the role of individual portions of the DPN molecule in this interaction. They also unequivocally demonstrate the feasibility of extending the application of nuclear resonance to the study of specific molecular interactions at biochemically meaningful concentrations.

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Adenosine Triphosphatase Activity of Glycerinated Muscle Fibres in a Mechanical Field

MUSCLE is a 'mechano-chemical' system which can convert chemical energy into mechanical energy. The glycerinated muscle fibre is a typical example of the model system where the mechanical work (contraction) is closely coupled with the chemical reaction (dephosphorylation of adenosine triphosphate (ATP)). In such a mechano-chemical system, it is expected that the chemical reaction is also closely coupled with the mechanical work which is applied to the system.

Using the glycerinated muscle fibre, such a mechano-chemical coupling is studied, and it is observed that the ATP-ase activity of the muscle fibre is markedly accelerated under mechanical field.

Glycerinated psoas muscle fibres of rabbits were prepared by the method of Szent-Gyorgyi[1]. Seven fibre bundles (each 0·05–0·1 mm in diameter, 30 mm in length) are mounted between two levers which can stretch a fibre with various frequency and various amplitude. After washing the fibre with a medium (0·15 M potassium chloride and 0·002 M magnesium chloride buffered at pH 7·0), the reaction is started by immersing the fibre into 10 ml. of the same medium containing 2 mM ATP. The solution is continuously stirred and the temperature is kept constant by thermo-electronic elements[2] (Komatsu Electronics Inc., Tokyo). An aliquot of 0·5 ml. of the medium is taken out at the proper intervals and an inorganic phosphate liberated is assayed by the Hexanol–Celite column method[3].

When the lever is fixed, a steady rate of dephosphorylation is observed after an initial burst of dephosphorylation[4]. If the fibre is repeatedly stretched by the lever (frequency: 1·5 c/s, amplitude: 2 mm), the rate is immediately increased as shown in Fig. 1. This acceleration disappears when the movement of the lever is stopped, and the dephosphorylation rate returns to that of the original state.

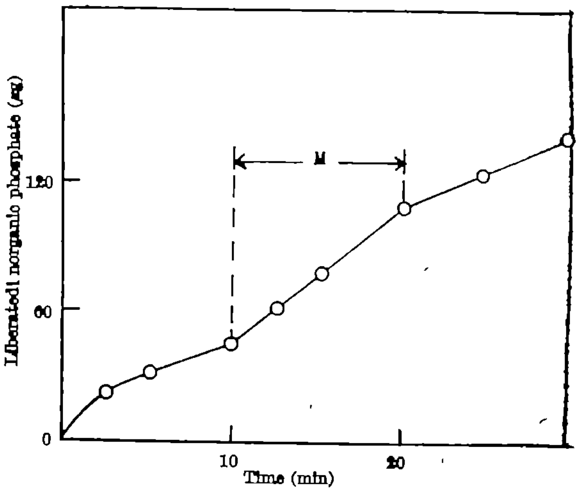

Fig. 1. Effect of repeated stretching on the dephosphorylation of ATP. Glycerinated muscle fibres (seven bundles, each 0·05–0·1 mm diam., 30 mm long) were immersed in 0·15 M potassium chloride, 0·002 M magnesium chloride, pH 7·0, and 0·002 M ATP. Temp., 20° C. *M* indicates repeated stretching (frequency, 1·5 c/s; amplitude, 2 mm)

The mechanical acceleration of the ATPase is also observed in muscle fibres under sonic vibration field just as observed in *F*-actin solution by Asakura[5]. When myofibrils prepared from the glycerinated muscle fibre (or rabbit muscle fibre) were put in the sonic field of a magneto-strictive sonic oscillator (frequency: 10 kc/s; input power of the final amplifier of the generator is about 15 W), the ATPase activity is accelerated (Fig. 2).

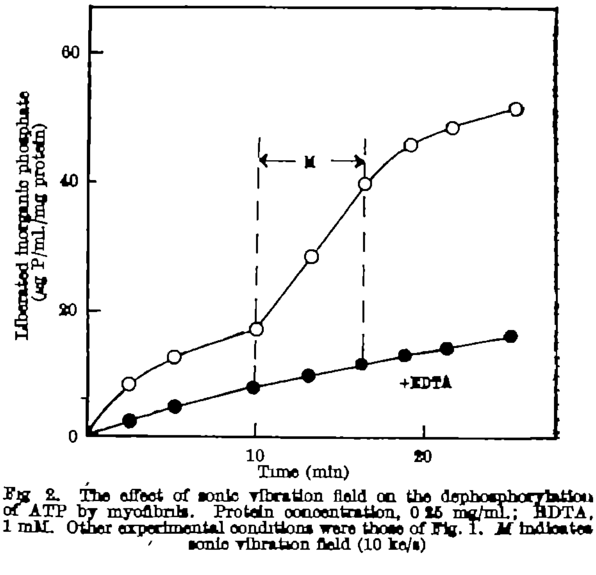

Fig. 2. The effect of sonic vibration field on the dephosphorylation of ATP by myofibrils. Protein concentration, 0·25 mg/ml.; EDTA, 1 mM. Other experimental conditions were those of Fig. 1. *M* indicates sonic vibration field (10 kc/s)

In the presence of EDTA (muscle-relaxing reagent), the ATPase activity of the myofibrils is suppressed and the mechanical acceleration disappears. The accelerated ATPase activities in Figs. 1 and 2 are nearly equal to the ATPase activity at the initial contraction process (initial burst). Therefore, it is probable that the interaction between myosin and actin filaments at the contracted steady state is enhanced by the mechanical work and that the ATPase activity is accelerated to the level at the initial contraction process.

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PHYSIOLOGY

Measurements of Tubular Fluid pH *in vivo* in Rats

IN recently reported micropuncture experiments on the mechanism of urine acidification in the rat, *p*H of tubular fluid was measured after the fluid had been withdrawn from the nephron[1–4]. A theoretical objection to this method is that carbonic acid may not be in equilibrium with carbon dioxide in the tubular lumen[5,6]. Withdrawal of the fluid would allow the reaction $CO_2+H_2O \rightleftharpoons H_2CO_3$ to reach equilibrium quickly with the result that both the concentration of carbonic acid and the *p*H would change. That this may be the case is suggested by recent estimates of tubular fluid *p*H *in vivo* by Rector, using a series of indicator dyes[7]. His results suggest a much greater degree of acidification in the proximal tubule than previously thought from *in vitro* measurements.

In the experiments reported here, a new method devised in this laboratory was used to obtain direct measurements of tubular fluid *p*H in the lumen without disturbing the physiological state of the nephron. Two micropipettes were inserted into the same tubular lumen along its longitudinal axis. One of the micropipettes contained a quinhydrone electrode and the other a silver–silver chloride reference electrode. Liquid junction was made via Ringer's solution. Tubular fluid was withdrawn under anaerobic conditions into the micropipette containing the quinhydrone electrode and *p*H measured as soon as electrical contact with the electrode was made. Then, this pipette was withdrawn from the lumen, the tip sealed with potassium chloride-agar, and *p*H determined *in vitro* at 37° C with a calomel reference electrode. The results are shown in Table 1.

Table 1. COMPARISON OF TUBULAR FLUID *p*H *in vivo* AND *in vitro* DURING 0·5 M SODIUM SULPHATE INFUSION

Exp. No.	*In vivo p*H	*In vitro p*H	Blood *p*H	Bladder urine *p*H
		Proximal tubule		
1	5·40	7·03	—	—
2	5·04	7·02	7·45	5·35
	5·96	6·95	7·45	5·35
3	5·08	6·84	7·44	4·87
	5·45	7·02	7·44	4·87
	4·65	7·00	7·44	4·87
4	4·73	7·31	7·53	—
5	5·95	6·94	7·47	4·75
	4·60	7·03	7·47	4·75
6*	5·81	6·60	7·40	5·37
	5·75	6·68	7·40	5·37
7*	5·98	6·08	7·57	4·83
	6·00	7·00	7·57	4·83
	5·96	7·08	7·57	4·83
		Distal tubule		
2	5·60	5·49	7·45	5·35
4	4·96	5·62	7·53	—
6*	5·08	5·25	7·40	5·37
	5·25	6·06	7·40	5·37

* In these experiments, carbonic anhydrase was administered intravenously; 6·4 mg was given as prime and 0·13 mg/min in the sodium sulphate infusion. Assay of bladder urine by the method of Philpot and Philpot (ref. 8) showed the enzyme to be present.

These results confirm the findings of Rector that tubular fluid *p*H *in vivo* is much more acid than plasma and considerably more acid than *in vitro*. The fact that *p*H rose after the fluid had been withdrawn from the nephron is most readily explained by a fall in the concentration of carbonic acid. This in turn is compatible with the view that carbonic acid is in a state of disequilibrium in the tubular lumen.

Two hypotheses, diametrically opposed to one another, to account for acidification of the urine are at present being held[5,6]. One proposes that bicarbonate ions are removed from the tubular fluid by a specific transport process, and the other that hydrogen ions are secreted by the epithelial cells. Either of these two mechanisms is compatible with the low *in vivo p*H values found in the experiments reported here. However, the fact that *p*H rose after the fluid had been withdrawn from the nephron favours the hydrogen ion secretion theory. If bicarbonate removal were the primary event, the reactions taking place in the tubular lumen would be:

$$(1)\ H_2CO_3 \rightarrow H^+ + HCO_3^-$$

$$(2)\ CO_2 + H_2O \rightarrow H_2CO_3$$

The concentration of carbonic acid might be expected to rise, therefore, after the fluid had been withdrawn from the nephron if the hydration of carbon dioxide (equation 2) had not reached equilibrium *in vivo*. The *p*H measured *in vitro* would be more acid in these circumstances than *in vivo*. In contrast, if hydrogen ion secretion is the basic mechanism, reactions 1 and 2 would be proceeding toward the left in the tubular lumen as hydrogen ions combined with bicarbonate to form carbonic acid. Removal of the fluid from the nephron would allow the dehydration of carbonic acid to continue to equilibrium with the result that carbonic acid concentration would fall and *in vitro p*H would be higher than *in vivo*.

It is of interest that carbonic anhydrase, in amounts large enough to cause its excretion in the urine, was unable to accelerate the dehydration of carbonic acid sufficiently for equilibrium to be reached *in vivo*. Larger amounts might have been more effective, but it is also conceivable that factors other than presence or absence of this enzyme, such as the rate of hydrogen ion secretion, the geometrical relationship between luminal surface area and fluid volume, and the rate of flow along the nephron preclude a state of equilibrium for carbonic acid.

The concentration of carbonic acid in the tubular lumen can be approximated from the equation: $pH = 3{\cdot}2 + \log HCO_3^-/H_2CO_3$. At a *p*H of 5·5 and a bicarbonate concentration of 25 mm/l. carbonic acid would be 0·1 mm/l. or almost 100 times that of plasma. At the same *p*H but lower bicarbonate concentrations, carbonic acid concentration would be proportionally smaller. This could account for the fact that *in vivo* and *in vitro p*H were much closer to one another in the case of distal tubular samples than with proximal samples. If most bicarbonate is reabsorbed in the proximal tubule and little reaches the distal tubule, relatively small amounts of carbonic acid would be generated distally and the dehydration reaction could more easily approach equilibrium *in vivo*.

In previous experiments reported from this laboratory[4], a correlation was made between proximal tubular fluid *p*H measured *in vitro* and trans-tubular electrical potentials, using the simplified Nernst equation: $Em = 61{\cdot}5 \log C_1/C_2$. The results suggested that hydrogen ions are distributed passively between proximal fluid and plasma in response to the electrical potential. On the basis of the present findings, however, it seems certain that an active transport mechanism for hydrogen ions must be in operation since the highest recorded proximal electrical potentials can account for H+ gradients of less than 1 *p*H unit.

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Vitamin B_{12} in the Serum of the Rhesus Monkey

MOST investigations of the physiology of vitamin B_{12} have been carried out in man or laboratory animals other than the sub-human primates. Indeed, it has been specifically noted that vitamin B_{12} deficiency has not been produced experimentally in monkeys[1-3], and those macrocytic anæmias which have been induced are known to respond to treatment with folic acid (sometimes in association with ascorbic acid) but not to vitamin B_{12} (ref. 4).

Recently, two rhesus monkeys in our colony developed a paralysis which called to mind sub-acute combined degeneration of the cord in man and which improved following treatment with parenteral vitamin B_{12}. This led us to measure the serum-levels of the vitamin in other monkeys in the colony. Using the microbiological assay method with *Euglena gracilis*[5], the level of vitamin B_{12} was estimated in the sera of three male and 18 female normal untreated adult rhesus monkeys (*Macaca mulatta*) which had been in the colony for 1–15 years. The mean figure of 56·6 $\mu\mu$g/ml. (*S.D.* 20·8) is very low compared with the levels found in other mammals (not less than 200 $\mu\mu$g/ml. by the same technique[6]), and with the range of normal in man (mean value 390 $\mu\mu$g/ml.[5]); in fact, it is the value to be expected in cases of pernicious anæmia in relapse.

Low levels similar to those which we have found have already been observed by Wilson and Pitney[7] in 17 young rhesus monkeys which had been on a standard laboratory diet for many months. The mean serum-level of 120 $\mu\mu$g/ml. was accepted by them as normal for the species. Das Gupta *et al.*[8] found even lower levels in six healthy young rhesus monkeys (mean value 56·8 $\mu\mu$g/ml.) living on the "usual animal room diet", and they commented that the somewhat higher figures obtained by Wilson and Pitney[7] might have been due to the higher vitamin B_{12} content of their 'natural' laboratory diet.

Repetitions of the tests on our monkeys have shown that the levels of vitamin B_{12} remain consistently low. On the other hand, monkeys that have been in the laboratory environment for only a short period showed higher levels at the time of the first test, but the values fell to the low figure characteristic of the rest of the colony within three months. Estimates on a group of 29 monkeys examined within five weeks of captivity and immediately on arrival in Britain gave a mean value of 208·2 $\mu\mu$g/ml. (*S.D.* 93·2). These findings imply that monkeys in their natural habitat have satisfactory amounts of vitamin B_{12} in their serum as judged by the standards of man and other mammals, perhaps as the result of a more varied diet in the wild state.

The ordinary diet fed to our monkeys consists of potatoes, bread, carrots and other root vegetables, green vegetables and fresh fruit, and is supplemented with 'Bemax', ascorbic acid and halibut liver oil. This diet has been thought to be adequate since monkeys have been kept on it in apparently good health for long periods of time—up to 24 years—and since hæmatological examinations have never revealed any notable changes. Further examinations of the blood from these monkeys now known to have low vitamin B_{12}-levels have also failed to show any significant evidence of anæmia.

This overall picture of very low levels of serum vitamin B_{12}, usually without obvious signs or symptoms of deficiency, in monkeys on a vegetarian diet seems closely to resemble the findings in strict vegetarians[9,10], where low serum B_{12} values are not necessarily associated with anæmia or gross neurological changes. In many other species, however, there is evidence that vitamin B_{12} deficiency may lead to a range of disorders including retardation of growth, congenital deformity, increased incidence of demyelinating disease of the nervous system and a number of lesser effects (for review see ref. 2). Special mention may also be made of the many reports that the deficiency has some ill-defined influence on the reproductive processes[11-14]. We intend particularly to study the influence of vitamin B_{12} supplements in our group of breeding females the fertility of which has proved to be disappointingly low, and would suggest that other users of laboratory primates might consider the possible effect of low levels of vitamin B_{12} in the serum in the interpretation of their results.

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Serum Iron in Normal and Castrated Mammals

MOST authors are agreed that there is a sexual difference in serum iron values in man.

Previous work[1,2] carried out on six kinds of mammals has not, however, shown this difference. On the other hand, it seems beyond doubt that the gonads have a definite effect on iron metabolism in some species. The castration of rats[3] and rabbits[4] modifies the level of serum iron and in the former affects hepatical deposit[5].

The injection of sex hormones has also a certain influence on other species, and it has been seen how the œstrogens reduce the serum iron in rats[4] and in rabbits[6].

In the work recorded here, I have examined the influence of gonads on the serum iron values and the total iron-binding capacity in 5 species of mammals—bull, horse, ass, lamb and pig. For this purpose the values for normal and castrated specimens are compared. The results obtained with the first three species have already been given[7].

The serum iron values and the total iron-binding capacity are determined using Ramsay's methods[8,9]. The average values of both the normal and castrated specimens have been submitted to a test of statistical significance (Student's *t*).

Table 1. AVERAGE VALUES FOR SERUM IRON AND TOTAL IRON-BINDING CAPACITY (TIBC) FOR THE SPECIES CONCERNED

	Normal			Castrated		
Species	No.	Serum iron γ Fe (per cent)	TIBC γ Fe (per cent)	No.	Serum iron γ Fe (per cent)	TIBC γ Fe (per cent)
Bovine ♂	20	102 – 6 (45 – 156) σ = 28	368 – 13 (225 – 472) σ = 54	20	91 – 8 (40 – 156) σ = 34	317 – 21 (135 – 517) σ = 98
Equine ♂	15	99 – 7 (60 – 160) σ = 28	317 – 15 (217 – 445) σ = 55	20	94 – 3 (65 – 120) σ = 14	293 – 11 (210 – 420) σ = 47
Ass ♂	20	84 – 6 (45 – 180) σ = 29	253 – 8 (195 – 337) σ = 36	20	94 – 4 (50 – 125) σ = 17	264 – 9 (187 – 345) σ = 38
Sheep ♂	20	131 – 8 (70 – 180) σ = 31	328 – 20 (200 – 600) σ = 85	19	125 – 9 (56 – 250) σ = 39	274 – 14 (185 – 428) σ = 60
Porcine ♂	8	96 – 11 (40 – 130) σ = 28	432 – 21 (330 – 408) σ = 55	20	90 – 5 (40 – 140) σ = 24	464 – 18 (336 – 630) σ = 78
Porcine ♀	20	94 – 6 (40 – 185) σ = 24	491 – 23 (332 – 735) σ = 97	20	85 – 5 (50 – 140) σ = 22	507 – 18 (345 – 630) σ = 78

The results given in Table 1 shows that in the species studied no clear influence of castration on the serum iron values is noted. In general a decrease in values can be observed, but the differences which appear are not statistically significant. An evident decrease in serum iron following castration has been quoted in the horse[10], but the average of only 5 specimens was taken. With respect to the values of the total iron-binding capacity we have only found a significant difference existing in the bovine species ($t = 2{,}181$), being lower in castrated than in the normal types.

The effect of castration on serum iron values does not seem to be uniform in the different mammals referred to, and for the moment every attempt to generalize the action of sexual hormones on iron metabolism should be treated with reserve.

The opinion of certain authors[11] that it is difficult to obtain an explanation of the sexual difference in man from the endocrinous effects found in other mammals now appears to me more justified than ever.

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HÆMATOLOGY

Types of Haptoglobins in Araucanian Indians of Chile

SINCE the discovery of the polymorphism among haptoglobins in human serum made by Smithies[1] with starch-gel electrophoresis, information concerning geographical distribution has increased rapidly.

The Hp 1 gene is known to be common in Africa, but rare in Asia, especially India. In Europe, from east to west Hp 1 increases slowly. In America, Sutton[2] has noticed an increase from Mongolian and Amerindian population from south-east Asia (0·22 in the Japanese), through Alaska (0·33) and North America (0·45 in Navajos and 0·59 in Apaches) to Central (0·59 in the Mayas) and South America. In the last-named area the values are: Venezuelan Indians (0·55) and Peruvian Indians (0·73). A limited series of 34 Mapuche Indians from Temuco has been reported by Parker and Bearn[3] finding 0·74.

Serum specimens were obtained from 229 'full blooded' Araucanian Indians of the south of Chile: 113 of the Pehuenche sub-group and 116 of the Mapuche sub-type. They speak the same language, but their habitat and customs are quite different. The Pehuenches examined live in Lonquimay, in a hidden valley of the Andes; they are very isolated and originally were nomads. Their admixture with white is very low, as can be seen from their blood groups (Table 1)[4]. The population is rather scanty and very endogamic. A very high incidence of goitre exists in this region[5].

Table 1. CLASSIC BLOOD GROUPS OF ARAUCANIAN INDIANS OF CHILE

	Total individuals	O	A	B	AB
Mapuches (Temuco)	450	358 (79·6)	50 (11·1)	42 (9·3)	0 (0)
Pehuenches (Lonquimay)	182	174 (95·6)	6 (3·4)	2 (1·1)	0 (0)

Percentages in parentheses.

The Mapuche Indians examined were from the Central Valley of Chile, near Temuco. This population constitutes the main group of the Araucanian Indians. They are classified as an agricultural and sedentary population since the pre-Columbian period. Their proximity with white cities has elevated admixture to 30 per cent more or less, an estimation based on their blood groups. These findings are consistent with earlier surveys made in the same region in relation to blood groups[6].

The Negro admixture of the two groups must be very low, if any. Neither glucose-6-phosphate dehydrogenase deficiency nor abnormal hæmoglobins were found[4].

Serum was separated the same day, and stored at 20° C for 20–60 days when starch-gel electrophoretical analysis was performed in a vertical set, with borate buffer (*p*H 8·6), and stained half with amido black 10*B* and half with benzidine-peroxide reagent. Hæmoglobin was added to all samples except when they were obviously hæmolysed.

Table 2. HAPTOGLOBINS OF ARAUCANIAN INDIANS OF CHILE

		Haptoglobin types 1–1	2–1	2–2	Total individuals	*p*Hp 1	*S.E.*
(I) Pehuenches (Lonquimay)							
(*a*) Without goitre							
Unrelated		22	14	1	37		
Related	2	8	6	1	15		
	3	5	1	0	6		
	4	4	0	0	4		
					62	0·78	0·04
(*b*) With goitre							
Unrelated		25	14	0	39		
Related	2	4	3	1	8		
	4	3	1	0	4		
					51	0·80	0·04
					113	0·78	0·02
(II) Mapuches (Temuco)							
Unrelated		65	39	12	116	0·72	0·02

Table 2 shows the results with both Pehuenche and Mapuche groups. The Pehuenches are divided according to consanguinity and size of the family, and according to presence of goitre. The Mapuche were all unrelated and had no goitre. The differences among these two groups reflect the endogamic and endemic goitre characteristic of the first group. As the population examined was partially related, we calculated the gene frequency according to Cotterman's method[7].

The highest frequency of gene Hp 1 was found in Lonquimay, where we suspect a low admixture with white. In Temuco, instead, where the admixture is very high, the frequency falls. There are sufficient facts to suggest that all the Araucanian sub-types were very much related before the arrival of the Spaniards, so that subsequent admixture seems to be the best explanation for the differences found in the Hp 1 gene frequency. This suggests also that the primitive Araucanian Indians of the pre-Columbian period must have had an Hp 1 gene frequency of more than 0·80.

Our results are in very good agreement with what would be expected under Hardy–Weimberg equilibrium (*P* more than 0·30).

In summary, the Hp 1 frequency of the Araucanian Indians is reported as being 0·72 (*S.E.* 0·02) for the Mapuche and 0·78 (*S.E.* 0·02) for the Pehuenches, in accordance with the increase described from south-east Asia to South America. Endemic goitre does not seem to be related to this gene distribution.

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Inactivation of Thrombin by Normal Human Urine

CONTINUING our investigations of thrombin inactivation in blood and other body fluids[1–4], in order to determine their antithrombin activity, we decided to examine the influence of normal human urine on the activity of added thrombin.

(1) The standard thrombin solution is obtained by dissolving a small quantity of thrombin pulver ('Thrombofort' Richter) in physiological sodium chloride solution. By addition either of thrombin or physiological saline we fixed the activity of thrombin solution, that is, 0·1 ml. mixed with 0·1 ml. of physiological saline coagulated 0·2 ml. of standard dry plasma solution during 30 ± 2 sec. (2) The plasma substrate is prepared by dissolving the dry, lyophilized plasma (produced at the Blood Centre, Krakow, Nowa Huta) in distilled water in gravimetric proportion 7·5 : 100·0. This concentration corresponded to that used for intravenous infusions. (3) The urine is obtained from normal, fasting, healthy adults of both sexes. The urine is centrifuged (10 min, 3,000 r.p.m.) in order to eliminate the morphological elements. During further examination only the upper layer of centrifuged urine is used. We examined: reaction (whether acid, neutral, alkaline), specific gravity, colour, transparence, contents of proteins, sugar and a microscopic picture of sediment. A more exact determination of *p*H was made with universal indicator paper (Labo Moderne, Paris 1e), having a sensitivity of *p*H 0·1. The findings did not differ from normal values. We examined 35 specimens of urine with *p*H's of 5·0–10·0.

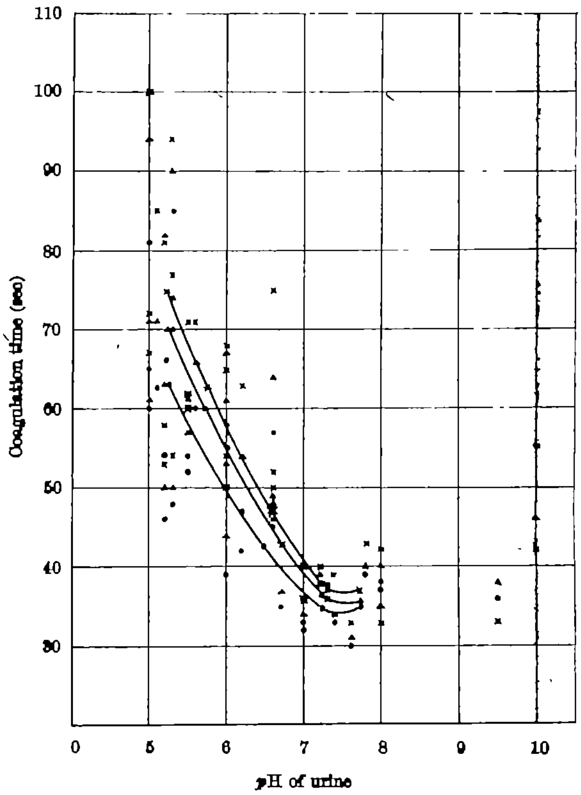

Fig. 1. ○, Values of coagulation time after 0-min. incubation; △, values of coagulation time after 10-min. incubation; ×, values of coagulation time after 20-min incubation. The figures are based on the mean values of coagulation times of the *p*H limits: 5·0–6·0, 6·0–7·0 and 7·0–8·0

To determine thrombin inactivation, 0·1 ml. of thrombin standard solution was mixed with 0·1 ml. of examined urine, and immediately after (10–15 sec) 0·2 ml. of plasma substrate was added and the coagulation time of the whole mixture determined. The same volumes of thrombin solution and urine were mixed and incubated in room temperature for 10 and 20 min. also. After that time 0·2 ml. of plasma substrate was added again and the time of coagulation determined. In the control reaction instead of 0·1 ml. of urine the same quantity of physiological saline was used. All results are given in seconds. These are presented in Fig. 1. It shows that when thrombin is mixed with urine the *p*H of which is less than 7·0 (acid urines) the time of coagulation becomes immediately prolonged as compared with its control values (0 min incubation). The degree of prolongation depends on *p*H; the nearer to its neutral value of 7·0, the lesser becomes the prolongation of coagulation time. Besides this relationship one can observe that the urines having the same value of *p*H do not prolong identically the coagulation time as compared with its control values.

When the urine is neutral or slightly alkaline and the *p*H equal to or higher than 7·0 the coagulation time undergoes only small or no prolongation. The strongly alkaline urines cause a moderate prolongation of the coagulation time.

The 10- and 20-min. incubation period of all urines prolongs little or not at all the values of times of coagulation in comparison with initial value (0 min incubation).

The influence of *p*H on the coagulation of blood and plasma and the thrombin activity is well known[4–6]. Various investigators find the optimal activity of thrombin at the *p*H 7·2–7·5; the maximal activity of thrombin appears at the neutral or slightly alkaline *p*H (7·0–8·0). The further from those values and the nearer to strongly acid or alkaline *p*H the weaker becomes the action of thrombin. Our results confirm those already published.

Schulz[10] investigated among other problems the influence of normal human urine, obtained in the morning from fasting individuals, on the coagulation time of recalcified, oxalated blood and plasma and did not find any action of urine on the recalcification time under the conditions of his experiments.

Conclusions. (1) Normal examples of human urine with acid or strongly alkaline pH inactivate immediately the added thrombin. The degree of inactivation depends in great part on the distance of urine pH from its neutral value. This inactivation is very small or none when the urine pH is neutral or slightly alkaline. The incubation of urine with thrombin increases little (or does not increase at all) the initial inactivation. (2) The results of *in vitro* investigations prove that the alkalization of urine obtained by appropriated diet or pharmacological remedies may *in vivo* exercise a favourable influence on threatening or already existing hæmorrhages of the urine-excreting system.

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HISTOCHEMISTRY

Histochemical Demonstration of Aminopeptidase Activity in the Cells of the Blood and Bone Marrow from Various Hæmatological Disorders

Aminopeptidase activity has been demonstrated histochemically in the leucocytes of the blood and bone marrow of man using alanyl β-naphthylamide as the substrate[1,2]. Cytoplasmic aminopeptidase activity was localized in the neutrophils, monocytes and eosinophils while lymphocytes and basophils failed to exhibit a positive reaction for aminopeptidase activity[1]. During granulopoiesis, aminopeptidase activity appeared initially in the late *B* myelocytes and gradually increased during later stages of maturation; erythrocytic elements were non-reactive.

The procedure used in this investigation has been detailed by Ackerman[1] and entails the fixation of air-dried films with cold 0·5–1 per cent osmium tetroxide in dimethylformamide for 2 min, washing in running water and placing the films in an incubation medium containing 4 mg of alanyl β-naphthylamide dissolved in 5 ml. of distilled water, 5 ml. of 0·1 M Sorenson's phosphate buffer at pH 6·7, 1 mg of magnesium sulphate and 5 mg of fast garnet *GBC*. Incubation periods of 6–18 h at room temperature were used routinely. Following incubation the films were washed in running water, blotted dry and mounted in glycerine or glycerogel; counter-staining with 0·5 per cent aqueous methyl green was optional. Alanyl β-naphthylamide hydrochloride used as the substrate was synthesized in our laboratory using the method outlined by Burstone[3,4].

Blood and bone marrow films obtained from patients with various hæmatological disorders were processed with blood and/or bone marrow films from individuals exhibiting no hæmatological disfunction. Ninety-one patients with hæmatological dyscrasias were examined: chronic myelocytic leukæmia (12); acute–sub-acute myelocytic leukæmia (5); acute–sub-acute monocytic leukæmia (15); chronic lymphocytic leukæmia (16); leuco-lymphosarcoma (14); Hodgkin's disease (4); lymphoma (3); polycythæmia vera (3); secondary polycythæmia (2); thrombocytopenic purpura (5); pancytopenia (3); iron deficiency and hæmolytic anæmia (6) and infectious mononucleosis (3). The relative activity, judged by the intensity of the reaction, of the cellular elements in the hæmatological disorders were evaluated and compared with similar cells from the normal individual. The processing of normal films with abnormal blood and/or bone marrow films permitted an important controlling factor minimizing possible variations in the histochemical procedure.

In general, increased aminopeptidase activity was observed in the myelocytes and mature neutrophils in chronic myelocytic leukæmia as well as in the younger myelocytes in acute–sub-acute myelocytic leukæmia; myeloblasts and promyelocytes were non-reactive. The mature neutrophils exhibited relatively normal reactions for aminopeptidase in acute-sub-acute myelocytic leukæmia while the neutrophils and developing granulocytes from patients with chronic myelocytic leukæmia receiving antileukæmic therapy exhibited normal to slightly decreased aminopeptidase activity with the procedures used in this investigation. In acute–sub-acute monocytic leukæmia, the young and relatively mature monocytes usually exhibited increased aminopeptidase activity as compared with normal mature monocytes; monoblasts were non-reactive and the mature neutrophils exhibited an essentially normal aminopeptidase reaction. During the differentiation of the neutrophilic myelocytes and monocytes, aminopeptidase activity appeared initially in the region of the cytocentrum and became more generalized during maturation. Aminopeptidase activity was demonstrable only after the development of peroxidase activity in these cells.

Lymphocytic cells present in chronic lymphocytic leukæmia and lymphosarcoma were non-reactive, and the mature neutrophils and developing myelocytes present in the bone marrow of these individuals were normal in their histochemical reactivity. Normal reactions for aminopeptidase were observed in the neutrophils and myelocytes from individuals with polycythæmia, hæmolytic and iron-deficiency anæmias, thrombocytopenic purpura, pancytopenia, Hodgkin's disease, lymphoma and infectious mononucleosis. Increased aminopeptidase activity was noted in the neutrophils from patients with pyogenic infections.

No attempt was made to place the histochemical reactions for aminopeptidase activity observed in the cells of the blood and bone marrow on the same level of semi-quantitative evaluation as has been utilized in the study of leucocyte alkaline phosphatase activity since our experience indicated the capriciousness of the reaction and the not infrequent variation in cellular reactivity in different areas of the same preparation. In spite of these inherent difficulties, our investigations revealed what appear to be significant variations in leucocyte aminopeptidase activity in certain hæmatological disorders, for example, myelocytic and monocytic leukæmias and in pyogenic infections. Differences between aminopeptidase, alkaline phosphatase and peroxidase activities were evident and of interest. Although the present histochemical procedure for aminopeptidase activity may not assume a significant role in diagnosis of various hæmatological disorders, it does merit further study and adds to our knowledge concerning the histochemical alterations occurring in the cells of the blood and bone marrow in disease. Improvements and refinements in both the histochemical substrates and techniques can be expected to improve the sensitivity and reliability of methods for the histochemical determination of aminopeptidase activity.

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Nuclear Deoxyribonucleic Acid Content in Corona Radiata of the Ovary of the Rat

THE mere situation of the corona radiata in the follicle is highly suggestive of the existence of inter-relationship between the granulosa and the oocyte. Experimental work revealed that the granulosa cells assume a role in transporting hormonal[1–4] and nutritional[5] substances towards the oocyte. Furthermore, morphological evidence of a pathway between both was given recently[4].

Numerous investigations, especially in endocrine organs, showed the DNA content of the nuclei to be closely related with cell function[6]. Since the variations of the DNA content of the components in the rat ovary were particularly marked[7–10], I tested whether the DNA content of the granulosa cells of the follicle wall would differ from that present in the corona radiata. For that purpose the DNA content in interphasic nuclei of both elements was determined by Lison's histophotometrical method[11] on Feulgen-stained ovaries of rats in procestrus and cestrus containing w ll-developed tertiary follicles. After fixation in a mixture of alcohol 75 per cent, formalin 20 per cent and acetic acid 5 per cent, the ovaries were treated under the usual standardized conditions[6].

The determinations of DNA were carried out in 9 tertiary follicles out of 3 series of ovaries. In each series the individual values were assembled in a pool (Table 1). In every case significant differences were noted between the corona radiata and the granulosa of the follicle wall: in the first series the DNA content of the periovocytary nuclei is 35 per cent ($P < 0·001$) higher than in the peripheral nuclei; in the second and third series the differences are, respectively, 30·5 ($P < 0·001$) and 24·9 ($P < 0·001$) per cent. This difference seems to be limited to tertiary follicles. No significant distinction in DNA content appeared between the granulosa cells surrounding the oocyte and those belonging to peripheral layers in secondary follicles without antrum formation (Table 2). The difference of 4·6 per cent falls within the limits of error.

In order to check possible gonadotropic influences, the work was extended to the hypophysectomized animal. Five days after hypophysectomy, the difference in DNA content between the nuclei of the corona radiata and the peripheral ones of the follicle wall in some well-preserved tertiary follicles was only 16 per cent and had no statistical significance ($0·2 < P < 0·3$) (Table 3). However, a significant difference seems to be restored after gonadotropic replacement. After daily administration of chorionic and serous gonadotropin to the hypophysectomized animal during 5 days the difference of the DNA content again attains, respectively, 35·9 ($P < 0·001$) and 34·7 ($P < 0·001$) per cent.

Table 1

		n	$\bar{x}$	$\log \bar{x}$	s	$s_{\log \bar{x}}$
Series I (*KR* 14–15–16)	Follicle wall	119	1,000	3·0000	0·093	0·0082
	Periovocytes	65	1,350	3·1303	0·106	0·0132
Series II (*KR* 18–19–21)	Follicle wall	151	1,000	3·0000	0·093	0·0082
	Periovocytes	97	1,305	3·1156	0·106	0·0132
Series III (*KR* 111–115–123)	Follicle wall	146	1,000	3·0000	0·068	0·0077
	Periovocytes	54	1,249	3·0966	0·078	0·0107

Table 2

KR 19 and *KR* 21	n	$\bar{x}$	$\log \bar{x}$	s	$s_{\log \bar{x}}$
Peripheral	67	1,000	3·0000	0·133	0·0142
Periovocytes	29	954	2·9797	0·106	0·0097

Table 3

		n	$\bar{x}$	$\log \bar{x}$	s	$s_{\log \bar{x}}$
Hypophysectomy	Follicle wall	203	1,000	3·0000	0·088	0·0062
	Periovocytes	46	1,163	3·0656	0·311	0·0046
Hypophysectomy + chorionic gonadotropin	Follicle wall	199	1,000	3·0000	0·085	0·0079
	Periovocytes	49	1,359	3·1332	0·112	0·0123
Hypophysectomy + serous gonadotropin	Follicle wall	152	1,000	3·0000	0·085	0·0069
	Periovocytes	65	1,347	3·1293	0·121	0·0150

By means of the histophotometrical determination of nuclear DNA in the granulosa cells of cavitary follicles in the rat, a fair distinction can be made between the cells of the follicle wall and the corona radiata. This finding argues in favour of a functional distinction between these follicular components. This distinction, which is probably related with the special transfer functions of the corona radiata, seems to depend on gonadotropic influences.

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Soluble Glucokinase Activity in Bovine Tissues

PREVIOUS investigations of glucokinase activity in ruminant tissues have involved the measurement of glucose or oxygen uptake (in the presence of glucose as substrate) by tissue homogenates, mitochondria or supernatants after centrifugation of homogenates at 9,000*g* [1–3]. These methods showed glucokinase activity in brain and intestinal mucosa, but not in the liver of sheep. The apparent lack of glucokinase activity in the liver preparations may have been due to the presence of powerful glucose-6-phosphatase activity[4]. The interfering enzyme can be removed by centrifugation of homogenates at 100,000*g*, and this step has revealed appreciable activity of soluble glucokinase in rat liver[5], which was previously thought to have only slight glucokinase activity. This technical development suggested that glucokinase activity in ruminant tissues should be re-evaluated, for it is of interest in at least two respects. First, from the comparative view, overall glucose utilization is less in intact ruminants than in other mammals[6], so low glucokinase activities may be expected in some tissues. Secondly, some metabolic diseases, such as pregnancy toxæmia in sheep and acetonæmia in cows, resemble diabetes in some regards, and the hepatic glucokinase activity of fasting or alloxan-diabetic rats is only about 40 per cent that of normal, fed rats[5].

The present method was based on those of DiPietro and Weinhouse[5] and Grignani and Lohr[7]. Tissues were taken from seven adult cows at a slaughter-house within 10 min after stunning, and from five adult female rats which had been fasted overnight. Homogenates were prepared by disintegrating 1 g of tissue in 10 ml. of ice-cold buffer (0·15 M potassium chloride, 5 mM sodium ethylenediamine tetraacetate, 5 mM magnesium chloride, *p*H 7·0) in a Waring blender. Supernatants were obtained by centrifuging the homogenates at 100,000*g* for 30 min in a Beckman/Spinco model *L* centrifuge.

Assay of the soluble glucokinase involved measurement of the rate of increase of optical density at 340 mμ of a reaction mixture in a Beckman *DU* spectrophotometer. It depended on the generation of reduced nicotinamide-adeninedinucleotide-phosphate (NADPH) in the presence of added glucose-6-phosphate dehydrogenase. Final concentrations in the reaction mixture were 50 mM glycylglycine at *p*H 7·6, 8 mM magnesium chloride, 1 mM sodium fluoride, 0·75 mM adenosine triphosphate (ATP) except in the blanks, 0·25 mM NADP, 2 mM EDTA, and in addition each cell contained at least 0·02 Kornberg units of glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.). The final volume was 2·1 ml., and up to 0·3 ml. of supernatant could be included. The reaction was started by the addition of ATP except in the blanks. Increase in optical density was linear for at least 10 min. Micromoles of NADPH generated were calculated from a molar extinction coefficient of 6·22 × 10⁶ cm²/mole.

The results are shown in Table 1. All tissues tested had soluble glucokinase activity. In bovine tissues, the activity was high in salivary gland, mammary gland and duodenum, moderate in brain, liver and kidney, and low in muscle and adipose. Glucokinase activity in rat tissues were 2·3 to 6·3 times higher than in corresponding bovine tissues.

Table 1. SOLUBLE GLUCOKINASE ACTIVITY*

Tissue	Cows, 7	Rats, 7
Salivary gland	30·7 ± 4·8†	—
Mammary gland	36·0 ± 2·2	—
Duodenum	36·2 ± 2·3	81·0 ± 13·5
Brain	28·4 ± 3·3	139·6 ± 9·3
Kidney	25·2 ± 2·2	100·4 ± 9·3
Liver	17·4 ± 1·3	44·7 ± 4·5
Muscle	8·6 ± 0·8	34·2 ± 6·1
Adipose	3·1 ± 0·3	19·5 ± 2·8

* One unit represents the formation of 0·01 μmole NADPH/min/g fresh tissue under the conditions described in the text.
† *S.E.*

The comparative significance of these results is difficult to assess because of the problem of body-size. If a relation to the three-fourths power of body-weight which holds for oxygen consumption of intact animals[8] applies here also, the rat tissues should have about 7 times the glucokinase activity of the cow tissues. Since this is approached only in the case of adipose tissue, it seems that the generalization does not apply. Krebs[9] has found this also to be the case for oxygen consumption of tissues *in vitro*. At present, we can only note that the ruminant glucokinase activities are less than those of the rat, without deciding on the relative influence of body-size and of different patterns of metabolism.

The results do tell us something, however, about the pattern of ruminant glucose metabolism. The tissues which appear to utilize little glucose are skeletal muscle and adipose. Reid[10] has suggested that acetate uptake spares the utilization of plasma glucose by muscle in sheep. Acetate might also be taken up by the adipose tissue and be synthesized into fatty acids in a way similar to mammary lipogenesis in the cow. The uptake of plasma glucose by the mammary gland and its utilization for synthesis of lactose and glycerol are well known[11]; but we have found no information on glucose metabolism by salivary gland. The high activity of duodenal glucokinase is provocative, since the glucose-6-phosphatase activity is low[4]. Perhaps glucose taken up by intestinal mucosal cells is transformed during the process of absorption. The presence of soluble hepatic glucokinase supports the suspicion that the deficiency of glucokinase activity found by Gallagher and Buttery[3] was due to glucose-6-phosphatase activity in their preparations[1,3]. Further work is in progress on tissues from normal sheep, and from cases of pregnancy toxæmia and acetonæmia.

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Thermal Injuries and Formation of Mucopolysaccharides in the Skin

WE have recently demonstrated that in tuberculous tissue[1], in carcinoma[2], in varicose ulcer[3] and in silicotic tissue[4] large amounts of a periodic acid–Schiff-positive material are found which—according to different histochemical and chemical control examinations—in all probability correspond to neutral mucopolysaccharides[5]. Further examinations disclosed that under physiological and pathological conditions the thyroid gland has an important role in the regulation of the mucopolysaccharide contents of the organism[6–7]. It is probable that the mucopolysaccharide metabolism of a tissue *in situ* is dependent on its vascular supply. As a consequence of a poor blood supply a state of hypoxia or anoxia ensues and oxybiosis must give place to a more primitive kind of metabolism which will then ensure for a certain time the energy supply of the damaged tissues. In the course of this new metabolism, neutral mucopolysaccharides arise as metabolic derivatives[8]. Neutral mucopolysaccharides are produced in tissues whenever a stage of hypoxia or anoxia sets in in the tissues, but the degree of the histological damage is not so severe as to involve the immediate destruction of the entire tissue.

Knowing that injuries of the skin caused by burning or freezing lead to hypoxia or anoxia, we carried out experiments to determine the alteration of the metabolism with special regard to the formation of neutral mucopolysaccharides.

Our experiments were performed on 15 guinea pigs and 10 rats. The skin of the guinea pigs was burned or frozen under general anæsthesia. The rats were submitted to scalding (4 sec). Freezing was carried out by applying dry ice (120 sec). The effect of time factor of the injuries was studied on specimens of skin examined daily from animals of each series, for a period of a week.

The histological preparations were stained by Ritter–Oleson's, hæmatoxylin–eosin, and Van Gieson's method. The staining of Ritter–Oleson was supplemented by enzyme digestion, acetylation and studies of metachromasia. In rats determinations of serum hexosamine were also made.

Our experimental results seem to give strong support to our presumption that in injuries of the skin caused by burning or freezing, especially in the vesicles and in the cutis, large quantities of periodic acid–Schiff-positive material arise which in all probability correspond to neutral mucopolysaccharides. Shortly following the injury, increase of acid mucopolysaccharides may be observed, but beginning with the second or third day following the injury, the local appearance and the increase of neutral mucopolysaccharides are striking. The hexosamine content of the blood was found also to double shortly after the injury and slowly decrease from the third or fourth day following the injury.

We think that our results add support to our statement that the histological appearance *in situ* of neutral mucopolysaccharides is the consequence of a metabolic process following a general biological rule—a process which seems to occur whenever a local hypoxia or anoxia arises in the tissues without the immediate destruction of the whole tissue.

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HISTOLOGY

Collagen and a Cellulose-like Substance in Fossil Dentine and Bone

The first direct evidence that certain organic components are retained in the fossil remains of vertebrates comes from the analysis by paper chromatography of specimens of *Dinichthys* of Middle Devonian age (380 million years old)[1]. This work has shown that degradation products of collagen can still be recognized. More recently[2], it has been demonstrated that fossil dentine and bone-like tissues in Devonian ostracoderms are capable of being decalcified and sectioned on a microtome. Further work at the Royal Dental Hospital, London, using normal histological staining techniques, has now indicated the presence of polysaccharides as well as collagen in the dentine and bone-like tissue of the ostracoderms. Using fossil bone belonging to a bison from the most recent Ice Age, and to *Dremotherium* from the Miocene (25 million years old), electron microscope photographs have been obtained at the Nuffield Orthopaedic Centre, Oxford, showing collagen fibrils and the intact walls of canaliculi[3]. Furthermore, X-ray diffraction of the same material has established that it is the tubular form of collagen that is present.

In the work now in progress, an attempt has been made to isolate the organic content in the bone of a prosauropod dinosaur from the Upper Trias (200 million years old), and in the dermal armour of further Devonian ostracoderms. One of the main difficulties encountered, however, has been that since only dilute acids or alkalis at room temperature can be used to isolate the collagen, although most of the mineral content can be removed, certain minerals have so far resisted solution. In the dinosaur, a manganese phosphate has been the major obstacle, while in the ostracoderms there is a residual aluminium silicate. These minerals have been identified by their X-ray diffraction patterns.

Nevertheless, electron microscope investigations of patches of almost completely demineralized bone in the dinosaur, and dentine and bone-like tissue in the ostracoderms, reveal a fibrillar structure; an electron-diffraction examination of the same material shows a mixture of mineral and collagen patterns. In addition, in the dentine of the dermal armour of the ostracoderms, electron microscopy shows that the dentine tubules are intact, while the electron-diffraction pattern indicates that their walls are composed of an organic compound. The material is similar in appearance and staining characteristics to other mammalian tissue components, which have been isolated for X-ray diffraction and have given a cellulose-like pattern.

Our sincere thanks are due to Prof. J. Trueta of the Nuffield Orthopaedic Centre, Oxford, and to Prof. R. B. Lucas of the Royal Dental Hospital, London, for the provision of facilities. Financial assistance from the Nuffield Foundation is gratefully acknowledged by one of us (L. B. H. T.).

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PATHOLOGY

Paired Helical Filaments in Electron Microscopy of Alzheimer's Disease

In the light microscope, the characteristic change in the neurones of the cerebral cortex in Alzheimer's disease is the presence of dense bundles of argyrophilic fibrils. They are coiled into skeins or 'squash racket' shapes, and appear to fill most of the perikaryal cytoplasm, sometimes giving the cell a swollen appearance. It has been suggested by Divry[1] that the fibrils are extra-cellular except in the later stages of the change. The fibrils stain with congo red, and this results in a type of birefringence suggesting the presence of longitudinally arranged micelles in the fibrillar bundles. These well-known findings would lead one to expect the presence of fine filaments associated with these cells in the electron microscope, and if intra-cellular, these might be neurofilaments, which are suggested by Gray and Guillery[2] to be the basis of neurofibrillary argyrophilia.

This is a preliminary report of observations in the electron microscope on cortical biopsies from three cases of Alzheimer's disease, confirmed by light microscopy. In each case the biopsy was taken from the parietal lobe through a burr hole prior to ventriculography. The specimen was cooled rapidly below 20° C after removal and cut into small pieces in the fixative within 5 min of removal. The fixatives were 1 per cent OsO_4 or 0·6 per cent $KMnO_4$ buffered to pH 7·2, and fixation was continued for 4 h. The tissue was then dehydrated in alcohol and embedded in 'Araldite'. Most of the observations were made on material which had been stained in 1 per cent alcoholic phosphotungstic acid before embedding.

Most of the neurones seen were normal, being similar to those seen in biopsies from normal animals and from human cortex exposed for removal of tumours. A number of neurones, however, were very different from normal.

They contained thick bundles of parallel filaments of indefinite length, sweeping round the nucleus, often leaving only a thin rim of normal cytoplasm near the cell membrane. In places the filaments diverged or formed whorls, and some planes of section gave the impression of a ring. They could also be found in cell processes in the neuropil. In the later stages of the change the cytoplasm outside the bundles still appeared normal in fine structure, but compressed against the cell membrane, so that the ribosomes appeared as a dense mass. In the middle of the bundles, however, some lamellated bodies were found, similar to those described by Webster[3] as degenerating mitochondria. Other bodies were found in the bundles consisting of vesicles 500–1000 Å in diameter embedded in a dense granular mass of irregular shape, about 1μ in diameter.

At high magnifications the filaments composing the bundles were seen to be double helices. These consisted of two 100 Å filaments 150 Å from centre to centre, and completing one full turn in 1600 Å. Their appearance varied with the thickness and obliquity of the section. When parallel to the plane of section they appeared as helices, though this single appearance could be interpreted as a regular series of fusiform swellings, or at high packing densities, as tubules, as described by Terry[4]. In thick oblique sections they appeared as asymmetrical shapes, rough on one side and smooth on the other. Thin oblique or transverse sections did not show dots or circles, as would be shown by single filaments, tubes, or fusiform swellings, but dots paired with short curved segments, or two curved segments, or, very occasionally, a pair of dots. All these forms could be explained by a uniform helix with the dimensions described.

It is difficult to speculate on the nature of these structures. It is possible that they are neurofilaments occurring in vastly increased numbers and associated in pairs; though the individual filaments of each pair seem to be of higher density than neurofilaments, especially in unstained preparations. The tendency of these pathological filaments to stain with congo red suggests a similarity to amyloid, but the latter is known to be extra-cellular, and these filaments are intra-cellular.

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Relationship of Gamma-Globulin to the Fibrils of Secondary Human Amyloid

AMYLOIDOSIS is the accumulation in characteristic locations of an eosinophilic substance or substances which are homogeneous when viewed with visible light and which often show characteristic staining reactions, consisting of metachromasia with crystal violet and positive staining with congo red. Recent electron microscopic investigations indicating that amyloid of diverse origins has a finely fibrillar ultrastructure[1] have been substantiated in a number of laboratories[2]. The exact nature of amyloid, however, remains obscure. Since amyloidosis often occurs in human beings in relationship to chronic inflammatory disease and in animals subjected to repeated antigenic stimulation, it has been suggested that there is a close relationship between immunological mechanisms and the pathogenesis of this disorder. The fluorescent antibody investigations of several workers[3,4] demonstrating the presence of γ-globulin in association with amyloid deposits has suggested to some that γ-globulin is an integral component of amyloid. Immunochemical work[5], however, as well as investigations of the disappearance time of human γ-globulin labelled with iodine-131 injected into patients with severe amyloidosis[6], have suggested that γ-globulin may not constitute a significant part of the structure of amyloid.

The isolation of the fibrillar component of amyloid in relatively pure form[7] and the recent development of a method of conjugating immune globulins to ferritin[8] provide a more precise method of detecting and localizing γ-globulin. The purpose of this communication is to report the use of the latter technique in investigating the interaction of anti-human γ-globulin with isolated amyloid fibrils.

Amyloid-laden liver, obtained at post-mortem examination from a patient with secondary amyloidosis, was homogenized, spun at 5,000 r.p.m. and the layer of amyloid fibrils removed and washed five times in saline, all operations being carried out in the cold. The conjugation of anti-human γ-globulin to ferritin was accomplished by the use of meta-xylylene diisocyanate according to the technique of Singer[8]. Conjugation was verified by the use of paper and immunoelectrophoresis; retention of antibody specificity and potency by Ouchterlony plates. Dialysed, lyophilized amyloid fibrils were suspended in saline, incubated with ferritin conjugated anti-human γ-globulin for 30 min at room temperature and then centrifuged for 10 min at 3,500 r.p.m. The supernate was discarded, and the precipitate was washed twice in cold physiological saline and twice in double-distilled water. In control experiments, the fibril suspension was incubated with unconjugated anti-human γ-globulin prior to incubation with the conjugate in an effort to block any reaction between the latter and fibrils. Also, incubation of fibrils with unconjugated ferritin was carried out. As a positive control, typhoid–paratyphoid vaccine was agglutinated with hyperimmune human serum; these coated organisms were afterwards treated in the same fashion as was the suspension of amyloid fibrils.

In all test systems aliquots of the final suspensions were suspended in 1–2 ml. of double-distilled water, a drop of suspension placed on an Athene grid for 2·5 min, and then treated with 1 per cent phosphotungstic acid by the hang-

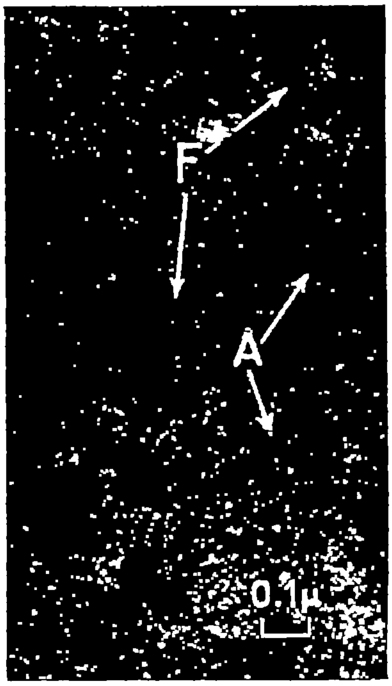

Fig. 1. Unshadowed preparation of human amyloid fibrils (*A*) exposed to ferritin conjugated anti-human γ-globulin (*F*). Note the random relationship of the ferritin conjugated antibody to the fibrils. (× 60,000)

ing drop method. After drying, grids were examined in an RCA-*EMU*-3*F* or a Siemens Elmiskop I electron microscope.

Fig. 1 illustrates the results of the exposure of isolated amyloid fibrils to ferritin conjugated anti-human γ-globulin. The labelled material (*F*) has only a random relationship to the amyloid fibrils (*A*), indicating that the former is not specifically bound to the latter. In the positive control specific adherence of the tagged antibody to the typhoid bacilli could be demonstrated. These results have been obtained consistently in other amyloid-laden tissues. These experiments support the hypothesis that undenatured human γ-globulin is not an intrinsic part of the amyloid fibril of secondary human amyloidosis.

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Excretion of Orotic Acid and Orotidine in Heterozygotes of Congenital Orotic Aciduria

A NUMBER of purine and pyrimidine bases have been demonstrated in human urine in health or in disease[1-3]. Urinary excretion of orotic acid was found in studies of the propositus and so far only described case of congenital orotic aciduria[4]. Both orotic acid and orotidine (formed by the irreversible dephosphorylation of orotidine-5′-phosphate) are excreted in large amounts during therapy with 6-azauracil or 6-azauridine, compounds which on conversion to 6-azauridylic acid competitively inhibit the decarboxylation of orotidine-5′-phosphate to form uridine-5′-phosphate[5]. In work reported here evidence is presented for urinary excretion of orotic acid and orotidine by normal subjects, and for an abnormal pattern in presumed heterozygotes of congenital orotic aciduria.

Twenty-four urine collections (under toluene or in hydrochloric acid) were obtained from five previously described subjects with the erythrocyte enzyme pattern of heterozygous congenital orotic aciduria[6], from two subjects with clinical gout, and from seven normal controls. After preliminary observations failed to show any dietary influence the majority of determinations were made without dietary control except for the '*R*' family, who were examined while on a low purine–pyrimidine diet. Orotidine-^{14}C was prepared from orotidine-5′-phosphate labelled with carbon-14 (ref. 6) using *E. coli* alkaline phosphatase; orotic acid-^{14}C was obtained from commercial sources. For isotope dilution studies labelled orotic acid-^{14}C (2·5 μmole) and orotidine-^{14}C (2 μmole) were both added to a 10 per cent aliquot of a 24-h urine specimen and were initially purified by concentration on a charcoal column and chromatography on a 'Dowex 1-chloride' column as previously described[7]. The radioactive peaks, again concentrated on charcoal, were purified to constant specific activity by repeated ascending paper chromatographic separations, using several systems: *n*-propanol/formic acid/water (70 : 10 : 20) R_F's: orotic 0·39, orotidine 0·31; *n*-butanol/ethanol/formic acid/water (50 : 15 : 10 : 25) R_F's: orotic 0·41, orotidine 0·30; *n*-propanol/water (60 : 40) R_F's: orotic 0·53, orotidine 0·47. Concentrations of purified orotic acid and orotidine were determined spectrophotometrically in 0·1 N hydrochloric acid at 280 mμ and 266 mμ respectively, and radioactivity measured in a Packard Tri-Carb scintillation counter. The validity of the isotope dilution technique as used was supported by: similarity of chromatographic and ultra-violet spectroscopic characteristics of products purified to constant specific activity with those of authentic orotic acid and orotidine; recovery experiments with added authentic compounds; reproducibility of results using varying aliquots of urine; and conversion of recovered orotidine

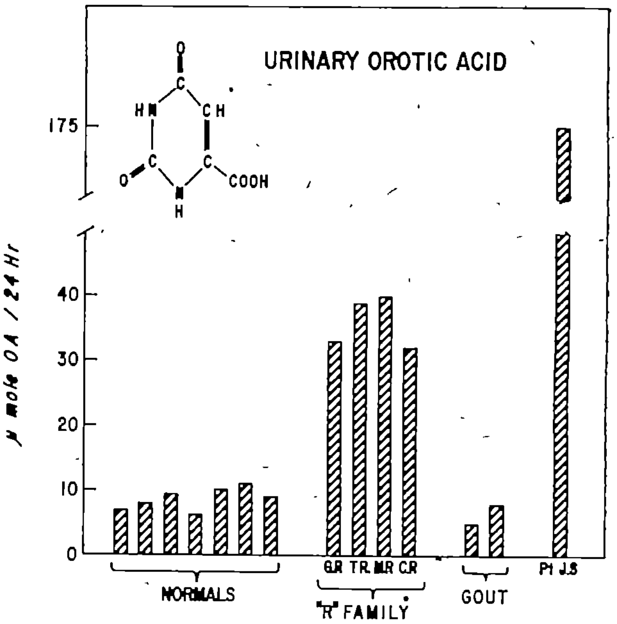

Fig. 1. Twenty-four-hour urinary excretion of orotic acid by control subjects, two patients with idiopathic gout, the parents and two siblings of the propositus of congenital orotic aciduria ('*R*' family), and by J. S., an additional subject with the erythrocyte enzyme defect of congenital orotic aciduria

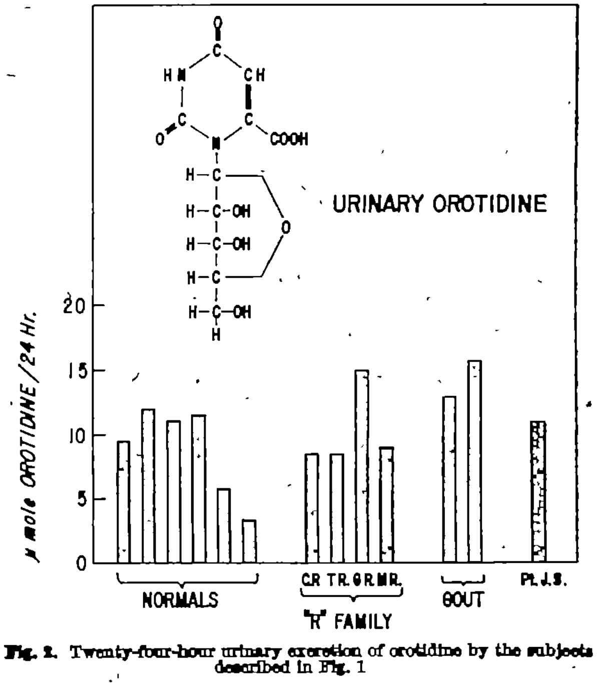

Fig. 2. Twenty-four-hour urinary excretion of orotidine by the subjects described in Fig. 1

to orotic acid of the same specific activity by acid hydrolysis (2·5 N sulphuric acid, 100° C, 1 h).

Normal urinary excretion of orotic acid was found to be approximately 9 μmole (1·4 mg) per 24 h (Fig. 1). Members of the '*R*' family, that is, the parents and two siblings of the propositus of congenital orotic aciduria, excreted orotic acid at approximately four times the normal rate. In subject J. S., unrelated to the '*R*' family but previously demonstrated to exhibit decreased erythrocyte activity of orotidylic pyrophosphorylase and orotidylic decarboxylase[6], urinary orotic acid was found to be 165–177 μmole/24 h on 8 repeated determinations on two urine collections. Excretion of orotidine averaged 9 μmole (2·5 mg) per 24 h in control subjects, with no abnormality observed in the five presumed heterozygotes of congenital orotic aciduria (Fig. 2). Alterations in the pattern of trace purines and increased excretion of 5-ribosyluracil (pseudouridine) have been described in primary gout[8]. No change in urinary orotic acid or orotidine was found in two gouty patients despite one collection being obtained during an untreated attack of acute gouty arthritis.

In summary, the normal urinary excretion of orotic acid and orotidine is in the range found for a number of other purine and pyrimidine compounds. Each represents approximately 0·1 per cent of the estimated daily rate of *de novo* pyrimidine synthesis[7]. The increased urinary orotic acid in presumed heterozygotes of congenital orotic aciduria is consistent with the previously described decreased activities of orotidylic pyrophosphorylase and orotidylic decarboxylase in erythrocytes from these subjects, and in contrast to the simultaneous increase in urinary orotic acid and orotidine produced by specific inhibition of orotidylic decarboxylase with 6-azauridine.

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Plasma Triglycerides and Thrombosis in Rats

THROMBOSIS of the cardiac chambers occurs in rats given hypercholesterolæmic diets containing 40 per cent butter, 5 per cent cholesterol, 2 per cent cholic acid and 0·3 per cent thiouracil[1]. If butter is replaced by arachis oil, thrombosis does not occur, but fibrous atherosclerosis of the proximal aorta is seen instead[2]. Other fats besides butter, such as beef fat and hydrogenated arachis oil, are also thrombogenic, and the essential factor is a triglyceride containing a saturated fatty acid such as distearin-mono-olein[3]. The required triglyceride for atherosclerosis is less clear, but linoleic acid may be involved. Butter alone is not thrombogenic, and cholesterol, cholic acid and thiouracil are all essential for thrombosis, but thiouracil can be omitted if a highly saturated fat such as cocoa butter[4] is used.

Later work has concentrated on finding the essential biochemical differences between the atherogenic and thrombogenic systems. Major differences were found between experimental and normal rats but not between rats showing thrombosis or atherosclerosis. In both groups an increase in coagulability was demonstrated, caused by an elevation of coagulation factors and decreased fibrinolysis[4]. Biochemical examination of the plasma showed markedly increased cholesterol[5] and phospholipids[6] and total glycerides (triglycerides and phosphoglycerides)[4]. Of the total plasma fatty acids, arachidonic acid was diminished and oleic acid was increased[6]. In the present experiments attention has been given to the plasma triglycerides. High levels have been found only in those rats given a diet which cause thrombosis.

Groups of 8 rats were given various hypercholesterolæmic diets containing different fats previously investigated (Table 1)[2–4]. After one month of feeding, the rats were fasted for 24 h, killed with carbon dioxide and blood taken from the inferior vena cava using heparin as anticoagulant[4]. Plasma triglycerides were separated from the phospholipids using the method of Handel and Zilversmidt and estimations of triglycerides[7], cholesterol[4] and phospholipids carried out on the appropriate eluant from the silicic acid column. Free fatty acids were also estimated according to the method of Dole[8]. The results given in Table 1 also include pathological data from previous experiments in which the rats were given the diets until death or on conclusion of the experiment at 6 months[2–4]. There is a marked correlation between the ability of a diet to produce thrombosis and its effect on plasma triglycerides but no obvious correlation with cholesterol, phospholipid or free fatty acid levels. Thus the atherogenic arachis oil group has normal triglycerides. When thiouracil is omitted from the diet high levels are seen only with cocoa butter but not with butter.

Although comparison of the thrombotic system in the rat and the disease of coronary thrombosis in man has evoked some criticism[9], our group has always maintained that the system might provide valuable leads to future research in the field of thrombosis[10]. It is of interest, therefore, to note that the major lipid abnormality in patients with coronary heart disease is an increase in triglycerides[11], and it has been suggested that the incidence of the disease may be linked with plasma triglyceride levels[12]. The current results emphasize the importance of glycerides, and current investigations are concerned

Table 1. EFFECT OF HYPERCHOLESTEROLÆMIC DIETS ON VASCULAR PATHOLOGY AND PLASMA LIPIDS

Group	Diet*	Vascular pathology		Plasma lipids			Free fatty acids
		Thrombosis	Atherosclerosis	Triglycerides	Cholesterol	Phospholipids	
				mg/100 ml.			μequiv./100 ml.
1	Laboratory rat cubes	–	–	80	80	131	32·4
2	40 per cent butter†	+	–	469‡	2,810‡	670‡	38·8
3	40 per cent arachis oil†	–	+	75	1,600‡	406‡	24·8
4	40 per cent hydrogenated arachis oil†	+	–	297‡	2,380‡	424‡	–
5	40 per cent butter without thiouracil§	–	–	105	504‡	175	–
6	40 per cent cocoa butter without thiouracil§	+	–	194‡	1,810‡	627‡	–
7	40 per cent butter alone¶	–	–	77	95	171	–

* Other constituents given in Gresham and Howard (ref. 3). † Also including 5 per cent cholesterol, 2 per cent cholic acid, 0·3 per cent thiouracil. ‡ $P > 0·001$ comparison with group 1. § Also including 5 per cent cholesterol, 2 per cent cholic acid. ¶ Not including cholesterol, cholic acid or thiouracil. + Lesion present. – Lesion absent.

with the mechanism by which they may exert their thrombotic action.

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A Factor in the Serum of Tumour-bearing Rats which is Deleterious to Cells in Tissue Culture

In attempts to improve the growth of a transplantable rat carcinoma (*S.O.K.*1)* in tissue culture by the addition of rat serum, it was observed that sera from rats bearing *S.O.K.*1 did in fact exert a deleterious effect on the cells. This observation was investigated.

*S.O.K.*1 is a squamous cell kidney carcinoma which arose spontaneously in 'John's' isogenic strain of rats. The tumour is regularly transplanted subcutaneously in these rats and up to the time of writing has been through 23 transfers. Primary cultures of the tumour were grown in a lactalbumin hydrolysate medium with 20 per cent calf serum and all tests were put up in a modified Parker's 199 medium[1]. Cultures were grown for 24 h or 48 h before addition of the rat serum and results were recorded after a further 24 h incubation.

The effect of serum from *S.O.K.*1 bearing rats on cells in culture was to cause them to lose their ability to adhere to the glass. Ultimately the cells died, but it was impossible to tell whether death was due directly to some deleterious factor in the serum or to the inability of the cells to survive in an unattached state. Some cell lysis occurred, but again this could not be definitely attributed to this factor.

In order to test the point, 32 sera from tumour-bearing rats have been examined as 10 per cent solutions in 199 medium and compared with a normal serum control. Results are shown in Fig. 1. It can be seen that the effectiveness of the sera against *S.O.K.*1 cells in culture increased with the tumour size. Fig. 2 illustrates the results of a single experiment.

Experiments showed that the tumour factor was also active against other cell lines, namely, rat embryo, *R.I.B.5** (a transplantable rat sarcoma) and *Hep.* 2.

Sera of rats examined after operative removal of *S.O.K.*1 showed the usual activity in tissue culture immediately after operation but it was completely lost after 7 days. The activity was, however, restored by a regrowth.

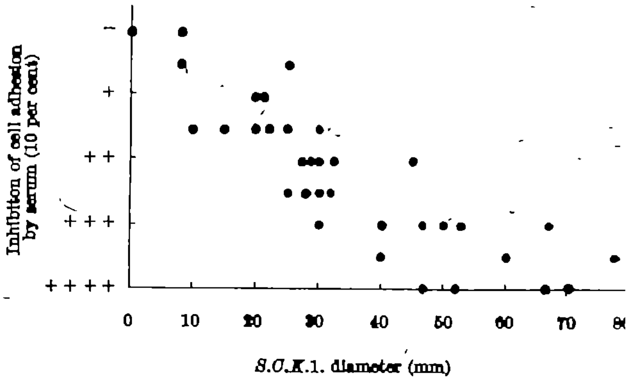

Fig. 1. Correlation between size of tumour and inhibition of adhesion of cells in culture by serum. + + + +, Complete inhibition; + + +, + +, +, approximately 75, 50 and 25 per cent inhibition, respectively

Interstitial fluid from *S.O.K.*1 caused complete or almost complete inhibition of cell adhesion when added to cultured cells. Normal tissue fluids tested did not have this effect. This gives further confirmation of results reported here. The factor was also found on dialysis to appear in the dialysate so presumably is small enough to pass fairly freely into the rats' circulation.

A similar dialysable factor which is cytotoxic for strain *L* cells in culture has been demonstrated by

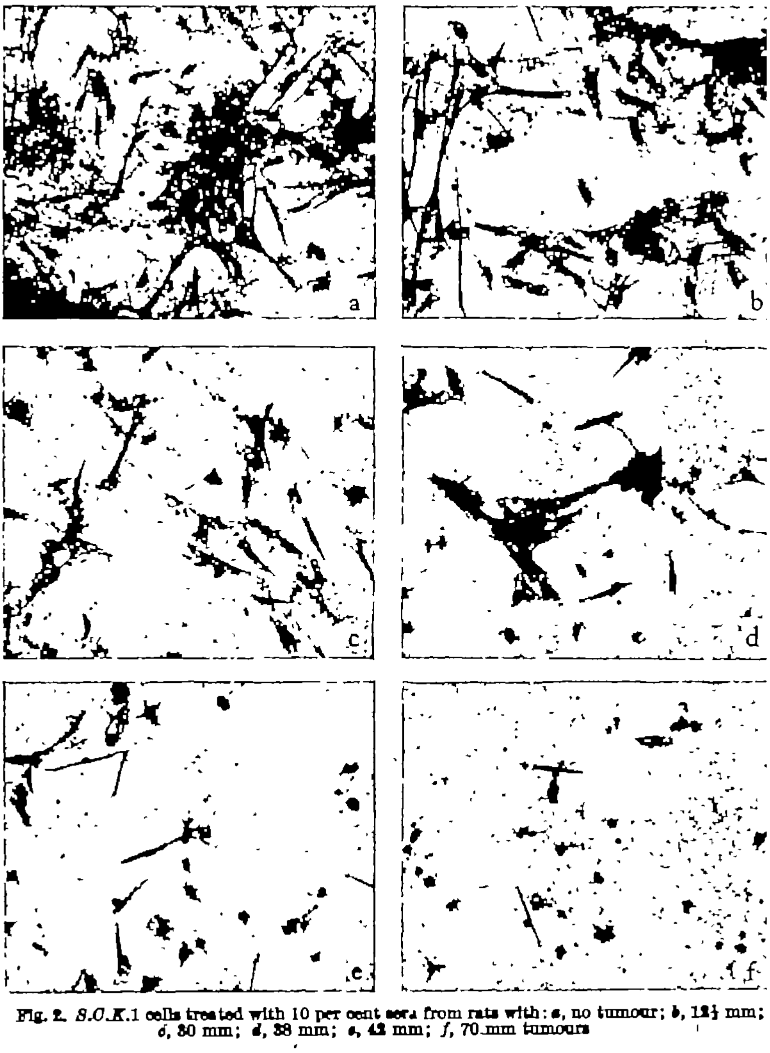

Fig. 2. *S.O.K.*1 cells treated with 10 per cent sera from rats with: *a*, no tumour; *b*, 12½ mm; *c*, 30 mm; *d*, 38 mm; *e*, 42 mm; *f*, 70 mm tumours

B. Holmberg[2] in Walker carcinoma and mouse Erhlich–Landschutz hyperdiploid transplants.

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Renal Disease associated with Positive Lupus Erythematosus Tests in a Cross-bred Strain of Mice

A STRAIN of mice *NZB/BL* suffering from an auto-immune type of hæmolytic anæmia has been described[1,2]. Since then it has been shown that approximately 4 per cent of this strain have positive lupus erythematosus cell tests and that many of the animals show renal changes resembling those seen in human systemic lupus erythematosus[2,3]. In this paper it was also shown that F_1 and F_2 progeny derived from a cross between mice of the *NZB/BL* and the *NZY/BL* strains showed a low incidence of hæmolytic anæmia but 25 per cent of the animals had positive lupus erythematosus cell tests and all had renal changes resembling lupus nephritis. These animals died from renal failure within 18–24 months of age.

Recently, hybrids derived from a cross between the *NZB/BL* strain and an inbred strain of white mice known as *NZW* have been shown to die of renal failure within 8–10 months. All animals show positive lupus erythematosus cell tests and all have florid renal changes showing close identity with those seen in human lupus nephritis.

The kidneys may appear enlarged, dusky pink in colour, flecked with multiple red specks, or contracted, typically *café au lait* coloured and granular. Early glomerular changes are hypercellularity with unfolding and thickening of the capillary walls to form typical wire-loop deformities. Lymphoid deposits around the hilar vessels spread along their course towards the cortex and are usually heavily populated with plasma cells and Russell body forms. The renal tubules contain hyaline casts of periodic acid–Schiff-positive material and often granules or droplets of similar material may be seen in the cytoplasm of the proximal convoluted tubules.

Other lesions are found according to the stage or intensity of the disease process. In the most florid cases large areas of fibrinoid necrosis involve the basement membrane and cellular elements of the glomerulus, while others show numerous so-called hyaline thrombi and occasional hæmatoxylin bodies. Approximately one-third of cases show fibrinoid necrotic lesions in the walls of medium and small renal vessels. In these cases similar vascular changes have been noted in the spleen, lymph nodes, stomach, thyroid and myocardium.

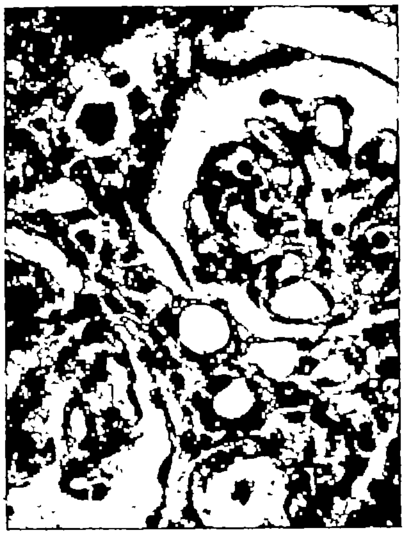

Fig. 1. Wire-loop deformity of glomeruli (hæmatoxylin–eosin, × c. 320)

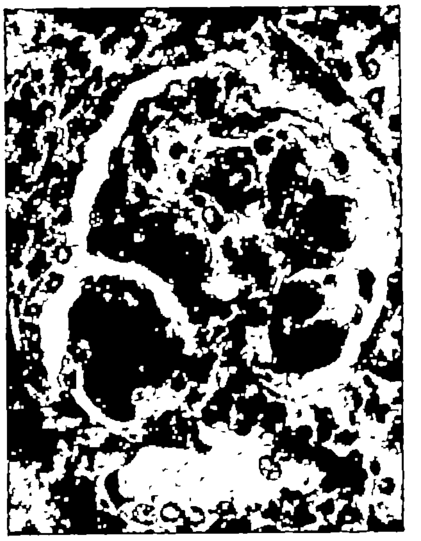

Fig. 2. Hypercellularity and fibrinoid necrosis of glomerular tuft (hæmatoxylin–eosin, × c. 320)

It is believed that this hybrid strain will prove invaluable in research into the nature and therapy of systemic lupus erythematosus.

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RADIOBIOLOGY

Cæsium-137 in Dried Milk Products in Relation to Phytoclimatic Zones

A RECENT evaluation of radiological data gathered by the U.S. Atomic Energy Commission's Health and Safety Laboratory includes a statement that the concentration of fission products in commercial milk is essentially independent of total rainfall[1,2]. However, the precipitation values were presented as arbitrary ranges and the landscape units used were not strictly biological regions. The purpose of this communication is to show that if radiological data from the same source are arranged according to natural landscape units a relationship between phytoclimatic zones and the cæsium-137 content of milk is indicated.

For our purpose, the geographical region considered is the north-western portion of the United States where broad areas are mapped as distinctive natural vegetation zones[3]. Such broad areas are necessary to allow reasonable assurances that milk sample stations actually derive milk from an area representative of a particular vegetation zone.

Ten of eleven milk sampling stations in the north-west region were grouped into one of three natural vegetation zones (Table 1). Portland, Oregon and Burlington, Washington, were assigned to the Pacific north-west coniferous forest zone. Bismarck, Bottineau, and Cando, North Dakota; and Mitchell, South Dakota, were assigned to the grassland zone. Sunnyside, Washington; Payette and Idaho Falls, Idaho; and Monroe, Utah, were assigned to the sage-brush zone. Because of its proximity to the Wasatch Mountains, which provided a diversity of vegetation zones within a small area, the milk sampling station of Ogden, Utah, was excluded.

Cæsium-137 is deposited on aerially exposed plant parts directly from the atmosphere and the amounts ingested and assimilated by grazing animals are dominantly from this source rather than from plant uptake via contaminated soils[4]. Total precipitation is regarded as important in depositing cæsium-137 over landscapes and the amounts deposited are related to the amount of precipitation; moist regions receive more cæsium-137 than regions of less moisture[5].

The cæsium-137 content of milk samples in pc./g potassium from stations grouped according to vegetation zones is presented in Table 1. Milk products from sampling stations in the coniferous zone averaged 90 pc./g potassium as compared with only 54 and 37 for grassland and sage-brush zones. Total precipitation from weather stations near the milk sampling stations showed the coniferous forest zone stations as receiving an average of 61 in. from January 1, 1959, until June 1960. This was compared with an average of only 29 and 12 in. from grassland and sage-brush zones respectively. It is realized that precipitation readings from a few stations probably do not represent an accurate measurement of the precipitation over the entire milk harvest areas; nevertheless, natural vegetation reflects the biological effectiveness of precipitation and the boundaries of these may be used to delimit long-term biological climates. It has long been recognized by plant ecologists that total annual precipitation in the north-western United States is not sufficient to differentiate sage-brush and grassland zone climates[6]. The seasonal distribution of precipitation in the sage-brush zone is concentrated in winter months, while that of the grassland zone is concentrated in spring and summer (Table 1). Seasonal precipitation could greatly influence the amounts of cæsium-137 deposited on foliar surfaces. In temperate climates having a definite seasonal agricultural yield foliar deposited cæsium-137 reflects the amounts largely accumulated during the summer photosynthetic period. Forages grown in the semi-arid sage-brush zone seldom experience summer precipitation and growth water is supplied by irrigation. In the grassland zone growth of forage crops and summer precipitation coincide to yield higher cæsium-137 values in contrast to the non-synchrony of biological and meteorological events and lower cæsium-137 values in the sage-brush zone.

Table 1. AVERAGE CÆSIUM-137 PC./G POTASSIUM IN DRIED MILK PRODUCTS AND PRECIPITATION FOR MILK SAMPLING STATIONS IN THE NORTH-WESTERN UNITED STATES, JANUARY 1959–JUNE 1960, GROUPED ACCORDING TO NATURAL VEGETATION ZONES

Vegetation zone	Sample station	No. of samples	avg. ^{137}Cs pc./g K	Precipitation total inches	% of ppt in winter months
Coniferous forest	Portland, Oreg.	65	89	67	52
	Burlington, Wash.	75	92	55	47
			avg. 90	avg. 61	
Grassland	Bismarck, N.D.	67	65	20	10
	Bottineau, N.D.	77	51	29	5
	Mitchell, N.D.	58	42	37	12
	Cando, N.D.	31	48	—	—
			avg. 54	avg. 29	
Sage-brush	Sunnyside, Wash.	78	22	9	55
	Payette, Ida.	70	43	18	49
	Idaho Falls, Ida.	73	49	9	28
	Monroe, Utah	73	34	—	—
			avg. 37	avg. 12	

The higher average cæsium-137 content of dried milk products produced in the moist forest zone appears related to the greater precipitation in this zone in contrast to the grassland and sage-brush zones. The lower average cæsium values in milk products produced in the sage-brush zone in contrast to grassland zone appear as a result of a non-synchrony of phenological and meteorological events induced by land management practices.

To test statistically the effect of vegetation zones in relation to the cæsium-137 content of milk an analysis of variance was performed which included seasonal effects The hypothesis of no zonal effect was rejected at the 1 per cent level of significance. Additional inferences were obtained by subsequent tests for judging contrasts[7]. All three contrasts between means were significant at the 1 per cent level. The computed values: $\psi_1 > 12$ pc. cæsium-137/g potassium; $\psi_2 > 32$ cæsium-137 pc./g potassium; where ψ_1 is the difference between the means of the grassland zone and the sage-brush zone and ψ_2 is the difference between the means of the forest and grassland zones.

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Decreased Incorporation of Phosphorus-32 in Radiosensitive Organs after Whole-body Irradiation in Rats

CONSIDERING the significant role of phosphorus in transferring and in passing energy into the organism, we investigated the distribution of the intravenously applied $Na_2H^{32}PO_4$ in the individual organs of the non-irradiated group of five rats and the group of five rats exposed to an X-ray dose of 600 r. Irradiation was carried out with the Mackrophos 250 X-ray apparatus of 200 kV, 15 m.amp, filtration 0·5 mm copper + 0·5 mm aluminium, focus distance of 120 cm. dose rate of 10 r./min. The irradiation doses were controlled by a Mekapion dosimeter.

$Na_2H^{32}PO_4$ was applied to the experimental animals, 25 μc./200 g weight with an original specific activity of 13·1 mg phosphorus/ml., *p*H 7·2.

The rats were divided into groups (each group consisting of five rats) and the application was made for one day for all animals. The rats were killed by decapitation after 24 h, 3, 7, 14 days and the activity in blood, bone, marrow, brain, thymus, spleen, liver, kidney, small intestine, muscles, testes and bone compacta was examined. The activity was determined individually after scoring organs by means of the end-window Geiger–Müller tube (type 33/50), window thickness 4·0–5·2 mg/cm^2.

The mean error was calculated from the formula $\delta = \pm \sqrt{\frac{\Sigma \Delta^2}{n(n-1)}}$; the mean error is shown in Fig. 1, without making use of the samples of bone marrow, since we were short of sufficient material for the investigation and therefore we summarized samples of five animals.

The results obtained may therefore be divided into three groups. The first group consists of the radiosensitive organs: bone marrow, thymus, spleen and intestine As is apparent from Fig. 1, the percentage of the incorporated phosphorus-32 in these organs of irradiated animals is significantly smaller during the whole period of investigation than in the case of non-irradiated animals

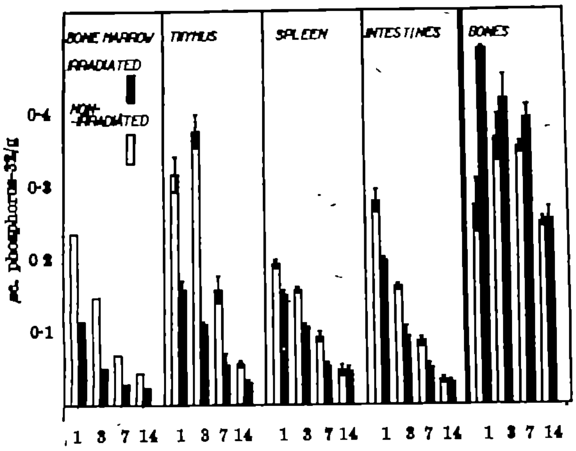

Fig. 1. Incorporated phosphorus-32 in radiosensitive organs and bones in controls and whole-body irradiated rats

Together with the progressive regeneration of the post-irradiation changes we found, on the fourteenth day of the investigation, that the values of the incorporated phosphorus-32 were normal.

A rather different character of incorporation is observed in the compact bone where the degree of uptake of phosphorus-32 is greater during the whole period of investigation in the irradiated animals.

The level of phosphorus-32 in the blood is significantly higher the first day after the irradiation, and from the seventh day it increases substantially.

Our results are in agreement with the findings of Olson *et al.*[1] and Florsheim and Morton[2]; but they differ from the findings of Sherman and Forssberg[3], who observed an increase of phosphorus compounds (sodium phosphate—for labile phosphates in the acid medium, ATP and for soluble phosphates in the acid medium) in liver.

The increased values of phosphorus-32 in the blood of irradiated animals are in agreement with the results obtained (to be published) concerning the excretion of phosphorus-32 by the urine in the irradiated rats[4].

With regard to the phosphorylation processes, the decrease of the phosphorus content in the deoxyribonucleic acid after irradiation[5,6], we conclude that our results are in agreement with the aforementioned findings.

It has been shown that the radiosensitive organs are unable to make use of the applied phosphorus metabolically after irradiation.

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Factors modifying the Post-Radiation Effects of Homologous Spleen

IN 1954 Barnes and Loutit[1], investigating an unrelated problem, observed that splenic cell suspensions from infant mice can protect adult homologous recipients from the early death induced by supra-lethal X-radiation. The protected animals died after 30 days apparently from what is now recognized as 'secondary disease'. Using different mouse strain combinations, Schwartz *et al.*[2] noted that administration of adult parental splenic cells into supra-lethally X-radiated F_1 hybrid animals results in an early death of the injected recipients, to the extent that they die even earlier than the uninoculated irradiated controls. A similar phenomenon of early mortality was also observed by Cole *et al.*[3], who showed that parental adult mouse spleen cells induce death within a week when given to sub-lethally X-radiated F_1 hybrids. Splenic cells from the same parental strain were also able to induce death within 8 weeks in some of the injected F_1 non-irradiated recipients. Makinodan *et al.*[4], using completely homologous combinations and supra-lethal X-radiation, were also able to confirm the observations of the early death induced by the infusion of the splenic adult cells.

As an explanation for the 'killing effect' induced by injection of homologous adult spleen cells the hypothesis was advanced that immunologically competent donor cells are rapidly established within the defenceless host and kill it by an overwhelming graft-versus-host reaction. With infusion of infant homologous spleen cells the early 'killing effect' is avoided presumably because the donor cells are immature and incapable of immunological reaction. The recipients are protected and survive the early lethal effects of acute X-radiation; they succumb, however, later as a result of a secondary disease appearing as soon as maturation restores the immunological competence of the donor cells. In this case the sequence of events bears a striking similarity to the phenomena observed when adult homologous bone marrow is infused in similar circumstances. Adult marrow differs from adult spleen in that the former contains only few immunologically reactive elements. Similarly, and in contrast to the adult spleen, the infant spleen consists largely of hæmopoietic elements while the immunologically active cells are limited in number. It is, therefore, conceivable that the chronological difference in the appearance of the graft-versus-host reaction produced by the respective infusion of adult and infant splenic cells in a homologous recipient is merely an expression of the difference in the number of immunologically competent cells present in the two organs. The differences, in other words, between adult and infant spleens in their behaviour as homologous donors could be explained on a simple quantitative basis.

In order to investigate this hypothesis it is necessary to examine parameters influencing the phenomenon of the 'splenic death': (*a*) dose of irradiation; (*b*) degree of histo-incompatibility; (*c*) time of the post-radiation splenic injection; (*d*) dose of splenic cells injected; (*e*) age of the donor of splenic cells. This communication deals with the examination of the first three parameters.

BDF_1 female mice 18–25 weeks old were used as recipients. Splenic cell suspensions were prepared from female *CBA*, *A* and C_3H mice. The animals were irradiated with a clinical X-ray unit operating at 200 kVp, 20 m.amp. with 0·50 mm aluminium and 0·25 mm copper added filtration. Target distance was 7 cm and dose rate 118–120 r./min. Total doses used are referred to in the text. All splenic infusions were given at a standard dose of $12·5 \times 10^6$ cells via tail vein, within the first 24 h after exposure. Non-infused animals served as controls. No supportive therapy was given. The animals were observed for a period of 60 days.

Table 1 summarizes the effects of various X-ray exposure-levels on the 'killing effect' of the spleen. With doses varying from 400 to 500 r. no deaths were observed either in the control or among the treated animals. Exposures at 600 r. caused death within three weeks to all the animals treated with *CBA* or *A* spleen cells. During the same period the animals infused with C_3H spleen cells as well as the control animals survived. With C_3H spleen cells the 'killing phenomenon' was demonstrated at the 675-r. X-radiation level. All recipients

Table 1. CUMULATIVE DATA ON THE SURVIVAL OF MICE INFUSED WITH DIFFERENT HOMOLOGOUS SPLEEN CELLS FOLLOWING VARIOUS EXPOSURES TO X-RADIATION

X-ray dose (r.)	No. of BDF_1 recipients	Donor strain 12 5 × 10⁶ spleen cells	Survival expressed as dead/alive animals at the end of days 8	15	21	30	60
400	110	None	All animals survived				
450	88	*CBA*					
500	44	*A*					
	44	*C_3H*					
600	44	None	0/44	0/44	0/44	3/41	9/35
	66	*CBA*	16/50	57/9	66/0	—	—
	66	*A*	23/43	52/14	66/0	—	—
	60	*C_3H*	0/60	0/60	0/60	2/58	5/55
675	33	None	0/33	0/33	5/28	12/21	17/16
	44	*CBA*	19/25	37/7	44/0	—	—
	33	*A*	15/18	25/8	33/0	—	—
	33	*C_3H*	7/26	27/6	33/0	—	—
750	22	None	0/22	2/20	8/14	11/11	11/11
	33	*CBA*	21/12	29/4	4/0	—	—
	33	*A*	26/7	30/3	33/0	—	—
	22	*C_3H*	12/10	22/0	—	—	—
800	143	None	0/143	10/133	28/115	75/68	136/7
	11	*CBA*	7/4	11/0	—	—	—
	22	*A*	15/7	21/1	22/0	—	—
	22	*C_3H*	7/15	21/1	22/0	—	—
900	44	None	9/35	34/10	44/0	—	—
	22	*CBA*	15/7	22/0	—	—	—
	11	*A*	9/2	11/0	—	—	—
	11	*C_3H*	8/3	11/0	—	—	—

were dead within three weeks in contrast to the radiation control animals, which showed an insignificant rate of mortality during the same period of time. Above this radiation-level, no homologous spleen recipient survived more than 21 days. In fact the majority of the animals died within the first 15 days. The results suggest the existence of a radiation threshold, above which all injected animals die. Within the given strain combinations, the phenomenon is reproducible when the splenic cell dose and the post-irradiation time of injections are kept constant.

In mice, apart from other apparently non-critical systemic damages, sub-lethal radiation seems to inflict its major effect on the reticuloendothelial system of the animals. The difference between survival and death after varying amounts of X-radiation may depend on the degree of damage to the immunologially competent *RE* cells in the host. When immunological responsiveness was not sufficiently impaired by X-radiation the host could reject the donor spleen cells and survive. Larger amounts of irradiation presumably destroyed this capacity to react against the donor spleen cells, which were therefore able to act against the host. This postulate is attractive, and it could be greatly enhanced if the X-radiation threshold proved to be dependent on the number of splenic cells infused. Should this not be the case, however, other explanations must be sought, for example, a possible 'all-or-none' damaging effect of sub-lethal X-radiation on the *RE*-host system, which either survives or is destroyed as a whole.

The results also indicate that histo-incompatibility plays an essential part in the induction of the 'killing effect'. They also indicate that the *H*–2 locus is not the only locus which determines the degree of histo-incompatibility. Spleens from *CBA* and *A* animals although of varied *H*–2 locus composition show identical X-radiation threshold as to the 'killing effect'; on the other hand, *CBA* and *C_3H* donor spleen have different thresholds in spite of their $H\text{-}2^k$ similarity.

Another aspect of interest is that the 'killing effect' depends on the time of the post-radiation administration of the homologous spleen suspensions (Table 2). The 'killing effect' is always present when infusions are made within 20 h following X-radiation. The phenomenon disappears when the spleen cells are given 5 days after exposure to the threshold dose of X-rays. It is interestir to note that the lethal effect of homologous spleen is sti present 5 days later if the recipient is exposed to high X-ray doses (Table 2). An explanation for this 'tim effect could be that the recovery of the host *RE* syste allows the rejection of the splenic graft. Supporting th explanation is also the fact that mice infused at day 5 n only survive but do not develop secondary disease at later stage.

Table 2. EFFECTS OF THE POST-X-RADIATION INFUSION TIME AND THE X-RADIATION DOSE ON THE LETHAL EFFECT OF HOMOLOGOUS SPLEEN

X-ray dose (r.)	Donor strain of 12·5 × 10⁶ spleen cells	No. of BDF_1 recipients	Post-rad. infusion time	Survival for 21 days
600	*A*	22	24 h	none
600	*A*	22	5 days	all
675	*A*	11	24 h	none
675	*A*	11	5 days	none

Addendum: During the preparation of this manuscript dublication (*Transp. Bull.*, 30, 29; 1962) by G. E. Cosgrov *et al.* appeared describing similar results, in parental *F* hybrid mice combinations.

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Unfavourable Response of Radiation Nephritis to Administration of L-Triiodothyronine

RADIATION injury is a dynamic state, not a static one and therefore feasibly modifiable[1]. Numerous compound have been tested for possible advantageous effects o radiation injury, but with the exception of 3,5,3′-L-tri iodothyronine (T-3), administration of these agents witl established damage has not been beneficial. It is o interest that in spite of the apparent property of T-3 t sensitize tissues to irradiation[2,3], this same material i able to alter favourably tissue injured by irradiation.

L-Triiodothyronine was initially selected for investiga tion by Glicksman *et al.* as a possible modifier of radiatio injury on the basis of observations that thyroid analogue caused decrease in established radiation damage of ski and subcutaneous tissues in patients previously irradiate for carcinoma of the thyroid. Their recent reports[4,] demonstrate a favourable response of radiation ski necrosis, ulceration, and fibrosis in humans and experi mental animals to T-3 administration.

T-3 causes marked decrease in subcutaneous fibrosi and improvement in the blood supply. Since the respons of kidneys to high doses of radiation is, as in the skin primarily a fibrotic and atrophic process, it seeme reasonable that T-3 might also have a salubrious effec on heavily irradiated kidneys.

To test this hypothesis the left kidneys of 14 youn adult New Zealand rabbits, weighing an average o 1·9 kg, were irradiated *in vivo* to a total dose of 3,90 rads (three treatments of 1,300 rads a week apart) wit cobalt-60. Eight weeks after conclusion of irradiatio the animals were randomly divided into two groups Thereafter, the first group of rabbits received 25 μg/k T-3 subcutaneously daily. The other group was com posed of control animals and received no further treat ment.

Table 1. STRIPPED KIDNEY WEIGHTS (G) AT NECROPSY

Weeks post-irradiation	Control group Left kidney	Right kidney	T-3-treated group Left kidney	Right kidne
20	4·0	16 8	1·6	12·2
20	4·5	7 7	2·3	11·2
20	4·0	6·4	2·8	11·0
26	1·8	12·0	1·9	13·0
26	4 2	18 0	0 5	12·5
26	2·6	9·4	1·0	10 6
26	2 4	13 2	2·3	10·0
Average	3 4	11·9	1 8	11·7

At necropsy, 20 and 26 weeks post-irradiation, the stripped kidney weights were recorded. As shown in Table 1, the average weight of the control left kidneys was 3·4 g and that of the kidneys from the T-3 treated animals was 1·8 g. The difference in weights is statistically significant ($P < 0·01$) in spite of the small number of animals. The average weights of the right kidneys were 11·9 g and 11·7 g respectively. At necropsy the control animals were slightly heavier (2·9 kg average) than the animals treated with T-3 (2·5 kg average); but the absence of difference in the right kidney weights excludes the importance of this discrepancy.

Evaluation of histological change in the kidneys of the two groups is in progress. Preliminary interpretation suggests correlation with the gross findings; that is, more severe histopathologic change in the irradiated kidneys of the T-3 treated animals than in the irradiated kidneys of the control group.

One can argue that the dose of T-3 used was too large to alter the severe radiation nephritis produced. However, Glicksman and associates[4,5] kept their patients and experimental animals at near toxic levels to achieve modification of the skin damage. In spite of T-3 administration the rabbits in our experiment maintained their average weight of 2·5 kg, so the dosage was not apparently excessive.

Possibly at lower radiation doses there would be less severe reaction which could be altered by T-3. However, the apparent detrimental effect at the radiation dose used in this series belies this supposition.

We have no reasonable explanation for this unexpected deleterious effect of 3,5,3′-L-triiodothyronine. Nevertheless, the presented results suggest that T-3 is not an effective modifier of radiation damage, at least that involving the kidneys of rabbits.

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BIOLOGY

Occurrence of a Warty Layer in Vascular Cells of Plants

Electron microscopic observations have revealed that mature tracheids of conifers are lined on the inside with a warty layer. It consists of more or less spherical particles of an average diameter of 0·1–0·3μ, which are often covered by an amorphous layer. The appearance and properties of the warty layer vary according to species[1–5]. It develops during the last stages of cell differentiation and consists of the remnants of the protoplast. Consequently the existence of a warty layer should not be limited only to the conifer tracheids. Light microscopical observations have shown that vessels, tracheids and fibres of many hardwood species have also a granular appearance[6]. Our investigations with the electron microscope demonstrate that this structure corresponds to the warty layer (Fig. 1).

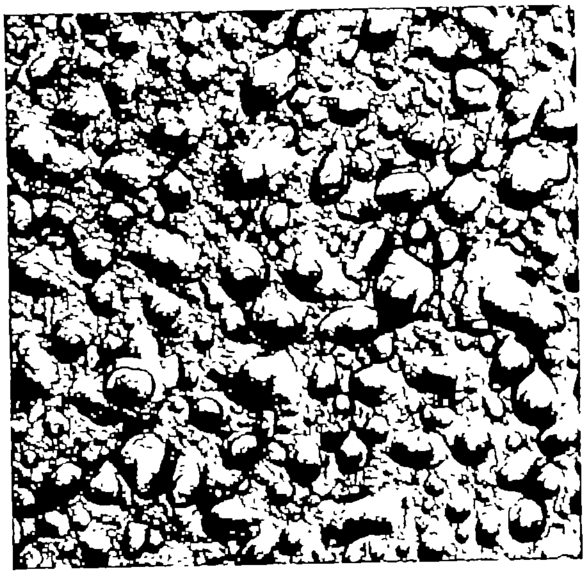

Fig. 1. Warty layer covering the inner side of a vessel wall of *Fagus silvatica* (× 10,000)

The presence of a warty layer has been reported so far only for lignified cell elements of woody species. If the origin of this layer is connected with the death of protoplasm in the final stage of differentiation of vascular cells, it might exist in such cells of herbaceous plants as well. Observations with the light microscope of longitudinal sections of several genera such as *Fuchsia*, *Geranium*, *Helleborus*, *Lavendula*, *Malva*, *Mesembrianthemum*, *Plantago*, *Rosmarinus*, *Saxifraga*, *Vicia*, have established that vascular cells in stems and leaves are covered with a granular layer. In the different plants it can be seen with varying degrees of clarity. For a more detailed investigation *Plantago major* L. was selected. Vascular bundles from petioles and leaves were fixed in osmium tetroxide, embedded in epoxy resin, and the sections were stained with lead acetate.

Vessels and also fibres show in the late stages of differentiation a distinct lining of the wall, which consists of two

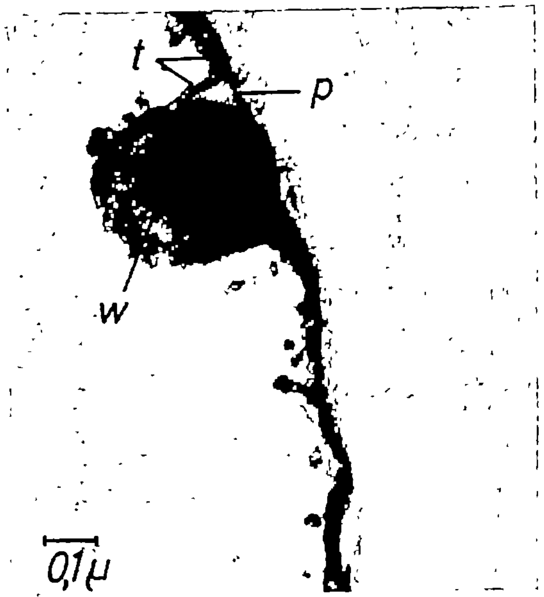

Fig. 2. Portion of vessel wall in section from *Plantago major* showing wart (*w*) which rests on the plasma membrane (*p*) and is covered by the tonoplast (*t*) (× 66,000)

membranes—the plasma membrane and the tonoplast—with the remaining cytoplasm between them. Particles of varying size are present within the dying cytoplasm. With progressive differentiation the cytoplasm condenses and the tonoplast is brought close to the plasma membrane. The particles which remain are sandwiched between these membranes and form excrescences into the cell lumen (Fig. 2). In surface view these particles give the appearance of a warty layer. Thus the origin of the warty layer in *Plantago* is similar to that recently found by Wardrop and Davies[7] for the tracheids of *Actinostrobus*. However, in the case of *Plantago* we do not regard the warts as local thickenings of the cell wall, even in part, but rather as components of the cytoplasm which have become cemented to the wall in the last stages of differentiation.

The observations recorded here have shown that the warty layer is not only present in the lignified cells of woody species, but also in the vascular cells of herbaceous plants. Therefore its occurrence can be regarded as a general structural feature of plant cells, which is derived from the dying protoplast.

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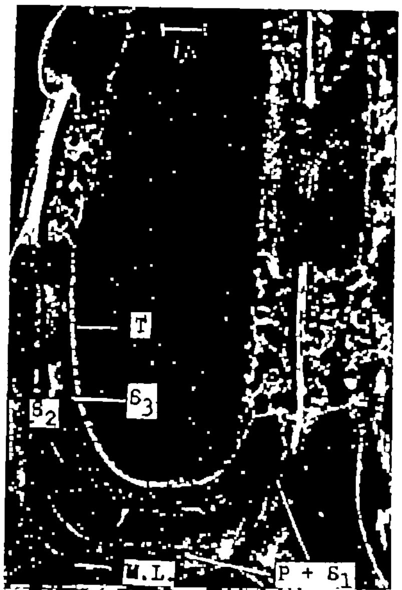
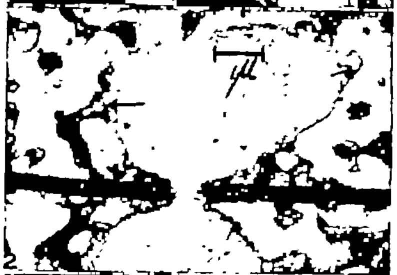
Nature of Vestured Pits in Angiosperms

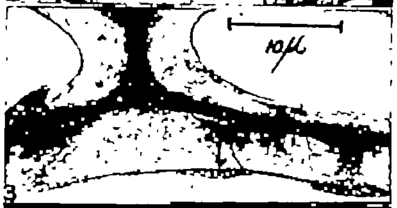
VESTURED (cribriform) pits occur in many families of the dicotyledons and in many instances serve as taxonomic criteria between families. The nature of the vestures was first recognized by I. W. Bailey[1] as "minute outgrowths from the free surfaces of the secondary wall". General confirmation of this view has been obtained in a recent electronmicroscopic study of the vestured pits of *Eugenia* sp. In Fig. 1 the general form of the vestures and their attachment to the cell wall are apparent. In the unshadowed section in Fig. 2 it can be seen that the vestures represent outgrowths of the secondary cell wall and in some instances appear to arise from the pit membrane as well. It may be noted from Figs. 1 and 2 that the texture of the vestures is heterogeneous in that the outgrowth of the cell wall appears to be covered by a membrane which is extremely dense to electrons. This membrane is continuous over the vestures and forms a lining around the inner surface of the cell wall adjacent to the lumen (Fig. 1).

Sections cut after maceration with hdryogen peroxide acetic acid showed that this treatment removed the lining membrane, but the enclosed outgrowth of the cell wall forming the vesture was attacked at apparently the same rate as the cell wall itself. The lining membrane (Figs. 1 and 2) was strongly absorbing in ultra-violet light. In some instances the vestures extended laterally beyond the aperture into the cell lumen (Fig. 3).

In view of recent investigations in this laboratory into the nature of the wart structure of gymnosperm tracheids which showed it to consist of a number of outgrowths of the inner layer of the secondary wall, it is reasonable to suppose that the vestures represent an analogous type of cell wall sculpturing in angiosperms. In cells where the vesturing extended beyond the pit aperture structures closely resembling the wart structure of gymnosperms were observed (Fig. 4). The wart structure in angiosperms has already been recognized by Liese and by Harada (private communications*). It is assumed, by analogy with studies of the development of the wart structure[2], that the dense membrane lining the cell lumen (Figs. 1 and 2) corresponds to the "terminal lamella" of Frey-Wyssling[3], and, as pointed out elsewhere[4], arises from the denatured membranous elements of the cytoplasm.







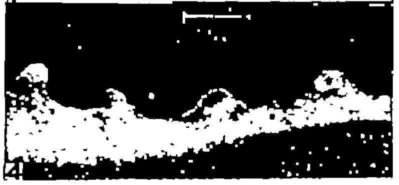


Fig. 1. *Eugenia* sp. A transverse section showing vestured pits between two vessels (right) and a vessel and parenchyma cell (left). Shadow-cast with platinum–palladium. $P + S_1$, Primary wall and outer layer of the secondary wall; S_2, middle layer of the secondary wall; S_3, inner layer of the secondary wall; T, terminal lamella

Fig. 2. An unshadowed section similar to Fig. 1 showing the continuity of the vestures with the cell wall (arrows). Note the difference in scattering power of the primary wall in the pit membrane as compared with the cell wall

Fig. 3. *Eucalyptus regnans*. An ultra-violet photomicrograph of a transverse section through part of a vessel and adjacent tracheids showing the ultra-violet absorption of the vestures and their lateral extension beyond the pit apertures of the vessel

Fig. 4. *Eugenia* sp. Part of a transverse section of the cell wall of a vessel similar to that in Fig. 1, showing the wart structure. Shadow-cast with platinum–palladium

An investigation of the development of vestured pits a special aspect of the general problem of cell wall rmation is in progress.

Incidental to the foregoing observations, it may be noted Fig. 1 that at the pit border the inner (S_2) and outer ,) layers of the secondary wall are not confluent.

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[See also preceding communication.—Editor.]

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Soluble Components of the Cambial Zone of *Eucalyptus regnans* F. Muell.

SEVERAL investigators have shown that the nutrient ids in the phloem and, in some instances, the liquors in e cambium of arborescent gymnosperms and angio-erms contain some of the following constituents[1]: mono-ccharides, chiefly glucose and fructose with traces of ntoses (mainly xylose); uronic acids, sorbitol and nnitol; disaccharides, chiefly sucrose; trisaccharides, including raffinose and 1^F-β-fructosylsucrose; higher oligosaccharides such as stachyose and verbascose; phenolic glycosides; oxalic, succinic, citric and lactic acids; amino-acids and peptides; inorganic salts: etc.

The work reported here represents an attempt to identify and to estimate quantitatively the major soluble carbohydrate substances of the cambial zone of *Eucalyptus regnans*. Shortly after the vernal resurgence of cambial activity, the bark was stripped from freshly felled trunks of several 18-year-old trees of *E. regnans*. Immediately after removal of the bark, the soft outer layer of the logs was collected by means of a 'Perspex' scraper[2]. The scrapings, which included the cambium and adjacent differentiating xylary elements (that is, the cambial zone), were plunged into boiling ethanol (final concentration 80 per cent) in order to inhibit enzyme activity. The subsequent examination of the cambial material and the results obtained are outlined in Fig. 1. The sub-division of soluble materials into acidic, neutral and basic fractions was achieved by the use of ion exchange resins 'Amberlite *IR* 120' (H) and 'Duolite *A* 4' (OH).

The substances present in acidic fraction I were identified by paper chromatography. The presence of a spot corresponding to glucuronolactone and the absence of a brick-red colour when the uronic acid spot was sprayed with the basic lead acetate reagent of Gee and McCready[3] indicated that the uronic acid detected was glucuronic acid rather than galacturonic acid. The occurrence of

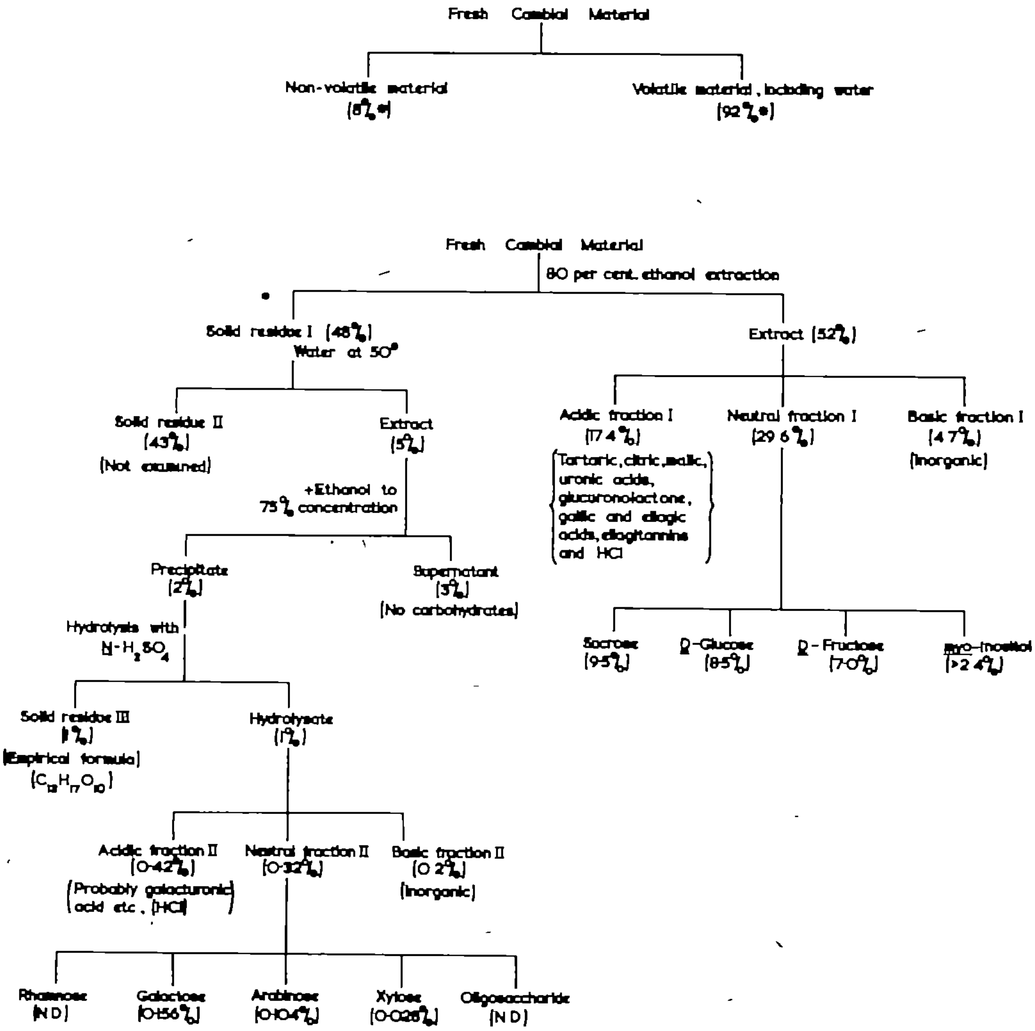

Fig. 1. Fractionation of extracts from the cambial zone of *E. regnans*

* Expressed on the basis of fresh weight of material; all other results are on the basis of oven-dry material.
N.D., Not determined.

appreciable quantities of malic, citric and tartaric acids may be associated with the operation of the citric acid cycle either within the areas of secondary growth or within photosynthesizing cells; in the latter case excess production of these acids may result in their excretion from the photosynthesizing cells and their accumulation, after translocation, in the cambial zone.

Sucrose, D-glucose, D-fructose and *myo*-inositol, which were determined quantitatively by chromatography[4], represent more than 90 per cent of neutral fraction I; they were characterized by the formation of derivatives, by physical properties and by infra-red spectroscopy. *myo*-Inositol has not been isolated previously from cambial samples, and is of particular interest as a possible intermediate between carbohydrates and aromatic compounds and as a possible precursor of D-glucuronic acid[5].

The water extract of solid residue II contained material which could be precipitated on the addition of ethanol. The precipitate, during hydrolysis with dilute acids, yielded substances which were chromatographically identical with rhamnose, galactose, arabinose, xylose and galacturonic acid. The relatively large amounts of galactose and arabinose suggest that pectic substances may be present in the water-soluble polymer.

It has been suggested that substances with reactive groups are transported, generally in an inactivated state, to the regions of active secondary growth[6]. For example, glucose, fructose and galactose are usually translocated, in the assimilate stream, as sucrose and as higher oligosaccharides of the raffinose series of sugars[7]. Hence the water-soluble polymer may represent an inactivated mobile complex of monosaccharide residues which could be utilized, following enzymatic hydrolysis in the cambial regions, as base units which are immediately synthesized into cell-wall polysaccharides.

The water-soluble polymer appears to contain certain very reactive units because, during hydrolysis with dilute acids (even at room temperature), the insoluble material (solid residue III) is formed rapidly. Once formed it will not redissolve either in organic solvents or in aqueous solutions of acids or of alkalis. It does not seem to be related to lignin because it gives a negative Wiesner test[8], it is not aromatic (from infra-red spectroscopy) and it contains very few methoxyl groups. Infra-red spectroscopy indicated that the insoluble material is predominantly organic in nature and contains carbonyl and hydroxyl groups but does not contain carboxyl groups. The empirical formula $(C_{12}H_{17}O_{10})_x$ of the insoluble substance considered in conjunction with the foregoing evidence suggests that it may be formed by the acid-catalysed condensation of very reactive carbohydrate units.

We thank the Australian Micro-analytical Service for microanalyses, Mr. K. J. Harrington and Mr. H. G. Higgins for help in the preparation and interpretation of infra-red spectra, and Miss Ann Carle for help with the chromatography of polyphenolic substances.

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Induction of Callus on Decapitated Hypocotyl of *Helianthus* by *Escherichia coli* and other Bacteria

In vitro cultures of angiosperm organs in association wit several common free-living bacteria have resulted in callu formation. The gross morphology and histology of thes calluses are similar to crown gall tumours produced the same time under similar conditions. Controls lackin the associated bacterium show little proliferation of cu surfaces.

Seeds of Giant Russian annual sunflower were surfac sterilized by removal of testa and immersion for 20 min 20 vol. hydrogen peroxide and germinated on 25 c.c. complete White's medium agar in tubes. When th etiolated seedlings had expanded the cotyledons, the hyp cotyls were severed below the cotyledonary node and th cut surface was inoculated with a loopful of a 48-h-o nutrient broth culture of the bacterium under test. F comparison, hypocotyls were also inoculated with *Agr bacterium tumefaciens* and controls consisted of cu hypocotyls either with a drop of nutrient broth or wit no treatment. The tubes were incubated at 25° C in th dark.

Within a week the hypocotyls with *Agrobacteriu* showed evident callus formation; both sets of controls ha either formed wound tissue with no further proliferation the terminal tissue had died. Over the same period rather low proportion (about 25 per cent) of the cut ends the hypocotyls inoculated with the bacteria under te began to proliferate and, by the time the nutrients in th agar were exhausted, had formed calluses comparab in size to those of the crown gall (Fig. 1). The bacter producing positive results were *E. coli*, *Pseudomon aeruginosa*, *Sarcina lutea* and *Aerobacter aerogenes*.

Since *E. coli* produced the most pronounced effects, was chosen for further study. Strains of this bacteriu re-isolated from the callus proved more virulent. The induced a high proportion (more than 75 per cent) positive results and the callus grew more rapidly.

Examples of the callus produced by *E. coli* and *Agr bacterium* were fixed and sectioned. In both there we similar proliferations of the vascular tissue. The prolife ation induced in the ground tissue by *E. coli* appear more regular but included irregular differentiation tracheary elements. Occasionally associated with th

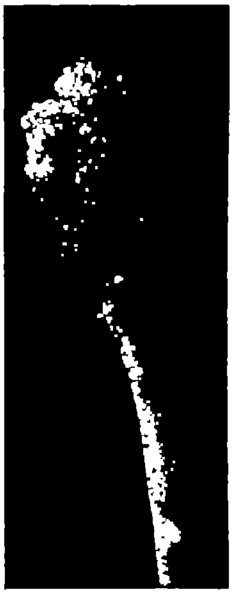

Fig. 1. Proliferation of decapitated hypocotyl of *Helianthus* inoculated with *Escherichia coli*

callusing induced by several bacteria was a distension of the hypocotyl, which might reach a maximum some distance below the cut surface. A full description of the morphogenetic responses will be published elsewhere.

In the early stages of these experiments, while the cut surfaces were still moist, the controls formed wound-healing tissue which simulated a small callus. This is consistent with the proliferation of wounded surfaces of many plants in humid atmospheres[1]. It seemed possible, therefore, that the bacterial cultures merely allowed callusing to continue by maintaining moisture at the cut surfaces. When the work was repeated with the surfaces of the controls kept moist, greater proliferation resulted, but nothing comparable with the bacteria-induced callus was produced.

Attempts to induce callus formation by inoculation of wounds on intact sunflower seedlings in glasshouse conditions were unsuccessful with *E. coli*, though 100 per cent successful with crown gall. This ineffectuality of *E. coli* we attribute to rapid wound healing in relatively dry air. Decapitated seedlings of sunflower grown on White's medium in the light, and intact seedlings grown on White's medium in the light in a humid atmosphere, have produced local stem swellings when a wound was inoculated with *E. coli*.

No reports of callus-induction by non-pathogenic bacteria are known to us and comments on these results would be appreciated. Zielinski[2] reports accelerated growth of carrot tissue cultures in association with an unnamed bacterium and Fallot[3] records increased rate of growth of tissue cultures of *Vitis rupestris* in association with *Bacillus megaterium*.

We thank Miss L. Williams, Department of Microbiology, University of Queensland, for confirming the identity of the cultures of *E. coli* used in our original inoculations and also those recovered from induced callus.

We also acknowledge the controlled environment facilities made available to one of us (D. E. G. S.) by the New Zealand Universities' Grants Committee.

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Succession of Nematophagous Fungi during the Decomposition of Organic Matter in the Soil

ALTHOUGH nematophagous fungi have been intensively studied in agar cultures containing nematodes, and soil organic matter has commonly been used as a source of these fungi[1,2], little seems to be known of the succession of fungi in this biological group during the decomposition of organic matter in the soil. Preliminary work has accordingly been done to determine the nature of any succession with the view of further investigation.

A soil with a wide variety of species of indigenous nematophagous fungi was selected for examination. These comprised three retiary trap-formers, *Arthrobotrys oligospora*, *Dactylaria psychrophila*, and *Trichothecium cystosporium; Dactylella cionopaga*, a fungus producing modified adhesive hyphal branches as trapping organs; an unidentified zoopagaceous fungus with aseptate adhesive hyphae; an unidentified fungus with constricting-ring snares and three endozoic parasites *Acrostalagmus obovatus*, *Harposporium anguillulae* and *Nematoctonus leptosporus*. In pure culture and on nematode-infested agar the retiary trap-forming fungi grew vigorously and sporulated intensely. *Dactylella cionopaga* grew much more slowly and sporulation was relatively sparse. Neither of the unidentified fungi was isolated in pure culture but on agar containing nematodes both showed sparse and slow growth.

The soil was used fresh and unsterilized after being passed through a 7-mm mesh sieve and its moisture content was maintained as 50–55 per cent of the total water-holding capacity. Direct observation of the fungi in the soil was made using the agar disk technique[3]. A characteristic succession pattern was noted during the decomposition of both green manure, in the form of cabbage-leaf tissue, and carbohydrates as glucose or sucrose. The period during which the fungi were seen to be predaceously active continued for 7–11 weeks after the addition of amendments to the soil.

The nematophagous activity of the retiary trap-forming fungi increased, and, having reached a peak, declined during the first half of this period. The zoopagaceous fungus and *Dactylella cionopaga*, having played a subsidiary part as nematode-destroyers during this time, then increased in activity during the second half of the period and became the predominant components of the nematophagous complex. After reaching a peak of activity these in turn declined. The ring-forming organism was rarely observed and then only in the final stages of the succession. The endozoic parasites appeared sporadically during the whole of the succession, attacking a proportion of the nematodes available for infection.

Excluding the endozoic parasites the pattern of succession appears to bear no relation to the numbers of nematodes in the soil at any particular time and would seem rather to depend on growth rate and spore-production, organisms more vigorous in these respects appearing first followed by those less vigorous. However, the sequence may be related more fundamentally to the changes in the nutritional status of the soil microhabitats with time. Investigation of the nutritional requirements of the nematode-trapping fungi may help elucidate the problem.

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Chemical and Immunological Comparisons of Allelic Immobilization Antigens in *Paramecium aurelia*

PURIFIED immobilization antigens of *Paramecium aurelia* (syngen 1) were obtained from stocks containing different alleles at the *D* locus[1] and compared by the technique of 'fingerprinting'[2,3]. Previously[4,5] the antigen has been shown to be a protein of molecular weight approximately 250,000. It was extracted by the salt–alcohol method of Preer[4] and purified by ammonium sulphate precipitation and chromatography on carboxymethyl cellulose. Tryptic digests were prepared from antigen denatured by reduction with 2-mercaptoethanol, alkylated with iodoacetic acid[6] and 'fingerprinted' by the method of Katz, Dreyer and Anfinsen[3], except that the chromatographic solvent was *n*-butanol/water/acetic acid (17 : 9 : 4) and the electrophoresis buffer had a *p*H of 4·2.

Nine naturally occurring allelic D types were examined and all were found to differ in the peptide patterns produced. The patterns were of the same general form, however, in contrast to two G serotypes (90G and 60G, controlled by alleles at another locus) which showed a radically different distribution of peptide spots. This

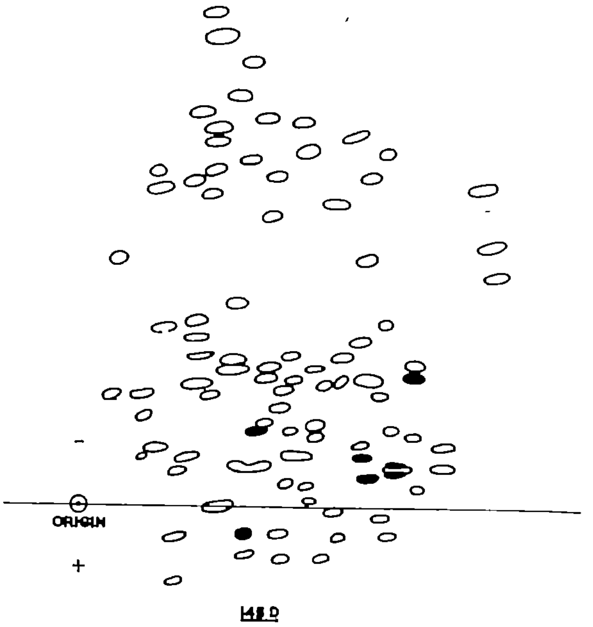

Fig. 1. Tracing of a fingerprint of antigen 145D. Chromatography horizontally and electrophoresis vertically, Shaded spots contain tryptophan

confirms the observations of Steers[7] with the A, B and D serotypes of syngen 4 of *P. aurelia*.

In our study of the D antigens, we found that genes at the *D* locus alone were concerned in specifying the structure of the isolated protein. This was shown by comparing two preparations of antigen 90D: one from stock 90 itself and the other from a strain containing the allele D^{90}, which, by repeated back-crossing, had been made nearly isogenic with the unrelated stock 60. The peptide patterns of the two were indistinguishable.

Since the patterns contain about 80 spots (Fig. 1), detailed analysis has so far been impracticable. However, comparisons were made between the nine D antigens by counting the numbers of differently situated spots, and these results are shown in Table 1. These numbers are assumed to be twice the number of altered peptides but represent only a minimum estimate of amino-acid replacements.

The immunological relationship between the various antigens was investigated by observing cross-reactions by the immobilization test. These results are shown in Table 2. The correlation between immunological diversity and number of peptide differences is quite evident. All members of the group of five D types which are indistinguishable from 60D show only small differences in peptide pattern while the types which are clearly distinguishable immunologically have many more peptide differences. The type 145D, which cross-reacts very strongly with 60D but can be differentiated, shows an intermediate number of differences.

As shown in Table 2 the antigens investigated were derived from material of exceedingly diverse geographical origin, and it is assumed that they can have only a very remote evolutionary connexion. It would thus be expected that mutations involving portions of the molecule not concerned with its antigenic specificity would be randomly distributed throughout the stocks. That so few peptide differences were found between types indistinguishable immunologically but of independent origin suggests that the antigenic site is a major portion of the molecule. Otherwise the number of peptide differences would be more nearly independent of the antigenic relationship.

Table 1. Pairwise Comparisons of 'Fingerprints' of Allelic Immobilization Antigens showing Numbers of Differently Situated Spots
(Blank spaces indicate no comparisons made)

	60D	33D	90D	103D
178D	2			
181D	4			
521D	3			
535D	6			
145D	10	20	20	20
33D	20	—	—	—
90D	20	10	—	—
103D	20	20	20	—

Table 2. Immunological Relations of Nine Different D-Type Immobilization Antigens in *P. aurelia*

Antigens	Origin of stocks	Antibodies Anti-60D (192)	Anti-41D (74)	Anti-103D (172)	Anti-33D (92)	Anti-90D (178)	(181
60D 178D 181D 521D 535D	Vermont Santiago, Chile Tokyo Paris Adelaide	*100*	*100*	< 5	< 5	< 5	< 5
145D	Paris	*100*	*100*	20	< 5	< 5	< 5
33D	Baltimore	80	10	< 5	*100*	25	< 5
90D	Pennsylvania	< 5	10	35	20	*100*	*100*
103D	Philadelphia	< 5	< 5	*100*	< 5	< 5	10

Cross-reactions are indicated as percentages of the homologous reactions (The numbers in parentheses under Anti-60D, etc., are serum numbers.)

It appears, therefore, that antigenic specificity is here due, not to a small number of chemical groups as is known for certain polysaccharide antigens, but to the cumulative effect of many amino-acids.

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Hypergammaglobulinæmia in Mink Initiated by a Cell-free Filtrate

Aleutian disease in mink is a spontaneous, transmissible disease which can be used as a model for studying the pathogenetic mechanisms in certain immunological and connective tissue diseases[1]. Hypergammaglobulinæmia is a constant feature of the disease[1,2,3], and the severity of the natural and experimental disease appears to parallel the rise in γ-globulin[2,3]. The histological[5,6] and histochemical[7] changes resemble those of the connective tissue diseases of man. A genetic predisposition[4] as well as a familial occurrence[8] have been demonstrated.

We have shown that hypergammaglobulinæmia can be experimentally produced by the injection of tissue suspensions from naturally affected mink[2]. The active principle was found to be resistant to 0·3 per cent formalin at 5° C for 2 weeks but not for 40 weeks[2]. The work reported here was carried out to characterize further the active principle by filtration and ultra-centrifugation of affected tissue suspensions.

The mink used in these trials were from our own herd in which Aleutian disease has never naturally occurred. The number of mink in each treatment group as well as the genotypes are given in Table 1. The genotypes are designated as homozygous recessive (*aa*), heterozygous (*Aa*) or homozygous dominant (*AA*) for the Aleutian gene[4].

The tissues used for the preparation of the mocula were from *aa* mink with the natural disease. The spleens, livers, and kidneys were collected immediately after death and kept at − 26° C until used. Portions of these organs were ground in a Waring blender and diluted to 10^{-1} with phosphate buffered saline (*p*H 7·5). The suspension was then centrifuged for 1 h at 10,000 r.p.m. in a Servall odel RC-2 refrigerated centrifuge with an SS-34 rotor 0,500g) and the supernatant fluid removed. The pernatant fluid was then filtered through a Krueger 'E' d and the filtrate was centrifuged for 1 h at 35,000 p m. in a model L Spinco ultracentrifuge with a 40·3 gle head rotor (95,000g). The supernatant fluid was moved and the pellet resuspended in an equal volume phosphate buffered saline.

All mink except the non-injected controls received 2 ml. bcutaneously of inoculum. The original 10 per cent ude tissue suspension was injected into group I. Follow- g filtration and ultracentrifugation at 35,000 r.p.m., roup II received the supernatant fluid and group III ceived the resuspended pellet. Group IV served as non- jected controls.

Serum samples were collected prior to inoculation and gain when the trial was terminated 2 months following oculation. Filter-paper zone electrophoresis and total rum protein determinations were carried out as prev- usly described[1]. All the mink had normal serum protein alues before inoculation.

The post-injection serum protein values were com- ared with those of 19 experimentally affected animals and 9 non-affected animals originating from our herd[2]. The iteria for hypergammaglobulinæmia included: (1) γ- lobulin values of 2·0 g or more per 100 ml. of serum ontrol mean + 2 $S.D.$ = 1·99 g/100 ml.) with α- and β- lobulins within the normal range; (2) normal or elevated tal serum protein values; (3) normal or decreased bumin levels; and (4) albumin/globulin ratios of 1·0 or ss. Gross and histological studies on all mink will be e subject of another report.

ble 1. TOTAL NUMBER OF ANIMALS BY GENOTYPE IN EACH TREATMENT GROUP AND THE NUMBER DEVELOPING HYPERGAMMAGLOBULINÆMIA

roup	Treatment	Recipient genotypes Affected/No. injected *aa*	*Aa*	*AA*	Total No. Affected/total injected
I	Crude tissue suspension	5/5	1/3	2/2	8/10
II	Supernatant fluid following filtration and ultracentrifugation	1/5	0/4	1/6	2/15
III	Resuspended pellet following filtration and ultracentrifugation	5/5	3/4	2/6	10/15
IV	Non-injected controls	0/4	0/5	0/7	0/16

The results are summarized in Table 1 and indicate that n elevation of γ-globulin occurred in treatment groups I, I and III. All the homozygous recessive mink in groups I nd III showed a rise in γ-globulin, but only part of the eterozygous and homozygous mink responded in this anner. This difference in genotype susceptibility has een previously noted[3]. Two of 15 mink in group II had ypergammaglobulinæmia. One was an aa mink and the ther an AA. The mean serum protein values and the andard deviations of all groups are given in Table 2. tatistical analysis using analysis of variance[8] revealed a gnificant increase ($P < 0·01$) in γ-globulin and a signifi- ant decrease ($P < 0·01$) in albumin in groups I and III.

The filter excluded viable tissue cells and bacteria. If e cause of Aleutian disease were a soluble substance, ch as a soluble protein, it is probable that the active action would remain in the supernatant fluid. A part- ulate substance would be expected to settle during the cond centrifugation (95,000g for 1 h).

Changes in serum protein occurred in 66 per cent of the nimals given the sediment and in 80 per cent of the nimals given crude tissue suspension. The supernatant uid evoked a response in 13 per cent of the mink. The ncentration of the initiating factor, therefore, was higher in the sediment than in the supernatant fluid and the activity of the sediment was close to that of the crude tissue suspension. It would appear that the cause of hypergammaglobulinæmia in mink is a filterable substance which can be almost completely removed from aqueous suspension by ultracentrifugation at 95,000g for 1 h. It seems possible that this filterable substance could produce hypergammaglobulinæmia and other changes through one of several possible mechanisms: (1) stimulation of primitive mesenchymal cells to proliferate and form many plasma cells which produce hypergammaglobulinæmia; (2) initiation of an auto-immune response; or (3) initiation of an alteration which in turn is responsible for the pathological changes. At present, it is not known whether the elevated γ-globulin is antibody directed against a tissue or other antigen, but initial studies to characterize this γ-globulin indicate that it has an ultracentrifugation constant of approximately 7 S[5].

The finding of a filterable, cell-free substance which initiates changes in mink that resemble certain connective tissue diseases in man raises speculation as to possible similar mechanisms involved in these human diseases.

This investigation was supported in part by U.S. Public Health Service grant $A5400$, training grant $2G$–414 and the Mink Farmers' Research Foundation.

Note. While this work was in progress, Dr. J. D. Russell, Madison, Wisconsin, was able to cause hypergammaglobulinæmia in mink using Berkefeld N filtrates.

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Vitamin B_{12} and the Detoxification of Hepatotoxic Pyrrolizidine Alkaloids in Rumen Liquor

IN Australia chronic liver disease, with sometimes a high death rate, has occurred in flocks of sheep which have grazed pastures containing the summer-growing annual plant, *Heliotropium europaeum* L., for two or more consecutive seasons. Pyrrolizidine alkaloids have been isolated from the plant and their structures determined[1,2]. The plant may contain up to 3 per cent of alkaloid, mostly as N-oxides[3]. We have studied the disease in grazing sheep[4–6] and by administration of the purified individual alkaloids to rats[7–9], and we have defined the essential pathology as an atrophic hepatosis with megalocytosis of the parenchyma cells.

By extending these investigations to a wide range of pyrrolizidine alkaloids we have concluded that only those which are allylic esters are hepatotoxic, and have put

Table 2. MEAN SERUM PROTEIN VALUES ACCORDING TO TREATMENT GROUPS EXPRESSED AS G/100 ML. OF SERUM

oup	Treatment	Total serum protein	Albumin	α-Globulin	β-Globulin	γ-Globulin	α/γ
I	Crude tissue suspension	7·40±0·55*	2·85±0·72	0·62±0·11	1·26±0·44	2·63±0·79	0·6
I	Supernatant fluid	7·39±0·48	3·95±0·47	0·75±0·10	1·13±0·17	1·52±0·79	1·1
I	Resuspended pellet	7·55±0·66	3·29±0·59	0·70±0·10	1·24±0·14	2·37±0·60	0·7
	Non-injected controls	7·53±0·69	4·17±0·61	0·71±0·28	1·25±0·27	1·37±0·31	1·2

* Mean ± $S.D.$

forward the hypothesis that they act in the tissue cell as alkylating agents by a mechanism involving alkyl-oxygen fission[10].

The relatively small amount of liver damage suffered by sheep after grazing on pure stands of *H. europaeum* for only one season suggested to us that much of the alkaloid must be destroyed in the rumen, and we have, therefore, sought to determine the products arising from heliotrine, in the rumen contents of sheep several hours after an oral dose. We found the major product to be a 1-methylene derivative, 7-α-hydroxy-1-methylene-8α pyrrolizidine (1), together with some unchanged heliotrine and the hydrolysis product, heliotridine. We had isolated the optical enantiomer of the base (1) from *Crotalaria goreensis*[11] and found it devoid of hepatotoxic properties both in acute tests and in a prolonged course of repeated injection in rats. We have synthesized (1) and found it likewise to have no acute hepatotoxic action. Although chronic testing of this enantiomer is not yet completed, no pyrrolizidine alkaloid has been found to cause a hepatosis if it did not produce liver necrosis in the acute test.

To study this conversion *in vitro* we incubated heliotrine with rumen liquor under conditions commonly used in artificial rumen studies. A sample of rumen contents was taken from a sheep with a rumen fistula, filtered through cheese-cloth, lightly centrifuged (400*g*, 3 min) and incubated at 39° C with a constant stream of carbon dioxide bubbling through it. Under these conditions the heliotrine (0·5 mg/ml.) was recovered unchanged even after 48 h incubation. However, since the administration of cobalt by mouth to sheep, which materially raises the vitamin B_{12} content in the rumen[12], has been clearly shown to protect sheep against *Phalaris* staggers[13] and since this disease is believed to be due to a toxic substance produced by the plant *Phalaris tuberosa*[14], we examined the effect of adding 0·4 μg/ml. vitamin B_{12} (Glaxo–Allenbury's preparation of cyanocobalamin, 'Cytamen 1000') to the prepared rumen liquor with which the heliotrine was incubated.

Under these conditions the heliotrine was all converted within 24 h to the 1-methylene derivative (1), which was isolated and identified by gas chromatography and infrared spectrum. We also found that there was a partial conversion without added B_{12} in rumen liquor that was strained but from which the B_{12}-rich particulate matter was not removed by light centrifuging. On the other hand, if all the micro-organisms were removed by centrifuging at 4,000*g* for 1 h the heliotrine was recovered unchanged after 24 h incubation even when 1 μgm B_{12}/ml. had been added. The rate of conversion of heliotrine to (1) was increased as the B_{12} concentration was increased up to 1 μgm/ml. It was decreased as the *p*H was lowered from 6·7. In all instances there was a lag period of 6–10 h during which no breakdown of heliotrine occurred. Neither the rate of conversion nor length of the lag-period was affected by addition of glucose. The lag-period was not eliminated by pre-incubation of the rumen liquor with B_{12} for 24 h before addition of heliotrine. Vitamin B_{12b} (hydroxocobalamin) was less effective in concentrations up to 5 μgm/ml.

Other pyrrolizidine alkaloids have been examined by incubation for 24 h with filtered and lightly centrifuged rumen contents with and without the addition of 1 μgm/ml. of B_{12}. The alkaloids were broken down only in the pots to which B_{12} was added, and in each instance in which the product has been characterized it has proved to be the 1-methylene derivative. Thus, of the other alkaloids from *H. europaeum*, lasiocarpine was converted to 7α-angelyloxy-1-methylene-8α-pyrrolizidine; supinine and heleurine, esters of supinidine rather than heliotridine, yielded 1-methylenepyrrolizidine. Heliotrine-*N*-oxide was reduced to heliotrine in the absence of added B_{12}, but in its presence (1) was the only product.

Two retronecine bases tested, echimidine, isolated from *Echium plantagineum* L.[15], and monocrotaline, from *Crotalaria retusa* L.[16], were stable in our test system unles B_{12} was added, and then they were converted to 1-methylene derivatives as judged by colour reactions on pape chromatograms.

These experimental observations are being continue and extended and detailed results will be publishe elsewhere, but this preliminary report is made because c the possible practical applications which they suggest.

Since it has been shown that adequate cobalt is provide for the protection of sheep against *Phalaris* staggers ove extended periods by the deposition of heavy pellets containing cobalt in the 'rumen-reticulum'[17], our result suggest that these pellets, which are available commercially, may well protect sheep and cattle from chroni intoxication by pyrrolizidine alkaloid-containing plant such as *Heliotropium*, *Senecio* and *Crotalaria* species. W have set up field trials and pen experiments to assess th protective action of cobalt pellets in sheep exposed t heliotrope poisoning, but since the chronic diseas develops only slowly, results will not be available for a least two years.

We thank Glaxo–Allenbury's (Aust.) Pty., Ltd., for gift of vitamin B_{12b}.

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Axenic Culture of Two Trichomycetes from Californian Mosquitoes

THE Trichomycetes are a group of primitive fungi which with a few exceptions, live attached by holdfasts to the gut linings of arthropods. We have encountered twc Trichomycetes, *Rubetella culicis* (Tuzet et Manier, 1947 and *R.* sp., inhabiting the rectums of mosquito larvæ in California; both these fungi have been found infesting several species of mosquitoes.

To our knowledge, the only successful axenic culture heretofore made with any member of the Trichomycetes was by Whisler[1], who cultured the ectocommensal *Amoebidium parasiticum*, from *Cladocera* on a tryptone agar medium. Tuzet, Rioux and Manier[2] attempted tc culture *R. culicis* and observed some elongation of hypha filaments; however, they did not obtain germination o conidia.

Our first isolation of *R. culicis* (Fig. 1) was made from a fourth-instar larva of *Ædes melanimon* Dyar collected from an irrigated pasture in Merced County. *Rubetella* sp (Fig. 2) was first isolated from a fourth-instar larva o *Culiseta incidens* (Thomson) collected from rock pools in the foothills of the Sierra Nevada Mountains in Fresnc County.

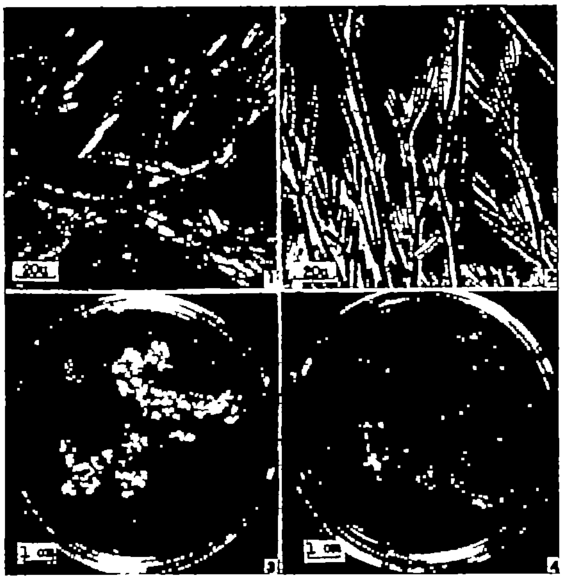

Fig. 1. *Rubetella culicis* from the rectum of *Aëdes melanimon*
Fig. 2. *Rubetella* sp. from the rectum of *Culiseta incidens*
Fig. 3. Culture of *Rubetella culicis* in thioglycollate broth
Fig. 4. Culture of *Rubetella* sp. in thioglycollate broth

Heavily infested larvæ were surface disinfected with a 1 : 1,250 concentration of 'Roccal' solution (Withrop Laboratories, New York), rinsed in sterile distilled water, and blotted on sterile filter paper. The rectums of the arvæ were removed by dissection and placed in a drop of 0·675 per cent saline solution, containing about 1,000 µg of streptomycin and 3,000 units of penicillin *G* per c.c. The rectums were transferred to a sterile slide where they were dissected open with fine needles; individual thalli 1–2 mm n diameter were scraped free from the chitinous rectal ining and transferred through 5 successive baths of sterile saline solutions containing antibiotics. Finally, the thalli were placed in culture medium.

Our first successful culture was established on *SNB*-9 medium, a biphasic blood agar medium developed by Diamond and Herman[3] for the culture of hæmoflagellates. Equally good growth was later obtained on Difco brain-heart infusion agar slants, using a broth overlayer composed of 2 per cent neo-peptone and 0·675 per cent sodium chloride in water; good cultures were also obtained using Difco *N.I.H.* thioglycollate broth.

Initial isolates required about 2 weeks to reach 5 mm in diameter; conidial production usually occurred after the cultures were a month old. The production of aberrant conidia was common; however, sub-cultures were readily accomplished, and mosquito larvæ were readily infested in the laboratory by exposing them to older cultures containng heavily conidiating thalli.

The colonies of both species of *Rubetella* appeared soft, white, and compact on agar; however, each species took on a characteristic form in broth. *R. culicis* colonies were almost spherical and billowy (Fig. 3), while *Rubetella* sp. colonies were more stringy or lacy in form (Fig. 4).

The axenic culture of these fungi has provided an imporant step in the elucidation of their nutritional requirements, life-cycles and relationships with their hosts.

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MICROBIOLOGY

Cellulose Acetate Membranes for detecting Peroxidase in Mycobacteria

A QUICK and simple test for detecting peroxidase in cultures of mycobacteria has been outlined recently[1]. The test consists in exposing micro-organisms in or on a suitable culture medium to a reaction mixture of catechol and hydrogen peroxide in an acetate buffer at a *p*H of 4·0. In the presence of oxygen the catechol is oxidized by peroxidase, forming a dark brown compound, probably benzoquinone. This change in colour sharply distinguishes peroxidase-positive organisms from peroxidase-negative ones, which remain colourless. The test can also be carried out in a liquid medium, but where suitable solid or semi-solid media are used, colonies of each type can be distinguished in mixed cultures. As the presence or absence of peroxidase is intimately bound up with susceptibility or resistance to isoniazid, the demonstration of sensitive and resistant colonies in a mixed population without exposure to isoniazid is made easy.

In experimental work where artificial mixtures of isoniazid-sensitive and resistant strains have been used, best results were obtained where colonies have been numerous but discrete and uniform in size. In addition a medium is required which is easy to prepare, consistent in performance and capable of furnishing a rapid growth which is convenient to handle should further tests be needed. With the view of these requirements several media have been investigated, namely, Fisher[2], Kirchner[3], Tarshis[4], and Dubos and Davis[5]. Of these, the last has proved the most suitable.

The modification of Tirunarayanan and Vischer's method described here makes use of cellulose acetate membranes laid on a nutrient medium in the manner detailed by Knox and Smith[6] for the detection of penicillinase-producing organisms.

For the detection of peroxidase, Dubos and Davis medium containing 0·4 per cent bovine albumin and 0·5 per cent agar was used, poured into plastic Petri dishes and overlaid, when set, with 5-cm or 8-cm Oxoid membrane filters. Plates were allowed to dry (closed) overnight at 37° C and inoculated with 2 drops (0·04 ml.) from a mixed aqueous 10^{-4} dilution of 6–7 day-old stock Dubos cultures of *H*37*Rv* isoniazid-sensitive and resistant strains. These manipulations were carried out in an inoculating cabinet with air extraction. Incubation in large air-tight

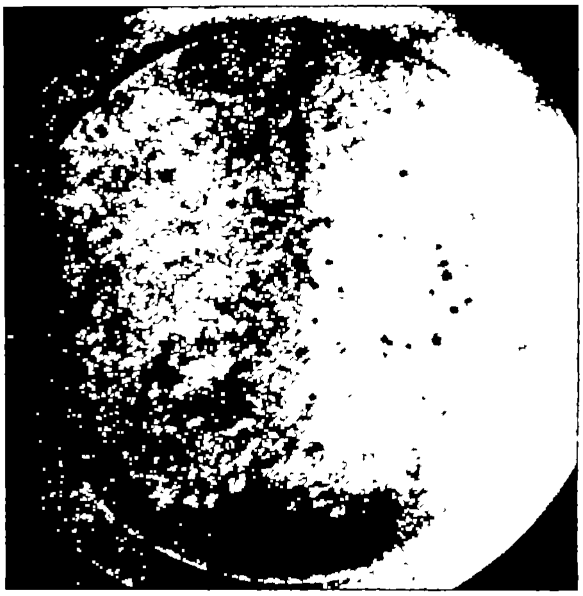

Fig. 1. (× *c*. 1·5

glass or metal containers has proved more successful and convenient than individual sealing of plates, and contamination rates have been very low. Sufficient growth for the catechol test has been obtained after 10 days' incubation, but in general a 14- to 21-day period is to be preferred. Colonies of tubercle bacilli grown on Dubos/membrane agar were smooth, convex, well separated and white in colour. With the strains used, colonies of peroxidase-negative, isoniazid-resistant cells were generally larger than colonies of the sensitive strain.

The catechol test was simply performed by transferring the growth-bearing membrane to a second Petri dish containing two or three thicknesses of 9-cm Whatman No. 1 filter papers impregnated with the reaction mixture, which was made up as follows: 0·2 M sodium acetate, 2 ml.: glacial acetic acid diluted 1 in 85, 8 ml.; 2 per cent catechol (aqueous), 2 ml.; 1 in 100 hydrogen peroxide (30 vol.), 1 ml. Darkening of peroxidase-positive colonies occurred after 15 min or so at room temperature, and the change in colour was at a maximum in 30 min–1 h. Peroxidase-negative colonies remained quite colourless and this contrast was maintained for several hours at least. Incubation at 37° C did not appear to hasten the reaction.

The sensitivity of the test is such that one peroxidase-positive colony is readily detectable among 400–500 peroxidase-negative colonies on a membrane of 8 cm size after 2–3 weeks' incubation, while one negative colony can be differentiated from some 200–300 positive under similar conditions. As peroxidase-positive colonies represent the isoniazid-sensitive cells, it will be seen that a small number of isoniazid-sensitive individuals may readily be detected among large numbers of isoniazid-resistant cells when neither of them has been exposed to the action of the drug.

Sub-cultures made from cells treated with the reaction mixture at the time when the test was read failed to grow, and it seems that exposure to the reagent kills them. It follows, therefore, that further testing involving growth is not possible with cells used in the reaction, but this disadvantage could be overcome by means of replica plating techniques.

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Reversal of o-Nitrobenzoic Acid Inhibition of Microbial Growth by Amino-Acids

SEVERAL benzene derivatives reportedly inhibit microbial growth by blocking essential metabolic reactions but, in some instances, the site of inhibition has not been established. High concentrations of *p*-aminobenzoic acid inhibit certain bacteria[1,2] and the bacteriostatic activity toward *Escherichia coli* strain *W* is reversed by *p*-hydroxybenzoic acid and, to a limited extent, by shikimic acid or 5-dehydroshikimic acid[1]. *p*-Aminobenzoic acid also suppresses growth of *Saccharomyces cerevisiae*, which can be reversed by aromatic amino-acids such as phenylalanine, tyrosine and tryptophan[3]. Utilization of *p*-aminobenzoic acid as an oxidizable substrate by a *Flavobacterium*[4] is competitively inhibited by *p*-aminosalicylic acid[5], and it has been suggested that *p*-aminosalicylic acid competes with the substrate for the specific transport mechanism(s)[6].

Inhibition of *Aspergillus niger* by *m*-dinitrobenzene is influenced by the age of the culture and can be reversed by casein hydrolysate or a mixture of amino-acids similar in composition to casein hydrolysate[7]. The toxic effect 2,4-dinitrophenol on *E. coli* can be counteracted by a amino-acid mixture similar in composition to that obtaine from cow's liver[8]. *m*-Nitrobenzoic acid inhibited oxidatio of *o*-nitrobenzoic acid by *Nocardia opaca* and utilizatio of *p*-nitrobenzoic acid by *N. erythropolis*[9]. Similar studie revealed the *para*-isomer did not inhibit oxidation *o*-nitrobenzoic acid and the *ortho*-isomer did not influenc oxidation of the *para* form.

Investigations have revealed that *o*-nitrobenzoic ac inhibits growth of a *Flavobacterium* capable of usin *p*-nitrobenzoic acid as the sole source of carbon an energy for aerobic growth. This investigation was undertaken to elucidate the mechanism of this antagonism.

Growth studies were conducted in a defined mediu composed of: sodium chloride, 0·1 per cent; potassiu dihydrogen phosphate, 0·32 per cent; dipotassiu hydrogen phosphate, 0·42 per cent; and *p*-nitrobenzoi acid as the carbon and nitrogen source. When succini acid replaced *p*-nitrobenzoic acid as the carbon an energy source, 0·2 per cent ammonium chloride wa added as the nitrogen source. All substrates were use in a final concentration of 0·02 M unless indicated otherwise. The medium was adjusted to *p*H 7·0, autoclave at 121° C for 15 min, and mineral salts added as previous described[6]. The amino-acids and other additives wer sterilized separately and added aseptically.

Inoculum cells were grown on *p*-nitrobenzoic acid aga slants, washed by centrifugation, suspended in steril 0·01 M potassium phosphate buffer (*p*H 7·0), and on drop added to each reaction tube. All tubes were incubated on a reciprocating shaker at 37° C. Growth wa followed by measuring optical density at 540 mμ in Bausch and Lomb Spectronic 20 spectrophotometer.

Addition of *o*-nitrobenzoic acid (0·06 M and 0·12 M) t the medium containing *p*-nitrobenzoic acid (0·02 M) a the source of carbon and energy resulted in a marke inhibition of growth (Fig. 1). The degree of suppressio is a function of the inhibitor concentration. Control indicated the organism was unable to utilize *o*-nitrobenzoic acid as a substrate for growth.

Investigations were made to determine if growth wa inhibited when compounds not structurally related to the

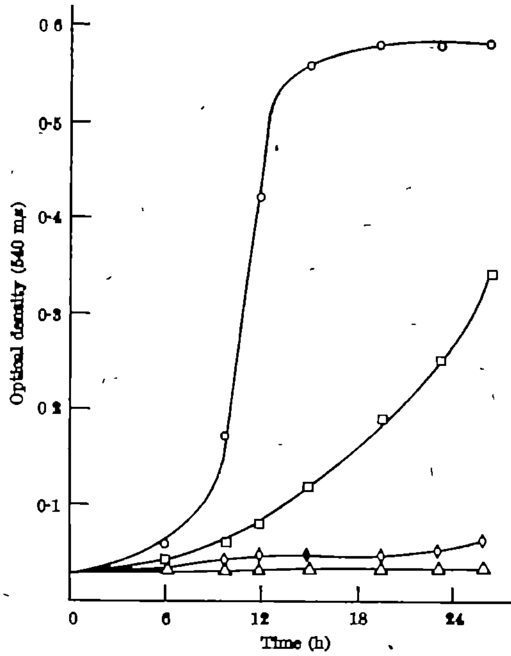

Fig. 1. Effect of *o*-nitrobenzoic acid on growth of *Flavobacterium* sp. using *p*-nitrobenzoic acid (0·02 M) as carbon and nitrogen source. ○, *p*-NO_2; □, *p*-NO_2 + *o*-NO_2 (0·06 M); ◇, *p*-NO_2 + *o*-NO_2 (0·12 M); △, *o*-NO_2 (0·12 M)

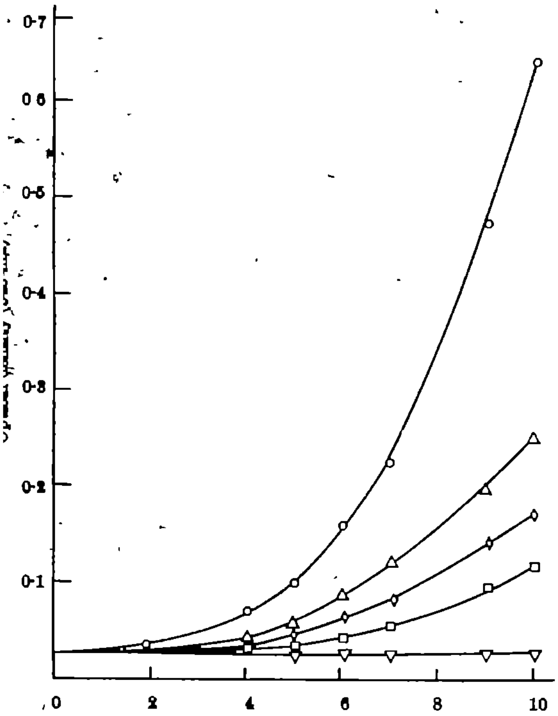

Fig. 2. Effect of *o*-nitrobenzoic acid on growth of *Flavobacterium* sp. using succinic acid (0·02 M) as carbon source. ○, Suc; △, Suc + *o*-NO₂ (0·02 M); ◇, Suc + *o*-NO₂ (0·04 M); □, Suc + *o*-NO₂ (0·06 M); ▽, *o*-NO₂ (0·06 M)

itrobenzoic acids were used as substrates. Fig. 2 shows ıat *o*-nitrobenzoic acid also inhibited growth when 02 M succinic acid was used as the substrate.

Viable cell counts were made during the 6 h immeately following inoculation of the medium to determine *o*-nitrobenzoic acid was killing the cells. Growth was easured spectrophotometrically and, concurrently, aliıots were withdrawn and the number of viable cells termined by plate counts on nutrient agar. No sigficant decrease in viable cells was observed in systems ntaining the inhibitor, denoting that *o*-nitrobenzoic acid es not kill the cells but impairs growth by interfering ith an essential metabolic process. Washed cell suspenons, used in respirometric studies, oxidized succinic or nitrobenzoic acid at the same rate in the presence or sence of *o*-nitrobenzoic acid. Thus, the inhibitor does t influence uptake of the substrate by the cells or the te of substrate oxidation.

Growth studies were conducted in which the substrate inhibitor ratio was varied. Results indicated that the gree of inhibition was related to inhibitor concentration igs. 1 and 2). Increasing the substrate concentration ither (succinic or *p*-nitrobenzoic acid) while maintaining e same inhibitor concentration did not decrease the hibition. Since similar inhibition trends were obtained gardless of concentration of substrate, these results tablish that the inhibition was dependent on the conntration of inhibitor and not the ratio of substrate to hibitor.

Reversal investigations with various additives were ıdertaken using the succinate–salts medium in the esence and absence of 0·04 M *o*-nitrobenzoic acid. esults indicated casein hydrolysate (0·05 per cent) or a ixture of 17 amino-acids completely reversed the inhibon. Additional studies demonstrated a combination of e amino-acids (DL-aspartic and L-glutamic acid, DL-ethionine, L-leucine, glycine) gave good reversal of the hibition but was not so effective as the mixture of amino-acids (Table 1). The results indicate that either glutamic or DL-aspartic acid could be omitted from the edium but not both. Growth was evident when either glycine, DL-methionine, or L-leucine was omitted from the mixture but reversal was not equivalent to the growth obtained with the five amino-acid mixture. Thus the presence of DL-methionine, L-leucine, and glycine enhances reversal but these amino-acids do not appear to be absolutely essential. L-Cystine or L-cysteine was unable to replace methionine in the mixture.

Table 1. INFLUENCE OF AMINO-ACIDS ON *o*-NITROBENZOIC ACID INHIBITION OF MICROBIAL GROWTH

Medium components*	Optical density 540 mμ	
	10 h	14 h
Suc	0·26	0·95
Suc + 17 amino-acid mixture†	0·28	1·00
Suc + *o*-NO₂	0·08	0·24
Suc + *o*-NO₂ + 17 amino-acid mixture†	0·32	0·94
Suc + *o*-NO₂ + Asp, Glut, Gly, Meth, Leu	0·20	0·70
Suc + *o*-NO₂ + Glut, Gly, Meth, Leu	0·17	0·58
Suc + *o*-NO₂ + Asp, Gly, Meth, Leu	0·17	0·59
Suc + *o*-NO₂ + Asp, Glut, Meth, Leu	0·13	0·41
Suc + *o*-NO₂ + Asp, Glut, Gly, Leu	0·12	0·40
Suc + *o*-NO₂ + Asp, Glut, Gly, Meth	0·15	0·42
Suc + *o*-NO₂ + Asp	0·13	0·38
Suc + *o*-NO₂ + Glut	0·14	0·36
Suc + *o*-NO₂ + Gly	0·05	0·16
Suc + *o*-NO₂ + Meth	0·07	0·19
Suc + *o*-NO₂ + Leu	0·06	0·17
Suc + *o*-NO₂ + Asp, Glut	0·14	0·40
Suc + *o*-NO₂ + Gly, Meth, Leu	0·08	0·28

* Succinic acid (Suc), 0·02 M; *o*-nitrobenzoic acid (*o*-NO₂), 0·04 M; DL-aspartic acid (Asp) and L-glutamic acid (Glut), 40 μg/ml.; L-leucine (Leu) and DL-methionine (Meth), 16 μg/ml.; and glycine (Gly), 8 μg/ml.

† 17 Amino-acid mixture. DL-alanine, DL-aspartic and L-glutamic acid (80 μg/ml.); L-arginine, DL-isoleucine, L-lysine, DL-methionine, DL-phenylalanine, DL-serine, DL-threonine, DL-tryptophan (16 μg/ml.); DL-valine, glycine, L-histidine, L-leucine, L-proline, L-tyrosine (8 μg/ml.).

Other diverse biological compounds including vitamins and co-factors were tested, but none significantly reversed the inhibition.

These findings establish that *o*-nitrobenzoic acid is not bactericidal to *Flavobacterium* cells but inhibits growth by interfering with an essential metabolic reaction(s). Since the inhibition can be reversed by certain amino-acids, one mechanism by which *o*-nitrobenzoic acid apparently suppresses microbial growth is by interfering with synthesis or interconversion of specific amino-acids.

This work was aided by contract *Nonr*-3443(00)*NR* 103–504 between the Office of Naval Research, Department of the Navy, and Oklahoma State University (Agricultural Experiment Station Project 1027).

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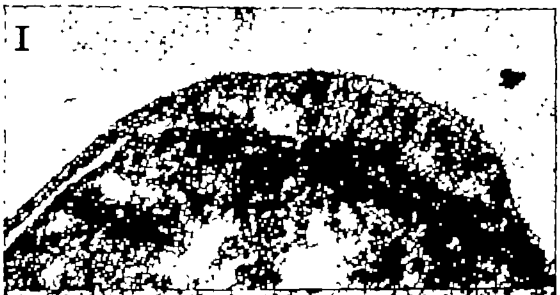
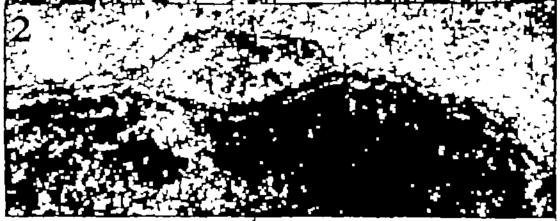
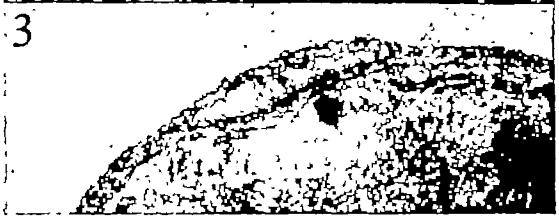
An Organelle confined within the Cell Wall of *Nitrosocystis oceanus* (Watson)

ONE of us isolated a nitrifying organism from the open ocean and called it *Nitrosocystis oceanus*[1]. In the course of examining this organism, observations were made on the fine structures shown in sections of embeddings made in 'Vestopal W' following the preparative prescriptions of Ryter and Kellenberger[2,3]. A number of structural peculiarities were observed, which will be described in a separate paper, and among them there was a structure that seems to be without precedent so far as we are aware. It was an organelle of regular and recognizable form lying between the major layers which make up the cell wall of this bacterium.

A high proportion of sections of these cells shows a peripheral separation of the layers of the cell wall con-







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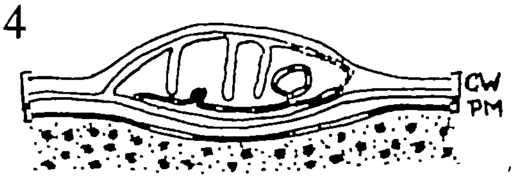

Fig. 1. A micrograph of a section (uranyl acetate stained) showing the cell wall splitting to surround the cell wall organelle with its vertical bars. Underneath lies the unit membrane of the plasma membrane. Some isolated unit membrane lies at the base of the organelle. (× c. 112,000)

Fig. 2. Clearer evidence of the components of the cell wall. (× c. 70,000)

Fig. 3. Micrograph showing participation of the inner cell wall membrane in the structure of the organelle. (× c. 70,000)

Fig. 4. Diagram based on the micrographs observed. *CW*, Cell wall; *PM*, plasma membrane

taining a number of more or less distinct bars of material running between inner and outer wall (Fig. 1). Many cells show two or three of these, and it must be assumed that each cell has several of them disposed over its surface. The ovoid profiles in the sections have a long axis of about 0·5μ and a short axis of about 0·1μ. These structures can be followed through serial sections, and several of them occupy 4–6 sections so that the other dimension would be of the order of 0·25–0·5μ. It would appear, then, that these structures are plaques within the cell wall and are oval or round in plan.

The absolute boundaries of the structure are formed by the outermost membranous component of the cell wall, which is a very thin layer of about 25 Å thickness, and the inner boundary consists of a triple structure (two dense layers separated by one of low density) that has an overall thickness of 70–95 Å. In the normal portion of the wall these two layers are separated by an interposed layer of about 60 Å thickness, and it seems obvious that this layer must thicken when this organelle is formed (Fig. 2). However, the organelle does have other components: membranous structures and whatever substance it is that separates them. Some sections show vertical bars consisting of two slightly separated unit membranes. Others show ring profiles close to the outer border, and a great many others look almost empty. A few of these latter wit little complicating structure show the possible origin o the bars or tubes as intrusions from the inner side of a expansion of the inner double membrane of the cell wal (Figs. 3 and 4). It may be a bit more complex than thi because in a few instances (Fig. 1) one can recognize tha there is a single or possibly double unit membrane laye lying just outside the inner border of the organelle as w have described it here. It may then be, since the spac ings are very similar, that the membranous componen of the inside of the structures is formed by intrusions of thi new layer and perhaps even that this layer encompasses th whole structure. It is somewhat difficult to decide eve in serial sections what forms the exact border of th structure and whether it is self-enclosed.

The chemical complexity of the bacterial cell wall ha long been recognized[4]. It is a structure in itself that ha some of the attributes of an organelle and may eve require some of itself as a starter or scaffold on whic the new structure is built[5]. Certainly it seems to play a essential part in cell division, and it seems to confer othe attributes such as shape on the cell, thus contributing t the individuality of this cell in nature. This seems t imply that it must not only have structure but also balanced anabolic and catabolic machinery within itse to deal with some of its own needs; to allow acceptanc and incorporation of the building blocks that the ce provides, and control of the edifice that is built. It is the perhaps less surprising that this discontinuous structur may be found within bacterial cell walls; or, perhaps on should say that it is surprising that discontinuous structures have not been found before within bacterial cell wall considering all the complicated things that bacteria hav to accomplish in order to wrest a living out of obdurat environments.

We have no idea of what the function of this particula structure might be. It seems to be regular and frequen enough to expect some function to be found. For th moment it must be added to the list of beautiful structure without obvious function, to be found in nearly all sort of living cells. One is reminded somewhat of the elegantl ordered fibres of the 'organite antapical' which wer described in *Thiovulum majus* by Fauré-Fremiet an Rouiller[6].

However, the work of de Boer, la Rivière and Houwink has shown more clearly than ever that this structure lie below the plasma membrane of the cell and that it divide so that the resulting daughter organelles are partitione with the cells at division.

Our cell wall organelle is definitely outside of the plasm membrane and has not yet been noted to show any patter symptomatic of division. Since it is not yet recognizabl defined in light microscopy it is rather hard to get evidenc on this latter point. Despite all the unknowns it seem certain that this is an unprecedented organelle because o the site in which it is formed and maintained.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 14

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 4.30 p.m.—Mr. J. M. Winchester and Mr. R. Pearson: "Development of Packages for Pesticides".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. B. Braithwaite: "Believing (as Opposed to Considering) Natural Laws".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION P.G.36 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. Thomson: "Modern Transducers with an Electrical Output".

UNIVERSITY OF LONDON (in the Eugenics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Paul Turan (University of Budapest): "Analysis and Diophantine Approximation".* (Further lectures on January 15 and 16.)

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Oil Mist Lubrication".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. E. J. H. Corner, F.R.S.: "The Royal Society Expedition to Kinabalu".

Tuesday, January 15

INSTITUTION OF AGRICULTURAL ENGINEERS (at the Royal Society of Arts, John Adam Street, London, W.C.2), at 11 a.m.—Whole-day Meeting—Technical Papers.

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. L. S. Penrose, F.R.S.: "Finger-prints, Palms and Chromosomes".*

SOCIETY OF CHEMICAL INDUSTRY, joint meeting of the LONDON SECTION and the SURFACE ACTIVITY GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. F. Stone: "Physical Methods of Examining Catalysts".

Wednesday, January 16

COLOUR GROUP (GREAT BRITAIN) (in the New Physics Department, Imperial College of Science and Technology, London, S.W.7), at 3 p.m.—Dr. B. H. Crawford: "Some Measurements of the Colour Temperature of Daylight at Teddington"; Mr. C. J. Chamberlin: "Notes on the Related Colour Temperature on North Daylight in Southern England"; Dr. S. T. Henderson: "The Spectrum of Daylight".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. V. D. Barnett and Mr. T. Lewis: "A Study of the Relation between G.C.E. and Degree Results".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. K. Hoselitz: "Masers".

BRITISH INSTITUTION OF RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Prof. P. Bruel: "Instruments in Acoustics".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. H. L. Penman, F.R.S.: "Irrigation in Great Britain" (Fernhurst Lecture).

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. K. Fletcher: "Precise Measurement of Temperature and Humidity".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. Baysung Hsu: "Some Physical Aspects of the Printability of Paper".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the Science Museum, South Kensington, London, S.W.7), at 7 p.m.—Lt.-Col. B. D. Shaw: "Chemistry is Fun" (Seventh Ladies' Evening).

Thursday, January 17

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. N. Korakas: "Magnetite Formation during Copper Matte Converting"; Dr. G. R. Davis: "Results of Comparative Sampling Methods at Kilembe Mine, Uganda, during the Exploration Phase" and "Observations on Sample Preparation".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. H. P. F. Swinnerton-Dyer: "Rational Points on Cubic Curves—Some Experimental Results".

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. F. H. Westheimer: "The Mechanism of the Enzymic Decarboxylation of Acetoacetic Acid" (Centenary Lecture.)

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Can We Design for Short Endurance Fatigue?"

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. P. B. Caplin: "The Manufacture and Use of Pitch Fibre Pipes".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. David Morley: "A Medical Service for Children under Five, in West Africa".

Friday, January 18

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), from 10.30 a.m. to 6.30 p.m.—Three-session meeting and discussion on "The Origin and Constitution of the Planets". Speakers: Sir Edward Bullard, F.R.S., Dr. R. W. Girdler, Sir Harold Jeffreys, F.R.S., Prof. W. H. McCrea, F.R.S., Dr. P. J. Message, Dr. H. W. Menard, Prof. S. K. Runcorn, Prof. H. Suess (Heidelberg), Dr. D. C. Tozer and Prof. Harold Urey (U.S.A.).*

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, W.1), at 5.30 p.m.—Mr. J. E. Clegg: "The Navigation of Satellites and their Launching Vehicles".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "How Should Engineering Education Take Account of Developments in the Newer Materials?—Item 1, Plastics".

Saturday, January 19

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. Eva Crane: "The World of Bees and Beekeeping".*

Monday, January 21

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Peter Salway: "Exploring the Roman Fenland".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. P. C. Clemmow: "Magnetohydrodynamic Waves in the Ionosphere".

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. B. E. Bunce: "Drying of Electroplated Parts".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Enfield Technical College, Queensway, Ponders End), at 7 p.m.—Dr. A. G. Maddock: "Actinide Chemistry".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT (with an honours degree in biochemistry or equivalent in AGRICULTURAL BIOCHEMISTRY—The Secretary, The University, Aberdeen (January 17).

UNIVERSITY RADIATION PROTECTION OFFICER (with a good honours degree, together with experience in work involving ionizing radiations); in addition to radiation protection duties, the Protection Officer will be expected to undertake a limited amount of teaching and/or research duties—The Registrar, The University, Liverpool, quoting Ref. No. CV/231/N (January 19).

ASSISTANT LECTURER (with an honours degree in chemistry or agricultural chemistry or equivalent, and preferably experience in biochemistry and/or animal nutrition) IN AGRICULTURAL CHEMISTRY, for duties which include lecturing and practical teaching to degree and diploma students and some advisory work—The Secretary, School of Agriculture, West Mains Road, Edinburgh (January 23).

ASSISTANT LECTURER or LECTURER IN PURE MATHEMATICS—The Registrar, The University, Sheffield (January 24).

LEVERHULME STUDENT (with a postgraduate diploma in chemical engineering or equivalent qualifications) IN CHEMICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (January 25).

PRINCIPAL SCIENTIFIC OFFICER (with a first- or second-class honours degree, evidence of high scientific calibre in locust or allied research, and considerable experience of control in the field) to take charge of the Field Research Section in the Anti-Locust Research Centre, London—The Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5603/63 (January 25).

LECTURER IN PHYSICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (January 31).

READER (with considerable research experience, preferably in solid state physics or in biophysics) IN PHYSICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (January 31).

SENIOR LECTURER (with a registrable medical qualification, preferably a higher qualification, and teaching and research experience in general medicine) IN MEDICINE at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 31).

EDWARD A. DEEDS JUNIOR FELLOW FOR RESEARCH IN ENGINEERING, CHEMISTRY, PHYSICS, MATHEMATICS or any cognate subject—The Secretary, University of St. Andrews, Queen's College, Dundee (February 2).

SENIOR LECTURER or LECTURER (candidates for the senior post should have several years experience of teaching and examining in botany in an academic institution) IN BOTANY at University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 2).

SENIOR LECTURER or LECTURER (with research qualifications in analysis) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (February 2).

EXPERIMENTAL OFFICER (preferably with experience in bacteriology), to assist in phytoplankton and microbiological research—The Director, Scottish Marine Biological Association, Marine Station, Millport, Isle of Cumbrae, Scotland (February 8).

LECTURER IN PURE MATHEMATICS, and a LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Manchester 13 (February 9).

CHAIR OF CHEMISTRY; CHAIR OF ZOOLOGY; CHAIR OF BOTANY; and CHAIR OF GEOGRAPHY at Fourah Bay College (The University College of Sierra Leone)—The Secretary, University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 15).

SENIOR LECTURER or LECTURER (with an appropriate honours degree or equivalent qualifications; and a higher degree for appointment as Senior Lecturer) IN MATHEMATICS at Wollongong University College, The University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (February 15).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY and PHARMACOLOGY or any related subjects—The Registrar, The University, Liverpool, quoting Ref. No. CV/234/N (February 23).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMICAL OCEANOGRAPHY, ENGINEERING or PHYSICS—The Secretary and Registrar, The University, Southampton (February 28).

LECTURER IN PURE MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (February 28).

LECTURER (medically qualified) IN BACTERIOLOGY IN THE DEPARTMENT OF MICROBIOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (February 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, METALLURGY, ENGINEERING, GEOLOGY, AGRICULTURAL SCIENCE or related subjects—The Registrar, The University of Wales, University Registry, Cathays Park, Cardiff (March 4).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY or PHYSICS—The Secretary, Royal College of Science and Technology, Glasgow (March 5).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS or CHEMICAL TECHNOLOGY (Chemical Engineering)—The Secretary, The University, Edinburgh (March 15).

ASSISTANT PHYSICIST (with a B.Sc. or higher degree) with the Wellington Hospital Board, Wellington, New Zealand—The High Commissioner for New Zealand, New Zealand House, 415 Strand, London, W.C.2.

BIOCHEMIST (graduate, with an honours degree in chemistry) IN THE CHEMICAL PATHOLOGY DEPARTMENT, for duties which will include routine investigation and participation in research—The Senior Consultant Pathologist, Queen Elizabeth Hospital for Children, Hackney Road, London, E.2.

BIOCHEMIST (probationary or basic grade) (with a first- or second-class honours degree in biochemistry or chemistry, or equivalent qualifications) IN THE DEPARTMENT OF PATHOLOGY—The Group Secretary, The Royal Hospital, Wolverhampton.

DEMONSTRATORSHIPS, RESEARCH ASSISTANTSHIPS; SCHOLARSHIPS, FELLOWSHIPS, BURSARIES, and STUDENTSHIPS IN THE DEPARTMENT OF CHEMISTRY, for students working for the Ph.D. degree—Prof. F. L. M. Pattison, Head, Department of Chemistry, University of Western Ontario, London, Ontario, Canada.

GRADUATE RESEARCH ASSISTANT, to work on the chemistry of medicinal natural products—Dr. G. E. Francis, Department of Biochemistry, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, E.C.1.

LECTURER IN ASTRONOMY, to establish a small department—The Principal, Victoria College, Victoria, B.C., Canada.

MIDLAND SILICONES RESEARCH STUDENT IN INORGANIC CHEMISTRY, for work which will involve the preparation and study of new types of compounds containing silicon—The Professor of Chemistry, Westfield College (University of London), Hampstead, London, N.W.3.

NATIONAL RESEARCH COUNCIL and other POSTDOCTORAL FELLOWSHIPS TENABLE IN THE DEPARTMENT OF PHYSICS—Prof. M. J. Johns, Chairman, Department of Physics, McMaster University, Hamilton, Ontario, Canada.

PHARMACOLOGIST or PHYSIOLOGIST (with an honours degree), to study active polypeptides in acute parasitic infections—The Administrative Secretary, School of Tropical Medicine (University of Liverpool), Pembroke Place, Liverpool 3.

PHYSICIST or ELECTRONIC ENGINEER (honours graduate) IN THE DEPARTMENT OF MEDICAL PHYSICS (University of Edinburgh and South-Eastern Regional Hospital Board), to work on a variety of electronics and instrumentation problems in the field of medical diagnosis and research—The Secretary, South-Eastern Regional Hospital Board, 11 Drumsheugh Gardens, Edinburgh 3.

PROFESSOR OF PHYSICS or ASSOCIATE PROFESSOR OF PHYSICS (with a strong background in the theory of atomic and molecular spectra, electron collision processes, etc.)—The Director, Geophysical Institute, Box 938, College, Alaska, U.S.A.

READER (with substantial research experience) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—Dr. J. H. Calderwood, Royal College of Advanced Technology, Salford, Lancs.

RESEARCH FELLOW IN NUCLEAR PHYSICS, for work at high or low energies—The Assistant Registrar, Faculty of Science, The University, Edgbaston, Birmingham 15.

RESEARCH STUDENT (with a good honours degree or G.R.I.C.) IN THE DEPARTMENT OF CHEMISTRY, for work on catalytic organic reactions—Dr. D. Bryce-Smith, The University, Reading.

SENIOR PHYSICIST—The Personnel Officer, St. Bartholomew's Hospital, London, E.C.1.

SENIOR TECHNICIAN (with technical experience in histology, or an interest in assisting with general animal experimental work) IN THE HENRY DALE LABORATORY FOR EXPERIMENTAL SURGERY at the Royal National Orthopaedic Hospital, Stanmore—The Secretary, Institute of Orthopaedics, 234 Great Portland Street, London, W.1.

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EDUCATION BY MASS MEDIA IN EMERGENT COUNTRIES

THE recently issued report of the Council for Technical Co-operation in South and South-East Asia on Techal Co-operation under the Colombo Plan in 1961–62* knowledges the help given by several private American undations, such as the Ford Foundation, in rekindling mmunity spirit and co-operation in depressed neighurhoods of large cities in India and Pakistan, and the ia Foundation in promoting social science research in kistan and basic agriculture and rural development in etnam. Particular reference is made to the Rockefeller undation's support of a programme for the improveent of English language teaching in the Philippines gun in 1957, for which a second grant of 430,500 dollars s been given. A major feature of the report is the phasis placed on technical training, and here even more an in the provision of experts and equipment the uestion of effective communication is all-important.

A survey of training facilities at the technician-level in e area made in 1960–61 (the Mills Report) and financed a generous grant from the Ford Foundation has not ly focused attention on the urgent need to improve the tput of middle-grade technicians in the area but has o emphasized the need to eliminate or minimize the ychological, administrative and other difficulties in the ay of maximum utilization of training facilities in the ea. Effective communication is no less important in inging home to the leaders and people of the countries the region the nature of the challenge which the accelered economic development they are seeking represents. e necessary conditions for a fuller and better life for e peoples of the area cannot be created without a wideread understanding of this moral challenge, any more an the shortage of trained manpower required for a gher tempo of economic development can be achieved ithout efficient communication.

In this context it should be noted that the report gards education as probably the most important single ctor in achieving rapid economic development and chnological progress, and that there has been a sigficant increase in the sharing of training facilities within e area—on which the Mills Report already mentioned rected particular attention. This Report, moreover, while ncerned especially with the economic advantages of ilizing skill and knowledge already available within the ea instead of seeking assistance from outside, did not il to emphasize the need to take due account of human ctors such as traditions and prejudices. This is all part the general problem of effective communication, and it against this background that the establishment of the homson Foundation is to be especially welcomed.

This Trust was established by Mr. Roy Thomson in ovember by a grant of £5 million essentially to enable ritain, with her facilities and wide experience through e Commonwealth, to help the developing countries train emselves in establishing effective media of mass comunication—extensive enough, and of adequate quality, satisfy the rising demand. The Trustees, of whom Lord Kilmuir is chairman, include Field Marshal Lord Alexander, Lord Williamson, Mr. K. R. Thomson and Mr. J. M. Coltart, and the Trust Deed defines the purpose of the Trust as "the provision of facilities for and the encouragement of education and technical and vocational training and for the dissemination of news and information regarding the affairs of the world for the purpose of promoting the general advancement of knowledge and mental and spiritual enlightenment of all peoples with a view to achieving closer understanding between them and encouraging and enabling them to play an informed and responsible part in their own affairs and in the affairs of the world". It includes also the provision, or maintenance for this purpose, of schools, technical colleges, training centres and other educational establishments and facilities, the provision of educational books, lectures, television and wireless programmes, and all other media for the dissemination of learning, and the provision of courses and facilities for training teachers, journalists and others employed in any industry or service connected with the dissemination of news or information to the public.

That the Trust is particularly intended to help the emergent nations is made clear in a leaflet which outlines the purpose and plan of the Trust. In the belief that education is the greatest need of the developing nations and that effective use of mass media is the best means of providing the education that will enable these hundreds of millions of normally backward peoples to make as much progress in the next decade as the Western World has made in the past century, the establishment of an honest information and truthful Press allied with radio and television stations, magazines and books, all imbued with the essential quality of integrity, is seen as the primary need. Illiteracy and isolation make conventional methods of teaching inadequate and too slow; television, radio and newspapers are regarded as the necessary effective weapons.

It is, of course, the urgency of the situation that leads the Foundation to stress the importance of the mass media, and the real purpose is to have available the literature that will be required by the millions of youngsters now learning to read and write as new countries adopt compulsory education. Moreover, while aid in this field requires experience as well as capital, it needs also honesty of purpose, and here the private foundation has an overwhelming advantage over Government aid. Television cannot build a relation of trust with the viewer unless it is seen to be disinterested and at the service of the public. For this reason the Foundation intends to devote much of its resources to television as an instrument of education, although it is also hoped to enable the new nations to develop their own Press and television.

The new television services overseas will need trained men from their own countries, and for these the Thomson Centre, which is being established near Glasgow, will primarily cater. It is designed so that the actual studios used in overseas operations are exactly recreated and trainees will take courses in programming, engineering and administration. It is also proposed to supplement the local training of journalists by co-operating in the establishment in Britain of a residential training centre near Glasgow, such as the National Council for the Training of Journalists advocates, where aspiring journalists can receive intensive training. It is also proposed to make available highly qualified journalists, newspaper technicians and administrators to assist countries in training the requisite personnel for establishing and developing newspapers, magazines and similar periodicals. The Foundation will itself produce television programmes, particularly for education, and make available television engineers, producers, etc., to assist in training the requisite personnel for establishing and developing television services. Besides assisting in the provision of all kinds of educational literature, including text-books, the Foundation will offer scholarships at British and Commonwealth universities to promising students expecting to take up careers in mass media in the hope that students who thus benefit from a wide and deep education will be firmer in their attachment to freedom and integrity and more fully aware of their responsibility to the public.

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* Department of Technical Co-operation. Technical Co-operation under Colombo Plan: Report for 1961–62 of the Council for Technical Cooration in South and South-East Asia, Colombo, October 1962. Pp. 92. ondon: H.M.S.O., 1962.) 5s. net. See also p. 237 of this issue of ture.

The magnitude of the contribution which the proposed Trust will make to a field as yet comparatively neglected may not be immediately apparent. However, the British Government's contribution under the Colombo Plan alone in 1961–62 of some £1,572,000 included £362,571 for equipment, and the £844,000 for trainees and £365,000 for experts covered 80 new training places out of 273 in the field of transport and communications under the Plan, which also supplied 253 experts in this field, of which telecommunications can only be a fraction; it is obvious that the Foundation could make a very significant contribution to a field which is still comparatively neglected. Moreover, technical assistance under the plan in this field is concerned in the main with the provision of equipment and experts for the actual transmission rather than with programmes and the effective use of the means of broadcasting and television.

It should not be forgotten that there is also the Overseas Visual Aids Centre, sponsored jointly by the Government and the Nuffield Foundation, which was established some four years ago with a grant from the Foundation, and in December 1961 the formation of the Centre for Educational Television Overseas, with matching contribution from the Nuffield Foundation and the Government of £100,000 over five years, and substantial help from the British Broadcasting Corporation, the independent television companies, the Gulbenkian Foundation, industry and commerce, was announced by the Secretary for Technical Co-operation. The main function of this Centre for the next five years is to be a clearing house for requests for programme material, advice and training facilities from any country that wishes to take educational television seriously. The Centre will help to meet these demands by arranging training courses, and by adapting or producing television material which will enable the teacher in the overseas studio to make the most of his own gifts and local knowledge.

The Thomson Foundation contemplates working in this way and over a much wider field. However, it should not be thought that the Foundation is limited to work in the British Commonwealth or even in under-developed countries. It is empowered to concern itself with raising standards of journalism in the Press and on television anywhere in the world. From what Lord Kilmuir has already written, the Foundation is seeking to help existing bodies in Britain to foster high standards of journalism and responsible use of the film and television screen and mass media generally—its scope could range over a the activities covered by the conference on "The Proble of Communication: Science and Industry" arranged April 1961 by the Department of Scientific and Indu trial Research*. If primarily concerned with educatin through informing the general citizen rather than th technologist, the scientist or the industrialist, the problem of communication often have much in common, and muc that was said in the discussions following the papers Dr. M. R. Hopkins on information as an educationa problem, the late Mr. P. Cudlipp on industry, science an the Press, Mr. F. Anstey on films to illuminate an persuade, or Dr. T. Margerison on television, is highl relevant to the work of the new Foundation.

It is well, therefore, that the Trustees of the Thomso Foundation propose to make the fullest possible use o joint effort and to stimulate other developments by othe people. The Foundation is now a pioneer effort thoug it will be working in a field comparatively neglected an undeveloped. Furthermore, little is known how permanent is the impact of educational broadcasting whether by sound or television, and there may be som danger that the urgency of this immediate problem o educational development in the emergent countries ma lead us to use or rely on the mass media to an exten that fuller knowledge will indicate to be unjustified However, this is no reason for rejecting the aid which th mass media can give. It points rather to the vital need fo co-operation with others working in this field and b other means, to the importance of circumspection and above all, to the urgency for further research into ne methods of teaching, their use and impact. If the aim of the Thomson Foundation are to be realized swiftl and fully, much will depend on the scale and urgenc with which educational research, and especially that concerning the use of the mass media, is carried out. Abov all, it should be remembered that high standards of professional conduct and practice are not rapidly achieved They take time to grow. It is not sufficient to propagat the idea of impartial and disinterested news, of tellin the truth in the belief that it will prevail, unless we see simultaneously to foster the climate of opinion in whic high standards of professional conduct are respected an encouraged.

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* Department of Scientific and Industrial Research. Proceedings Conference on Science and Industry—The Problem of Communicatio 18–19 April, 1961, Brangwyn Hall, Swansea. Pp. v+113. (London H.M.S.O., 1962.) 10s. See *Nature*, 190, 965, 1961.

COMPTON LECTURES ON THE UNIVERSE

The Universe

By Dr. Otto Struve. (Karl Taylor Compton Lectures 1959.) Pp. ix+159. (Cambridge, Mass.: The M.I.T Press, 1962.) 4.95 dollars.

SINCE their inception in 1957 the Karl Taylor Compto Lectures in honour of the ninth president of th Massachusetts Institute of Technology have attracted distinguished group of lecturers—Bohr, Struve, Lwof and Rabi. This book is the second series of six lecture delivered in 1959 on "The Universe" by Dr. Otto Struve

The audience must have been thrilled and stimulate by Struve's six talks. They are so beautifully done tha the speaker obviously had a particular interest in hi subjects. Indeed, Struve makes it clear in his preface tha the lectures were not intended to give a complete accoun f astronomical progress, but that the subjects were hosen because the topics were of special interest to him. e begins with the origin and evolution of the solar ystem, then deals with stellar evolution, galaxies, radio stronomy, binary stars and variables, and concludes with talk on man and the universe. The first two of these ctures appear to include the material which Struve ave in the two Condon Lectures in 1958.

Two features exemplify the lectures. They are sprinkled ith elementary calculations of orders of magnitude which nderline the essence of the problems considered, and their utlook is entirely contemporary. Although the book an be read without any particularly deep knowledge of stronomy, it is too specialized in its selection for those ho might expect the broad treatment indicated by the itle. For example, 8 of the 25 pages devoted to radio stronomy are concerned with flare stars of the *UV Ceti* ype and a discussion of the possibility and consequences f detecting radio emission from them, and a further third f the chapter describes the condition of the central region f the galaxy as observed by the Leyden 21-cm work nd the 4-cm observations at Green Bank. Both these opics are of great importance, but readers from other isciplines might well form an odd impression of the alance of the researches of the world community of radio stronomers. This chapter contains the somewhat parochial claim that in his pioneer pre-War observations Reber "was thus first in having discovered discrete radio sources other than the so-called Sagittarius A source. . . ." This can be allowed only as hindsight. Neither Reber nor any other astronomers recognized the significance of the concentration of the radio isophotes in Cygnus and Cassiopeia in terms of the reality of a discrete or localized source, until Hey and his colleagues observed the intensity fluctuations and the Cygnus source was afterwards isolated by Bolton and Stanley in Australia.

The futuristic outlook which Struve displays in the last chapter—and indeed throughout the book—is remarkable in a scientist of his generation. There is no doubt that the pleasure which Struve gave to his audiences will now be communicated to a wide circle through the publication of this book.

A. C. B. Lovell

VEMA OCEANOGRAPHIC RESEARCH SERIES

Abyssal Crustacea

By J. Laurens Barnard, Robert J. Menzies and Mihai C. Bačescu. Pp. ix+223. (New York and London: Columbia University Press, 1962.) 10 dollars; 80*s*.

OVER the past eight years a major part of the Lamont Geological Observatory's research programme has been based on data gathered by the Columbia University research vessel *Vema*. Her seventeen wide-ranging cruises, primarily for the study of marine geophysics and submarine geology, were planned to permit of co-operation with vessels and scientists of other nations; and future expeditions are envisaged. Limited attention was also given to areas of oceanography and marine biology with an application to the main fields of interest. Previously, work done on the *Vema* was reported in standard scientific journals. Now, through the generosity of the Trustees of the Vetlesen Foundation, funds are available for the publication of a new *Vema Research Series* of reports.

It is rather odd, therefore, to find this, the first number of the series, masquerading as a separate book with the misleading title of *Abyssal Crustacea*. It is not a general account of abyssal crustaceans as the unwary might be led to suppose. Indeed, such a work would be premature when much of the material collected on recent deep-sea expeditions still awaits determination. Nor does it deal with more than a fraction of the astonishingly rich *Vema* collections of abyssal amphipods, isopods and cumaceans. This in no way detracts from the merits of these three first reports, each by a competent specialist.

Dr. Laurens Barnard's paper entitled "South Atlantic Abyssal Amphipods collected by *R. V. Vema*" is the first of a series on the extensive amphipod collections from several abyssal areas such as the Caribbean, eastern Pacific, Indian Ocean and Red Sea. He briefly discusses adaptations to abyssal conditions, the food of some abyssal species, endemism in the deep-sea, the relationship of the abyssal to the shallow-water fauna, and lists all previously recorded abyssal species of Amphipoda from depths exceeding 2,000 metres. Nine new genera and thirty-eight new species of Gammaridea are described and fourteen other species are recorded.

Dr. Menzie's "The Isopods of Abyssal Depths in the Atlantic Ocean" covers a wider region and includes Russian data from the Arctic polar basin. A reasonably complete list of previously known abyssal benthic isopods from all oceans is given by region and the "excluded probable pelagic species" are listed separately. Nine new genera and 107 new species are described and 69 other species are included. The ecology, zoogeography and phylogenetic relationships of these isopods will be dealt with in a subsequent report.

Dr. Bačescu's short paper is devoted to a study of the abyssal cumacean genus *Makrokylindrus* Stebbing; five new species are described in detail, and a key to the determination of the twenty-five species at present assigned to this genus is appended.

The price seems reasonable for a bound volume copiously illustrated with excellent line drawings. Other numbers will be awaited with interest.

Isabella Gordon

PROBLEMS AND RESEARCH IN THE HISTORY OF SCIENCE

History of Science

An Annual Review of Literature, Research and Teaching, Vol. 1. Edited by A. C. Crombie and M. A. Hoskin. Pp. vii+133. (Cambridge: W. Heffer and Sons, Ltd., 1962.) 30*s*. net.

THE first volume of this annual review appeared within a few days of the launching of the first issue of the *British Journal for the History of Science*. This coincidence may be welcomed in the light of the increasing interest in the subject throughout Britain. The scope of *History of Science* is revealed in its sub-title: *An Annual Review of Literature, Research and Teaching*. The editors point out that the aim of the *Annual* is to perform a function not systematically carried out by any of the existing journals in this field. "It is intended not to be a competitor to these older journals in publishing original historical articles, but to complement them by systematically providing critical reviews both of problems and materials for research in specific fields and of recent publications."

A study of the first volume shows how ably the editors have achieved their objective. In the first half of the book six articles appear covering a variety of problems: "The Physical Sciences in the First Half of the Nineteenth Century: Problems and Sources" by L. Pearce Williams; "The Expanding World of Newtonian Research" by D. T. Whiteside; "Science and Technology in the Eighteenth Century" by D. S. L. Cardwell; "The History of Medicine in 1960–61" by F. N. L. Poynter; "History and Philosophy of Science at Oxford" by A. C. Crombie; "History and Philosophy of Science at Cambridge" by Gerd Buchdahl. An example of the critical approach, and its value, may be seen in the following quotation from Dr. Whiteside's contribution: "The fallacy of all summaries of the history of ideas by the analysis of Great Books is that the great thinker is made to stand or fall by one expression of his thought, and we see none of the hesitancy, vagueness and quick changes of opinion as his thought develops".

Not least important of these articles are the two dealing with the history and philosophy of science at Oxford and Cambridge respectively. They provide thought-provoking themes especially with regard to the relation of history and philosophy of science to other disciplines. Indeed, they raise questions of fundamental importance in the teaching of students and the training of future instructors.

The remainder of the book is devoted to essay reviews of nine books. Here again the value to the reader is the critical nature of the review, which may extend to as many as ten pages. Shorter reviews of some twenty other books occupy the last few pages of the volume, which also include a note on the importance to historians of science of manuscript records of scientists, a list of doctoral theses in preparation, and of books received.

The book is well—and very legibly—produced, and fills an obvious gap in the record of the history of science.

H. D. ANTHONY

GENETICS AND HUMAN BEINGS

Genetic Mechanisms in Human Disease

Chromosomal Aberrations. Edited by M. F. Ashley Montagu. Pp. xviii+592. (Springfield, Ill.: Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1961.) 156s.

AN explosive burst of fundamental discoveries about abnormal chromosome constitutions in human beings occurred in 1959–60, as a consequence of the new techniques for short-term cell culture (bone marrow, skin and peripheral blood). A collection of these papers has been made by M. F. Montagu, and they make most exciting reading.

Fifty-five papers have been collected from various international journals, and as one might have expected, more than half of these appeared in *The Lancet*. But a few are from less-accessible sources, and one must welcome this effort at bringing these together into one handy volume.

Evidently, the responsibility of the editor is to select the most important and fundamental discoveries, to put them in a sequence for the most coherent reading, to select, if necessary, such additional papers from this field or closely related fields (medicine) which may make the presentation fuller and place the discoveries in their proper relief, and lastly, to see that the selected papers are presented in the best way, technically.

Although the editor of the present volume has collected most of the basic contributions throwing light on the chromosomal origin of a variety of 'diseases' in man, one cannot help feeling that a better collection might have been made. Some papers have been included to the embarrassment of the authors who no longer would subscribe to their suggested interpretations. Other articles discussing topics without sufficient general knowledge of genetics and cytology should not require a repeated publication, while other, more informative discourses, well deserve this repetition.

The papers appear almost chronologically, which tends to destroy the possible cohesion one might have obtained by bringing them into some sectional arrangement. An introductory paper by K. Hirschhorn and H. L. Cooper attempts to give enough basic information so that the uninformed reader may understand the following pages.

It is remarkable that so few of the workers in the field of human cytology have any experience from plants or lower animals in cytology and genetics. This has led to a number of suggestions and interpretations strangely unreal to the cytogeneticist—suggestions which since the papers appeared have been utterly refuted. Similarly, terminology in the non-cytological papers is not always *au pair* with present-day concepts in cytogenetics. Detailed criticism is, however, beyond the scope of this review.

In spite of some lack of critical judgment in selecting the papers, the fundamental contributions are so exciting and outstanding that they override the few less illustrious ones. The discoveries of trisomy in mongolism, the Turner and Klinefelter syndromes explained as sex chromosome aberrations, the superfemale, the chromosome constitutions of true intersexes and of testicular feminization, a new trisomy syndrome (chromosome 18) previously not recognized as a clinical entity, Barr's work on sex chromatin, and finally, the chromosomal basis of chronic myeloid leukaemia—all represent outstanding contributions to our knowledge.

One cannot but welcome this effort of making available in a single volume so many of the original articles, and the type and illustrations surpass in many instances the original presentations.

S. MULDAL

NOT ALL MY EYE

The Eye

Edited by Hugh Davson. Vol. 1: Vegetative Physiology and Biochemistry. Pp. xv+440. 100s. net. Vol. 2: The Visual Processes. Pp. xviii+796. 157s. 6d. Vol. 3: Muscular Mechanisms. Pp. xiv+323. 86s. Vol. 4: Visual Optics and the Optical Space Sense. Pp. xvii+432. 100s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.)

THE Academic Press assembled a galaxy of stars, planets and satellites and produced this useful source book on the eye. Divided into four volumes covering ocular physiology and biochemistry, vision, intrinsic and extrinsic ocular muscles, and optics and space perception respectively, the book is further subdivided into separate sections, written by eighteen authorities. One rejoices over the splendid section on ocular protection—much neglected in other comparable texts, and happily plods through the lucid pages on optics. The sections on the cornea and intra-ocular pressure show far and away the best balance, with those on electrophysiology and photobiology of the visual pigments as runners-up. The difficult tasks of describing anatomical, embryological and comparative ideas have also been tackled, although one begins to suspect in Volume 1 that collaboration between the sundry authors might have been closer: by the time one has finished with Volume 4 this suspicion has matured to certainty. Excellent though the work is, it is incomplete. The eye has been atomized, and no one has bothered to pick up the pieces. C. P. Snow is fortunate in having only two cultures to worry about. In this text the eighteen authors barely understand one another's language. The reader may be able to overcome these barriers, though when he reaches the discussion by Pirenne and Dartnall respectively on the absorption of light he will be in as many difficulties as when he juxtaposes what Alpern and van Heyningen have to say on the crystalline lens.

Granit's contribution to Volume 2 is magisterial. Here writes not only a research worker but also a teacher. The introduction to the visual system (not referred to in the section on anatomy in Volume 1) is classical, and that to the electrical behaviour of the visual system a model. Anyone unfamiliar with the detailed ideas underlying many of the experiments mentioned will be grateful to the writer for his pains, although his ready acceptance of some criteria—like the amplitude of the *b*-wave when the whole ERG should be scrutinized—may one day necessitate some changes in emphasis.

One is a little uncertain as regards the audience for whom these volumes are destined. This is a common difficulty. Thus the optics section contains ophthalmological applications, and the sections on the lens (the approach to which could be more up to date) and on binocular vision have also much to interest the clinician. If knowledge of the eye is assumed, the section on anatomy and embryology is redundant; if it is not assumed, more information would be helpful—especially on the lateral geniculate body and beyond.

Most of the sections are thoughtful, and the reader is unlikely to find any part dull. He may, however, differ from the authors in opinion or in interpretation, as when Ogle reaches a questionable conclusion from a wholly inadequate theory of the Pulfrich effect. Again, Pirenne, quoting Fick (with a faulty reference), relates chromatic aberration of the eye to visual sensitivity, but fails to note that his argument need not apply to hypermetropes. The section on vision is bedevilled by deference to rather ambiguous results obtained by fundus reflectometry, and unnecessary repetition of the associated fallacious statements. Turning to Volume 3 the reader will readily notice that Alpern soft-pedals acceptance of Fincham's capsular theory of accommodation because we know of no quantitative data on capsular elasticity, yet peddles Gullstrand's theory although we have no quantitative data on the functional size of the ciliary muscle. This is no isolated example of bias, and the reader may well decide that, in this section, the references form the focal point of interest. The volumes reveal several lacunæ: Stark's and Bleichert's important work on the pupil is forgotten, nor is there any mention of Stone's on lens and eye implants, or of Gaze's experiments on optic nerve regeneration. The recent work on the extent to which *in vivo* photochemistry governs the gamut of visual intensity is not discussed, nor is the fact that the number of retinal receptors stimulated is more important than retinal area as shown by Ten Doesschate.

When these points are made, however, all that is adverse has been said. When they have been considered, one may almost find oneself in a position to answer the question: Who will read it? Biochemists will scarcely look at the chapters on biochemistry—of interest to other specialists—but will learn a lot from the other sections. Visual psychologists will (please!) read Volume 2 before embarking on their next experiment. Ophthalmic surgeons will want to browse through Pirenne's chapters before interpreting 'normal' visual fields. In short, there is a bite here for everyone. If the connoisseur gets up from table without the fond illusion that he has been served a meal at the Parisian Savarin, at least he will not feel starved.

R. A. Weale

SUMMIT DISCUSSIONS ON DEOXYRIBONUCLEIC ACID

Deoxyribonucleic Acid, Structure, Synthesis and Function

Proceedings of the 11th Annual Reunion of the Société de Chimie Physique, June 1961. Pp. xi+235. (Oxford, London, New York and Paris: Symposium Publications Division, Pergamon Press, 1962.) 60s.

THE organizers of this very successful conference deserve special credit on two counts: first, for holding their meeting in such an original place, in the bracing mountain air of Haute-Savoie; and secondly, for initially publishing the proceedings (in the *Journal de Chimie Physique*) only a few months after the meeting. On the second count, it is therefore lamentable that the publishers of this bound edition, which differs mainly from the earlier proceedings in having an index, should have taken a further nine months or so to produce it. The interest of the volume will thus be to some readers mainly historical, since the broad field to which it refers has undergone a major convulsion since the papers were presented. There was at that time no public announcement of the dramatic results obtained in the United States on protein synthesis with synthetic polymers as 'messenger molecules'; these results added a great deal of knowledge, and helped to confirm what were previously only hypotheses, about the way in which the genetic code is translated from a sequence of purine and pyrimidine bases along a nucleic acid chain, into a sequence of amino-acids in a protein.

The volume is redeemed from obsolescence by the high quality of the papers it contains. They are written by an exceptionally representative group of authors, and most aspects of DNA are covered. The thorny question of whether DNA molecules contain sub-units is discussed by Sadron, while Levinthal shows that the DNA of bacteriophage *T2* can be isolated in a single giant molecule. Wilkins reviews X-ray diffraction work, and explains why the Watson–Crick scheme for purine–pyrimidine base pairs is preferable to various alternative schemes. Luzzati reports low-angle X-ray diffraction. Theoretical papers deal with the electronic structure of DNA bases, and with their interaction when stacked together in a helix. Hurwitz *et al.* contribute a paper on the biosynthesis of RNA (ribonucleic acid) by DNA—part of an initial trickle of papers, now become a raging torrent, which has confirmed beyond reasonable doubt the first half of the famous central dogma which says that 'DNA makes RNA'. This dogma, first publicized by Crick, has always aroused strong feelings in the breasts of nucleic acid workers, either of enthusiasm or of irritation. The second half of the dogma—'and RNA makes protein'—was really beyond the scope of the conference, but was implicit in much of what was said. Its proof or disproof has absorbed the energies of an increasing number of workers since then.

The ingenious application of physical chemistry to DNA is described in a paper read by Marmur: quite simple techniques have been used to make hybrid molecules of DNA, in which one of the two strands comes from one strain of bacteria and the other from a related strain. The bearing this work has on genetic crossing-over is obvious. In this connexion, Stahl provided what appeared at first to be intelligent light relief by inviting the conference to consider a hypothetical chromosome structure consisting of a series of linked circles. When the amusement had subsided it became clear that nobody could seriously fault the model, and it remains open for further study. Work on many other important topics was also reported, including the biosynthesis of DNA and the action of various external agents on DNA structure. This latter aspect has been studied with a wide variety of techniques, too numerous to list here. The volume contains papers from many workers prominent in this field.

One may reflect while looking down the list of participants in this conference on the remarkable variety of backgrounds which they display. The conclusion seems inevitable that really comprehensive work on nucleic acids and their function cannot be expected to flourish easily in universities where all the research has to be fitted into compartments labelled "Chemistry", "Biology", "Physics" and so on. In Britain, unfortunately, such universities are still very much in the majority.

M. Spencer

MOLECULAR BIOLOGY FOR THE CYTOLOGIST

Organization of Cellular Activity

By Prof. Ch. M. A. Kuyper. Elsevier Monographs: Biochemical Section. Pp. x+272 (135 figures). (Amsterdam and New York: Elsevier Publishing Company, 1962.) D.fl.20.

THE author has made a valuable attempt to bridge the gap between morphological observations and biochemical data, the accent being placed on cell physiology. Prof. Kuyper points out that morphology and biochemistry should supplement each other, and he tries to break down the still existing barriers of mistrust between morphologists and biochemists. He places the emphasis on what is now being called 'molecular biology' rather than on 'classical' biochemistry: little is said about the description of natural substances, except, of course, nucleic acids and proteins. If Prof. Kuyper's book succeeds in breaking down existing barriers between cytologists and molecular biologists, it will have served a most useful purpose. The best way, for molecular biologists, to get the confidence of the cytologists is to show them that the structure of the nucleic acids and their role in the synthesis of specific proteins can be understood without too much effort. Cytologists must realize that the concepts of molecular biology must be transferred from the '*E. coli* ± phage' system to real cells.

The book begins with a general introduction: the main cytochemical methods, the chemical composition of the cells (with the accent placed on nucleic acids and proteins, as already mentioned) and a brief description of the cell are successively examined. Chapter 2 deals with the production and storage of energy: ATP, enzymes, co-enzymes, glycolysis, tricarboxylic acid cycle, oxidative phosphorylation, mitochondria, hexose-monophosphate shunt, photosynthesis and chloroplasts are well presented. Chapter 3 is devoted to the "organization of frontiers": the main topics are, of course, the cell membrane, its permeability, active transport, pinocytosis and related processes. In Chapter 4 the author deals with protein synthesis: the role of nucleic acids and ribosomes, the genetic control, the secretion of proteins—including the possible role of the Golgi apparatus—are discussed. Chapter 5 (the organization of mechanical activity) is devoted to the structure of muscle and the mechanism of its contraction; cilia, flagellæ and the mitotic spindle are compared with muscle cells. The next chapter deals with mitosis and meiosis, chromosomes, DNA structure and reduplication. In Chapter 7, Prof. Kuyper discusses the organization of specificity and diversity: biochemical genetics, transfer of information from the nucleus, the organization of the interphase nucleus, giant and lamp-brush chromosomes, differentiation are presented in little more than 20 pages (excluding the figures). Chapter 8 deals with the organization of regulation (induction of enzymes, regulation of their activity, Pasteur effect, cellular homeostasis, hormonal regulations). The concluding remarks end with the wish that more theoretical chemists and solid-state physicists might become interested in the organization of cellular activity.

The aims of the author are obviously excellent. However, criticisms cannot be avoided: if the presentation of the book (there are many good electron micrographs) is pleasant—despite too many misprints—its reading must often be confusing for beginners, university students in particular: for example, mitochondria, Golgi, periodic acid Schiff Feulgen 'coloration', DNA, RNA, RNase, DNase are mentioned on pages 6 and 12; but one must wait until p. 33 before nucleic acids are discussed and until p. 41 before the general morphology of the cell is described. Another very serious drawback—which is admitted by the author himself—is the almost complete absence of bibliography: references are limited to reviews and books, and the reader is left with a dogmatic impression.

There are very few factual errors; but the lack of quotations makes it almost impossible to check doubtful statements. For example, to which species might belong the gastrula, made of 128 cells only, in which nuclear transplantations were made (p. 242): not the amphibian gastrulæ studied by Briggs, King, Moore and Fischberg, I hope. The attitude of the author towards biological and cytological problems is often over-sceptical: he does not believe much in the virtues of histological fixation or autoradiography with tritium-labelled precursors. Although he does not admit it, Prof. Kuyper obviously retains some sympathy for a "vital force". Therefore, viruses—which are not "known" for certain to be "living" —have been barred from the book. It is true that they are "decidedly not cellular in nature", but . . . they multiply in cells and change the organization of the latter after infection. Unfortunately for the author, the book was written and published a little too early: messenger RNA is barely mentioned, the Monod–Jacob theory of gene regulation is not discussed and the work on coding of Nirenberg and the Ochoa school was not yet known.

But Prof. Kuyper is certainly not to blame, if molecular biology is advancing at full speed, too fast for writers of scientific books. Despite a number of shortcomings, the present book is very welcome and should interest molecular biologists as well as cytologists.

J. BRACHET

AIRBORNE RADAR AND WEAPONS SYSTEMS

Airborne Radar

By Donald J. Povejsil, Robert S. Raven and Peter Waterman. (Principles of Guided Missile Design.) Pp. xx + 823. (Princeton, N. J.: D. Van Nostrand Company) Inc.; London: D. Van Nostrand Company, Ltd., 1961., 131*s*. 6*d*.

THE books issued in the series *Principles of Guided Missile Design* are, in many respects, successors to the *Radiation Laboratory Series* of the 1948/49 period. In the early series the emphasis was on the techniques of radar and engineering design details were given, but in this present series the emphasis is on the principles of operation. This volume on *Airborne Radar* is an excellent book, which should be of great value to designers of airborne radar systems and to serving officers, giving them a good insight into concepts of weapon systems.

The mathematical treatment is well handled, and the physical significance is clearly brought out in many examples. The volume is most up to date, and it is probable that because only the general principles of the systems and devices are described, security clearance for publication has been given. There is a possibility that attempts have been made to cover too much ground and descriptions are given of many devices, the omission of which would not have lessened the value of the book.

After briefly describing the general characteristics of radar and the types of operation of which they are capable, the importance of considering radar as a major component in an overall weapon system is stressed. In the example dealing with air interception the inter-relationships of the many sub-systems are examined and the requirements of the air interception radar derived.

The range performances of various radar systems are examined, taking into account the propagation factors, including the effect of the atmosphere and the back-scattering effect of the terrain and precipitation. A concise theoretical treatment is given of signal and noise analysis with many examples. The general properties of many different types of radar are given, and their advantages and disadvantages are clearly brought out.

In the following chapters the principles of operation of the radar system are discussed with the topics including: the receiver; automatic gain control; automatic frequency control; angle stabilization during searching and tracking; automatic angle and range tracking; the microwave properties of aerials, radomes and waveguides; power generation using magnetrons and klystrons; the basic features of display systems and their inter-relation with the human operator; the mechanical design and packaging of airborne equipments. Many chapters are concluded with references to new techniques and devices which may have possible future application. In the final chapter, the application of radar and infra-red radiation is described for use in airborne navigation and air to ground surveillance systems.

R. ASPINALL

AN EXPERIMENT IN SCIENTIFIC COMMUNICATION

By Dr. ALMA HOWARD*

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THE large number of papers offered at present-day congresses, and the arrangement of the programme so as to make the contributed material widely available, present a major difficulty for the organization of international meetings. At the second International Congress of Radiation Research, held at Harrogate during August 5–11, 1962, an attempt was made to find a means of solving this problem.

The Congress was sponsored by the Association for Radiation Research. The proposal to hold the Congress in Great Britain was first put forward at a meeting of the Association in September 1959. At that meeting a number of members expressed dissatisfaction with the usual arrangement of the scientific programme at large meetings, in which most of the time is devoted to the presentation of numbers of short papers in multiple parallel sessions. Prof. J. Rotblat suggested that the rapporteur method should be used, and described his experience of it at one or two meetings which he had attended. Members were impressed with his enthusiastic endorsement of the method. The Congress Organizing Committee thus began its work with a recommendation from the sponsoring body to try to devise a plan for the programme which would reduce the number of parallel sessions, and to consider the use of the rapporteur method for this purpose.

In its early meetings some of the advantages and difficulties of the rapporteur method were weighed and discussed by the Committee. It was recognized that some time would be saved by the presentation of papers by a rapporteur. Papers read by their authors could be presented at the rate of four an hour; in a rapporteur session this rate could be increased by a factor of two or perhaps more. It was also recognized that the method had particular advantages for topics of widespread interest which would attract large audiences, and therefore that plenary rapporteur sessions would be appropriate. In an attempt to meet the wishes of the Association, to give the rapporteur method a fair trial, and at the same time to exercise caution by retaining a conventional plan for part of the programme, the Committee decided to devote the five mornings of the Congress week to plenary rapporteur sessions, and to arrange the remaining papers in parallel sessions on the four afternoons.

Radiation research workers all over the world were invited to send abstracts of papers describing their recent unpublished work, on the understanding that if their papers were chosen for the rapporteur sessions they would be asked to submit a full version before the Congress. Abstracts were due by January 15, 1962, seven months before the Congress; most of them arrived by January 25. During February 9–14 fifteen members of the Scientific Programme Committee met at a hotel. Each abstract was assigned to 3–6 members for reading and classification under a number of headings. When all the material was classified, groups of abstracts were selected and examined by small sub-committees of two or three members. In these small groups the subject-matter, scope and pattern of sessions and groups of sessions began to emerge, and the substance and arrangement of the rapporteur programme grew out of the new work submitted by the Congress members. Rapporteurs, all of them workers in Great Britain, were suggested and approached, and abstracts were submitted to them. Usually a few more abstracts were sent to a rapporteur than he was expected to cover, giving him the opportunity to refuse particular papers.

The total number of abstracts received (772) was nearly double the number expected, making both possible and desirable an increased number of rapporteur sessions. Nine separate programmes running in parallel were required on each afternoon, and one of these comprised three rapporteur sessions. Altogether twenty-five rapporteur sessions were arranged, containing initially 387 papers. The number of papers reported on by a rapporteur ranged from ten to nineteen. There were three reasons for putting more papers into each rapporteur's list than had been originally planned. One was the pressure of the number of abstracts submitted; a second was the impression that some of the abstracts represented rather slight pieces of work, which would justify no more than a brief reference; and a third was the expectation that a considerable proportion of authors would fail to submit their papers when requested. This last expectation proved to be exaggerated; in fact, of the 383 papers requested, 314 were received in time to be included in the rapporteurs' reports. Moreover, it became clear that the extent and value of a piece of experimental work is not always explicit in its abstract. The result was that some of the rapporteurs had to deal with a greater bulk of material than they felt was desirable, even though at the start each rapporteur consciously accepted, on the basis of the abstracts, all the papers on which he finally had to report.

Abstracts of all papers, arranged in their sessions, were printed and circulated to all Congress members six weeks before the meeting†. Full papers were requested by June 1, 1962; most had been received by June 15. Rapporteurs were asked to produce the manuscripts of their reports by July 20, and most of them did so. Meetings were arranged between authors and rapporteurs so that questions could be raised before the time of the session. By good fortune, all twenty-five rapporteurs were able to attend the Congress, and to deliver their reports in person. All rapporteur sessions were held in the Royal Hall, Harrogate, with a four-language simultaneous translation service in operation. Each report was scheduled to occupy 30 min of a 1-h session. The remaining time was allotted to discussion. The leading authors of the papers included in the report sat on the platform, and in many sessions their comments were given priority in the discussion. A discussion secretary was appointed for each session, with the duty of writing an account of the discussion for publication in the Congress *Proceedings*, in which the rapporteurs' reports, but not the original papers, will also appear‡.

On the fourth and fifth days of the Congress week, members were asked to write down their comments on the suitability of the rapporteur system for large congresses. Owing to an unfortunate accident some of the replies were lost. Of the 214 available for analysis (and believed to be a random sample), 174 (81 per cent) expressed the view that the system was suitable. 21 regarded it as unsuitable, and 19 did not answer the question.

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† Abstracts volume, available from the Hon. Secretary, Association for Radiation Research, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester 20. 10s.

‡ *Radiation Effects in Physics, Chemistry and Biology*, edit. by Ebert, M., and Howard, A. (North Holland Publishing Co., Amsterdam, in the press).

Among those members who were not in favour of the rapporteur method, the most common reason was that an audience would gain more from a review of the field by an expert who was not encumbered by having to report on the papers which were submitted to the Congress. Some members also felt that the lack of contact between audience and the individual authors was an overriding disadvantage of the method. A few thought it intolerable for an author to have his work reported by someone else.

Many of the replies expressing approval of the rapporteur method contained comments and suggestions on how it might be more effectively used. The most common criticism of the Harrogate practice was that the number of papers per session was too large. Many members thought that it should be not more than ten. There was also a large body of opinion in favour of a more rigorous selection of papers with the aim of greater uniformity in material, methods and approach. Some members thought that only the poorer papers should be put into rapporteur sessions; others, only the best.

With regard to the rapporteur himself, almost everyone pointed out the obvious fact that everything depends on his quality, and many expressed their appreciation of the high level of performance attained at the Congress. Rapporteurs had been asked to introduce their subject and to synthesize the results of the several authors. A number of members thought it very important that this should be done; a few were of the opposite opinion. It was suggested that the rapporteur's report should be printed and circulated to all authors in the session, or even to all congress members. Some members would have liked to have had fuller abstracts or pre-prints of the full papers, to read at, or before, the Congress. Some felt that authors should be allotted time to speak in the discussions, and an equal number thought that the authors had been given too much time, and that more of the discussion should involve the audience.

The Harrogate experiment showed clearly that if, as is undoubtedly true, new techniques must be found to solve the problem of communication at congresses where large numbers of papers are offered, a possible and acceptable one is the rapporteur method. As practised at the Second International Congress of Radiation Research, it had the effect of providing up-to-the-minute reviews and discussions on topics chosen because they were shown by the proffered papers to be the growing points of radiation research. For the non-specialized audience interested in keeping abreast of new developments on a broad front, this was of great advantage in a field which covers a very wide range of disciplines. The comments of Congress members showed that as listeners the great majority found the rapporteur sessions valuable. As authors and would-be speakers, they were much less favourably inclined.

For those at least who attend congresses primarily to listen and to learn, the rapporteur method offers an attractive alternative to the multiple parallel sessions arrangement of congress programmes.

INTERACTION OF TECHNOLOGIES*

By L. HOLLIDAY

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SPECIALIZATION increases inexorably in the world of technology. In the Stone Age there were no specialists and every man in the tribe was fully trained to carry out the laborious tasks which were necessary to keep him alive. Every man was concerned with the problems of food, of defence, of shelter, and with the straightforward issue of survival. Specialization began at the time of the neolithic revolution in the Middle East, when farming began and urban communities sprang up. This was accompanied by the emergence of craftsmen—of potters, weavers, smiths and farmers. It had taken about one million years for specialization to begin.

Within the space of about ten thousand years, we have reached the stage of specialization in technology at which we operate to-day. We have brick technologists who know nothing about plastics; we have plastics technologists who know nothing about metals; we have metals technologists who know nothing about wood; we have wood technologists who know nothing about textiles, and so it goes on. This is an exaggeration, of course, but it is the pattern which seems to be emerging. Can we therefore look forward to a time when we have specialization in low-density polyethylene of density 0·925, or of polyurethane foams made from polypropylene glycols? If this were to happen, progress would slow down because communication between technologists would be further impeded, and technologists would be trying to solve an increasing number of problems with unsuitable raw materials. Quite apart from this, to attempt to organize the field of technology into broader disciplines represents a fascinating intellectual challenge.

If we step aside and look at the field of science for a moment, the situation is quite different. The human mind has an urge to understand the external world, and to produce order and system out of facts. The scientist wants above all to produce statements and laws of the widest possible validity. A consequence of this is that a coherent and interrelated structure has been built up in the sciences. It is true that, viewed from Olympus, the realm of science would seem to be a continuum, and the branches of physical and biological science would appear to merge into one another and would seem to extend into further fields of knowledge and activity such as mathematics, philosophy, psychology and so on. However, even though the boundaries which exist between the sciences are arbitrary, there is an order and a system in science which is lacking in technology.

Problems of Technology

One of the first problems in technology is a problem in semantics. The word 'technology' is of necessity vague, since it embraces a vast span of interests and activities. If we qualify it in some way, for example, by saying polymer technology, we immediately describe a subject with certain boundaries. These boundaries may then become restrictive and artificial. Technology is, in fact, the science of industry, and comprises the application of science and of scientific method to industry. The classical way in which technology has been subdivided has been to group together industrial activities, using as a key certain commercial and technical interests which they have in common. Thus we talk of space technology, glass technology, petroleum technology and of many others. This is the first stage of evolution of technology, and represents a major step forward, since it stimulates the internal development of each particular technology. It does not necessarily, however, lead to the stimulation of one technology by another, which is the second stage of technological evolution. If technologists are to advance more rapidly as the result of mutual interaction, it is necessary that technology should be subdivided differently and reorganized so that the maximum number of fundamental similarities emerge.

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* Substance of a paper read before Section B (Chemistry) of the British Association meeting in Manchester.

This need becomes even more obvious when one examines the list of research associations in receipt of Government grants. This includes the following:

Baking
Brush
Cast Iron
Coke
Cotton
Cutlery
Drop Forging
Electrical
Felt
Files
Flour
Food
Fruit and Vegetable Canning
Furniture
Gelatine and Glue
Glass
Heating
Hosiery
Hydromechanics
Internal Combustion Engines
Iron and Steel
Jute
Lace
Launderers
Leather
Lime
Linen
Motor
Paint
Paper
Printing
Rubber and Plastics
Scientific Instruments
Shipbuilding
Spring
Steel Castings
Tar
Timber
Water
Whiting
Wool

To what extent can one achieve a synthesis with these pieces? If they are part of a jig-saw puzzle, how can one best produce a recognizable picture? We need to-day someone to do for the evolution of technologies what Mendeléeff and Darwin did a hundred years ago for the growth of chemistry and biology. This raises the question: Who is responsible for producing order out of the chaos which exists in the field of technology, and who is to perceive the technological skeins which run through industry?

One approach is to classify technologies in relation to man's needs, in which case we achieve a measure of simplification as shown in Table 1.

Table 1 gives only examples, but serves to show how technologies can be grouped on a more rational basis. Some of these major technologies are already well recognized—for example, mining, food and chemical technologies. Others, such as materials technology and environmental technology, have not attained the same recognition.

Table 1. BASIC TECHNOLOGIES

Biological technology
  Agriculture
  Forestry
  Fishing

Mining technology
  Ores
  Oil
  Coal
  Gas

Chemical technology
  Chemicals
  Polymers
  Metals

Materials technology
  Plastics
  Glass
  Metals
  Ceramics

Energy technology
  Gas
  Steam raising
  Electricity
  Nuclear energy

Food technologies

Environmental technology
  Building
  Housing
  Lighting
  Heating and ventilation
  Acoustics

Transport technology
  Road
  Rail
  Air
  Sea
  Space

Communications technology
  Radio
  Telegraphy
  Printing

Materials Technology

Let us take one example—materials technology—and consider it in a little more detail in order to demonstrate that certain fundamental principles can be seen to apply to a number of technologies which at the moment are considered to be quite separate.

This is a field where there should be great possibilities of cross-fertilization and interaction, for example, in the manufacture of intermediate raw materials, such as plastics, glass, metals, bricks or paper. If it can be accepted that this is, in fact, but one large technology, this will already represent an enormous simplification in the technological field. Can this statement be justified?

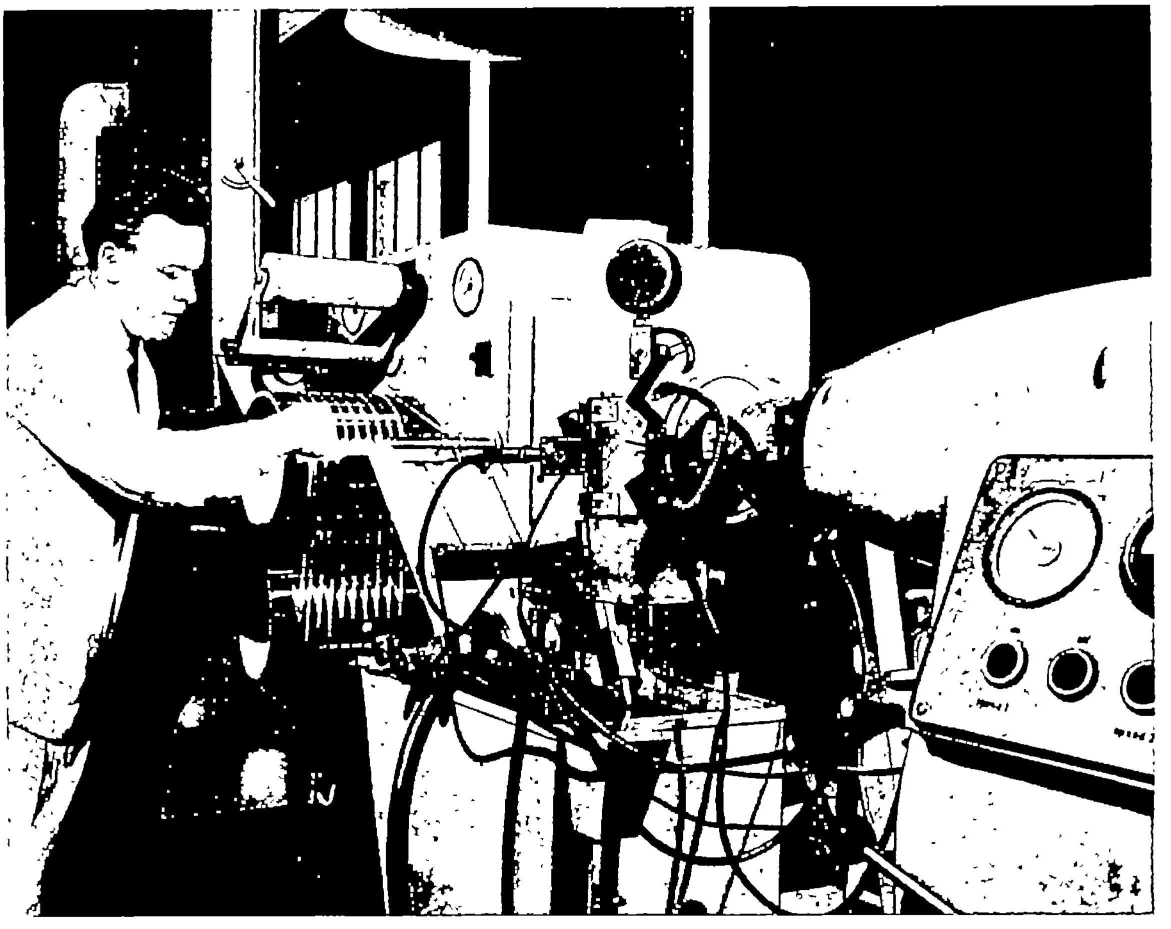

Fig 1. Extrusion of plastic monofilament. This is followed by quenching and hot drawing

There is a unity about simple and complex solid materials which is as fundamental as the unity which exists in the science of chemistry. The unity in chemistry is based on the concept of the atom and the molecule. The unity in materials science is based on the concept of a solid, since all solids by definition have certain features in common with all other solids. These features are the physical properties such as tensile strength, rigidity and hardness which determine whether an object is a solid or not. That there is a fundamental science of materials has only recently been recognized. It is true that there was already a considerable body of knowledge from metallurgy and crystallography which was waiting to be pressed into service, but the subject of materials science had not been investigated in a systematic way.

If materials science is the investigation of the properties of all solid materials, with the view of relating these properties to the ultimate molecular structure, there must also be a subject which we can call materials technology which deals with the fabrication and processing of these materials. Furthermore, since the physical properties of a solid are intimately bound up with the way in which it is made, it will be very difficult to draw a sharp line between materials science and technology. There are clearly two skeins or threads connecting together different branches of materials technology, (*a*) a process skein and (*b*) a product skein. Let us consider the process skein to begin with.

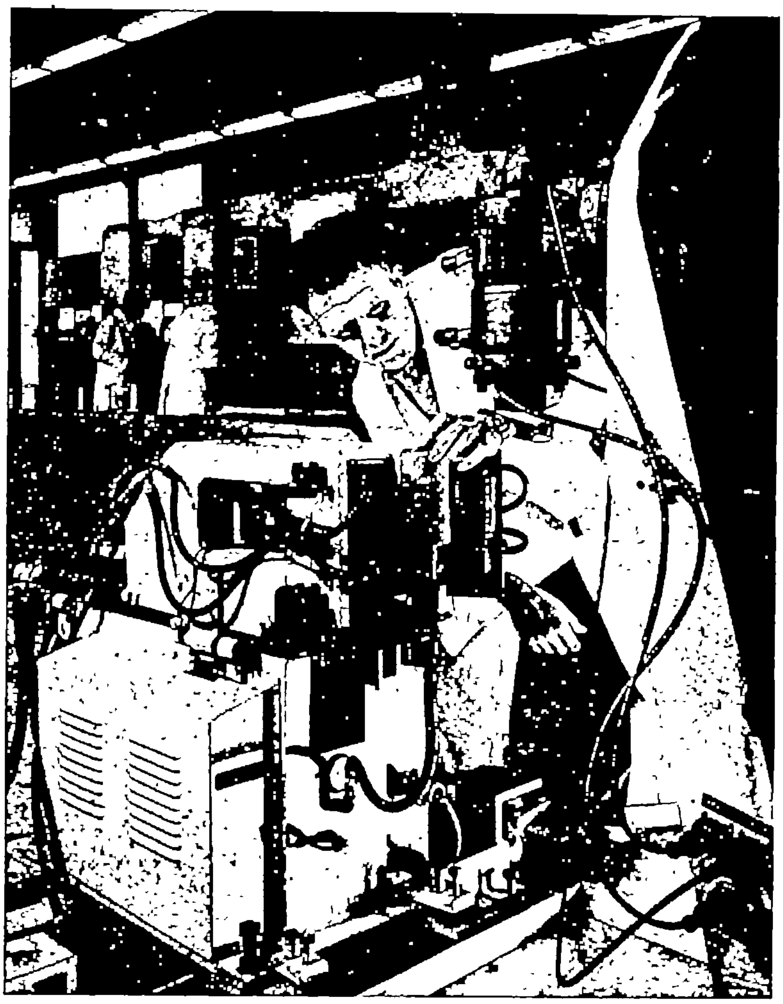

Fig. 2. Blowing of plastic bottles, showing a bottle being removed from the mould. The extruder, which produces a hot 'parison' tube, is shown on the right

Process Skeins—Unit-forming Processes

Ductile materials. A simple starting point is the processing of ductile materials—metals, thermoplastics and inorganic glasses. Inorganic glasses are not ductile at ambient temperatures, but are ductile at, say, 500° C. Metals and thermoplastics are not ductile at very low temperatures, but become ductile in the temperature-range 0–150° C. The property of ductility is, in fact, a function of temperature. Since similar processes are used for fabricating these materials, one can introduce the concept of 'unit forming processes' to describe them. Table 2 shows some processes which are in normal commercial use with these materials, from which it will be seen that similar processes are used, even though the temperature used varies. These processes divide themselves into true geometrical forming processes, and processes which set out to change the micro- or macro-structure of the material.

Although on an atomic or molecular scale the structures of these materials differ—from ordered crystalline lattices containing regions of disorder such as metals, to amorphous structures which show little order such as some polymers—they nevertheless show basic similarities in the ductile stage. In all cases, energy is required in order to change the physical form of the product. This work is required to overcome inter-molecular forces, and in some cases chemical bonds are broken in the process. If the molecules are large, as in the case of thermoplastics, the molecular orientation may be changed. In all cases, stresses may be set up in the product which may affect its physical properties, and these may have to be removed.

It will be seen that processes which have grown up in the older technologies of glasses and metals are being applied in many cases to thermoplastics (this shows the scope for the simplest form of invention—invention by analogy). Fig. 1 shows the manufacture of a thermoplastic monofilament and Fig. 2 the manufacture of plastic bottles. There has been an obvious interaction here, although it is not complete. It is surprising, for example, that three-dimensionally expanded metals (to quote one example) have not yet been developed commercially. There are other new developments in the field of forming processes which have not yet spread to all ductile materials, for example, extrusion accompanied by air blowing, the float glass process, impact extrusion and explosive forming.

Chemosetting materials. It will be seen that there is ample justification for studying unit-forming processes as a class, particularly since the concept of unit-forming processes can be further extended to other materials which at an earlier stage of manufacture will flow like ductile materials. These include bricks, ceramics, many thermosetting resins, rubber, concrete—the list is very long. This class of material is processed at some stage as highly viscous, non-Newtonian liquids which are shaped to the desired form. After they have been shaped, they are usually (but not always) heated in order to cure or set them, after which they are no longer ductile at any temperature. The transition from the liquid to the solid state is the result of a chemical reaction which is in sharp distinction to the class of ductile materials shown in the earlier table. This second class of material can be called 'chemosetting'. Chemosets in the liquid state may be homogeneous (as raw rubbers) or dispersions (as concrete). and the chemical reactions which are responsible for achieving the solid state are diverse—from the formation

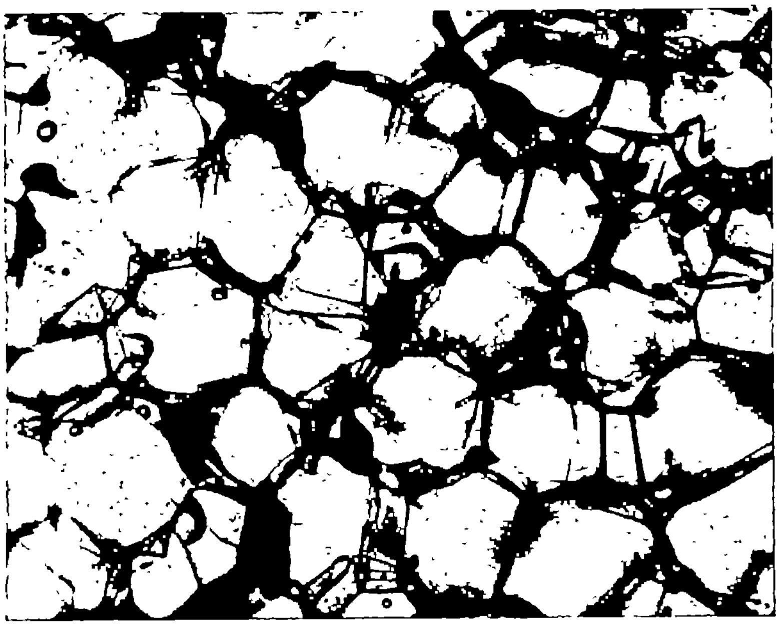

Fig. 3. Photomicrograph of cell structure of expanded polystyrene (× c. 75)

of carbon–sulphur bonds (in the case of rubber) to the formation of aluminates and silicates (in the case of concrete).

It is clear that a large number of unit-forming processes which are utilized for ductile materials can be used for chemosets, and vice versa. However, this analogy between bricks and ceramics on one hand, and metals or glasses on the other, has not been clearly seen. In fact, these have usually been regarded as being completely different classes of materials, which has inhibited the cross-fertilization of ideas which we are looking for. On general grounds it can be claimed that it will repay the brick technologist to study metals processing for example, and vice versa.

Filament processes. Clear analogies also exist in the field of weaving. All the ductile materials shown in Table 2 will form filaments which can be spun or woven. Woven metals, glasses and thermoplastics (like nylon, terylene and polypropylene) are important commercially. There has been very little interest in weaving chemosets, although this could be done. Presumably, the reason has been that woven materials in the great majority of cases are required to be flexible, and chemosets are usually rigid. However, since glass (which is a very rigid material) is woven commercially and rubber (which is a chemoset and very flexible) is also woven, it will be seen that the weaving of chemosets might repay study in special cases.

Table 2. Unit Forming Processes
Ductile materials

	Metals	Thermo-plastics	Inorganic glasses
(1) *Forming Processes*			
Melt processes			
Sintering	+	+	+
Casting	+	−	+
Flow Processes			
Moulding	+	+	+
Extrusion	+	+	+
Drawing	+	+	+
Stretching	−	+	−
Rolling/forging	+	+	−
Beating	+	−	−
Size Reduction			
Grinding	+	+	+
(2) *Structure-modifying Processes*			
Mixing Processes			
Alloying	+	+	−
Dispersion	+	+	+
Expanding	+	+	+
Surface Processes			
Case hardening	+	−	−
Surface treatment	+	+	+
Surface coating	+	+	+
Lamination	+	+	−
Heat Treatment Processes			
Annealing	+	+	+
Quenching	+	+	+

Matting processes. The formation of a mat or web—made up of fine filaments—is of importance in the manufacture of paper, and of growing importance in the case of non-woven fibres. Cellulose fibres, from which paper is made, are produced from wood by chemical digestions and extractions which remove the non-cellulosic materials, such as lignin. Their approximate dimensions are:

	Length	Width
Softwood	3–5 mm	40μ
Hardwood	1–3 mm	25μ

These fibres are thus very long and thin. The manufacture of paper and board depends on the fact that these fibres can readily be dispersed to form a suspension in water. When this suspension is filtered, a fibrous mat is formed which, on rolling and drying, produces a coherent sheet. This sheet can be built up in layers to produce a thick board of considerable mechanical strength. Superior mechanical properties can be obtained by impregnating this board with thermosetting materials such as urea-formaldehyde resins, which replace the air between the fibres by a rigid plastic.

The binding forces between the fibres in paper occur at the points where the fibres touch. Since the fibres are made up of cellulose, the binding consists of hydrogen bonds between the oxygen atom of a glucose unit in one fibre and a hydrogen atom of a glucose unit in an adjacent fibre. The result is a closely knit network of fibres.

It is apparent that a web or mat can be produced from any fibres of the correct geometry (that is, a high length/diameter ratio) if some self-bonding mechanism exists (as with paper) or some other bonding mechanism can be introduced, for example—the use of an adhesive. The key to using matting processes in a more general way is to recognize that the geometry of the system is not confined to cellulose fibres and the bonding forces are not restricted to hydrogen bonds. This has already been recognized in the case of non-woven fabrics, but the principle should be capable of extension.

Product Skelns

Materials may be simple or complex on a macroscopic scale. The simpler structures like metals, glasses or organic polymers may show either long-term or short-term order on a molecular or atomic scale. It is one task of materials science to relate observed physical properties to molecular structure, and good progress has been made in the past twenty years in the fields of metals and glasses.

There are, on the other hand, a large number of solids which are complex on a macroscopic scale. A typical example is expanded polystyrene (Fig. 3). Table 3 gives a few examples of such materials.

Table 3. Some Complex Solids which consist of Two or More Phases

	Continuous phase	Disperse phase
Concrete	Cement	Sand and gravel
Bitumen aggregates	Bitumen	Stone and air
Filled thermoplastics	Linear polymer	Inorganic filler
Reinforced rubbers	Cross-linked polymer	Carbon black, silica
Asbestos cement	Cement	Asbestos fibres
Laminates	Two continuous phases	
Glass fibre/resin systems	Cross-linked polymer	Glass fibres

One of the important features of these complex materials is their geometry, and phase relationship. They usually comprise a continuous phase and one or more disperse phases. Examples of complex materials with more than one continuous phase are rare, although they do exist (an open polyurethane foam has a continuous gas phase and a continuous polymer phase—expanded polystyrene, which has a closed pore structure, has a disperse gas phase and a continuous polymer phase).

Principle of geometrical similitudes. In understanding the properties of complex materials it is important to consider those aspects which are affected by the geometry of the system. This point can be illustrated by taking a particular example, although the approach is a general one. Let us consider a filled thermoplastic in which a thermoplastic material like polyethylene constitutes the continuous phase, and a filler like chalk or carbon black constitutes the disperse phase. The geometry is defined by such factors as particle size and shape, volume fraction of the disperse phase, size and concentration distribution, orientation if the particles are non-spherical, etc. The geometrical parameters are the same in a concrete road or a bitumen aggregate. If z_s is any physical property of the complex material, then:

$$z_s = f(z_a, z_b, z_{ab}, z_{bb}, c_b)$$

where z_a = physical property of continuous phase a; z_b = physical property of disperse phase b; z_{ab} = interaction term between the disperse and continuous phase; z_{bb} = interaction term between the particles of the disperse phase; c_b = concentration of disperse phase.

We can now enunciate some principles of geometrical similitude for complex materials: (1) the properties of complex materials are closely bound up with their geometry; (2) any complex material may exist theoretically in any geometry; (3) the effect of geometry is broadly similar in different systems.

Returning to the general equation, there are one or two physical properties which depend scarcely at all on interaction terms—for example, density and softening point. Nevertheless, it is the interaction terms which make complex materials so interesting.

Interaction term z_{ab}—between continuous and disperse phase. The magnitude of the interaction term z_{ab} depends on the particle size and concentration of the disperse phase, on the particle shape and surface area, and on the intermolecular forces at the interface between the particles and the continuous phase. In general, it increases as the particle size becomes smaller. It will be evident that not only are there a number of degrees of freedom which enable one to change the physical properties of the system by modifying the geometry but, in addition, it is possible to change the intermolecular forces at the interface by coating the particles before incorporating them into the matrix. This is done in such different fields as thermoplastics and bitumen aggregates.

Interaction term z_{bb}—between particles of the disperse phase. This interaction term is very dependent on particle size and concentration. It is therefore only significant in special cases. A typical example is in the reinforcement of rubber with carbon black or silica, where the particle size is below 1 micron. At below 10 per cent by volume, each particle is operating independently and only z_{ab} is important. As the volume concentration is increased, the particles begin to form filament-like aggregates and the term z_{bb} begins to play a part. This change in geometry shows up in the increased electrical conductivity of the system and in the elastic properties of the rubber.

Conclusions

These illustrations drawn from the field of materials technology illustrate the thesis—that benefits accrue from searching for the fundamental principles in individual technologies and for the relationships between them. This can only come from adopting a more analytical approach to technology as a whole, and by encouraging technologists to look beyond their individual specialities. What can be done to improve the situation?

(1) We should first look to the universities which have technological departments, and to the colleges of advanced technology, to bring together their technological departments into the largest possible units. As an example, instead of having in one university twenty professors of technological subjects each the head of an independent department, one should have a few large departments of technology, each with a number of professors. This is the way in which the Manchester College of Science and Technology is evolving. The department which now looks after textile chemistry and paper technology will in the future include polymer technology. We also need some professors of technology who are not concerned with narrow specialist fields, but who are concerned with industrial research and development as a whole, and with the philosophy of industrial research and development.

(2) There are too many technological societies and institutions, and too many technological journals. Some of these should be amalgamated.

(3) There is, in addition, a great need to cut down the number of industrial research associations and to bring them together into larger units. At the moment these research associations are scattered all over Britain in splinter groups. To have too many individual research associations means that the basic and fundamental research is frequently being carried out by teams which are too small to be really effective. The Department of Scientific and Industrial Research is aware of this fact, but will have a formidable task to improve the present situation. The situation in Holland, with its Toegepast Natuurwetenschappelijk Onderzoek Institutes, represents a pattern which Britain might follow. Most of the Toegepast Natuurwetenschappelijk Onderzoek Institutes are grouped together in one large laboratory complex, and this laboratory complex is situated on the campus of the technical university at Delft. Thus not only do the devotees of specialist technologies rub shoulders with each other but they also rub shoulders with the university scientists.

The Department of Scientific and Industrial Research, as a major spender of public research funds, has probably the key responsibility to promote the interaction of technologies. It is keenly aware of these responsibilities; but it will have to work hard even to stay abreast of a situation which daily becomes more complex.

RICHARD TOWNELEY AND BOYLE'S LAW

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THE year 1962 was the tercentenary of the publication of one of the best-known laws of science, Boyle's law, which stated that the volume of an enclosed gas was inversely proportional to the pressure applied to it[1]. As originally verified, the law only applied to air, which was at that time not understood to be a mixture of gases.

The law's establishment has been traditionally considered to be the work of Robert Boyle (1627–91), and it is one of the major researches on which his reputation as a principal scientist of the seventeenth century is based. Modern authorities, when commenting on the gas law, occasionally mention that Richard Towneley (1628–1706-7) has some claim to priority in the discovery of the gas law; but the evidence concerning his role has not been critically examined.

Both Boyle and Robert Hooke, his closest associate, referred to the gas law as "Mr. Towneley's hypothesis", and it is clear that it was on Towneley's initiative that they embarked on experiments which confirmed Towneley's suggested law.

Unfortunately, Towneley himself never released a treatise concerning air pressure, although Boyle referred to an incomplete work containing Towneley's views. He did not preserve any letters from Towneley, if, indeed, the latter did communicate with Boyle directly.

The first publication of the gas law occurred in the second edition of *New Experiments Physico-Mechanical Touching the Air* in 1662 [1]. There were no indications in the first edition of 1660 that Boyle was making investigations which would lead to the law, and close scrutiny of the 1662 edition impels the conclusion that the law was introduced as a corollary, rather than as a discovery of major importance. Consideration of the law was confined to Chapters 4 and 5 of Part 2 of the appendix to the work, in which Boyle was concerned with the demolition of Franciscus Linus's arguments or 'funicular hypothesis' disputing Boyle's explanation that the mercury column was supported by the pressure of air[2]. He found Towneley's hypothesis and experiments to be useful material in completing the arguments against Linus.

While writing his defence of the pressure or "spring of air" Boyle had beside him the results of experiments on the same subject by Richard Towneley and Henry Power (1623–68). These investigators had begun experiments using Toricelli tubes in 1653 in the Halifax region, after Power had reach Pecquet's *New Anatomical Experiments* . . .[3] which contained Pascal's views on air pressure as well as the famous observations on the lymphatic system.

Publication of Boyle's work in 1660 created a new impetus to their activities, and they performed a new series of experiments which were completed in April 1661. These were transcribed by Power and forwarded part by part to William Croone, the registrar of the newly formed society meeting in London, which was shortly to become the Royal Society. As was the custom of this group of scientists, the manuscripts were passed on to interested persons, and Croone wrote to Power informing him that Boyle was in possession of the manuscripts concerning the 1660–61 experiments[4]. Further, he informed Power that Boyle was using the information from the experiments using "weather glasses" in preparing his invective against Linus.

The title which Power used for the experiments which particularly interested Boyle was "Additional experiments made at Townley Hall, in the years 1660 and 1661, by the advice and assistance of that Heroick and Worthy Gentleman, Richard Townley, Esqr. and those Ingenious Gentlemen Mr. John, and Mr. Charles Townley, and Mr. George Kemp"[5]. Although the manuscript emanated from Power, it is quite possible that Boyle overlooked Power's part in the work, which would not be easily guessed from the title. Examination of Chapters 4 and 5 of Boyle's appendix show that the author leant heavily on the information from the foregoing manuscripts.

Power himself was mentioned by Boyle, when the latter quoted from a manuscript which he received at a different time from *Additional Experiments* . . .[6]. Boyle quoted a section of "A Confutation of this Funicular Hypothesis of Linus, by Henry Power Mae, Dr."[7].

The above manuscripts shortly became part of the collection of articles which were published as Part 2 of Power's only published work[8].

Because of priority of publication of Boyle's over Power's work, the second part of *Experimental Philosophy* has been neglected, its contents apparently lacking novelty in the light of Boyle's illustrious publication. Even Power's succinct microscopical observations were shortly to be eclipsed by Hooke's rambling *Micrographia*[9].

Towneley and Power's experiments do indicate the main stages which led to the apprehension of the gas law. In 1653 Power introduced into England Pascal's famous experiment in which the decrease in air pressure with increase in altitude could be illustrated by carrying a Torricelli tube up a hill. He measured the fall in the mercury-level with the ascent of Halifax Hill near his home at Elland.

In the same year the authors repeated Pecquet's experiment in which a small volume of air was enclosed above mercury in a Torricelli tube and they noted that it caused a lowering of the mercury column owing to two causes: "Ayr, besides its gravity has a nobler rarefactive faculty, by which it forces the Quicksilver to so considerable a descent, . . ."[10].

On April 27, 1661, they combined the effects of the two previous experiments by enclosing a volume of air above mercury and measuring its change at two atmospheric pressures, either at the foot or apex of Pendle Hill in Lancashire. They repeated the experiment using a tube of different length, and they measured the atmospheric pressure using a simple Torricelli tube containing a 29-in. column of mercury supported in a 42-in. tube.

They compared the pressure exerted on the enclosed air ($p1$) and its consequent volume ($v1$), with the same measurements made at a different pressure ($p2$ $v2$). They called these figures four "proportionals". They examined the relationship between these figures, which was made a little difficult by their use of two different units, either inches or smaller arbitrary divisions of the Torricelli tube. This necessitated conversion of the values into a common unit.

At the end of their experiments they were able to express the rather cryptic conclusion: "So that here is now four Proportionals, and by any three given, you may strike out the fourth, by Conversion, Transposition, and Division of them. So that by these Analogies you may prognosticate the effects, which follow in all Mercurial Experiments, and predemonstrate them, by calculation, before the senses give an Experimental (eviction) thereof"[11].

Thus they consider that if four such proportionals as those mentioned previously are known, they will have the following relationship:

$$p1v1 = p2v2$$

If any one is unknown, then transposition followed by division will enable it to be calculated. For example, if $p1$ is unknown:

$$p1 = \frac{p2v2}{v1}$$

There follow from these equations the common expressions of Boyle's law:

$$pv = K \ (K = \text{constant})$$

$$p = \frac{K}{v}$$

Power and Towneley stated only their experimental results; but they did include sufficient information in their tables and diagrams for the simple calculations to be made. The results shown here are after my conversion of all figures to Power's arbitrary units. It is not possible to convert the figures into inches, using the information given in the experiments. The main barrier in making the calculations was the profuse typographical errors which bedevilled the whole published work.

Locality and barometric height		Pressure (p)	Volume of air (v)	Constant $pv = k$
Exp. 1				
Pendle Hill Beacon (1,800 ft.)	66·58	55·32	84·75	4,687·4
Barlow (800 ft.)	69·01	57·23	83·80	4,795·9
Exp. 2				
Pendle Hill Beacon	32·66	18·60	17·80	334·04
Barlow	33·86	19·49	17·35	337·63
Barlow	33·86	19·66	17·58	345·63

The variation from the constant in these results is small enough to be accounted for by experimental error, and the authors themselves were well aware of frequent fluctuations in barometric height and they considered temperature to be one of its causes[12]. Boyle pointed out that their tubes were liable to be of non-uniform bore[13].

Examination of the evidence summarized here suggests that Richard Towneley and his collaborators played an important part in the postulation and experimental support of Boyle's law, and that Boyle was well justified in expressing his respect for the work of these minor scientific authors. It is overlooked that both Boyle and Hooke utilized the same apparatus, devised by Power and Towneley, in performing their own experiments on the gas law. They modified its use for their laboratory experiments, and obtained satisfactory results with it, although this original apparatus is less well known than the new apparatus which Boyle devised in which air was enclosed beneath mercury, in the short arm of a J tube. While Towneley's apparatus allowed the influence of reduced pressure on the volume of air to be measured, Boyle's J tube enabled the pressure to be increased.

Boyle's role in the establishment of the gas law remains important; his experimental results were accurate and extensive; the apparatus which he devised has become the standard one for illustrating the gas law; most significantly he gave the weight of his great reputation to the law, and published it in a highly influential work. He became the publicist of the gas law, which would have remained unnoticed in Power's work and Towneley's correspondence with Boyle.

However, the process of history has led to a loss of perspective in the examination of the achievements of such major figures as Boyle, leading to their excessive glorification, with the complementary neglect of such authors as Towneley and Power.

[1] Boyle R., *New Experiments Physico-Mechanical Touching the Air*, sec. ed. (Oxford, 1662).

[2] Linus, Franciscus, *Tractatus de corporum inseparabilitate* (London, 1661).

[3] Pecquet, J., *Experimenta Nova Anatomica, Quibus Incognitum Lacteus Chyli Receptaculum*, (Paris, 1651; English translation, London 1653).

[4] Croone, W., Letter to H. Power, September 14, 1661. Brit. Mus. Sloane MSS. 1326, f. 25. Letter to H. Power, January 9, 1661—[2] Brit. Mus. Sloane MSS. 1326, f. 28–29.

[5] Croone, W., Letter to H. Power, July 20, 1661. Brit. Mus. Sloane MSS 1326, f. 26. He requested Power to send his recently performed "Mercurial Experiments".

[6] Power, H., Letter to W. Croone, November 27, 1661. Brit. Mus. Sloane MSS 1326, f. 28. Power mentions that he has sent this work to Croone.

[7] Power H., *Confutation of Linus*. Brit. Mus. Sloane MSS. 1393, f. 166.

[8] Power, H., *Experimental Philosophy, in three Books: Containing New Experiments, Microscopical, Mercurial, Magnetical* (London, 1663).

[9] Hooke, R., *Micrographia: or some Physiological Descriptions of Minute Bodies* (London, 1665).

[10] Power, H., *Experimental Philosophy*, 114.

[11] Power, H., *Experimental Philosophy*, 130.

[12] Power, H., *Experimental Philosophy*, 128.

[13] Boyle, R., *New Experiments Physico-Mechanical* ... *in Works of the Honourable Robert Boyle*, edit. by Birch, T., 1 102 (London, 1744)

COMETARY COLLISIONS AND TEKTITES

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In a recent communication to *Nature*, Adams and Huffaker[1] have presented arguments purporting to show that tektites cannot originate from the Earth on mechanical grounds. They argue that it is not possible to propel small objects through the atmosphere of the Earth to high altitudes because of the enormous accelerations that are required. They also discussed the question of whether very large objects could be propelled from the Earth's surface through the atmosphere. Such proposals are so obviously impossible that it has seemed to me unnecessary that they should even be discussed, and no proposals that they could occur have appeared in the literature. But since the *Scientific American* has recently accepted these arguments as valid, it may be well to discuss the subject briefly.

I proposed that a cometary collision produced the tektites[2]. It was pointed out that a collision of a comet head with the Earth might be an exceedingly energetic event. It was estimated that one such event could occur every 50 million years. Prof. Z. Kopal (personal communication) pointed out that this was much too low an estimate for the frequency of such events. The qualitative description of this process quite clearly envisaged that the cometary material and the atmosphere would be propelled to a very large distance with high velocities and would probably carry with it some material from the surface of the Earth. No suggestion that small objects would be propelled through the atmosphere was made. It was suggested that a comet head broke up and that the fragments landed at different points resulting in a wide distribution. This suggestion is difficult to reconcile with the very similar compositions of tektites from the widely separated tektite areas as Pinson[3] has shown and as pointed out by him.

Recently, Janet Bainbridge[4] has made numerical calculations in regard to this question and finds that the cometary material would behave approximately as has been assumed. Calculations were made for a cylindrical comet of density 0·01 g/cm^3 approaching an olivine surface in vacuum at velocities appropriate for collisions with the Earth or Moon. Velocities of the colliding object after collisions both in the backward and horizontal directions were found to be nearly equal to the velocities of approach. In the presence of an atmosphere, the behaviour of small comet heads would be quite different but large objects would follow a pattern much like that for the objects in vacuum. The assumptions made above for the cometary object appear to be reasonable.

The proponents of an extraterrestrial origin of the tektites rest their arguments on the fact that the australites are often button-shaped and that these button-like structures have been produced by rapid flight through the atmosphere and on the difficulties encountered in matching the composition of the tektites exactly with that of common terrestrial materials.

Chapman[5] estimated the velocity of entry of the button-shaped australites as about the escape velocity of the Earth and hence reasoned that the tektites came from the Moon. Adams and Huffaker[1] estimate the entry velocity as possibly as low as 6·5 km/sec, and hence postulate a circum-terrestrial orbit which enters the atmosphere at glancing angles. No attempt is made to explain how large objects, that is, 1,700 tons, got from the Moon into such very specialized orbits. A criticism of this suggestion has been made recently[6]. No one has ever questioned that the button-like structures of the australites were made by the passage of these objects through gas at high velocity or at least that high velocity gases flowed over the objects in some way. But there seems to be a complete neglect of all the other evidence in regard to this problem by proponents of the lunar origin. The reader's attention is directed to a recent paper by Taylor[7] on the chemical composition of the australites in which it is concluded that their composition agrees closely with certain materials from the surface of the Earth, which were produced by all the complicated geological processes characteristic of a planet like the Earth. This is a conclusion entirely similar to one that came to my attention quite a number of years ago from the work of O. Joensuu at the University of Chicago in the early fifties. This work was not published but was referred to by me[9]. The large tektites of the Muong-Nong type have been discussed by Barnes and Pitakpaivan[9] recently. They are mixed with a laterite soil which the authors believe has evolved since the objects were produced by fusion of the surface of the Earth. Craters have been discussed by Cohen[10] which might well be the collision sites for cometary objects. The short time that the tektites have been in space has been pointed out by Viste and Anders[11]. The similarity of composition of tektites to certain soils has been discussed by Schwartz[12]. The impossibility of transferring material from the Moon to the Earth with the distributions that are observed was discussed by me in 1955 and again in 1962 [4,8]. The very uniform isotopic composition of the tektites as pointed out by Taylor and Epstein[13], and the very similar compositions of tektites from different areas as shown by Lowman[14] are difficult questions but perhaps not insurmountable for terrestrial origin. The very low nickel content is not easily reconciled with a lunar origin.

In connexion with the cometary collision idea, it is interesting to point out that the recent studies on the Tungussky catastrophe of 1908 are most informative[15]. The evidence is that an object having the energy of 4×10^{23} ergs (10 megatons of TNT equivalent) exploded five or more kilometres above the surface of the Earth. The radiated energy was about 1·1–2·8 times 10^{23} ergs. The estimated energy of the collision is seven times that estimated for the Canyon Diablio crater, yet no crater was observed. Interestingly enough, a forest remained standing directly under the point of explosion. There is considerable uncertainty in regard to the mass and velocity of the object. It apparently arrived at a low angle and Zolotov[15] believes at a low velocity, that is, 3–4 km sec^{-1}. Such a low velocity is not consistent with a high energy unless the energy was internal, that is, chemical energy. Krinov[16] mentions higher velocities. It scarcely could have stopped in the atmosphere if its mass per unit area had been much larger than the mass per unit area of the air through which it passed. But if the object were moving in an initial orbit about the Earth, its velocity would be minimal at 7·9 km sec^{-1}, and thus if its velocity of approach were only half this it must have lost three-quarters of its energy. Its path would have been from the south over some of the most populous regions of the Earth. It is difficult to believe that it would not have been seen by someone at seven o'clock in the morning. The velocity mentioned by Zolotov must be in error for some reason which is not evident from his paper. These uncertainties make it difficult to estimate its physical properties.

It is not possible to estimate the frequency of such collisions from one event. No event of such magnitude could have occurred in civilized regions of the world without some record having come down to us, but such areas have constituted only very small fractions of the Earth's surface. As noted previously[8], several astronomical objects have passed near the Earth during this century, and it is most likely that the inventory is not complete. A rough estimate of the probability of collision with the Earth indicates that this might occur within some millions of years or less. The estimated energy of collision is very much larger than 4×10^{23}, possibly some 10^{28} ergs. An object of this kind, if it had a high density, would pass through the atmosphere almost unobstructed and would produce a large crater. However, comet heads of low density would be stopped to a large degree by the atmosphere and no large deep craters would result. Since large craters are not numerous on the Earth's surface, these objects may well be comet heads of low density.

Suggestions have been made that the Tungussky object was contraterrene matter which is far too extreme a suggestion for serious consideration. This object was a small comet head according to Krinov[16]. If the object had had sufficient mass per unit area, it would have penetrated completely to the surface of the Earth, probably melted the surface of the Earth, and produced a very shallow crater. In other words, the Tungussky collision is an example of precisely the kind of collision that has been discussed in connexion with the origin of tektites, though smaller in mass than those suggested. Larger comet heads are discussed in the astronomical literature and such larger heads may well have produced the craters discussed by Cohen[10]. Both the probable cometary collision of an object only some 200 metres or so in dimension and these larger objects some kilometres in dimension moving near the Earth are consistent with the hypothesis that cometary collisions could be sufficiently frequent and of such character that tektites could be produced in this way. (Cohen at the Third International Space Science Symposium, May 1962, quoted a private communication from W. Gentner, H. J. Lippolt and O. A. Schaeffer to the effect that the potassium–argon age of a glass sample from the Ries crater and a moldavite tektite both have the age within experimental limits of error of 15 million years.)

Many features of tektites are accounted for by the cometary hypothesis. The chemical composition of the tektites is very like that of the more acid terrestrial materials. A mechanism for dispersal to great distances is provided by the cometary collision. High temperatures for rapid and incomplete melting are provided. The decreased estimate for the re-entry velocity, that is, 6·5 km sec^{-1}, makes it possible to account for the second melting of the australites. The types without flanges are those which did not re-enter at high velocities. The Muong-Nong types are residues of the melted pools produced at the collision sites and not scattered to great distances. A comet head collision has been observed in this century. Larger ones should melt the Earth's surface, produce craters and scatter material to great distances. All detailed features of tektites are not immediately explained by this theory, but its successes are so great that it would be well for authors to study possibilities for fitting their data into the general features of this theory. It is evident that a cometary collision is a very complicated event and that it is not possible to describe all its details with certainty.

It is evident that material of approximately tektitic composition is widespread on the Earth, though Taylor and Epstein's[13], Lowman's[14], Pinson's[6] and Taylor's[7] discussions show that only selected samples would account exactly for their composition. However, melting and distribution by a comet head collision would probably be highly selective. Thus, very fluid melts would scarcely remain as objects of the size of tektites in a blast of hot gases, and hence only very acid and viscous materials, that is, tektites and Libyan glass, would survive. Also basic melts would crystallize rapidly and would weather away in short periods of time. Even acidic materials melted at high temperatures would not survive. Only such material which had been barely melted, such as the tektites, would survive the blast of gases and would remain as uncrystallized and unweathered glassy objects after long periods of time. In this way the very selected compositions discussed by Lowman[14] can be understood possibly. Also, it should be noted that the present cometary collision hypothesis assumes that all the tektitic fields of south-east Asia and Australia originated from one site and hence their similarity is naturally explained. The general exclusion of Americanites and the Darwin and Libyan glasses from the classification of tektites make them a more uniform group than they are when these are included. Finally, melting by cometary collisions may be more selective than we are able to deduce from our general estimates in regard to such processes.

The postulate that tektites come from the Moon has never been discussed in an objective way. Varsavsky[17] tried to show that South Australia could be covered by objects from the Moon while North Australia did not receive such objects. He did not account for the other tektite areas nor for objects which missed the Earth on the first pass. O'Keefe has postulated objects moving in spiral orbits from the Moon and in no way considers the high probability, and in fact, certainty that many objects released from the lunar surface by some violent processes would move in very different orbits and arrive at all points of the Earth's surface. It is difficult indeed to recall any proposal discussed in the scientific literature in recent years which is so enmeshed in special pleading for nearly miraculous events as this proposal for the lunar-origin of tektites. Criticisms of these arguments have been made previously[18].

If comet heads collide with the Earth, they must also collide with the Moon, and some of the lunar craters must have been produced in this way. The central peaks with their apparent volcanic craters and the general "scooped out" shape are not inconsistent with qualitative ideas of the results of a gaseous collision such as that studied by Mrs. Bainbridge. These calculations indicate that a mild 'bounce' of the lunar surface at the collision point is possible. Such a rebound might explain the craters in the tops of many central peaks. The propellent and non-penetrating character of high-velocity gases and low-density solids rather than the more 'detonating' and penetrating character of high density silicate and metal objects might well account for some of the lunar craters. It is not evident that there are two classes of lunar craters. At present this suggestion is scarcely more than an intuitive estimate in regard to these phenomena.

I thank Drs. Albert and Celeste Engel for discussions of this subject, and Dr. Herbert York for discussion on the Tungussky collision.

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OLD MORTALITY

By Prof. LANCELOT HOGBEN, F.R.S.

DURING the early 'thirties, R. R. Kuczynski introduced then young research workers of Great Britain to the uses of the Actuarial Life Table (A.L.T.) as a substitute for standardized rates hitherto used for interpretation of vital statistics in the best sense of the term. Since the end of the Second World War there has been an agonizing reappraisal, largely due to the use of *cohort analysis* for the study of mortality and morbidity by R. A. M. Case[1] and for that of fertility by W. Taylor[2] and by P. K. Whelpton[3]. Thus the issue of a monograph by Dr. Case *et al.* on the Cohort Life Table (C.L.T.) from the Chester Beatty Research Institute on the eve of the Royal Society tercentenary celebrations of the publication of the *Observations . . . upon the Bills of Mortality* by John Graunt[4] is a timely event *. It is also greatly to the credit of the Institute. For like other inquiries by its senior author, his work in this field has the hall-mark of perceptive originality.

The A.L.T. is like the composite face which Galton obtained by photographic superposition in pursuit of Quetelet's Average Man. That is to say, it tells us what would be the number of survivors out of 1,000 births in each year of life, if age specific death-rates (mortality in each year of life related to the corresponding population at risk) were to remain constant as at the calendar year which dates the Table. No single C.L.T. is referable to one calendar year in this sense. Each such table pertains to the birth year of a particular cohort, that is, assemblage born in the same calendar year. Whereas each entry in the A.L.T. refers to a different cohort in that sense, successive entries of a C.L.T. thus record the life experience of one and the same assemblage, except in so far as migration distorts the picture. In short, the C.L.T. is as factual a record of a unique observable occurrence as is the photograph of an individual face.

Needless to say, the C.L.T., and cohort analysis in general, has two limitations. At the time of writing, one can know nothing about mortality of 14-year-old children born in 1950, and standards of diagnosis may well have changed drastically during the life experience of the now-extinct 1860 cohort. None the less, the outcome of focusing attention on the life experience of the cohort as a unit of study is salutary from a medical point of view, if only because it compels us to take stock of hitherto insufficiently recognized shortcomings of the A.L.T. and of its applications.

Consider first a type of situation characteristic of Western Europe throughout the past half-century, during which mortality in all age-groups has diminished, or at worst remained static at the tail-end of life. Thus the younger age-groups now alive have suffered exposure to less health hazards than corresponding age-groups of the parental generation. In such a set-up, we may expect what this monograph clearly discloses: for nearly all (if not all) age-groups, the A.L.T. for the calendar year X will cite a lower proportion of survivors than the C.L.T. referable to birth-date X. In a uniformly improving situation, use of the A.L.T. to estimate the risk of death before 60 of persons thirty years old at the calendar date to which its figures refer therefore discloses an unduly pessimistic prospect. If, as some people idly imagine, the current A.L.T. actually dictated the premium policy of an Insurance Corporation, circumstances would be prejudicial to the candidate for a life policy and, *ceteris paribus* (that is, with no change of the cost of living), favourable to the purchaser of an annuity.

Alternatively, consider a situation in which risk of contracting a particular killing disease is increasing *pari passu* with net diminution of death risk from all so-called causes. This is indeed what some claim to be true of lung carcinoma with due allowance for changing diagnostic standards. In this *milieu*, cohort analysis exposes what should rightly go into the text-books as the "Robert Case Illusion". Briefly stated it is this: a plot of case incidence in each year of life, expressed as a ratio referable to the corresponding population at risk in one and the same calendar year, may (and often does) disclose a peak followed by a fall suggestive of declining liability in later age-groups. This conclusion is inescapable only if we assume that the risk of onset (or death, as the case may be) does not depend on the duration of the exposure to harmful circumstances or that the latter remain constant throughout the life-time of an individual. By following out the life-story of a cohort, as Case[1] has done elsewhere, it may be possible to show that neither assumption is permissible. Comparison of data yielded by successive cohorts may then divulge that a peak is a non-existent nose of a composite face.

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* Chester Beatty Research Institute Serial Abridged Life Tables, England and Wales, 1841–1960. Part 1: Tables, Preface and Notes. Compiled by R. A. M. Case, Christine Coghill, Joyce L. Harley and Joan T. Pearson. Pp. xxvi+87. (London: The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, 1962.)

Foregoing considerations put the spotlight on how much gamesmanship the actuarial mystique with its insistence on arbitrarily painstaking interpolations conceals from public scrutiny. The belief that laborious refinements prescribed by A.L.T. priestcraft are essential to extraction of whatever useful information the A.L.T. can convey may account for a prevalent reluctance to construct home-made up-to-date tables without the *nihil obstat et imprimatur* of the Registrar-General. It is therefore worthy of comment that educational authorities (including the University Grants Committee) were relying a few years back on estimates of the *bulge* based on what was then the last official table—at that date several decades behind the times.

For some years, a few of us have known or at least suspected that life-tables of the sort Case calls conventional computed from age specific mortality rates in the *Annual Review* of the Registrar-General by the light of common sense (so-called "Greville's Method"), and at the expenditure of half a working day with a machine, differ very little from those computed the hard way. Dr. Case and his collaborators have exhibited in tabular form relevant facts which fully confirm this conviction. As he himself rightly comments: "A consideration of the large discrepancies between the results of conventional methods and actuality . . . and the trivial differences between simply constructed abridged tables and the meticulously graduated official English Life Tables makes one wonder what purpose is really fulfilled by such a display of actuarial expertise !"

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SEQUENTIAL ENZYME INDUCTION: A NEW APPROACH TO THE STRUCTURE OF COMPLEX MUCOPROTEINS

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AND
DR. J. W. HOPTON
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SINCE Monod's[1] classical work on the utilization of sugar mixtures by micro-organisms, many examples of sequential utilization of substrate components have been reported. For a particular organism, the sequence is predictable, and it is now well established that the enzyme system required to initiate the attack of a component is not elaborated until other components are preferentially exhausted.

We have been able to show that if the complex mucoprotein, orosomucoid, is included in a medium which will support growth of *Rhodopseudomonas palustris*, development of the culture is accompanied by a sequential release (Fig. 1) of constituent sugars of the polysaccharide moiety of the muco-protein. At any one time, only one enzyme system is operative, and in this respect, there is an obvious similarity to that behaviour of individual substrate specific enzymes which characterizes multiphasic growth. Orosomucoid can be conceived as a composite inducer for enzymes which cleave links between sugar constituents of the polysaccharide. However, the products of the enzyme actions cannot be utilized by the organism (orosomucoid will not serve as sole source of carbon for growth of the organism) and accumulate in the medium. Hence, from the results of qualitative and quantitative analyses of the products of this stepwise degradation, precise conclusions concerning the arrangement of sugar residues in the molecule can be drawn. Furthermore, it has been possible to deduce the configuration and mode of linkage of certain of the sugar residues in the carbohydrate moiety from the specificity of the induced enzymes and the response of cells to inducers of known configuration.

Sequential release can be observed in a single culture over a period of a week, but normal logarithmic growth is certainly not proceeding throughout the whole of this period. Indeed, it is likely that for most of this time, the behaviour of the culture is analogous to that of a resting cell suspension. However, if orosomucoid 'cores' left at the termination of release of a constituent are recovered by dialysis of the centrifuged culture, and incorporated in a fresh culture, the sequential effect can be reproduced and the overall process accelerated.

The electrophoretically and immunologically pure orosomucoid used in this investigation was isolated[2] from nephrotic urine and contained 10·8 per cent *N*-acetyl neuraminic acid (thiobarbituric acid—periodate assay[3]), 1·2 per cent L-fucose (cysteine hydrochloride–sulphuric acid assay[4]), 6·5 per cent D-galactose (D-galactose oxidase[5]), 16·6 per cent *N*-acetyl glucosamine (*p*-dimethylaminobenzaldehyde[6]) and 6·9 per cent mannose (by difference between total hexoses assayed with orcinol[7] and the galactose content[5]).

Washed succinate-cultured[8] cells of *R. palustris* 3574 were re-suspended in sterile fresh medium (5 ml., *p*H 7 adjusted with KOH) containing per litre succinic acid (6 g), $(NH_4)_2SO_4$ (0·015 mole), $MgSO_4$ (0·001 mole) and K_2HPO_4 (0·02 mole) and added to orosomucoid (5 mg) in a sterile flask (25 ml.). The culture was incubated and shaken at 35°. Paper chromatographic analysis of aliquots taken at intervals showed that *N*-acetyl neuraminic acid, *N*-acetyl mannosamine, galactose and oligosaccharides with R_{glucose} 0·18 and 0·27 in pyridine–*n*-pentyl alcohol–water (1 : 1 : 1) appeared successively in the culture medium. Any fucose liberated was below the level of detection.

Quantitative assays[3–10] of the sugars were made as appropriate on the whole culture, on the dialysable sugars and on the non-dialysable mucoprotein 'cores'

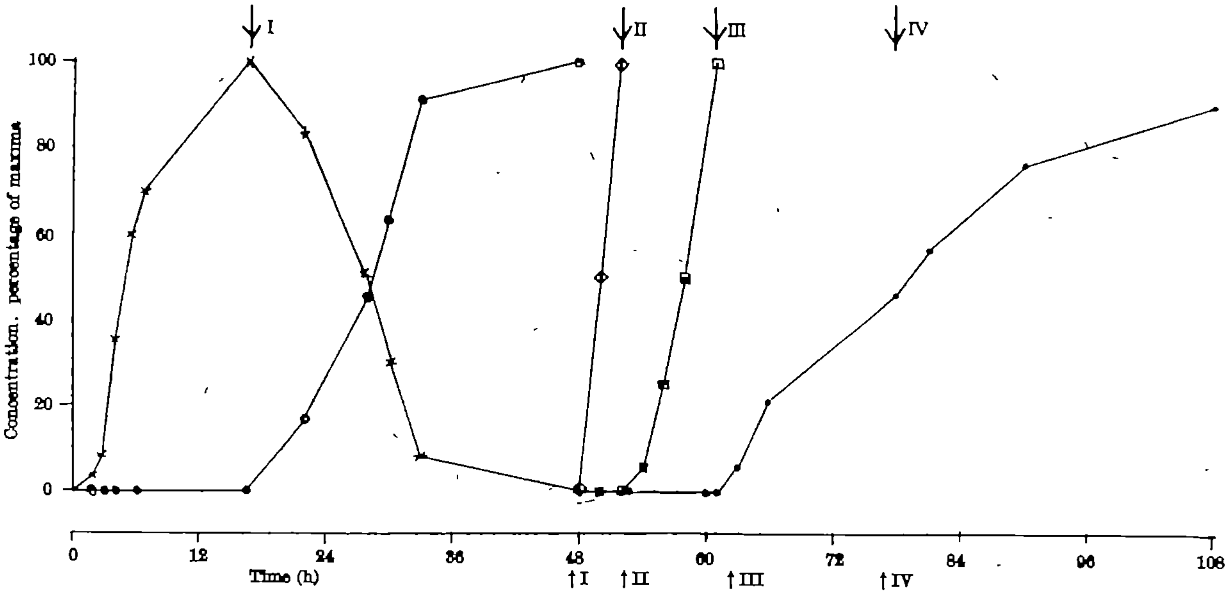

Fig. 1. Sequential cleavage of sugars from orosomucoid by successive enzyme induction. ×, *N*-acetyl neuraminic acid; ○, *N*-acetyl mannosamine; ◇, L-fucose; □, D-galactose; ●, oligosaccharides containing mannose and *N*-acetyl glucosamine. ↓ I, etc., indicates times at which appropriate non-dialysable cores were taken. ↑ I, etc., indicates times at which inductions were made with appropriate non-dialysable cores.

after removal of the cells. Corrections were applied in the analysis of the dialysable fractions for the effect of the constituents of the culture medium and for the presence of other sugars. To eliminate the effect of *N*-acetyl mannosamine, a neuraminic acid-free 'core' was used as an inducer for the later stages of degradation. The results were plotted (Fig. 1) as a percentage of the maximum content of each constituent sugar in the orosomucoid. The time-scale shown was an accelerated one achieved by the use of appropriate mucoprotein 'cores' dissolved in a fresh suspension of cells at the times indicated.

All the *N*-acetyl neuraminic acid (NANA) originally present in the orosomucoid is rapidly released by neuraminidase, the first induced enzyme. This shows that this sugar exists either as isolated end-groups or as a repetitive sequence of NANA residues at the extremities of the chains. Since periodate oxidation[11] of orosomucoid yields one mole of formaldehyde for each mole of NANA the former possibility is more likely. The released NANA (10·6 per cent) was converted to an equimolar amount of *N*-acetyl mannosamine (7·5 per cent) presumably by an aldolase action.

The total amount of L-fucose present in the orosomucoid was next released. Induction experiments with methyl α- and β-L-fucopyranosides showed that only an α-L-fucopyranosidase can be induced in *R. palustris*. Furthermore, this enzyme cleaved at least four-fifths of the fucose in orosomucoid while the NANA remained untouched. We can therefore conclude that NANA and fucose exist as separate end groups and, again, the α-L-fucopyranoside residues can exist as isolated end groups or as a repetitive sequence of fucose residues. Linkage through the 3-position is excluded because the fucose is destroyed by periodate oxidation of orosomucoid[11].

Orosomucoid itself does not cross-react with anti-Type XIV *Pneumococcus* sera which are known[12] to have a pronounced immunological specificity for β-D-galactopyranose end groups. However, after cleavage of the NANA and fucose the residual orosomucoid 'core' did show a cross-reaction with anti-Type XIV which disappeared concomitantly with the removal of all the galactose by the next induced enzyme (Fig. 1) in the sequence. This enzyme had the specificity of a β-D-galacto-pyranosidase since it could cleave lactose but not melibiose. Galactose oxidase is known[9] to react with bound galactose present as non-reducing end groups. Orosomucoid itself showed no reaction with galactose oxidase, a slight reaction after selective removal of fucose, and a strong reaction after removal of neuraminic acid. No reaction was given by a mucoprotein core from which all the galactose had been removed. The foregoing evidence suggested that β-D-galactopyranosyl end groups had been uncovered by the NANA cleavage, and that this residue was the next sugar along the chain.

The final stage of carbohydrate disruption was the liberation of two oligosaccharides. When used as an inducer, the first oligosaccharide R_{glucose} 0·18 gave the trisaccharide R_{glucose} 0·27 which contains two molecules of *N*-acetyl glucosamine and one of mannose. One of the *N*-acetyl glucosamine residues carries the reducing group in the trisaccharide since periodate oxidation followed by acid hydrolysis gave glucosamine before but not after sodium borohydride reduction. The mannose residue was always destroyed by periodate oxidation. The enzyme induced was therefore a glucosaminidase. Subsequent use of the trisaccharide as an inducer gave *N*-acetyl glucosamine and a component with the mobility (R_{glucose} 0·65) of a disaccharide. No free mannose was ever detected in the culture medium. The foregoing evidence, together with analyses of the dialysable oligosaccharide mixtures which consistently showed two *N*-acetyl glucosamine residues for each mannose residue, suggests that the oligosaccharide R_{glucose} 0·18 is a hexasaccharide—the dimer of the trisaccharide.

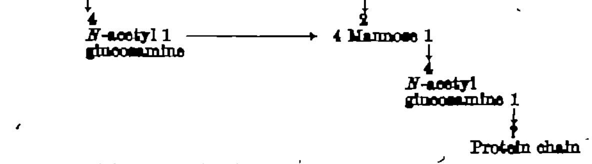

Fig. 2. Partial structure of α_1-glycoprotein

Urinary orosomucoid has been found[13] to be immunologically identical with serum acid α_1-glycoprotein and both show similar sugar analyses. The structure of part of the carbohydrate moiety of α_1-glycoprotein has recently been reported[14] and is shown in Fig. 2. All the foregoing evidence obtained from sequential enzyme induction is consistent with this structure although more details have yet to be established. The powerful tool now at our disposal allows us to stop the degradation at any stage in order to simplify the application of classical techniques of structural determination, and in order that the important relations between structure and biological activities of the complex molecules can be determined. Whether the method is applicable to other macromolecules has yet to be determined.

We thank the Medical Research Council for financial support and the award of a scholarship to one of us (G. L. P.). The culture used was obtained through the kindness of Dr. A. M. Torriani.

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NEWS and VIEWS

Contributions to *Nature*, 1962

The following is a list of special research articles and "Letters to the Editor", indicating numbers and countries of origin, which were published in *Nature* during 1962. These figures do not include the more general articles, reviews, etc.; but exclusively those which are mainly preliminary announcements of new discoveries or individual expressions of scientific opinion.

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Canada	121
Chile	9
China	1
Congo	1
Czechoslovakia	18
Denmark	15
Eire	15
Egypt	6
Ethiopia	1
Finland	18
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Indonesia	1
Israel	31
Italy	42
Jamaica	7
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Malaya	10
Malta	2
Mauritius	1
Mexico	1
Morocco	1
New Zealand	44
Nigeria	8
Norway	20
Nyasaland	3
Pakistan	1
Peru	1
Poland	23
Portugal	1
Puerto Rico	1
Rhodesia, North	2
Rhodesia, South	6
Roumania	5
Sarawak	1
Sierra Leone	2
South Africa	38
Spain	16
Sweden	57
Switzerland	20
Tanganyika	4
Trinidad	5
Turkey	1
Uganda	8
United Kingdom	1,076
United States	950
U.S.S.R.	28
Venezuela	3
Yugoslavia	10
Total No. of countries	71
Total No. of communications	3,296

Division of Reactors of the International Atomic Energy Agency: Dr. F. T. Miles

Dr. Francis T. Miles, who was until recently deputy chairman of the Nuclear Engineering Department of the Brookhaven National Laboratory, has been appointed director of the Division of Reactors of the International Atomic Energy Agency in succession to Dr. C. Sánchez del Río. He took up his duties at the Agency's headquarters in Vienna on December 27. Born in Princeton, New Jersey, in 1909, Dr. Miles studied at Princeton University, where he gained his A.M. in 1932 and his Ph.D. in organic chemistry in 1936. He also studied at the University of Basle during 1934–35. From 1936 until 1943, Dr. Miles was a research chemist with a chemical firm in Alabama, and then joined Columbia University, where he worked with Dr. W. F. Libby on the gaseous diffusion process for isotope separation. In 1946 he joined Clinton Laboratory (now Oak Ridge National Laboratory) as a group leader supervising research on the extraction of uranium-235 from irradiated reactor fuel, and in 1948 went to Brookhaven to supervise research on the separation of uranium-233 by neutron irradiation of thorium. Later he became deputy chairman of the Nuclear Engineering Department of this Laboratory. Apart from isotope separation processes connected with nuclear energy applications, Dr. Miles has been actively interested in several other aspects of reactor technology. From 1959 until 1962 he was a member of the Board of Directors of the American Nuclear Society. For some years he was also an associate editor of *Nuclear Science and Engineering*.

Experimental Fluid Mechanics at the College of Aeronautics, Cranfield: Prof. G. M. Lilley

A chair of experimental fluid mechanics has been established at the College of Aeronautics, Cranfield, in the Department of Aerodynamics. Mr. G. M. Lilley has been appointed to be the first professor of experimental fluid mechanics, with effect from January 1. The new chair will enable the experimental facilities available in the Department of Aerodynamics at Cranfield to be fully utilized in a developing programme of research. Prof. Lilley, a graduate of the University of London, has been a resident member of the teaching staff at Cranfield since 1946. First appointed as a lecturer in the Department of Aerodynamics, he was promoted to senior lecturer in 1950 and to deputy head of the Department in 1955. He has played a major part in the development of the Aerodynamics Laboratory and its research activities. Prof. Lilley is a recognized authority on turbulent flow and its relationship to aerodynamic noise. He is a member of a number of committees of the Aeronautical Research Council.

The Royal Society of London: Annual Report

The report of the Council of the Royal Society for the year ended September 30, 1962, indicates that the Council expects to make the first two appointments to the new research professorships which the Government is enabling the Society to create early in 1963 (Pp. 53. London: The Royal Society, 1962). An *ad hoc* committee has been appointed to consider the nomination procedure and the methods of selection of candidates for the fellowship, as well as the procedure for the election of foreign members. After taking legal advice and consulting the Charity Commission, which ruled that Rutherford scholarships can no longer be awarded to students from South Africa now that South Africa has ceased to be a member of the British Commonwealth, the Council has altered the regulations accordingly and also to exclude students from Eire. Discussions are still proceeding with Government departments and others regarding accommodation for the Society, and meanwhile members of the Society's staff dealing with geophysical and space research work and the international secretariat for the International Year of the Quiet Sun are being housed in rooms at Cornwall Terrace, Regent's Park. The Council has also agreed to co-operate with the Leverhulme Trust Fund in a scheme to establish Royal Society Leverhulme professorships to enable distinguished scientists to visit India. Of Parliamentary grants-in-aid administered by the Society during the year amounting to £162,640, £87,500 was spent on research, £63,000 on international research and scientific congresses and £7,500 on scientific publications. Besides some notes in the report itself on antarctic research, oceanic research, space research and geophysical research, reports are appended from the twenty-four research professors and research Fellows directly supported by the Society describing some of the results achieved, mostly with lists of publications.

Vice-presidents

THE president of the Royal Society, Sir Howard Florey, has appointed the following vice-presidents for the year ending November 30, 1963: Lord Fleck, treasurer of the Royal Society, formerly chairman of Imperial Chemical Industries, Ltd.; Sir Lindor Brown, biological secretary of the Royal Society, Waynflete professor of physiology in the University of Oxford; Sir William Hodge, physical secretary of the Royal Society, Lowndean professor of astronomy and geometry in the University of Cambridge; Sir Patrick Linstead, foreign secretary of the Royal Society, rector of the Imperial College of Science and Technology, London; Prof. W. T. J. Morgan, deputy director of the Lister Institute and professor of biochemistry in the University of London; Sir Gordon Sutherland, director of the National Physical Laboratory.

The International Calcium Silicate Products Research Organization

THE production of calcium silicate bonded bricks and other building products has developed rapidly in recent years. Yet production of sand-lime and flint-lime bricks, the most well-known form in Britain, still only amounts to about 3 per cent of the total brick output, but as a result of research a high proportion of these are now of specially high strength or of facing qualities. In Germany, Holland and Russia they constitute a major proportion of the brick and block output. The process of manufacture, which is comparable in cost with that of clay bricks, has advantages as quality can be closely controlled and bricks of any required strength can be made from the same raw materials by simple adjustment of various factors in the process. At a meeting held in Amsterdam in December, agreement was reached between the Chalk Lime and Allied Industries Research Association, Welwyn, Herts, and the Associations concerned with research for the Calcium Silicate Brick and Block Industry in West Germany (the Hauptverband Kalksandsteinindustrie e.V.) and in Holland (Stichting Kalkzandsteen-Laboratorium), on the formation of a joint research organization to be known as the International Calcium Silicate Products Research Organization. For the first two years the Secretariat of the Organization will be provided by the Chalk Lime and Allied Industries Research Association at Welwyn.

Building Research at the Manchester College of Science and Technology

WORK has now started at the Manchester College of Science and Technology on a three-year study of the structure and economy of the building industry. First announced in September 1962, this project is being carried out for the Department of Scientific and Industrial Research at a cost of £48,000. The Council of the College has appointed Mr. Gordon Bayley as research director of the project. Mr. Bayley, who is forty, is a graduate of Manchester, and has been area secretary of the Eastern Federation of Building Trade Employers covering Essex and East Suffolk for some years.

A steering committee has been set up to control the general policy of the project and to advise the research director and his team. The committee is composed of the following members: Mr. A. P. H. Aitken, Textile Machinery Makers, Ltd. (chairman); Prof. D. Harper, professor of building, University of Manchester, and director of project; Mr. M. W. Bennitt, Ministry of Public Building and Works; Mr. R. Brech, Economics and Statistics Department, Unilever, Ltd.; Prof. C. F. Carter, professor of economics, University of Manchester, and vice-chancellor elect of the University of Lancaster; Mr. E. J. Cook, Richard Costain, Ltd.; Mr. E. D. France, Industrial Labour Officer, National Federation of Building Trades Operatives; Miss P. K. Piercy, Department of Scientific and Industrial Research; Prof. R. W. Revans, professor of industrial administration, University of Manchester; Mr. R. T. Walters, deputy director research and development, Ministry of Public Building and Works; Dr. J. C. Weston, deputy chief scientific officer, Department of Scientific and Industrial Research, Building Research Station.

The Wellcome Laboratory, Pirbright: Foot and Mouth Disease Vaccines

THE completion has recently been announced of the new Wellcome Laboratory erected within the perimeter of the Research Institute (Animal Virus Diseases), Pirbright. In accordance with the arrangements announced by the Parliamentary Secretary for Science in May 1961, the Laboratory will undertake the large-scale production of living foot and mouth disease vaccines. Research at Pirbright has been particularly directed towards the attenuation of strains of foot and mouth disease virus and the pilot-scale production of living attenuated vaccines. A series of trials in South and East Africa led to increasing demands for these vaccines, and the Wellcome Foundation, Ltd., was invited to set up a production unit at Pirbright so that the Institute could continue to devote its resources and facilities to research. Large quantities of two different living vaccines have already been produced in temporary accommodation at Pirbright and supplied to Government authorities in Africa and elsewhere. Special emphasis has been placed on the production of a vaccine suitable for combating the epidemic of African-type foot and mouth disease which has been raging in the Middle East during the past six months. The vaccine will not, however, be used for the prevention of foot and mouth disease in Britain, where slaughter of infected and in-contact animals is the tried and approved method of controlling periodic outbreaks.

Government Wage Policies in the Public Sector

IN Broadsheet No. 467 (November 19, 1962) issued by Political and Economic Planning as part of a three-year study of Trade Unions in a Changing Society, B. Donoughue reviews wage policies in the public sector (*Planning*, 28, 19 November, 1962. Pp. 363–402. London: Political and Economic Planning, 1962. 4*s*.). The public services contain the fastest growing areas of public employment and also present the most complex difficulties to wage negotiators. Since the Second World War the most persistent problem for the unions in these services has been to maintain income-levels comparable with those in equivalent private employment where bonus payments substantially increase the earnings taken home. For the Government the major problem has been the reconciliation of its role as an employer with its wider responsibilities not to allow wage increases for its own employees which might be detrimental to the nation's economy. The growing acceptance in negotiating and arbitration procedures of the principle of comparability and the application of 'fair comparisons' formulæ largely solved the unions' difficulty. However, this was called into question by the Government's insistence that Britain's economic difficulties would not be solved unless wage increases were restricted to what the country could afford, and that wages must not increase on average faster than the average increase in national productivity. Pointing out that the public sector could play an important part in a national wages policy, Mr. Donoughue notes also that three specific questions will arise: how to ensure that public servants do not suffer a pay disadvantage compared with production workers; the role, if any, of arbitration; and the fixing of wages for loss-making public industries, such as the railways.

The Supply of Science Teachers

In replying to a debate on science and mathematics teachers in the House of Commons on December 21, opened by Mr. A. Skeffington, the Parliamentary Secretary to the Ministry of Education, Mr. C. Chataway, said that in March 1961 there were 7,321 mathematics graduates and 12,734 science graduates in grant-aided schools and establishments, increases of nearly 10 per cent and nearly 15 per cent, respectively, in two years; in the maintained secondary schools the figures were 5,077 and 8,528, increases of 7·8 per cent and 13·2 per cent, respectively. The numbers of mathematics and science graduates entering university departments of education to train as teachers had risen from 715 in 1957–58 to 965 in 1961–62 and 1,110 in 1962–63. While the growth in numbers entering the schools had kept pace with the general increase in the graduate teaching force, men teachers had been leaving the schools more rapidly than normally expected, in consequence of the ending of deferment under National Service, and further education and the training colleges had been gradually increasing their demands for mathematics and science graduates as part of their expansion programme. Physics teachers were far harder to come by than teachers of biology, and the general situation for girls was worse than for boys. The Ministry's Curriculum Study Group had embarked on a study of typical curricula patterns for schools of different types in relation to the demand and supply of teachers of particular subjects. Closer links were being formed with university appointments officers and a small steering committee had been established on the supply of mathematics teachers, bringing together schools, universities and the Ministry. The three-year course had given the training colleges the opportunity to broaden and deepen the training given to non-graduate teachers of science and mathematics. For mathematics there were about 240 lecturers and another 60 were teaching mathematics part-time or in combination with another subject. Science lecturers were limited to about 45 colleges. An encouraging expansion of teaching on television was proceeding and agreement had been reached between the British Broadcasting Corporation, the Independent Television Authority and the Postmaster-General on adult education and the expansion of the time allocated to it.

Standardization of International Non-Proprietary Names for Drugs

The World Health Organization has long been conscious of the need for standardizing non-proprietary names for drugs and seeking to gain their international recognition. In far too many cases the proposed international non-proprietary name (for example, thalidomide) has not appeared on the package in addition to the proprietary name, and this has complicated the problem of preventing, if necessary, any further use of the drug. Since 1953, lists have been published at intervals in the *WHO Chronicle* of proposed international non-proprietary names, and their adoption by national authorities for use in official texts, regulations, national pharmacopœias and other collections of specifications is becoming increasingly common. The first eleven lists of proposed international non-proprietary names have now been collected together under the title of *International Non-proprietary Names for Pharmaceutical Preparations: Cumulative List 1962*, giving the non-proprietary names in Latin and English, accompanied by the chemical names or descriptions in English (Pp. 52. Geneva: World Health Organization; London: H.M.S.O., 1962. 3 Swiss francs; 5s.; 1 dollar). The procedure for the selection of international non-proprietary names and the general principles for devising such names are appended at the end of the list. This publication should be of considerable assistance to national control authorities and pharmaceutical manufacturers, as well as to pharmacists, doctors and editors of scientific literature.

The O.E.C.D. Observer

The first issue of *The O.E.C.D. Observer* (November 15, 1962), published bi-monthly by the Organization for Economic Co-operation and Development, includes an article by T. Kristensen, the secretary-general, describing the purpose of this new periodical and an article by Prof. I. Svennilson on the role of research and education in the growth of an economy. The issue contains an article by G. Rehn on manpower, adaptability and economic growth, and some account of the Organization's structure for aiding development, including technical and educational assistance to newly developing countries. Also presented is a supplement which describes the aims, structure and work of the Organization, including an organization chart which indicates the place of the Committees of Scientific Research, Scientific and Technical Personnel, the Directorates for Scientific Affairs and for Manpower and Social Affairs, and the European Nuclear Energy Agency. Copies of *The O.E.C.D. Observer* are obtainable from O.E.C.D. Information Service, Château de la Muette, Paris 16e.

The Geology of Edinburgh

Just more than a century ago, the Geological Survey of Scotland published the first edition of its memoir on *The Geology of the Neighbourhood of Edinburgh*. Fifty years later a second edition appeared, and now after another half-century a third edition has been published (Pp. xii + 159 + 4 plates. Edinburgh: H.M.S.O., 1962. 35s. net). Probably no city in the world has made as great a contribution to geological thought and theory as has the Scottish capital, and it is a measure of the continuation of this tradition that this latest work, compiled by Dr. G. H. Mitchell and Mr. W. Mykura, is almost wholly concerned with information acquired by the Geological Survey since 1910. Not a mention of Hutton and his associates is to be found in its pages! In one respect alone does the new memoir compare unfavourably with its predecessors: although half the length of the 1910 edition (which had a coloured map), it is nearly five times its price. Not long ago the Edinburgh Geological Society sponsored a model guide for geological tourists (*Edinburgh Geology: An Excursion Guide*. Pp. xv + 222 + 8 plates: Edinburgh, Oliver and Boyd Ltd., 1960. 12s. 6d. net); and the juxtaposition of these two new books brings little credit to the costing methods of H.M.S.O., which result in the publications of the British Geological Survey being more expensive than those of most comparable official organizations overseas.

Research on Food Preservation in Australia, 1961–62

Readers of *Food Preservation Quarterly* will doubtless be aware of the stream of useful work which has emanated from the Food Preservation Division of the Commonwealth Scientific and Industrial Research Organization, Sydney, Australia. The wide scope of the scientific work carried out, together with its practical applications, is reflected in the Division's annual report for 1961–62, which includes sections on physics, food chemistry, microbiology, muscle biochemistry, meat, fish, eggs, plant physiology, fruits, vegetables and canned and dried foods (Pp. iii + 36. Sydney, Melbourne and Canberra: Commonwealth Scientific and Industrial Research Organization, 1962). Theories relating to heat and mass transfer when a fluid flows at a high velocity over the surface 'wall' have been the subject of further investigations. Work on beef showed that the pasteurization of hindquarters using hot water (65°–70° C) caused the destruction of 90–99 per cent of psychrophilic organisms without serious effect on the appearance of the tissues. The storage and quality of fruit has been considered from a number of points of view, including investigation of the prevention of scald and post-harvest treatment against infestation due to the Queensland fruit-fly. Considerable attention has been devoted to the radiation sensitivity of the eggs and larvæ of the fly. Better control of the radiation dose has resulted from the use of a small underwater fuel element irradiator and cobalt-60 source. Future projects will include the use of gas chromatography and infra-red spectrophotometry for the study of the volatile flavouring constituents of foods. Also the radioactive isotopes carbon-14, sulphur-35, calcium-45 and tritium are to be used in a study of the chemical and biochemical changes which take place in animal products.

Science and the Human Condition: Public Lectures at Hatfield College

For the third year in succession, and in response to public demand, the Teaching Staff Association of Hatfield College of Technology has organized a series of eight public lectures on scientific and philosophical subjects, taking as its general theme this year "Science and the Human Condition". The lectures are as follows: January 25, Dr. S. Lilley, University of Nottingham, "The Scientific Revolution and Industrial Processes"; February 22, W. J. P. Webber (Transport Salaried Staffs Association), "The Scientific Revolution and Industrial Organisation"; March 15, Prof. John Cohen (University of Manchester), "The Scientific Revolution and Leisure"; May 10, Sir John Rothenstein (Tate Gallery, London), "The Scientific Revolution and the Arts"; May 17, Dr. P. F. R. Venables (College of Advanced Technology, Birmingham), "The Scientific Revolution and Education"; May 24, Prof. Colin Cherry (Imperial College of Science and Technology, London), "The Scientific Revolution and Communication"; June 7, Prof. C. F. Powell (University of Bristol), "The Scientific Revolution and Morals"; June 14, Prof. P. M. S. Blackett (Imperial College of Science and Technology, London), "Technology, Science and Wealth". The inclusive fee for the course is 30*s*. Further information can be obtained from the Secretary, Teaching Staff Association, Hatfield College of Technology, Hatfield, Herts.

The Night Sky in February

Full moon occurs on Feb. 8d. 14h. 52m. U.T. and new moon on Feb. 24d. 02h. 06m. The following conjunctions with the Moon take place: Feb. 8d. 06h., Mars 3° N.; Feb. 20d. 15h., Venus 0·8° N. In addition to these conjunctions with the Moon, Mercury is in conjunction with Saturn on Feb. 28d. 09h., Mercury being 0·8° S. Mercury is a morning star, but is not favourably situated for observation from northern latitudes. Venus is also a morning star, rising at 4h. 45m., 5h. 00m. and 5h. 05m. on Feb. 1, 15 and 28, respectively. Its stellar magnitude is $-3·8$; its distance increases during the month from 69 to 88 million miles, and the visible portion of the apparent disk increases from 0·545 to 0·661. Mars is in opposition on February 4, and is above the horizon all night. It is at a distance of 62 million miles at opposition, when it has an apparent diameter of 14 seconds of arc and its stellar magnitude is $-1·0$; it moves from Leo into Cancer on February 4. Jupiter sets about three hours after the Sun on February 1, and conditions later become unfavourable for observation. Saturn is too close to the Sun for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Feb. 1d. 19h. 39·5m., +11° 445 (*D*); Feb. 3d. 21h. 48·8m., 104 Tau. *m* (*D*); Feb. 4d. 21h. 08·3m., χ^2 Ori. (*D*); Feb. 5d. 22h. 17·5m., ζ Gem. (*D*); Feb. 6d. 19h. 10·2m., 85 Gem. (*D*). *D* refers to disappearance.

Announcements

Mr. C. C. Hentschel, formerly vice-principal of the Chelsea College of Science and Technology and recognized teacher of zoology in the University of London, has been appointed principal, in succession to Mr. N. M. Lightfoot, who died on November 20 (*Nature*, 197, 19; 1963).

The first British pest control conference, organized by the Industrial Pest Control Association, will be held at Lady Margaret Hall, Oxford, during April 1–3. Further information can be obtained from Mr. W. A. Williams, Industrial Pest Control Association, 86 Strand, London, W.C.2.

A general discussion on "The Structure of Electronically Excited Species in the Gas Phase", arranged by the Faraday Society, will be held at Queen's College, Dundee, during April 2–3. Further information can be obtained from the Assistant Secretary, the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

The Chemistry Division at Argonne National Laboratory expects to occupy its new 'hot' laboratory wing early this year. In celebration of this event, it is planned to hold a symposium on "The Transplutonium Elements" at the Laboratory in Argonne, Illinois, during May 15–17. Further information can be obtained from Dr. D. C. Stewart, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois.

A United Nations conference on "Application of Science and Technology for the Benefit of the Less Developed Areas" will be held in Geneva during February 4–20. Further information can be obtained from the United Nations, Geneva, or, concerning the British contribution, from Mr. D. Flook, Press Office, Department of Scientific and Industrial Research, State House, High Holborn, London, W.C.1.

The tenth Easter school in agricultural science will be held at the University of Nottingham School of Agriculture, Sutton Bonington, during April 8–11. The school will be the first of three discussions on crop ecology and will be concerned with the potato crop. Further information can be obtained from Mr. J. D. Ivins, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough.

The sixteenth Laboratory Animals Centre congress will be held in the University of Glasgow during April 2–3. The programme will include: a method for producing hairless mice in large numbers; further observations on the maintenance of a mouse colony for the production of suckling mice; use of inbred strains of mice for feeding trials; the establishment of a beagle colony for experimental purposes; the care of dogs used for research; some observations on the American opossum; the significance of laboratory animals in the toxicity testing of new drugs. Visits have been arranged to the Hannah Dairy Research Station, the Marine Biological Research Institute and the Veterinary Research Farm, Cochno, and the Veterinary School, Garscube. Further information can be obtained from the Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey.

TECHNICAL CO-OPERATION IN SOUTH AND SOUTH-EAST ASIA

THE report for 1961–62 of the Council for Technical Co-operation in South and South-East Asia emphasizes that, in moving into its second decade, the Colombo Plan has become truly international, its 22 members including ten non-Commonwealth countries, and, outside the region, Australia, Britain, Canada, Japan, New Zealand and the United States. More than 24,000 persons from the countries of the area have received training in other Colombo Plan countries, and more than 4,000 experts have visited the area. Scientific, technical and training equipment to the value of more than £32 million has been supplied to the area and the total value of technical assistance supplied under the Plan now exceeds £102 million. The proportion of expenditure allocated to experts, trainees and equipment has remained fairly stable during the past few years, but the distinction between the giving and receiving countries has become gradually blurred. An astonishing and inestimable reservoir of goodwill has been created among its makers and constituted one of the most successful post-war ventures in international trust and co-operation.

The United Kingdom provided 656 new training places during the year, bringing the total provided under the Plan to 4,536, compared with 9,335 by the United States, 4,071 by Australia, 1,863 by Canada, and 1,914 by India. The United Kingdom provided 47 new experts during the year, making a total of 493 under the Plan, compared with 1,833 provided by the United States, 488 by Australia, 425 by Japan, 293 by Canada, 446 by India, and 180 by New Zealand. The United Kingdom provided £362,571 worth of equipment during the year, making a total of £2,372,359 out of the £32,643,789 provided under the Plan. Altogether in 1961–62, the United Kingdom provided £1,571,807 on technical assistance under the Plan, making a total of £8,623,240, exclusive of aid given to North Borneo and Sarawak under the Colonial Development and Welfare Acts. The United Nations and its specialized agencies has also supplied 9,149 experts and 6,951 Fellows to the area during the period of the Plan, and of these experts supplied during the year under the Plan, 103 were in general and 27 in technical education, 72 medical and health, 122 food, agriculture and forestry, 194 engineering, 253 transport and communications, 63 administration, 60 industry and trade and 29 social services.

Apart from continuing as a normal function of the Colombo Plan Bureau the survey of training facilities at the technical level in the area provided by the Mills Report, the Bureau has directed the attention of members of the Council to a recommendation of the Sub-committee on Technical Co-operation for a survey of technical assistance needs in the region and has suggested that each country should survey its needs and send the reports to the Bureau for circulation through the Council. The report also stresses the need for the developing countries to think in terms of long-term economic growth based on a rational use of manpower and material resources rather than short-term expediency and for a continuing critical evaluation of the productivity of technical co-operation. The continuing importance of technical co-operation as a means of removing the shortage of the trained manpower needed for an increased tempo of economic development cannot, however, be overemphasized.

Of trends during the year the report notes that about 25 per cent of expenditure was on trainees, nearly 40 per cent on experts and advisers and the remainder on books, films and equipment. There was a marked increase in the number of training places provided by countries within the region and the United States provided almost half the total training awards made during the year. Long-term assignments for experts were increasingly popular but expenditure on all forms of technical co-operation is increasing.

The examples of British contributions to technical co-operation which follow will illustrate the range of effort covered. In agriculture the British Government repeated its offer of training in the technology of stored products, entomology and rodent control and a laboratory assistant from Malaya was accepted for a two-year course at the Central Veterinary Laboratory of the Ministry of Agriculture, Fisheries and Food, while equipment is being supplied for use at the Veterinary Research Laboratory in Burma. Forest officers from Ceylon, Pakistan and Burma have attended the postgraduate course for forestry officers from tropical countries at the Imperial Forestry Institute, Oxford, and Britain is also supplying equipment to the Pakistan Forest Institute at Peshawar.

The Atomic Energy Commission of Pakistan is to establish an Institute of Nuclear Science and Technology and also proposes to establish training centres at graduate and postgraduate level in different fields of nuclear energy. To implement this programme, six trainees are following courses at the University of Manchester. The Government of India has obtained for five years the services of the Deputy Director-General of the National Coal Board of Britain, who will advise the Indian Coal Development Corporation on the development of three underground coal mines at Jaraughdi, Katanka and Swant. Britain has also provided Burma with the services for two years of an expert in jute grading and baling to assist in supervision and training of personnel in the jute production drive of the Agriculture and Rural Development Corporation. In addition to providing eleven experts and training for eighteen officers for the Operations Division of the Ceylon Transport Board, the British Government has now provided two rolling-stock engineers for one year, several short training courses in bus operation and larger courses for engineers and depot managers, etc. A deputy superintendent of police from Pakistan received three months training in Britain with county and city police forces and training was arranged with British Railways for a senior mechanical engineer of the Railway Staff College, Baroda, the works manager, Central Workshops, Mysore, a district mechanical engineer of the South-Eastern Indian Railway and the mechanical engineer (operating) of the Eastern Railway. Training of six-months duration in aircraft maintenance was arranged for three trainees from Indonesia's Civil Aviation Academy at various institutions in Britain, and Britain is providing an expert, training facilities and equipment for the Telecommunications Department of the Federation of Malaya, while three engineers are being trained in transistor technology for the Community Listening Centres of the Government of India, and a technician of the Burma Broadcasting Service is taking a one-year course in electronics at the University of Southampton.

In health services the British Government arranged a two-year course in radiation physics to supply the physicist of the radiotherapy unit at the Cancer Institute, Meheragana, Ceylon, with specialized training and loaned a physiotherapist to the Rangoon General Hospital to re-organize the Physiotherapy Department and train personnel. Training in oral surgery is being provided

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[*] Department of Technical Co-operation. Technical Co-operation under the Colombo Plan: Report for 1961–62 of the Council for Technical Co-operation in South and South-East Asia, Colombo, October 1962. Pp. 92. (London: H.M.S.O., 1962.) 5s. net. [See also p. 215 of this issue of *Nature*.

at the Institute of Dental Surgery, London, and Mount Vernon Hospital, Middlesex, for the supervisor and instructor of Thailand's School of Dentistry, and an assistant surgeon of the Mandalay General Hospital is taking a three-year postgraduate course in dentistry at the Dental School, Guy's Hospital, while at the request of Thailand a one-year course in applied nutrition and food sciences was arranged at the London School of Hygiene. A health educator and public health inspector from Malaya is studying for a diploma in health education at the Institute of Education, London, and Pakistan has been assisted to start the F.R.C.S. (Primary) course of the Royal College of Surgeons, London, while to meet the shortage of qualified anæsthetists in Burma, a civil assistant surgeon is following a two-year course in anæsthesiology at the Postgraduate Medical School, London.

In education, Britain arranged a two-year course in concrete technology for an assistant professor of the College of Technology, Coinbatore; a librarian from Malaya is following a one-and-a-half year course in librarianship at the North-Western Polytechnic, London; five trainees from Malaya, Indonesia and the Philippines attended a course in tropical agriculture offered by the British Government, and an engineer from Nepal a postgraduate course in chemical engineering at Battersea College of Technology. Seven teachers from Indonesia's Science Teaching Centre, Bandung, are attending two- to three-year courses in teaching science at Sheffield; science laboratory equipment, chemicals and books were supplied to five State high schools in the Shan States of Burma. A postgraduate course for two trainees from the University of Philippines was arranged at the Royal College of Science and Technology, Glasgow, and arrangements made for the senior lecturer of the Physics Department, University of Sind, to undertake nuclear research at Trinity College, Oxford, while a trainee from the Birbal Sahni Institute of Palæobotany is taking a postgraduate course at Queen's University of Belfast. The President of Burma's Shan State Civil Service is being trained in public administration in Britain, while five Laotian trainees attended a six-month course at the Royal Institute of Public Administration.

THE UPPER CRETACEOUS OF CENTRAL EUROPE

THE Palæontological Section of the Geological Society in the German Democratic Republic held a symposium in Dresden during October 2–7, 1962, on "Problems of the Stratigraphy and Palæontology of the Cenomanian to Coniacian Stages in Central Europe". Three days of papers with discussion and three days of excursions in Saxony were organized by Dr. H. Prescher of the Staatliches Museum für Mineralogie und Geologie zu Dresden and Dr. K.-A. Tröger of the Geologisches Institut der Bergakademie Freiberg (Sachs). The meetings were attended by some thirty geologists, mostly from the German Democratic Republic, but also from Czechoslovakia, Hungary, Poland and Great Britain. The eighteen papers read will be published early in 1963 in the *Berichte der Geologischen Gesellschaft in der D.D.R.*

General reviews of the present state of knowledge of the Upper Cretaceous in Bohemia were given by F. Macák and V. Müller and in papers sent in by J. Soukup and J. Dvorák. A useful summary has already been published by Soukup in tabular form with German transliterations of all the Czech localities mentioned[1].

The extensive work by Polish geologists on the flysch-facies of the Carpathians, thrust northwards into southern Poland, has become well known in Britain in recent years, mainly through the work of M. Ksiazkiewicz and his colleagues at Krakow[2,3]. The post-war work on the boreal facies in Poland is, as yet, less well known. A general summary of this recent work was given by S. Cieśliński, who has already published work on the Albian and Cenomanian which has faunal and facies similarities to north-west Europe[4,5]. For the symposium he had prepared a map which showed the Cretaceous basins in Poland and the facies-succession distribution in each basin. The Silesian–north Sudetic Cretaceous, lying to the south-west of the Sudetenrand Fracture, Eule Gneiss and the North Sudetic Schiefergebirge, was discussed in more detail by J. Milewicz, and is distinct from the rest of Poland, being allied to the Saxony–Bohemian region.

Six of the papers were largely or entirely concerned with Saxony itself. Much detailed work has been done here since the comprehensive summary by Seifert[6]. The fresh-water beds, widely developed at the base, have already been studied by Prescher[7] and Barthel[8]. W. Krutzsch (president of the Palæontology Section) gave an account of the micro-flora obtained from a boring at Königstein. Of the marine formations, not only the Unterquader but also the Plenus Zone has commonly been included in the Cenomanian by Saxon geologists; the Czechs have considered the Plenus Zone to be Turonian and this conforms with British usage; some Czech and Saxon geologists now refer to it as a transitional zone between the two stages. In Saxony the Plenus Zone contains *Metoicoceras* spp., which show that it corresponds to the Actinocamax Plenus Zone in England.

Detailed isopachytes for the Unterquader plus Plenus Zone prepared by F. Decker show that Seifert's palæogeographical maps are misleading and must have depended much on guesswork, at least for land–sea distribution. Decker's map shows a general increase of thickness to the north-east of the Elbtalgebiet beneath the south-westerly overthrust Lausitzer granodiorite. The same granodiorite conceals sediments of the North Sudetic Basin as shown by borings near Spremberg reported on by W. Haller. One of these showed some 1,100 m of marly clays and fine sandstones embracing only the Turonian to Lower Campanian. The coring was sufficiently wide to allow recovery of a considerable fauna of ammonites and *Inoceramus* spp.

The considerable thicknesses of detrital shelf sediments in Saxony above the Cenomanian were noticeable in the excursions. In southern Saxony there are more than 500 m of orthoquartzites (the Quadersandstein) in the Turonian alone. The provenance of this sand is still a problem and H.-D. Beeger has found that the non-opaque heavy minerals are almost confined to the stable trio zircon, tourmaline and rutile. Current bedding is often not developed and does not give a consistent pattern. The contrast with north-west Europe is further shown by the scarcity of glauconite and rarity of phosphate.

The next session of the Geological Society in the German Democratic Republic will be in Leipzig during May 13–18, 1963, on the theme "Facies". Further information can be obtained from J. Lamprecht, Wiss. Sekretar der Geologischen Gesellschaft in der D.D.R., Berlin N4, Invalidenstrasse 44, German Democratic Republic.

J. M. HANCOCK

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MANAGEMENT IN ELECTRICITY SUPPLY

MR. C. T. MELLING'S inaugural address as president of the Institution of Electrical Engineers, delivered on October 11, might be said to constitute a study of management related particularly to the electricity supply industry.

Pointing out that electricity has become almost ubiquitous in its application, being nowadays basic to industrial, commercial, domestic and rural life, Mr. Melling observed that the electricity supply industry's future growth would influence powerfully Great Britain's development as a manufacturing nation. It was, he said, an industry characterized by very rapid technological development and a high rate of growth. These in themselves produced financial problems of great magnitude and, over and above, the industry had undergone several drastic changes of organization. The supply industry will have to solve technical, economic and financial problems of great complexity.

In fulfilling its fundamental purpose of supplying electricity to consumers, the industry operates as a business with public service responsibilities. Business management, combined with public service, formed the incentive in building up electricity supply in the various independent organizations, public and private, which, prior to nationalization, constituted in their aggregate the national system. Principles and practices so established duly influenced the terms of the Electricity Act of 1947 which, in creating a single nationalized system, exemplified in some ways the principle that in codifying what is to be done, legislation should incorporate the best of what is already being done.

In summarizing the general duties of a supply authority Mr. Melling distinguishes nine separate areas of activity. The first of these which, in some sense comprehends, by implication, all the others, may be quoted in full: "to ensure within the framework of a sound financial policy that, by forecast planning and construction, there is sufficient but not extravagant, generation transmission and distribution capacity to meet demands as they arise and that, consistent with reliability and good service, there is economy in capital and operating costs".

In other paragraphs are briefly stated the requirements of a sound tariff structure and a vigorous commercial policy together with the important duties of fostering good employer–employee relations and of good public relations. The pursuit of research, the improvement of efficiency, the development of a good consumer service and the achievement of co-ordination, constitute the subjects of the further paragraphs.

As a first example of a general management problem Mr. Melling took that of the organization of an area board following the nationalization of the industry in 1948. Nationalization, abolishing existing undertakings and establishing new bodies, might under one area board incorporate some forty separate undertakings. Any process of re-organization tends to produce, in employees who will be affected, a sense of uncertainty and possibly of apprehension, and such an operation on a national scale might well have been expected to be accompanied by some degree of disorganization. In fact the transition from the old system to the new was effected quite smoothly, a result to be attributed largely to the momentum of existing practices and to the competence and the good spirit of the people in the industry.

Decentralization of the consumer service was the keynote of the organization of the area boards. It was the aim that district managers should be truly responsible managers for their districts who, working within the framework of a sound practical policy, would have confidence and freedom in their day-to-day operations and in the exercise of their judgment in their relations with employees, consumers, local authorities and the public. Of paramount importance was the maintenance of communication and consultation to keep rank-and-file employees properly informed and to gain their active interest and co-operation in the efficient and economical working of the organization. There are, established by statute, organizations for the negotiation between management and employees representatives of wages, salaries and conditions of service and, similarly, for joint consultation on safety, health and welfare, education and training. These measures will, however, be ineffective unless they really 'permeate' the system. That is to say, the requirement is not met by formal compliance with an instruction to meet at specified intervals but only by top management directly and personally informing and consulting middle management which in turn will do the like with lower management. Thereafter, foremen and supervisors must inform and consult the employees under their direct control. Information must be transmitted promptly along the established chain of command, for the 'short circuiting' of authority at any level undermines confidence and engenders disaffection.

Numerous detailed changes were made in the organization of area boards in the years following nationalization. Unduly small districts were combined to form larger distribution units and experiments were made in the arrangements for repair workshops and stores. Operating practices were analysed and work studies undertaken.

By considering the effects of changes on individual employees and by avoiding undue haste, improvements were made more effectively and resulted in real rather than paper economies. Excluding the cost of bulk supplies of electricity the operating costs of area boards (pence per kWh sold) have been reduced in the ten years to March 1962 by about 16 per cent. This, taking into account the diminished value of money in the same period, represents a real saving of some 45 per cent.

The urge for further economies continues and there are new problems arising from developments in technology and new operating techniques and, underlying everyone of these, is the basic problem of human relations. While it is essential that there should be a dynamic and enterprising spirit in relation to improvements, it remains true that large-scale changes in internal organization are just as disturbing to many people as general changes imposed by legislation. It is to be hoped that there can now be a period of stability and consolidation.

Mr. Melling chose in his second example of a general management problem to deal with certain specific issues in commercial development. The provision of the correct generation, transmission and distribution capacity to meet future demand depends on accurate forecasting. The trend from which such forecasts must be made depends on the antecedent period over which the trend is determined and herein lies the matter of judgment. Periods of depression or of industrial recovery are reflected in an oscillation of the rate of growth curve but, of course, each cycle varies from its predecessor. Based on a ten-year trend curve a compound interest factor of 7·6 per cent is obtained, but owing to the more rapid rate of increase in demand during the past few years a figure of 7·9 per cent is at present being utilized.

The industry's annual capital investment is approaching £500 million, which represents some 10 per cent of the nation's total capital investment. While it is clear that the present rate of increase of demand cannot continue indefinitely, it is unlikely that estimates of growth during the next few years are over optimistic. Great Britain still lags considerably behind the United States in the *per* *capita* consumption of electricity. It is, however, interesting to note that if in six years the present forecast should prove to be over-optimistic by, say, 500 MW the additional capital changes would be nearly offset by savings in operating costs as a corresponding amount of the least efficient plant in operation could be taken out of service and scrapped.

Modern statistical techniques and computer studies are utilized on long- and medium-range forecasting. Distribution requirements having essentially a local and to some extent an individual character remain largely matters of judgment on the part of management.

Research is a primary factor in promoting the commercial development of the industry. For engineering research the supply industry has in the past relied largely on manufacturers and the co-operative work of the Electrical Research Association. It has, however, been realized that the industry requires to conduct a considerable research programme of its own and on this an amount of about £6 million is being spent per annum.

It is clear that insufficient national effort has been directed to matters in which the characteristics of the consumer are of importance. Physiological, psychological and economic factors are involved. For example, the use of 'off peak' supplies for domestic and industrial purposes is increasing rapidly. Electric floor warming introduces a number of novel problems. Not least among the problems requiring investigation is that of the economics of such novel types of load. From the point of view of management, judgment and appraisal have to be applied to determining the sums to be allocated to research in its various aspects, and to the weight to be given to scientific and engineering opinion in the wider context of commercial and social implications. Management must, in fact, endeavour to provide conditions in which engineers, physicists, mathematicians, economists and statisticians can co-operate fully and effectively in advancing the practice of electricity supply.

In the matter of tariffs, management has not only the overriding responsibility of ensuring an adequate total revenue but also a considerable power of influencing the development of demand in desirable directions. While there must be no undue preference for or discrimination against any consumer or class of consumer, there are opportunities for devising special tariffs which can be not only related to the special requirements but also can conduce by better distribution of demand to improvements in the overall operation of the system.

Mr. Melling's third example was the most fundamental one—that of ensuring a supply of managers for one or two decades ahead. In the direct exercise of their professions, engineers, physicists, accountants or lawyers might give quite a good indication of their promise within a few years of graduation. The development of the potential manager and the demonstration of his qualities is a slower process. In Mr. Melling's opinion engineering provides, for management, as good a background as any other profession.

The supply industry, in developing its future managers, utilizes techniques common to most progressive industries. Potential managers attend courses in the industry's own residential training establishments, in universities and elsewhere. At the Electricity Council's training centre in Surrey are provided two types of course, one designed to promote general executive development through the medium of tackling unfamiliar problems by the syndicate method. The other studies individual subjects of importance to managers such as communication, organization and finance. The Generating Board and individual area boards organize similar training courses to meet their own needs. The facilities of the Administrative Staff College at Henley are also used.

Within the actual operating organization itself a system of regular appraisal is being used. This is designed to assess the potential of individuals and to formulate a scheme of training appropriate to individual needs. Following appraisal, selected members of staff may be sent on courses, given 'cross-postings' to new types of work, or allocated to specific assignments. The aim is to provide exposure to some type of problem which has not been met before and which will exemplify human, as well as technical and economic, factors.

Many problems in any sphere of activity are necessarily those of the specialist, and management, in exercising judgment, is as often concerned to assess the qualities of the expert as the advice which he offers.

Academic qualifications, experience, and in some measure knowledge and intelligence are susceptible of direct assessment. The assessment of the qualities of the potential manager which relate to character and personality remains one of the most difficult of problems.

Mr. Melling completed his address by reviewing the role of the Institution of Electrical Engineers in fostering, through the Institution's educational requirements, the development of potential managers.

J. Greig

THE *DRAGON* HIGH-TEMPERATURE REACTOR PROJECT

The third annual report of the Organization for European Co-operation and Development High Temperature Reactor Project (*Dragon*), covering the period from April 1, 1961 to March 31, 1962, has been issued by the European Nuclear Energy Agency*. The participants in the Project are the United Kingdom Atomic Energy Authority; the Euratom Commission, consisting of the six countries, Belgium, France, Germany, Italy, Luxembourg and the Netherlands; the Austrian Government; the Danish Atomic Energy Commission; the Norwegian Institut for Atomenergi; *AB* Atomenergi of Sweden; and the Swiss Government. The Project, which is based at Winfrith, Dorset, has as its principal aims the design, construction and operation of a 20-MW high-temperature gas-cooled reactor, and a programme of research and development to explore the practical possibilities of this type of reactor and the technical problems resulting from operation with temperatures close to 1,500° C in the core of the reactor. The *Dragon* reactor is not the only high-temperature reactor under construction. A similar reactor *HGTR* is being built for the Philadelphia Electric Company at Peach Bottom in the United States, and there has been continuous and close collaboration between the members of the United States Atomic Energy Commission and members of the Project concerning materials for the reactor cores, reactor physics and common design problems. The *Dragon* reactor is likely to be the first of the high-temperature reactors to be brought into operation. Fuel loading was expected to commence towards the end of 1962.

For 1961–62 expenditure on the Project is estimated at about £4,000,000 and for 1962–63 about £5,400,000. The total cost of the original five-year programme ending in 1964 is now estimated at about £15,000,000, and a working party has been set up to examine the question of an extension of the five-year agreement. (It has recently been announced that the extension has been approved; *Nature*, 197, 129; 1963.)

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* Organization for Economic Co-operation and Development: European Nuclear Energy Agency. O.E.C.D. *Dragon* High Temperature Reactor Project—Third Annual Report, 1961–62. Pp. 196. (Paris: Organization for Economic Co-operation and Development: European Nuclear Energy Agency, 1962.)

The annual report is divided into three main sections, dealing with research and development, reactor design and construction, and administration, respectively. In general, the technical predictions on which the project was originally based continue to be confirmed by experience. No significant changes in the basic concept have been necessary, though an important change in the safety principles has been decided on. The inner containment, which consists of a steel shell containing the reactor core, is to be filled with air instead of nitrogen, but the design is such that a return to nitrogen filling, if required, can still be made. The reactor pressure vessel has been successfully pressure and leak tested and delivered to the site. Its erection within the reactor building is proceeding. Steady progress has been made in the design of the fission product and helium purification systems, and a contract for the purification plant is expected to be placed shortly. Construction of the reactor building has advanced rapidly and the control building has been completed.

Theoretical studies during the year have been concentrated on 'burn-up calculations' and power and temperature distributions. The distribution of radioactivity in the *Dragon* system has been estimated. The experimental reactor physics investigations carried out in the *Zenith* reactor continued until July, when the exclusive use of the *Zenith* reactor for the *Dragon* programme came to an end. Several measurements have been made and evaluated. Heat-transfer channel experiments simulating *Dragon* reactor conditions have been carried out at Risø, Denmark. Carbon transfer experiments have emphasized the importance of extremely pure graphite in the core. Barium has been found to have a strong catalytic influence on graphite corrosion while silica acts as an inhibitor. The graphite for the main reactor components other than the fuel elements has been delivered and is in the process of being machined. No firm decision has yet been taken as to whether a fission product emitting fuel or a fission product retaining fuel will be used for the first charge in the *Dragon* reactor, but development work has proceeded on both types and plans for manufacturing either type are in hand.

The report contains many other details of the core and graphite assemblies and control mechanisms together with numerous dimensional diagrams and photographs of the various components. Annexed to the report are charts showing the composition of the staff of the Project; the members of the Board of Management and the General Purposes Committee—the two international committees responsible for the direction of the Project; and a list of all major contracts placed up to March 31, 1962.

S. WEINTROUB

RADIOACTIVITY IN HUMAN DIET

EACH year, as a reminder of the hazards of the atomic age, reports are issued by the Agricultural Research Council Radiobiological Laboratory of levels of radioactivity in human diet. The annual report, 1961–62*, includes a review of research designed to give better understanding of factors which influence the passage of radioactive material from fall-out into plants and animals, both by direct contamination of foliage and via the soil, finally appearing in the mixed diet of the human population. The results of this work range from a demonstration that deposition of strontium-90 in soils may be reliably predicted from figures for annual rainfall, to a final indictment of the Brazil-nut as a concentrator of radium and thorium from the soil on which it is grown; about 1,000 times as much of these isotopes are found in Brazil-nuts as in other nuts and fruits.

The report gives the mean ratio of strontium-90 to calcium in the total mixed diet as 6·2 picocuries per gram of calcium during 1961 as compared with 6·4 in 1960 and 9·0 in 1959, the corresponding values for milk being 5·9, 6·4 and 9·7. It is stressed, however, that the figures obtained so far do not as yet reflect the full effects of the resumption of nuclear testing in 1961, and the most recent measurements support the forecast that the situation in 1962 will not differ greatly from that in 1959 after the tests in 1958.

Reference is made in the report to a 'working level', which is defined as 130 picocuries of strontium-90 per gramme of calcium. Indefinite exposure at this level would induce in bone amounts of strontium-90 of approximately half the maximum permissible specified by the Medical Research Council. In fact, the recommendation of the Medical Research Council's second report, ***The Hazards to Man of Nuclear and Allied Radiations*** (Cmnd. 1225, December 1960), was that "if the concentration of strontium-90 in bone calculated as an average for any age group, including the groups of infants and young children, was found to be rising continuously and to have reached the level of one-half that recommended as the maximum permissible level for the population as a whole, a re-assessment of the situation would be required". The derivation of a 'working level' in the diet, from a maximum permissible level in bone, is not explained in the report from the Agricultural Research Council, and the size of the margin of safety adopted is not given.

In view of this the implications of the term 'working level' are difficult to assess and at best this seems a most unfortunate choice of words.

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* Agricultural Research Council: Radiobiological Laboratory. Annual Report, 1961–62. Pp. xv+96. (London: H.M. Stationery Office, 1962.) 6s. 6d.

PETROLEUM MULCH

IN arboriculture the word 'mulch' is usually associated with the compost of wet leaves, straw, etc., laid as a protection to the roots of newly planted or young trees, especially on inherently dry soils. Petroleum mulch, however, is a relatively recent product of research in the industry and although in some respects its behaviour and purpose are similar to traditional soil-dressings, its potentialities are probably more far-reaching and durable. In the autumn number of the ***Esso Magazine*** (1962), an informative and well-illustrated account of a petroleum mulch evolved by Esso Research is given. The efficacy of this product is now being tested and proved on a large number of sites in many different countries. Already results are most encouraging and augur well for yet another application of petroleum to the well-being of mankind.

Briefly, this type of mulch is in fact an inexpensive emulsion of petroleum resins, used as an agricultural spray for improving the growth and yield of crops of all kinds. Its function is four-fold: to warm the soil, to reduce evaporation, to protect against soil-erosion, and to retard the dispersal by rain-wash and other agents of

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vital mineral and chemical constituents in the soil growth-zones wherever it is used. Most crops are planted in rows and when seeded can be sprayed with this petroleum mulch. The width of these bands may vary from three to ten inches or more, according to the nature of the crops, spacing, and soil conditions. In this way a thin, continuous protective film of the emulsion is formed on the top of the soil, adherent but not deeply penetrating. This film is rain- and wind-proof and remains intact for up to ten weeks. The effect is drastically to reduce the rate of moisture evaporation from the soil involved; also, because of its dark colour, sunlight is absorbed and heat trapped, so that in favourable circumstances soil temperatures can be raised by as much as 20° above normal.

Some significant results are quoted. Tests on cotton plants in Arizona in 1960 revealed that where this mulch was used, twelve days after planting, 121,900 plants per acre had emerged as against 8,710 per acre on the control area. The final yield in bales per acre was 2·2 with mulch and 1·65 without, an increase of 34 per cent. In comparable tests with melons, an increase of 17 per cent was attained, while with vegetables such as carrots, onions, lettuces, turnips and radishes, the increase ranged from 22 per cent to 67 per cent. Similar tests of the reaction of different plants to petroleum mulch under a great variety of climatic conditions, have been carried out in the United States, Europe, and arid and semi-arid areas in North Africa, with equally beneficial results. H. B. MILNER

METALLOGRAPHIC EXAMINATION OF THE PHASE DIAGRAM OF THE GALLIUM–TELLURIUM SYSTEM

By J. R. DALE

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KLEMM and von Vogel[1] obtained a phase diagram for the gallium–tellurium system by means of thermal and qualitative X-ray analysis. Newman, Brice and Wright[2] modified this by investigating the system by differential thermal analysis and by direct observation of the melting-points under controlled tellurium pressures. Since no attempt appears to have been made to check the phase diagram metallographically and no data on the microscopy of the system appear to have been published, a metallographic analysis of the system between 50 and 85 per cent tellurium (all compositions are quoted in atomic percentages) was undertaken.

The samples for microscopic analysis were prepared from 99·999 per cent gallium (from Aluminium Industrie Aktien Gesellschaft) and 99·999 per cent tellurium (from Mining and Chemical Products, Ltd.). The gallium was cleaned by etching in dilute hydrochloric acid before weighing and the tellurium in concentrated hydrochloric acid. After weighing the materials (total weight approximately 2 g) were placed in silica crucibles which were sealed under high vacuum to eliminate loss of tellurium. In general, the samples were reacted for 8 h at 840°–860° C followed by furnace cooling to give a cooling rate of approximately 4°–5° C/min. Some of the samples were vibrated at 50 cycles/sec at low amplitude to give better mixing and more rapid reaction.

To prevent deformation of the relatively soft GaTe and to facilitate sectioning, ingots were mounted in cold setting low-shrinkage epoxide resin blocks ('Bakelite Resin 19019, Hardener Q18814'). In most cases the whole ingot was potted so that a full macroscopic examination could be carried out to determine if any segregation or other inhomogeneities had occurred during the reaction and cooling procedure.

The ingots were prepared for metallographic examination by first lapping successively on 400 mesh, 600 mesh and precision grades of silicon carbide emery polishing papers (Union Glue and Gelatine Co., Ltd.) using paraffin as a lubricant. Intermediate polishing was carried out by lapping on successive grades of diamond paste (L. M. Van Moppes) from 16μ particle size to 0·25μ held on photographic paper pads. A final polish was carried out using a 'Selvyt' cloth pad rotating at 750 r.p.m. and impregnated with a dilute solution of gamma polishing alumina. This long lapping and polishing procedure was found necessary to prevent flowing and deformation of alloys containing less than 58 per cent tellurium, for those having a higher tellurium content the intermediate polishing procedure could be omitted.

Two etches were selected for the determination of the phase diagram by microscopical analysis. The compositions of these etches were:

No. 1 etch: 50 parts glacial acetic acid; 5 parts hydrofluoric acid (40 per cent); 5 parts nitric acid (sp.gr. 1·42).

Samples were etched for 1–2 sec, rapidly transferred to a water bath or quenched and then dipped in concentrated ammonia for a few seconds to remove any slight film formed. The samples were finally washed in de-ionized water and rinsed in ethyl alcohol to prevent staining. No. 1 etch attacked GaTe more rapidly than Ga_2Te_3.

No. 2 etch: 50 parts glacial acetic acid; 5 parts nitric acid (sp.gr. 1·42); 2 parts hydrofluoric acid (40 per cent); 2 parts hydrogen peroxide (100 vol.). This was used as a stain etch to identify the gallium–tellurium compounds in conjunction with No. 1 etch; this caused Ga_2Te_3 to be stained light brown but had very little effect on GaTe.

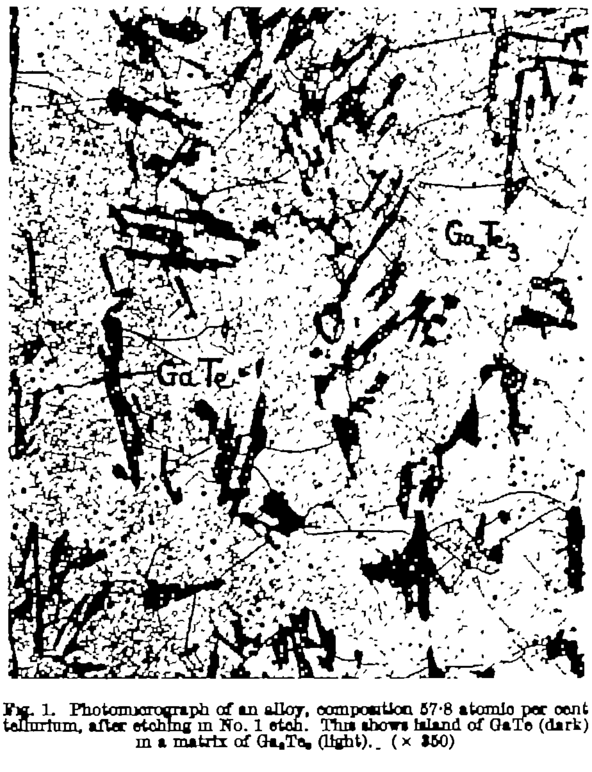

Fig. 1. Photomicrograph of an alloy, composition 57·8 atomic per cent tellurium, after etching in No. 1 etch. This shows island of GaTe (dark) in a matrix of Ga_2Te_3 (light). (× 350)

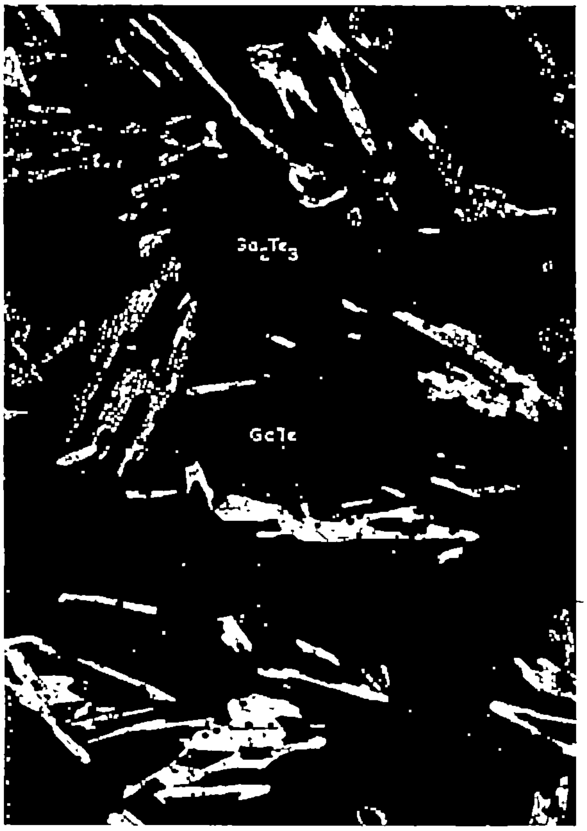

Fig. 2. Photomicrograph of the same section as in Fig. 1 but using etch No. 2. This shows islands of GaTe (light) in a matrix of Ga_2Te_3 (dark). (× 500)

The procedure normally used was to etch for 2–3 sec, wash in de-ionized water and finally rinse in ethyl alcohol.

The effects of these etches on similar sections of an ingot of 57·8 per cent tellurium can be compared in Fig. 1 etch No. 1) and Fig. 2 (etch No. 2). In Fig. 1 dark plates of GaTe can be seen in a light matrix of Ga_2Te_3, in Fig. 2 the matrix of Ga_2Te_3 is stained light brown and the GaTe unattacked.

All the samples examined were highly polycrystalline with a range of grain sizes from a few μ to almost 1 mm. A small number of ingots which showed segregation of the different phases were rejected when assessing the accuracy of the phase diagram. The type of segregation normally observed was that of plates of GaTe accumulating at the top free surface of the ingots with crystals of Ga_2Te_3 at the bottom.

A limited number of horizontally zone refined polycrystalline bars of Ga_2Te_3 grown under controlled tellurium pressures from $7{\cdot}4 \times 10^{-1}$ mm mercury to 5·2 mm mercury were examined for inhomogeneities and microhardness. In most cases when the tellurium pressure was less than approximately 2 mm mercury, free plates of GaTe were found in a matrix of Ga_2Te_3; as would be expected, the higher the tellurium pressure the smaller the concentration of GaTe formed. When only 5–10 per cent GaTe plates (by volume) were present they were found to segregate around the periphery of the bars leaving the centre single phase. When bars were grown under tellurium pressures greater than approximately 2 mm mercury both $GaTe_3$ and $GaTe_3$–Te eutectic were observed along grain boundaries of the Ga_2Te_3, generally near the centre of the bars and not close to the periphery.

Sections of ingots having compositions of 50–55 per cent tellurium showed strained primary plates of GaTe in a matrix of GaTe–Ga_2Te_3 eutectic. This can be seen in Fig. 3, which is a photomicrograph of a section of an alloy containing 54·9 per cent tellurium (loaded composition) after etching (No. 1 etch). In this case the microstructure of the eutectic shows it to be composed of a matrix of Ga_2Te_3 in which are set a large number of fine plates of GaTe.

Fig. 4 is a photomicrograph of an alloy containing 55·8 ± 0·1 per cent tellurium (by chemical analysis) after etching. The microstructure of the alloy shows a slight excess of GaTe from a homogeneous eutectic structure. Fig. 5 shows an alloy of 56·3 per cent tellurium (loaded composition) after a 30-day anneal at 760° C; this appears to have a slight excess of Ga_2Te_3 from a homogeneous eutectic structure.

From the microstructure of these ingots the composition of the GaTe–Ga_2Te_3 eutectic lies between 55·8 and 56·3 per cent tellurium, which is slightly lower than that obtained from differential thermal analysis measurements of Newman *et al.*[3] (they quote 56·5 per cent tellurium eutectic composition); this is not unreasonable since up to 1 or 2 per cent scatter of results was indicated.

Fig. 6 is a photomicrograph of an alloy of 58·4 per cent tellurium after etching. In this alloy the matrix consisted of strained crystals of Ga_2Te_3 surrounded by a large quantity of the GaTe–Ga_2Te_3 eutectic. In alloys having a tellurium content higher than 55–56 per cent, GaTe crystals had a needle-like appearance rather than the broad plates observed in alloys with less than 55 per cent tellurium.

When observed in the polished but unetched condition, GaTe (in alloys having a composition between 50 and 60 per cent tellurium) appeared as a light blue phase polished in relief.

Alloys containing 59·7 ± 0·1 per cent tellurium (by chemical analysis) were observed to consist of fairly large unstrained single-phase grains of Ga_2Te_3. On increasing the tellurium content to 60·0 ± 0·1 per cent (by chemical analysis), a highly strained but single-phase structure of Ga_2Te_3 was formed. This is shown in Fig. 7, where the grain boundaries (*g*) could be identified from mechanical polishing damage (*d*) by the change in orientation of the strain bands at the boundaries.

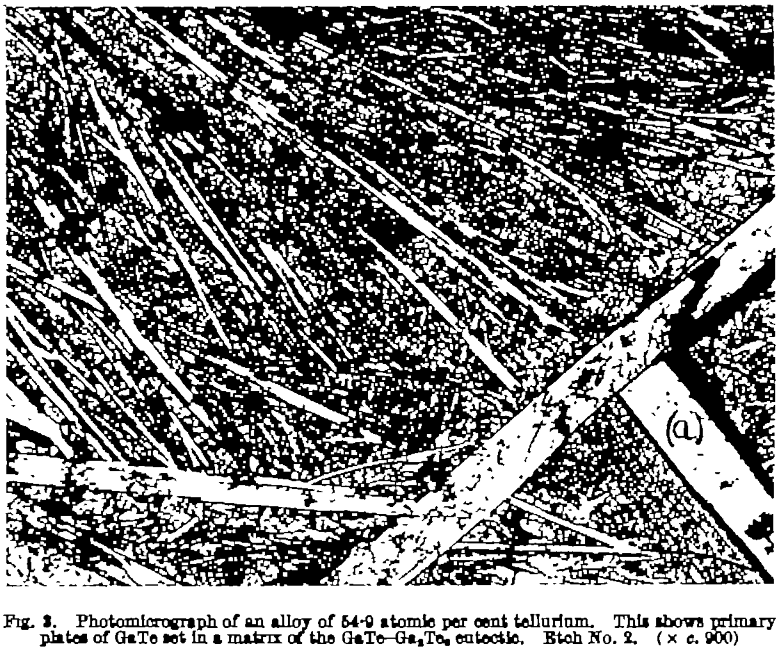

Fig. 3. Photomicrograph of an alloy of 54·9 atomic per cent tellurium. This shows primary plates of GaTe set in a matrix of the GaTe–Ga_2Te_3 eutectic. Etch No. 2. (× *c*. 900)

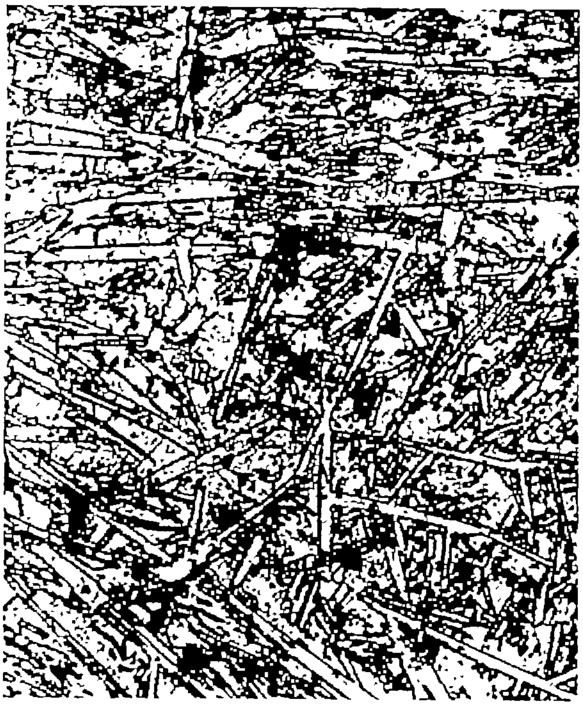

Fig. 4. Photomicrograph of an alloy of 55·8 atomic per cent tellurium. This shows the GaTe–Ga_2Te_3 eutectic. Etch No. 1. (× 180)

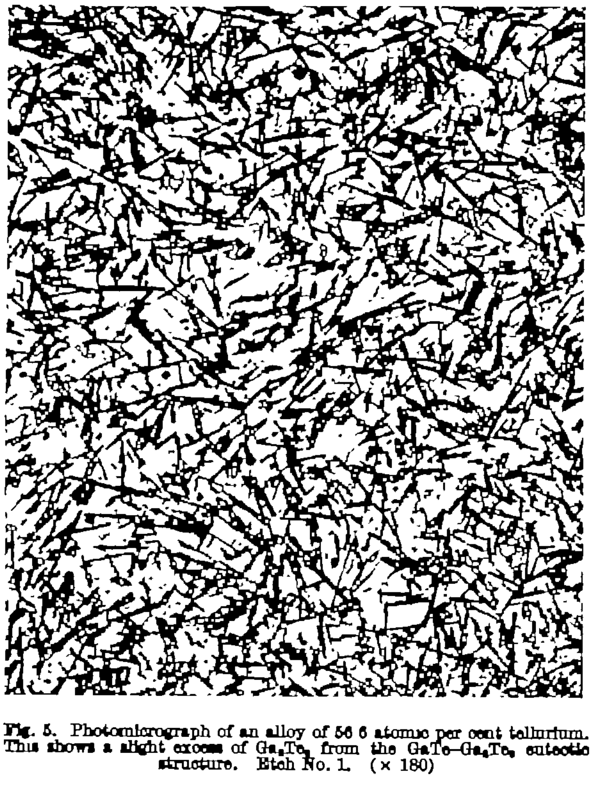

Fig. 5. Photomicrograph of an alloy of 56·6 atomic per cent tellurium. This shows a slight excess of Ga_2Te_3 from the GaTe–Ga_2Te_3 eutectic structure. Etch No. 1. (× 180)

Since these alloys were single phase it must be assumed that the compound Ga_2Te_3 has a finite range of solid solubility which appears to be of the order of at least 0·3 atomic per cent; it seems probable, however, since no strain bands were observed in the 59·7 per cent tellurium alloy, that the solubility range is much greater than 0·3 per cent. This observation of a wide composition range is also supported by the difference in resistivity of single-phase alloys of Ga_2Te_3: simple two-probe measurements showed a change from approximately 20 Ωcm for single-phase tellurium-rich alloys to more than 10^6 Ωcm for single-phase gallium-rich alloys. The wide range of hardness values obtained for the single-phase compound also supports these observations. These results differ from all previous work on the systems, which showed Ga_2Te_3 to exist only as a 'straight line' compound at 60 atomic per cent tellurium.

Fig. 8 is a photomicrograph of an alloy of 75·0 per cent tellurium after reacting at 460° C for 8 h and slow cooling; this indicates that equilibrium conditions have not been approached and show primary crystals of Ga_2Te_3 in a matrix thought to consist of dendrites of $GaTe_3$ and the $GaTe_3$–Te eutectic.

Fig. 9 shows the same alloy at a higher magnification; from the microstructure it can be seen that at 460° C

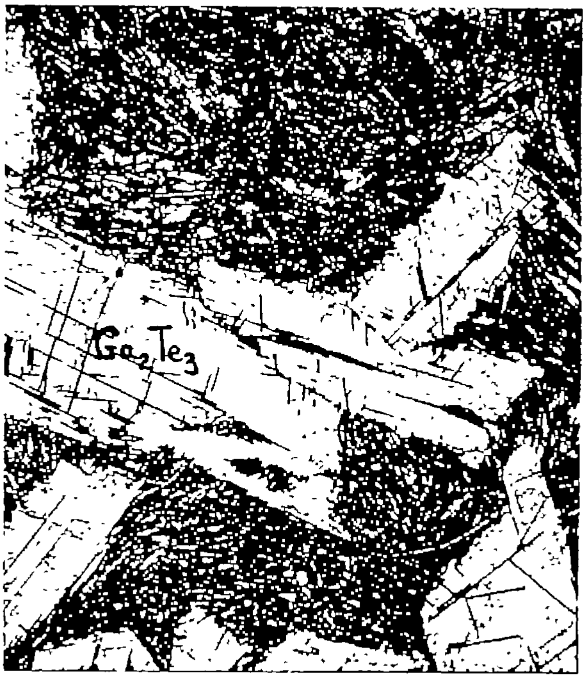

Fig. 6. Photomicrograph of an alloy of 58·4 atomic per cent tellurium This shows strained primary crystals of Ga_2Te_3 in a matrix of GaTe–Ga_2Te_3 eutectic. Etch No. 1. (× 180)

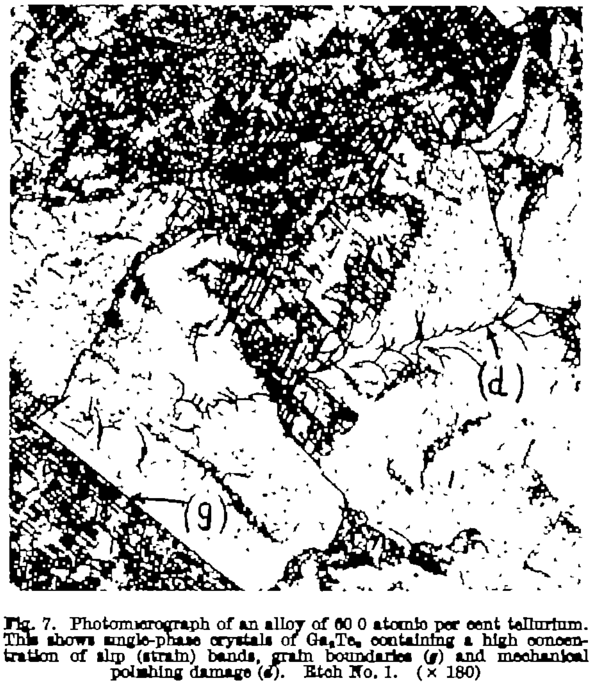

Fig. 7. Photomicrograph of an alloy of 60·0 atomic per cent tellurium. This shows single-phase crystals of Ga_2Te_3 containing a high concentration of slip (strain) bands, grain boundaries (*g*) and mechanical polishing damage (*d*). Etch No. 1. (× 180)

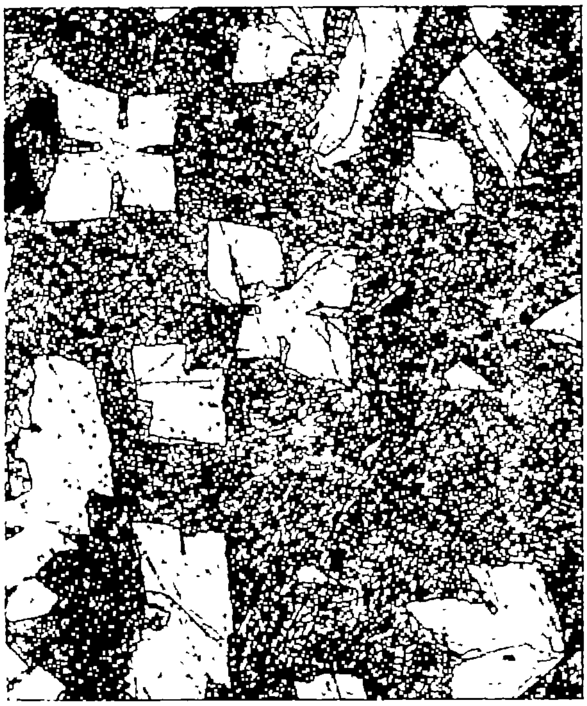

Fig. 8. Photomicrograph of an alloy of 75 atomic per cent tellurium. This shows primary crystals of Ga_2Te_3 in a matrix of $GaTe_3$ dendrites (light) and tellurium (dark). Etch No. 1. (× 100)

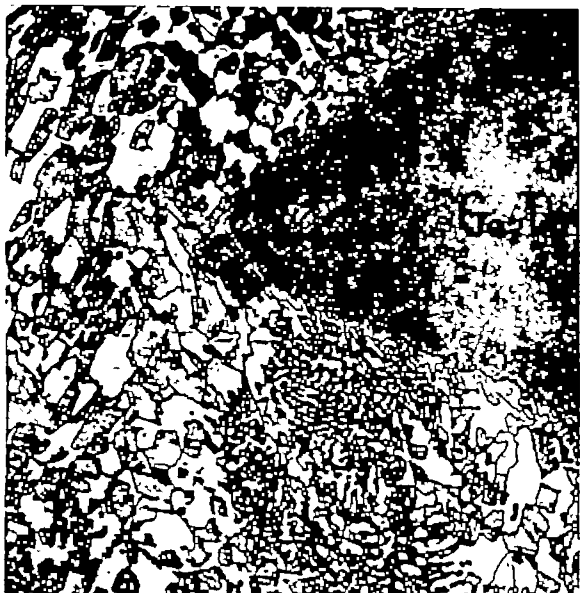

Fig. 9. As for Fig. 8 but showing more clearly the compound $GaTe_3$. (× 750)

crystals of Ga_2Te_3 were deposited, on cooling these reacted with the remaining liquid to form a single-phase component thought to be the compound $GaTe_3$. This peritectic reaction is also confirmed by the irregular edges of the Ga_2Te_3 crystals. The cruciform shape of the primary crystals of Ga_2Te_3 (Fig. 8) is probably due to their dissolution by the remaining liquid along preferred crystallographic planes.

The presence of approximately 20 per cent primary Ga_2Te_3 crystals in the ingot shows that equilibrium was not achieved, since, if the peritectic compound $GaTe_3$ was formed not more than 5 per cent Ga_2Te_3 would be expected. An attempt to obtain equilibrium conditions by reacting at 800° C, slow cooling from 435° C to 420° C at approximately 1° C/day (that is, through peritectic transformation) followed by quenching showed almost no increase in the size and quantity of the $GaTe_3$ crystallites. X-ray 'powder' patterns taken on crystalline and not powdered material proved the existence of the compound $GaTe_3$, which was also shown to be stable at room temperature over a period of almost one year. Powdered samples, however, did not exhibit the diffraction lines of $GaTe_3$, but it could not be established if this was due to a mechanical effect on crushing. This effect probably explains the observations of Newman *et al.*[4], who found $GaTe_3$ to be unstable.

Fig. 10 is a photomicrograph of a lightly etched alloy of 82·0 per cent tellurium; this shows a slight excess of $GaTe_3$ (light-coloured phase) from a homogeneous $GaTe_3$–Te eutectic.

Modifications suggested by these results have been been added to the phase diagram determined by Newman *et al.* This is shown in Fig. 11, where the amendments are indicated by dotted lines.

Microhardness measurements were carried out on a wide range of alloys to give additional information on the nature of the different phases in the system. The samples were prepared for testing in an identical way to those for microscopical analysis and were then given a light etch in No. 1 etch to delineate the different phases. Microhardness values were obtained by using a standard Vickers microhardness tester with loads of between 5 and 50 g, dependent on the nature of the phase being examined.

The values obtained for GaTe were inconsistent because of the high degree of deformation along cleavage and slip planes on application of the indenter. The high elasticity of the material also caused inconsistent results since the recovery of the GaTe after deformation on removal of the indenter load tended to give high values. The range of microhardness values obtained for GaTe was 40–70 Vickers pyramid numbers (V.P.N.), the lower number being probably near the true value.

A range of microhardness measurements were taken on individual crystals of Ga_2Te_3 grown from different melt compositions; these indicated that the higher the gallium

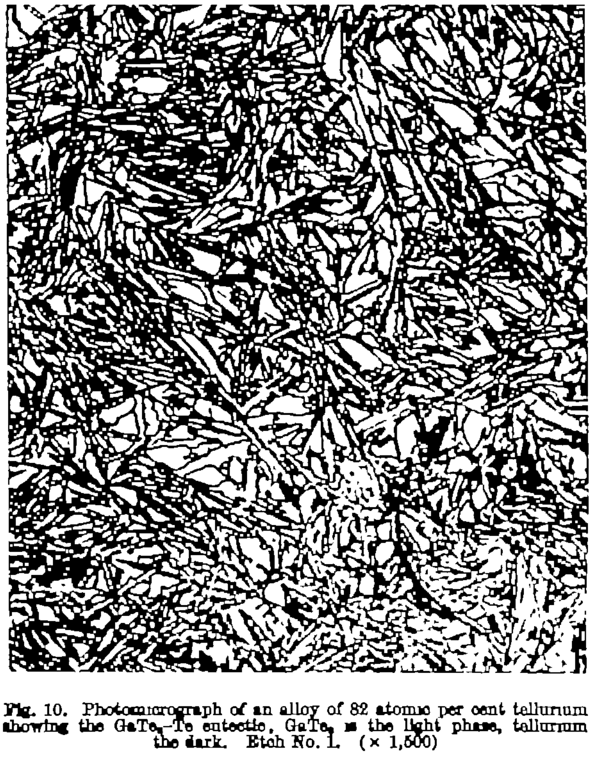

Fig. 10. Photomicrograph of an alloy of 82 atomic per cent tellurium showing the $GaTe_3$–Te eutectic. $GaTe_3$ is the light phase, tellurium the dark. Etch No. 1. (× 1,500)

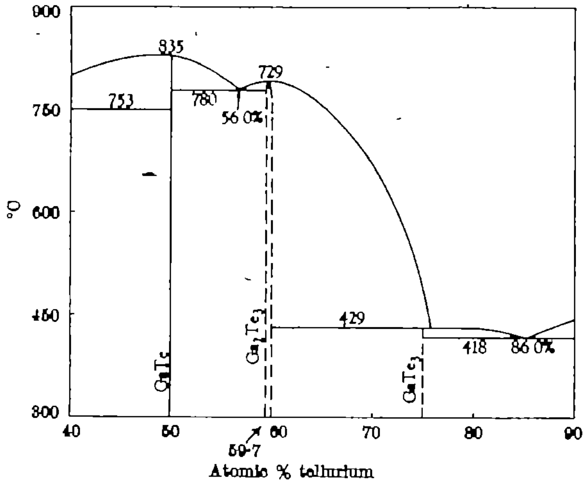

Fig. 11. Phase diagram of the gallium–tellurium system

content the higher the microhardness. Using a 20-g load applied for 15 sec the figures were:

Ga_2Te_3 primary crystals in GaTe–Ga_2Te_3 eutectic (total alloy composition 59 0 atomic per cent tellurium)	234 V.P.N.
Highest resistivity single-phase Ga_2Te_3 examined (not analysed)	198 V.P.N.
59·7 atomic per cent tellurium (single-phase alloy)	185 V.P.N.
60·0 atomic per cent tellurium (single-phase alloy)	174 V.P.N.

All the microhardness values were obtained from an average of at least 10 impressions with scatter of the order of ± 5 per cent.

The increase in microhardness with increasing gallium content was probably due to gallium atoms filling some of the vacant sites in the Ga_2Te_3 (Ga_2Te_3 has zincblende structure with vacancies on one-third of sites available for gallium atoms)[2]. The microhardness of Ga_2Te_3 primary crystals in tellurium rich matrices was measured and gave a range of microhardness figures of 180–200 V.P.N. This variation and relatively high value was thought to be due to the temperature of formation of the crystallites, the higher the temperature of formation the higher the hardness value was indicated. This effect would be expected if the Ga_2Te_3 solidus curved slightly from the maximum-melting point to the peritectic temperature and composition, that is, if the maximum melting-point of the compound is at a lower tellurium content than the stoichiometric composition. From the hardness measurements obtained on the tellurium rich Ga_2Te_3 this would appear to be nearer 59·7 than 60·0 atomic per cent.

This investigation has yielded evidence for modification of the gallium–tellurium phase diagram and has shown that the compound Ga_2Te_3 has a range of at least 0·3 atomic per cent solid solubility. Microhardness measurements on a wide range of alloys have shown that values vary from 40 V.P.N. for GaTe to 234 V.P.N. for Ga_2Te_3.

I thank Miss C. A. Fisher and J. A. Wilkinson for providing the alloys, J. A. Cundall for taking X-ray photographs, and Messrs. H. C. Wright, P. C. Newman and J. C. Brice for helpful discussions.

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TITANIUM COLOUR CENTRES IN ROSE QUARTZ

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ALTHOUGH several studies of the chemical content and the optical properties of rose quartz have appeared, no electron paramagnetic resonance investigations have yet been reported. We wish herein to describe several impurity 'colour' centres found in all the numerous samples studied by us.

We have prepared single, oriented crystals of rose quartz from various geographical locations (Table 1). These crystals in their virgin state do not (except for peaks from iron in some samples) show electron paramagnetic resonance at room temperature. A set of magnetic centres visible at 77° K in all crystals were created by X-irradiation at room temperature; these centres were already present in the virgin state of one sample (Custer) and were enhanced by the irradiation.

Of dominant interest in this article, we wish to report the observation by 3-cm electron paramagnetic resonance of a set $[\equiv A(\mathrm{Ti})]$ of new centres and the identification of their principal constituent as titanium existing substitutionally in silicon sites. These centres, stable at 300° K in the dark, could be bleached with ultra-violet light and restored by X-rays. The spectrum of each centre observed at 77° K consists basically of three primary lines (from equivalent unit cell sites made distinguishable by the magnetic field $\vec{H}$) which reduce to a single line for $\vec{H}$ along the three-fold symmetry axis ($\equiv Z$) and otherwise to two lines when $\vec{H}$ is located in the plane ($\equiv YZ$) perpendicular to any ($\equiv X$) of the three two-fold symmetry axes. The lines of one of the set, to be denoted by $A_{\mathrm{Ti}\text{-}M}$, exhibit four-line anisotropic nuclear hyperfine structure (Fig. 1, upper, and Table 2) indicating that an alkali atom M, most probably lithium, is contained in the centre. A second centre, tentatively denoted by $A_{\mathrm{Ti}\text{-}H}$, is very similar but has lines showing only two-line hyperfine structure which we attribute to interaction of the unpaired electron with a proton. In general, the lines of $A_{\mathrm{Ti}\text{-}M}$ were much more intense than those of $A_{\mathrm{Ti}\text{-}H}$ in the samples examined.

Table 1. Geographical Origin of Rose Quartz Crystals

Custer, S. D. (Frank Scott's Rose Quartz Mine), United States.
Hebron, Maine (courtesy of Chicago Museum of Natural History).
Paris, Maine, United States.
Nuevo, California (courtesy of Wheaton College), United States.
Madagascar, Africa.
Brazil, South America.
Salamanca, Spain.
Rabenstein, Bavaria, Germany (courtesy of Museum of Geology, Princeton University).

Besides these strong primary peaks, we have detected the weak anisotropic hyperfine structure arising from the low-abundance odd titanium isotopes, each line

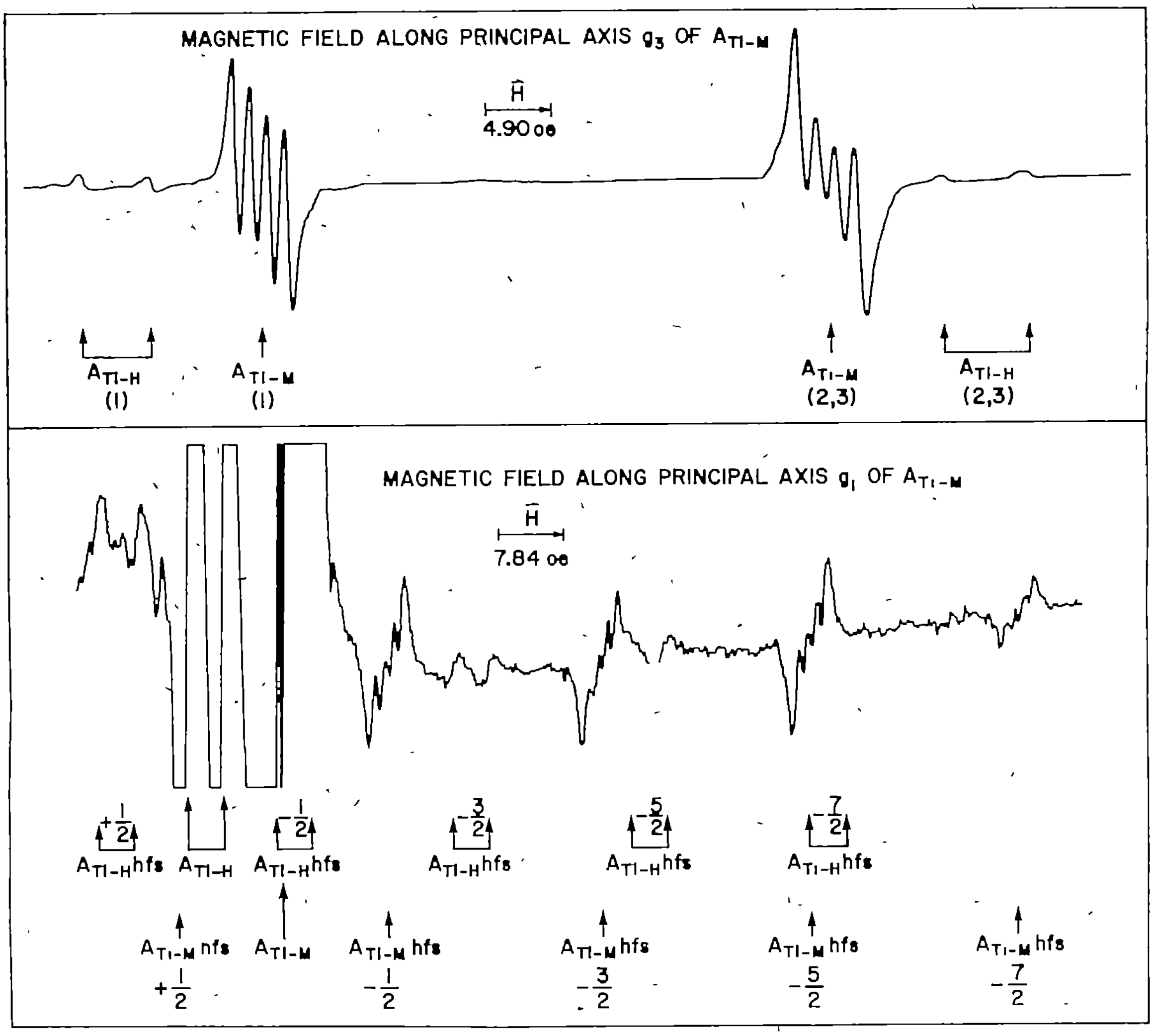

Fig. 1. The paramagnetic resonance spectrum of titanium centres in an X-irradiated rose quartz crystal at 77° K. Upper: The A_{Ti-M} and A_{Ti-H} centres. Lower: A portion of an amplified spectrum showing the weak titanium hyperfine structure

showing the alkali or proton peaks as secondary hyperfine structure. The relevant parameters are listed in Table 2. Because the hyperfine splitting goes as μ/I, which happens to be virtually identical for titanium-47 and titanium-49, the 6 lines of titanium-47 superimpose on the inner 6 of the 8 titanium-49 lines. The observed spectrum (Fig. 1, lower) shows exactly this form, making unequivocal the identification of titanium as the primary component of the centres; the relative intensities of these lines are also quantitatively as predicted. One previous report of titanium hyperfine structure has been made[1].

Elemental analysis by spectrochemical means, as well as by neutron activation and flame photometry, revealed the presence of titanium (5–15 p.p.m.), germanium (0·5–5 p.p.m.), aluminium, lithium and sodium in all the crystals of rose quartz.

The superposition of two of the three lines (Fig. 1, upper) from equivalent sites in the unit cell, when $\vec{H}$ lies in the plane YZ, indicates that one principal axis of the Zeeman splitting tensor $\bar{g}$ lies along the two-fold axis X. Moreover, then, only three lines will be seen at arbitrary orientations of $\vec{H}$, rather than the six expected for more general locations of the principal axes. These tensors, for both A_{Ti-M} and A_{Ti-H}, are given in Table 3, as are some hyperfine splittings along the principal g axes. Determination of the hyperfine tensors is difficult because of the overlapping of the numerous anisotropic lines.

Table 2. ABUNDANCE AND MAGNETIC HYPERFINE PARAMETERS FOR VARIOUS NUCLEI

	Abundance	I	μ (Bohr magnetons)	Relative intensity
^{1}H	99·98	1/2	2·79270	—
^{6}Li	7·43	1	0·82191	—
^{7}Li	92·57	3/2	3·2560	—
^{23}Na	100	3/2	2·2161	—
^{46,48,50}Ti	86·74	0	0	1
^{47}Ti	7·75	5/2	−0·78712	0·0219
^{49}Ti	5·51	7/2	−1·1023	0·00794

Table 3. ZEEMAN SPLITTING TENSOR $\bar{g}$ FOR THE CENTRES A_{Ti-M} AND A_{Ti-H}, AS WELL AS THE HYPERFINE SPLITTINGS AND LINE-WIDTHS (MAX TO MIN.) WHEN $\vec{H}$ IS ALONG THE g_1 OR g_3 AXIS

Centre	g	Hyperfine splitting $M(H)$	Hyperfine splitting Ti	Width
A_{Ti-M}	g_1 = 1·912, 70° from Z	1·16 oe	27·1 oe	0·68 oe
	g_2 = 1·930, X	—	—	—
	g_3 = 1·979, 20° from Z	1·35	—	0·67
A_{Ti-H}	g_1 = 1·916, 62° from Z	4·43	22·8	1·15
	g_2 = 1·936, X	—	—	—
	g_3 = 1·984, 28° from Z	5·0	—	1·11

The identification of lithium, rather than sodium, as the cation seen in A_{Ti-M} depends on the predominance of the former in the crystals, as given by the elemental analyses, and by analogy to the germanium centres (see following) in which lithium seems to form the more stable centres. Only one alkali centre is seen despite the presence of both lithium and sodium in the samples. Similarly, the correlation of the doublets with protons rests on the magnitude of the splitting and on the known presence of hydrogen in quartz crystals[2]. Experiments are under way to confirm these identifications.

Since the spectra of A(Ti) exhibit the point group symmetry (D_3) consistent with the quartz structure, it is clear that the titanium observed is not in the form of rutile needles (symmetry D_{4h}) embedded in the crystal. The orientation of the principal g axes, taken together with knowledge that titanium will form tetrahedral compounds, strongly suggests that the titanium atoms have replaced silicon atoms in their positions on the two-fold axes. In the solution studies of Ti^{3+} complexes[1], the electron paramagnetic resonance absorption was not observable at room temperature, but was strong at 228° K, with $g \simeq 1·95$ and titanium hyperfine splitting of 18·2 oe. Thus these spectra are very similar to ours and serve in the identification of the trivalent state for the titanium in the A(Ti) centres.

We have observed other colour centres, previously examined in other quartzes, in our samples of rose quartz. However, no manganese resonance was detected in any sample. The aluminium centres reported by Griffiths, Owen and Ward[3] were seen (at 77° K) in several virgin and all irradiated samples. All crystals, after irradiation, showed (at 77° and 300°) the germanium centres previously seen in germanium-doped synthetic quartz; three such centres ($A_{Ge\text{-}M}$, B_{Ge}, $C_{Ge\text{-}M}$) are obtained on irradiation at room temperature[4], whereas others occur after low-temperature irradiation[5]. A and C differ in the position of the alkali and are known with either sodium or lithium, but not with hydrogen; B is unstable and may be just a GeO_4^- site not stabilized by a positive ion.

A model suitable for the A(Ti) centres appears to be exactly analogous to that proposed by two of us for the germanium centre[4a,c]. We believe that an electron, dislodged from a source such as the aluminium–alkali sites[3], settles on the titanium central atom situated within the quartet of neighbouring oxygen atoms (which form a somewhat distorted tetrahedron in α-quartz). The alkali or hydrogen ion acts as charge compensator and is located in a nearby open space such as one of the c-axis channels. The small magnitude of the alkali and proton splitting indicates that the unpaired electron spends only a very small fraction of its time on the atom. Despite this, the alkali may have an important role in stabilizing the centre by interacting with the two nearest members of the oxygen quartet.

It seems likely that ions other than titanium and germanium, such as zirconium, tin, hafnium and lead will tend to form centres of the same type in quartz, although size limitations may become important.

The colour of rose quartz is still not completely understood. Since the early studies and the summary of the subject by Holden[6], who attributed the rose colour to traces of manganese, several investigations have been made which produced evidence that titanium is the important factor. Small rutile (TiO_2) needles have been shown to be present in all rose quartz, and give rise to light-scattering and absorption[7] in the ultra-violet. In addition, an absorption peak at 495·7 mμ is present and produces the colour. Our investigations show that substitutional titanium is present in addition to the colloidal rutile. However, its diamagnetic, tetravalent form will not cause the colour, and the paramagnetic trivalent titanium of the A(Ti) centres is destroyed by ultra-violet bleaching or heating without affecting the rose colour. It seems probable, as has also been suggested elsewhere[7b], that the colour is caused by a centre offering a more complex situation than titanium by itself, such as aluminium and titanium occurring as nearest neighbours in the lattice.

We are making detailed investigations of the action of ultra-violet light and X-rays on the concentration of the A(Ti) centres, of replacement of the alkali ions by electrodiffusion (as was done for the germanium centres[4c]), and of the hyperfine tensors. These results should lead to a more detailed quantum-mechanical model for the centres, and will be presented elsewhere.

This work was carried out under the auspices of the U.S. Atomic Energy Commission, and was presented at the 1962 Conference on Radiation Effects on Glass and Quartz, Rochester, New York. One of us (J. H. A.) was partially supported by the U.S. Air Force through the Air Force Office of Scientific Research of the Air Research and Development Command.

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POLYCARBOXYLIC AND PHOSPHONITRILIC ACIDS AS RADIOPROTECTORS: EFFECTIVENESS OF PYROMELLITIC ACID

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THE ability, previously reported by us[1], of inorganic condensed phosphates to protect mice against X-ray lethality when injected before irradiation has prompted us to test for this property other compounds having multiple acidic groups associated with a ring or short open-chain structure. In this work female mice of R stock weighing 20–25 g were used at the age of 14 weeks. The doses of the compounds administered in the radioprotection experiments were determined by their toxicity. After preliminary trials with single mice, increasing doses of each compound were given intraperitoneally to groups of 5 mice, which were observed for at least one week, until a dose resulting in at least one toxicity death in a group was reached. The next lower dose (Table 1, third col.) served as a guide to the dosage of the compound that could be used in irradiation experiments. This or a slightly lower dose was generally used at the outset. In these experiments, such doses frequently resulted in deaths during or shortly after irradiation: these were due to toxicity, as deaths from the dose of X-rays given do not occur before the fifth day. Some toxicity deaths would be expected statistically, especially since larger groups of animals were being used; however, enhancement of toxicity by X-rays is suspected though statistical proof is lacking. Where deaths occurred before the fifth day, these were avoided in subsequent experiments by using a lower dose of the compound.

Test acids were given intraperitoneally, as neutral aqueous solutions of the sodium salts, 10 min before exposing the mice in partitioned boxes to 1,025-r. X-rays under the following conditions of irradiation: 250 kV, 15 m.amp, half value layer 1·2 mm copper, distance to underside of box 60 cm, average dose-rate 73·6 r./min. The experiments were on the pattern previously described with internal controls[1], corresponding experimental and control groups being equal in size. 1,025 r. X-rays gave 100 per cent mortality in controls (injected with saline) during the course of these experiments, but gave 98 per cent mortality during one year. Survival at 30 days was taken as the measure of protective effect. The results are given in Table 1.

Table 1. PROTECTIVE EFFECTS OF POLYBASIC ACIDS (AS Na^+ SALTS) WHEN GIVEN 10 MIN BEFORE 1,025-R. X-RAYS

Acid	Basicity	Max. non-lethal dose in sample (m.moles/kg)	Dose used (m.moles/kg)	No of mice surviving/No. used
Triphosphonitrilic	3	70	60	4/10
Tetraphosphonitrilic	4	1·0	1·0	0/10
Phthalic	2	9	9	2/10
Hemimellitic	3	18	15	3/10
Trimesic	3	27	21	2/10
Mellophanic	4	1·75	1·0†	2/10
"			0·75	4/10
Pyromellitic*	4	12	12	5/20‡
"			9	26/40§
"			6	2/10
"			3	2/10
Naphthalene-1 : 4 : 5 : 8-tetracarboxylic	4	9	9†	6/10‡
"			6	1/10
Propane-1 : 2 : 3-tricarboxylic	3	21	18	2/10
Butane-1 : 2 : 3 : 4-tetracarboxylic	4	3	3	1/10

* Obtained from L. Light and Co. as the dihydrate (found: C, 41·6; H 3·55. Loss in wt. in 2 h at 100° *in vacuo*, 11·8. Calc. for $C_{10}H_6O_8.2H_2O$: C, 41·4; H, 3·47; $2H_2O$, 12·4 per cent).

† One toxicity death occurred before fifth day.

‡ Significant (ref. 2) by 2 × 2 contingency test using internal controls ($P < 0·02$).

§ Significant ($P < 10^{-4}$).

The structures of tri- and tetra-phosphonitrilates differ from those of cyclic metaphosphates only in that the ring O atoms are replaced by —NH—. This change alters the pharmacological properties, as shown by the marked effects on toxicity. Sodium tetraphosphonitrilate lacks radioprotective effect but the non-toxic triphosphonitrilate, in very high dosage, shows indication of effect which, however, is not significant by the severe test for significance used[2]. Evidently, in this group of substances the cyclic arrangement of anionic groups is not the sole requirement for radioprotective ability.

Of the polycarboxylic acids listed significant protective effect is shown only by naphthalene-1 : 4 : 5 : 8-tetracarboxylic and pyromellitic acids. In the former case the effect is shown only at a dose-level where marked toxic effects are also evident, resulting in one death due to toxicity in the irradiated group. The protective effect of pyromellitic acid is highly significant ($P < 10^{-4}$) at a dose-level of 9 m. moles/kg (conc. 0·3 M), when only a mild depressant effect is observable, which is not modified by exposure to X-rays, and the dose is substantially less than the maximum tolerated. It is of interest that this dose-level is optimal: the protective effect at 9 m.moles/kg is significantly greater ($P < 0·02$) than that at 12 m.moles/kg when the depressant effect is more marked. This indicates that the radioprotective effect is not merely a manifestation of toxicity. Pyromellitic acid is ineffective when given as long as 30 min before irradiation and has no influence on mortality when given immediately after 1,025 r.

No structure-activity relationships in the benzenepolycarboxylic acid series are discernible since any protective effects of the acids listed, other than pyromellitic, are non-significant with the numbers of mice used. Nor does radioprotective action appear to be related to the observed pharmacological effects in this series; thus, both mellophanic and trimesic acids share with pyromellitic acid a depressant property. Phthalic and hemimellitic acids produce enhanced irritability and seizures resembling the tetany due to calcium-complexing agents such as linear polyphosphates and ethylenediaminetetraacetic acid. We have as yet no evidence as to the mechanism of the protective action of pyromellitic acid.

Experiments with the hexabasic mellitic acid have been complicated by the fact that samples from different sources have shown different toxicities; however, a sample of low toxicity has shown significant radioprotective effect. Mellitic, benzenepentacarboxylic and related acids are at present being investigated.

We thank Mrs. D. M. R. Lowman for assistance, Mr. M. J. Corp for irradiations and Dr. D. R. Peck, of Albright and Wilson, Ltd., for gifts of sodium tri- and tetra-phosphonitrilates.

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AFFINITY OF STEROIDS FOR ELECTRONS WITH THERMAL ENERGIES

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THE vapours of most organic compounds are transparent to the passage of electrons with thermal energies; those few substances which absorb electrons strongly are frequently associated with the biological process of oxidative metabolism. Thus the alternate intermediates and coenzymes of the Krebs's cycle have unusually large coefficients for the absorption of thermal electrons and so do substances toxic to this system[1]. The possession of a high affinity for thermal electrons appears also to be a necessary molecular property of substances active in the hormonal control of metabolism, for example, the thyroid hormones. The steroid hormones are active in the control of metabolism; the measurement of their electron absorption was therefore potentially interesting to theories of the mechanism of their action. Recent advances in the analysis of steroids by gas chromatography[2,3] have made possible the presentation of the pure dilute vapour samples necessary for the measurement of electron attachment to steroid molecules in the vapour phase. Using these new techniques, the absorption coefficients for thermal electrons of steroids were measured and are reported here. The substances tested included members of the various hormone classes and the possible significance of the measurements in the chemistry and in the biological function of the steroids is discussed.

The technique used to measure electron absorption was closely similar to that previously described[1,4]. Briefly the test compound was vaporized in a stream of argon containing 5 per cent of methane at 250° C; the dilute vapour was passed through a 12-ft. chromatograph column containing a solid support coated with 1 per cent '*SE*-30' silicone stationary phase according to the method described[3] and then into an ionization chamber for the measurement of electron absorption. The chromatograph column served to separate from the test substances traces of electron absorbing contaminants. The ionization chamber contained a source of soft β radiation (tritium occluded in titanium foil) which set free from the carrier gas 8×10^{10} e/sec. At intervals of 50 μsec the concentration of free electrons in the chamber was sampled by the application of a pulse of potential to the chamber electrodes. This sampling pulse was 0·5 μsec in duration and 30 V in amplitude; it was sufficient to collect all the electrons present but too weak and brief to raise the electron energy appreciably above that of thermal equilibrium with the gas in the chamber. The presence of

5 per cent of methane in the argon carrier gas ensured the rapid removal of any excess energy gained by the electrons from the sampling pulse. In the presence of test vapour some of the electrons are removed to form negative molecular ions; these are too slow moving for collection during the brief sampling pulse and so the decrease in electron flow is a measure of the absorption of electrons by the test vapour. At the high temperatures needed to maintain the steroids in the vapour phase some loss by thermal decomposition or by irreversible absorption on the chromatographic column could take place.

To avoid possible errors in the calculation of absorption coefficients, the quantity of steroid surviving passage through the chromatograph column was measured directly by replacing the electron absorption chamber with a cross-section ionization detector[5,6]. This detector is precise and impartial in its response to the vapours of different substances. It was not possible to use this detector simultaneously with the measurement of electron absorption since it requires hydrogen or helium as the carrier gas, and neither of these is suitable for the measurement of electron absorption. As a further precaution against error, a reference standard substance, pyrene, was always included with each steroid tested and appeared as a separate peak on the chromatogram.

The absorption coefficients for thermal electrons of the steroids tested were calculated as follows: the total number of electrons attached to a known quantity of steroid was compared with the total number attached to a known quantity of the reference standard, pyrene. The numbers of electrons attached were calculated directly from the peak areas of the chromatograms under conditions where not more than 10 per cent of the free electrons were absorbed. In the interests of consistency with previous measurements, the reference standard, pyrene, was given an absorption coefficient of 6·0 in arbitrary units; an absorption coefficient of 1·0 on this scale corresponds approximately to a molecular cross-section for electron absorption of 10^{-16} cm^2. The probable error of the method was estimated by calibration with a single test steroid 4-androstene-3,17-dione and found to be ± 17 per cent. This lack of precision is less serious than it might at first appear to be. Electron absorption coefficients cover a range of at least 10^7 and ± 17 per cent error rarely affects the significance of comparisons between the absorption coefficients of different substances.

Table 1 lists the absorption coefficients of the steroids tested and also those of comparable substances from previous investigations. The absorption coefficients cover a range of at least 10^5 with the hydrocarbon steroids transparent to electrons and the unsaturated triketo steroids the most strongly absorbing.

A marked electron absorption in the steroids tested is found only with those possessing the structure $.CO.CH:CR_1R_2$; from previous measurements[1] it is known that this structure confers electron absorption provided that there is in the molecule an opportunity for further electronic interaction. For example, methyl vinyl ketone, 2-*cyclo*hexenone, and ethyl acrylate absorb free electrons weakly; whereas cinnamaldehyde, and fumarate esters absorb intensely. In the last two compounds the additional phenyl, and carbonyl components have respectively provided the necessary further 'conjugation' for a high degree of electron absorption.

It is not yet known experimentally what is the minimum requirement of the groups (R_1R_2) in the α β unsaturated keto structure above, for marked electron absorption; the results in Table 1, however, show that within the saturated part of the steroid framework these requirements are met. For example, 4-cholestene-3-one has an absorption coefficient of 11. Table 1 also indicates the relationship between structure and electron absorption in the saturated and unsaturated keto steroids. The presence of even three isolated keto groups in a saturated steroid confers little more absorption than the slight amount expected of a simple ketone, thus the absorption coefficients of androstane-3,17-dione and of allopregnane-3,11,20-trione are both less than 1. The introduction of a double bond at the 1 position in androstane-3,17-dione, although introducing the α β unsaturated keto structure, does not appreciably increase the electron absorption; a double bond at the 4 position in androstane-3,17-dione, however, increases the absorption 200-fold. Some of this large increase would appear to be attributable to interaction between the 4-ene 3-one system and the remote 17 keto group, for in cholestene 3-one and in testosterone where the 17 position is substituted respectively with an alkyl chain or a hydroxy group the absorption is three times less. The exact location of the remote keto group on the *cyclo*pentane ring does not appear to be important since the absorptions of progesterone, 4-androstene-3,17- and 3,16-diones are all similar. However, a keto group at the 11 position greatly enhances absorption, as in 4-pregnene-3,11,20-trione and 4-androstene-3,11,17-trione; these compounds absorb 2,500 times more strongly than does androstane-3,17-dione.

Table 1. ELECTRON ABSORPTION COEFFICIENTS OF THE STEROIDS AND OF RELATED COMPOUNDS

Compound class	Compound	Absorption coefficient
Hydrocarbons	Androstane	0·01
	Cholestane	0·01
Sterols	Cholesterol	0·03
Saturated ketones	Androstane-3,17-dione	0·10
	Allopregnane-3,11,20-trione	0·50
	i Cholestane-6-one	5·8
Unsaturated ketones	4-Cholestene-3-one	11·3
	Testosterone	8·4
	19-Nortestosterone	9 1
	3,5-Cholestadiene-7-one	96·5
	17β-Hydroxy-1,4,6-androstatriene-3-one	85·0
	1-Androstene-3,17-dione	0·21
	1,4-Androstadiene-3,17-dione	4·5
	4-Androstene-3,17-dione	23·5
	4-Androstene-3,16-dione	19 2
	Progesterone	22·2
	4-Androstene-11-ol-3,17-dione	76·0
	1,4,6-Androstatriene-3,17-dione	167
	Cortisone band	63·5
	6-Keto progesterone	143
	4-Androstene-3,11,17-trione	248
	4-Pregnene-3,11,20-trione	250
	1,4-Androstadiene-3,11,17-trione	535
	25-Keto-norcholesterol	0 03
	4,4′-Dimethyl-5-cholestene-3-one	0·03
	Oestrone	0·03
Comparable compounds	Methyl vinyl ketone	1·1
	Cinnamaldehyde	310
	Dimethyl fumarate	1,500
	Dinitrophenol	1,500
	Diiodobenzene	1,300

All values are relative to the absorption coefficient of chlorobenzene which is taken as unity and are for electrons with thermal energy.

Steroids containing the 1,4-diene-3-one system also provide interesting comparisons; 1,4-androstadiene-3,17-dione absorbs to only one-fifth the extent of 4-androstene-3,17-dione. When the 1,4-diene-3-one system accompanies a keto group at the 11 position, a vast increase in absorption is found; 1,4-androstadiene-3,11,17-trione with a coefficient of 535 is the most strongly absorbing of the steroids tested. The ene- and diene-one systems in other locations of the steroid framework may show electron absorption, for example, 3,5-cholestadiene-7-one; perhaps less expected is the absorption coefficient of 5·8 found for *i*-cholestane-6-one, which suggests an interaction between the 6 keto group and the *cyclo*propane bridged-ring. In general, electron absorption appears to be a sensitive indicator of electronic interaction between, for example, the orbitals of the 4-ene-3-one system and those of other keto or unsaturated systems elsewhere in the steroid molecule.

It may be significant that the highest absorption coefficients are found with compounds which are either steroid hormones or at least compounds having a substantial part of the structure of a hormone. The steroid hormones with the highest affinity for free electrons would appear to be the adrenocortical hormones; the androgens and progestogens, as exemplified by testosterone and progesterone respectively, absorb electrons moderately but the œstrogens represented by œstrone have no appreciable affinity for thermal electrons. The adrenocortical hormones themselves are unstable at high temperatures and tend to lose the side-chain attached to the 17 position[6]; it was not possible, therefore, to measure their absorption directly except with the chemically protected derivative *bis* methylene dioxy cortisone. This compound, with one less keto group than cortisone, nevertheless had an absorption coefficient of 72. The absorption coefficient of the thermal decomposition product of cortisone, 4-androstene-3,11,17-trione and also that of 4-pregnene-3,11,20-trione are both 250; and probably more truly representative of the absorption of cortisone itself. If 1 : 4-androstadiene-3,11,17-trione, the thermal decomposition product of prednisone, is representative of prednisone, then this substance is among the most intensely absorbing of the steroids tested.

So far the measurement of the absorption of electrons by organic substances has indicated that a marked degree of absorption (a coefficient greater than 10) is rare. Those naturally occurring substances which do react readily with thermal electrons are usually, however, directly or indirectly associated with the biological process of oxidative metabolism. The measurements reported here with the steroids are no exception to this rule.

It was previously suggested[1] that the presence of so many of the few simple carbon, hydrogen and oxygen compounds with a high affinity for free electrons among the intermediates of the process of oxidative metabolism was more than coincidental; and that this process required for its function the presence of an ordered sequence of reversible electron traps. If this idea is accepted, then it is possible to speculate further that the control of metabolic activity by the thyroid and some of the steroid hormones is directly connected with their undoubted capacity to function as electron traps. The fact that the œstrogens do not absorb electrons is not necessarily contradictory to this notion since their activity could be regarded as antagonistic to that of other steroid hormones and the œstrogens could possibly function by the competitive displacement of the electron-trapping steroids from their site of action.

We thank Dr. E. C. Horning for his advice.

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SYNTHESIS OF DEOXYRIBONUCLEIC ACID ON X-CHROMOSOMES OF AN XXXXY MALE

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CHROMOSOME labelling with tritiated thymidine has been shown to be a useful tool for discriminating between similar but non-homologous chromosomes in man[1]. The only example of significant difference in isotope incorporation between homologous chromosomes is in the *X*-chromosome pair in females[1-3]. At the end of synthesis of DNA (late-*S*) flash-labelling renders one of the *X*-chromosomes heavily labelled indicating not only a late synthesis but also a faster rate of synthesis than any other chromosome in the complement. We have previously suggested that this 'hot' *X*-chromosome is identical with the *X*-chromosome forming the Barr body (female sex chromatin) in interphase[1].

We have labelled leucocyte cultures from the young *XXXXY* male with 49 chromosomes previously described by three of us[4-6]. Prevalence of Barr bodies in smears of buccal mucosa from this patient was:

No. Barr bodies per nucleus	0	1	2	3	4	unclassified	Total
No. of nuclei	6	18	82	148	14	32	300

Blood cultures and their tritiated thymidine labelling were performed in Stockholm, and slides afterwards taken to Manchester for further processing and analysis. The autoradiographs show clear evidence of three 'hot' chromosomes in group III (Fig. 1) in virtually all cells examined.

Table 1. NUMBER OF AUTORADIOGRAPHIC GRAINS

Over all chromosomes	Over the three 'hot' chromosomes ('hot' *X*'s)			Total grains of three 'hot' *X*-chromosomes
43	11	9	5	25
62	12	5		17
67	11	10	10	31
84	18	14	10	42
87	14	13	9	36
92	19	17	11	47
93	26	20	12	58
100	19	18	8	45
104	26	21	16	63
105	17	12	9	38
106	32	15	14	61
144	18	9	6	33
147	26	19	18	63
154	21	19	12	52
163	21	14	12	47
205	27	24	22	73
208	28	24	19	71
230	32	25	22	79
286	35	32	32	99
289	24	23	19	66
289	29	23	17	69
305	30	23	22	75
3,361	496	389	309	1,194

Mean label grains over all chromosomes : 153
Mean percentage of grains over the three 'hot' *X*'s = 35·5
Mean percentage of grains over one 'hot' *X* = 11·84.

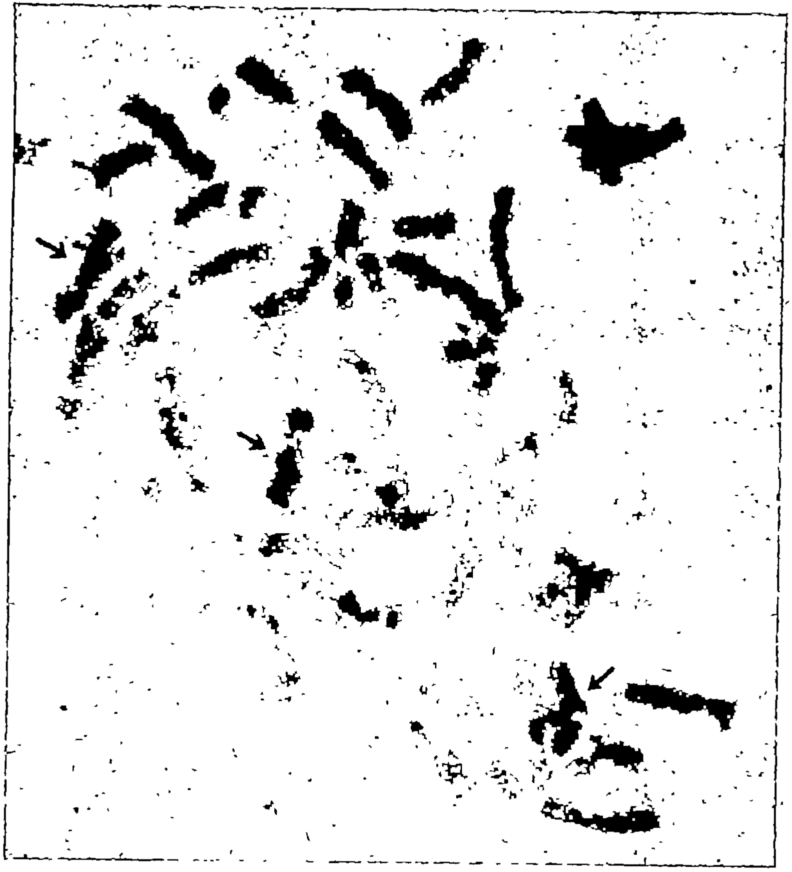

Fig. 1. Autoradiograph of end of *S* labelling (−3 h) of a typical cell from the *XXXXY* patient

The results of the grain counts for 22 cells of the *XXXXY* male are shown in Table 1. A detailed statistical analysis shows that the variation of the number of grains between the three 'hot' *X*-chromosomes in each cell is no more than that to be expected from the random nature of the formation of grains. We therefore conclude that all three 'hot' *X*-chromosomes in this cell synthesize in synchronism. The three 'hot' *X*-chromosomes account for 35·5 per cent of all the grains, or 11·8 per cent per 'hot' *X*. This value, however, cannot be directly compared with the 14·4 per cent on the 'hot' *X* in a normal female previously reported, as in that case there was only one 'hot' chromosome[1] while in this case there are three. If we assume that each 'hot' *X* and all the other chromosomes synthesize independently of each other then the ratio of the grains on one 'hot' *X* to the total grains over all other chromosomes excluding 'hot' *X*'s should be comparable. (The grains over the *Y* should also be excluded if complete identification of chromosomes is made.) This ratio for the *XXXXY* cells is 0·18 and for the *XX* cells 0·17.

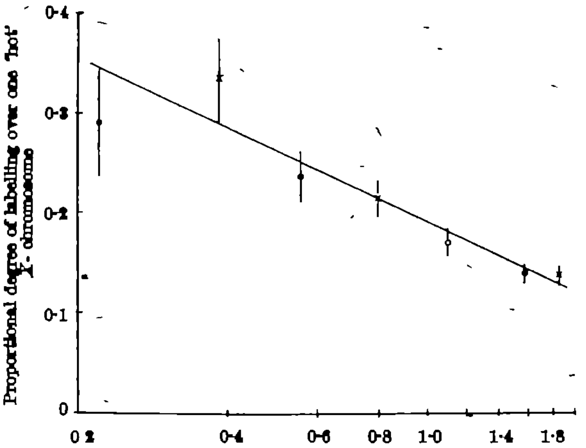

Fig. 2. Comparison of the 'hot' *X*-chromosomes in an *XX* and an *XXXXY* cell after flash labelling with tritiated thymidine at the end of *S*. Ordinate, the ratio of the autoradiographic grains over one 'hot' *X* to the total number of grains over all chromosomes except 'hot' *X*'s; abscissa, proportional to the number of grains over all chromosomes excepting the 'hot' *X*'s, the mean for each preparation being 1·0. The vertical lines indicate the estimated standard errors. (○, normal *XX* female; ×, *XXXXY* male (the three points correspond to the means of the three groups of cells in Table 1)

A further comparison can be made between the 'hot' *X* in these two cell types. It has previously been shown that the relative amount of labelling of the 'hot' *X* is dependent on the degree of labelling of the cell. This dependence for the *XXXXY* cells together with a replot of the previous data for the normal *XX* female cells is shown in Fig. 2. The abscissa is proportional to the grains over all chromosomes, excepting the 'hot' *X*'s, of the cells. This is chosen rather than the total number of grains because this is the set of chromosomes which is common to both types of cells and would be expected to serve as a better yardstick for the comparison of timing of the end of the *S* period. The constant of proportionality is chosen for each preparation to bring its overall average to the common point 1·0. The choice of a log scale is arbitrary, but has an important advantage that the dependence of the absolute number of grains per cell in irrelevant circumstances (for example, exposure time of the autoradiographs, specific activity of label, etc.) will only result in a lateral shift of the curve. A minor advantage is that the results are an approximate fit to a straight line on this plot. It will be seen that there is a remarkably close fit between the results from the two cell types. The slight displacement between the two sets of points is not really significant and could be either due to the *XXXXY* cells being slightly nearer the end of the *S* period, or, if the lateness in *S* period is the same for the two cell lines, due to an increased rate of synthesis of the 'hot' *X* in the *XXXXY* cell.

The overall conclusion is that there is a remarkable similarity of the pattern of synthesis of the 'hot' *X* chromosome at the end of the *S* period in such different material as a normal *XX* female, containing one 'hot' *X* chromosome and an abnormal *XXXXY* male containing three.

It appears that the late labelling (late-*S*) of chromosomes—by showing 'hot' chromosomes—is more informative about the nature of extra chromosomes than the mere determination of the number of Barr bodies, however, these two assessments should be used in conjunction whenever possible.

It has been reported previously[1] that the 'hot' *X*-chromosome represents a relatively inactive chromosome. The observation in this case of three 'hot' *X*-chromosomes may provide an explanation why supernumerary *X*-chromosomes have relatively little effect compared with extra autosomes.

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PROPERTIES OF THE SEPARATED CHAINS OF HUMAN γ-GLOBULIN

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ANTIBODIES in man and many animals are associated with a complex group of proteins known collectively as γ-globulin. This fraction was originally defined as the component which moves most slowly during electrophoresis of serum at alkaline *p*H. Serum proteins with the electrophoretic mobility of γ-globulin have certain common physico-chemical and immunological characteristics but are heterogeneous according to several criteria. In all species investigated the main component of γ-globulin has a molecular weight of about 150,000 and is usually referred to as *7S* γ-globulin; the heterogeneity of this fraction is shown by its wide range of electrophoretic mobility and diffuse spread on ion exchange chromatography as well as by the multiplicity of antibodies which it contains. About 5–10 per cent of γ-globulin consists of protein having a molecular weight of about 1,000,000 and referred to as 19*S* γ-globulin; this fraction can be identified by immunoelectrophoresis, when it is usually referred to as β_2M- or γ_1M-globulin. Immunoelectrophoresis has also led to the identification of a third type of γ-globulin known as γ_1A- or β_2A-globulin, which is mainly of similar molecular size to *7S* γ-globulin, but like the 19*S* fraction has a relatively high carbohydrate content. In multiple myelomatosis and certain related conditions in man, abnormal γ-globulins distinguished by their relative homogeneity are found in the serum; these pathological proteins may belong to any of the three main γ-globulin types.

Methods which have recently been used in the study of antibody structure provide a means for investigating the relationships between the three types of γ-globulin and the differences between normal and pathological γ-globulins. These methods involve a preliminary splitting of the protein by enzymatic digestion[1] or reduction of disulphide bonds[2]. When human *7S* γ-globulin is reduced in the presence of 6 M urea approximately 15 disulphide bonds are split and the molecular weight falls from about 150,000 to 50,000; the products of reduction are separable by chromatography but are biologically inactive and insoluble in aqueous solutions[3]. Reduction of γ-globulin in neutral aqueous solution splits a maximum of five disulphide bonds and subsequent dialysis against acetic or propionic acid dissociates two fractions which have been called *A* and *B* (ref. 4); these can be separated on 'Sephadex *G75*' columns and appear to correspond to the chains referred to as *H* and *L* (ref. 5) which are obtained after more extensive reduction. Fleischman, Pain and Porter[4] have shown that in human, horse and rabbit γ-globulin *B* chains comprise about 25 per cent of the total protein and have molecular weights of about 20,000.

Fig. 1. Electrophoresis in 8 M urea-formic acid starch gel of human *7S* γ-globulin (*U*), reduced human γ-globulin (*R*) and the *A* and *B* chains separated in 1 N acetic acid on a 'Sephadex *G75*' column

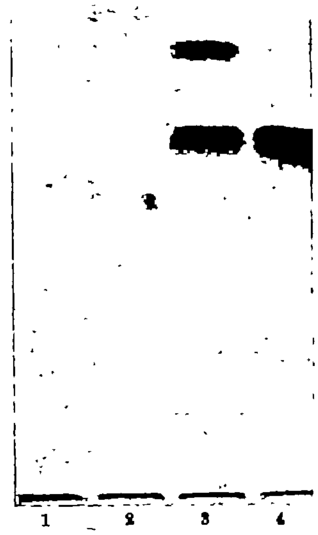

Fig. 2. Electrophoresis in 8 M urea-formic acid starch gel of reduced *7S* γ-globulin (4), *7S* myeloma globulin (3), pathological γ_1M-globulin (2) and γ_1A-myeloma globulin (1)

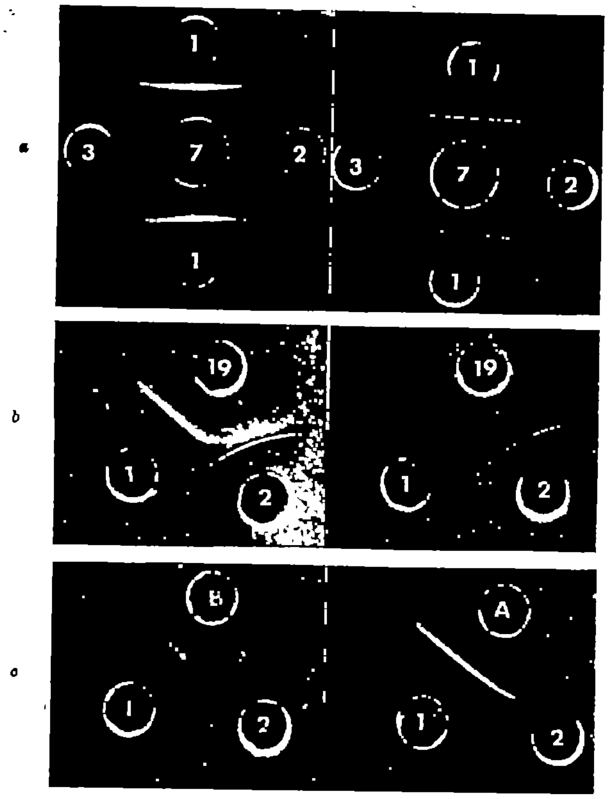

Fig. 3. *a*, Left, reaction of human $7S$ γ- (1), γ_1M- (2) and γ_1A-globulins (3) with rabbit anti-$7S$ serum (7), and right, with the same antiserum after absorption with $7S$ *B* chain. *b*, Left, reaction of normal human $7S$ γ- (1) and γ_1M-globulins (2) with rabbit anti-$19S$ serum (19), and right, with the same antiserum after absorption with $7S$ *B* chain. *c*, Left, reaction of normal human $7S$ γ- (1) and γ_1M-globulins (2) with rabbit anti-$7S$ *B* chain, and right, with anti-$7S$ *A* chain

The *A* chain which tends to precipitate at neutral *p*H comprises three-quarters of the original γ-globulin and has a maximum molecular weight of about 110,000. Some further properties of the *A* and *B* chains from various types of normal and pathological γ-globulins have been investigated. The results indicate that *B* chains carry those properties which are common to all γ-globulins while *A* chains are specific for each type.

Separation of A and B chains. Normal and pathological $7S$ γ-globulins were prepared by chromatography on diethylaminoethyl (DEAE) cellulose or by zone electrophoresis, and γ_1M-globulins by chromatography first on DEAE cellulose and then on 'Sephadex *G*200' columns. *A* and *B* chains were separated by the method of Fleischman *et al.*[4]. The yield of *B* from normal $7S$ and from pathological $7S$ and γ_1A-globulins was 24–29 per cent of the total protein.

Electrophoresis. The electrophoretic behaviour on a urea-formic acid starch gel of normal human $7S$ γ-globulin and the sub-fractions obtained after reduction is shown in Fig. 1. The *B* chains from all types of pathological γ-globulins differ from normal in their relative homogeneity[6], but all migrate within the range of normal $7S$ *B* on electrophoresis at acid *p*H (Fig. 2) or in alkaline buffers. The *A* chains of normal and pathological $7S$-globulins appear to be identical in urea-formic acid starch gels, but *A* chains from γ_1A- and γ_1M-globulins have slower electrophoretic mobilities (Fig. 2).

When *A* and *B* chains were prepared from γ_1 and γ_2 sub-fractions of $7S$-γ-globulin or from fast- and slow-moving pathological γ-globulins, it was found that the relative heterogeneity and mobility of the *A* chain at alkaline *p*H corresponded with that of the protein fraction from which it was derived. The mobility of the *B* chain, however, bore no relation to that of the molecule from which it was prepared.

Antigenic properties. *A* and *B* chains of $7S$ γ-globulin are antigenically distinct as judged by their reaction with rabbit anti-human $7S$ γ-globulin. Rabbit anti-$7S$ sera absorbed with $7S$ *B* chain do not react with γ_1A- and γ_1M-globulins (Fig. 3*a*). Similarly, anti-$19S$ sera absorbed with $7S$ *B* chain are specific for normal γ_1M-globulin (Fig. 3*b*). Antisera obtained by immunization of rabbits with human $7S$ *B* chain give a reaction of identity with normal $7S$ and γ_1M-globulins while antisera against the *A* chain react only with $7S$ γ-globulin (Fig. 3*c*). Similarly, *B* chains from pathological $7S$, γ_1A- and γ_1M-globulins give a reaction of complete or partial identity with normal $7S$ *B* when reacted with an antiserum to $7S$ *B* chain; antisera against $7S$ *A* chain, on the other hand, react only with *A* chains from abnormal $7S$ proteins and not with the *A*'s from pathological γ_1A- and γ_1M-globulins. These findings indicate that in both normal and pathological γ-globulins *B* chains carry antigenic determinants common to the three main types of γ-globulin while determinants specific for each are associated with their respective *A* chains.

Genotypic specificity. Recent investigations have established the presence within a single species of individual variants of γ-globulin which are antigenically distinct[7]. Variants of human γ-globulin have been distinguished from one another by differences in their ability to inhibit the agglutination of sensitized cells by selected sera containing substances serologically related to rheuma-

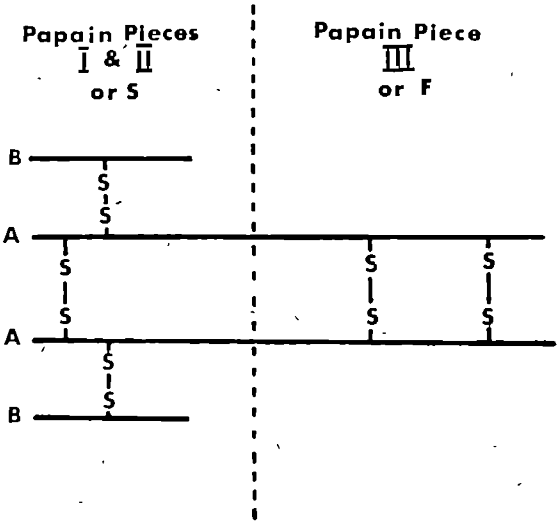

Fig. 4. Possible configuration of $7S$ γ-globulin The protein is shown made up of two *A* and two *B* chains. The dotted line indicates the probable site of papain cleavage which separates human γ-globulin into *S* and *F* fragments (ref. 12)

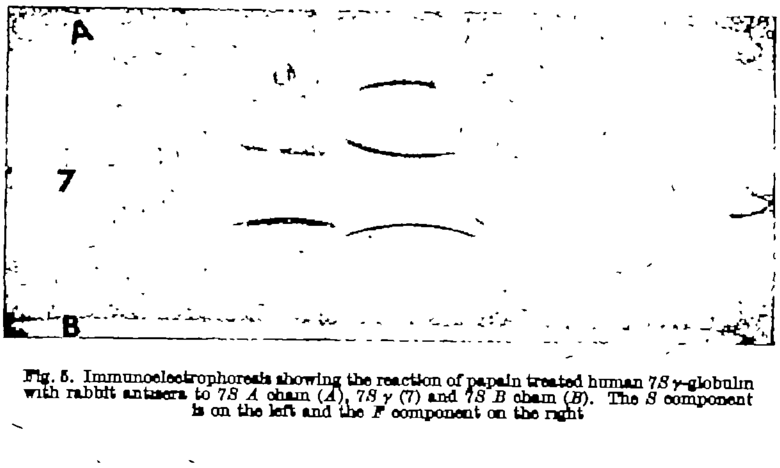

Fig. 5. Immunoelectrophoresis showing the reaction of papain treated human $7S$ γ-globulin with rabbit antisera to $7S$ *A* chain (*A*), $7S$ γ (7) and $7S$ *B* chain (*B*). The *S* component is on the left and the *F* component on the right

toid factors. Since the introduction of this technique[8] several genetically determined types of γ-globulin have been described. In Caucasians there are two independently segregating loci (Gm and Inv) which control the production of six well-defined γ-globulin variants. Gm activity is associated only with *7S* γ-globulin while the Inv factors are found on *7S*-, γ_1A and γ_1M-globulins[9,10].

A preparation of human *7S* γ-globulin from a Gm (a+b+) Inv (a+) individual was reduced and the *A* and *B* chains separated on 'Sephadex *G*200' in 1 N acetic acid; the *A* fraction prepared in this way is free of unreduced γ-globulin as judged by starch-gel electrophoresis. After separation of the chains Gm(a) and Gm(b) factors were detectable only on the *A* chain while Inv(a) activity was associated only with the *B* chain[11].

Relationship of γ-globulin chains to enzymatic pieces. Porter[1] has shown that papain activated by cysteine splits γ-globulin into three fragments separable by chromatography. One fragment (III in the rabbit, *F* in human) contains no antibody activity, while the other two (I and II in the rabbit, *S* fragments in human) each contain a single antibody combining site. Fleischman *et al.*[4] investigated the relationship between the papain pieces of rabbit γ-globulin and the chains separated by reduction. The *A* chain was found to react with antisera to piece I and piece III, while the *B* chain reacted only with anti-piece I. These findings indicated that pieces I and II contain the *B* chain and a portion of *A* chain, while fragment III contains only *A* chain, and this led Porter[12] to propose that *7S* γ-globulin has the general configuration shown in Fig. 4. The papain fragments of human γ-globulin have been studied by immunoelectrophoresis using rabbit antisera to *7S* *A* and *B* chains. The *S* fragment reacted only with anti-*B* and the *F* fragment only with anti-*A* (Fig. 5). Similarly, *S* purified by chromatography on diethylaminoethyl cellulose reacted with anti-*B*, but not with anti-*A* on gel diffusion. Since the *S* fragments comprise about two-thirds of the molecule and the *B* chains less than one-third, it appears that *S* must contain part of the *A* chain, but it has not been possible to demonstrate this with two different rabbit anti-*A* sera or with anti-*7S* sera absorbed with *B*; this suggests that the portion of the *A* chain included in the *S* fragment may be antigenically inactive in human γ-globulin.

Conclusion. Despite their diversity, all human γ-globulins have a basically similar structure and can be split by reduction into large *A* and smaller *B* chains. It is therefore logical to call the three main components of the system *7S*, γ_1A- and γ_1M-globulins and not to refer to the latter two as β-globulins.

The *B* chain of γ-globulin carries those antigenic and genotypic determinants which are common to *7S*, γ_1A- and γ_1M-globulins; *B* chain has been demonstrated in the papain fragment *S*, which is associated with the same common antigenic[13,14] and genotypic[9,10] factors (Table 1). These findings indicate that the three main types of γ-globulin contain *B* chains of similar structure.

Table 1. PROPERTIES OF THE SEPARATED CHAINS OF HUMAN *7S* γ-GLOBULIN

	A chain	*B* chain
Synonyms (ref. 5)	*H*	*L*
Total (per cent)	71–76	24–29
Max. mol. wt.	110,000	40,000
Elect. mobility (pH 8·8)	Related to original γ	Not related to original γ
Antigenic determinants		
Common to γ_1A and γ_1M	0	+
Specific for *7S*	+	0
Genotypic specificity		
Gm	+	0
Inv	0	+
Presence in papain pieces		
S	?	+
F	+	0
Myeloma proteins	Relatively homogeneous	Homogeneous

The *A* chains of *7S*, γ_1A- and γ_1M-globulins differ in electrophoretic behaviour and carry the antigenic determinants which are specific for each type of γ-globulin. Gml specificity, which is associated only with *7S* γ-globulin, is present on the *7S* *A* chain. The papain fragment, *F*, which is associated with the specific antigenic determinants of *7S* γ-globulin[13,14] and with Gm specificity[9,10], consists only of *A* chain. It appears therefore that the three main types of γ-globulin have different *A* chains; all the carbohydrate of the molecule is present in the *A* chains[4] and this may contribute to the differences which have been observed.

I thank Prof. R. R. Porter for his advice and Prof. N. H. Martin of St. George's Hospital and colleagues at St. Mary's Hospital for samples of pathological sera. The facilities for this work were provided by grants from the U.S. National Science Foundation, the U.S. Public Health Service, the Wellcome Trust and the Medical Research Council, London.

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NEUROCHEMICAL DIFFERENCES BETWEEN TWO STRAINS OF MICE

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PREVIOUS investigators[1-5] interested in genetic factors in behaviour have rather consistently found differences in behavioural measures between two strains of mice, the *BALB/cJ* and *C57BL/10J*. Briefly, these differences are as follows. The *BALB/cJ* mice are more fearful, as measured by the Hall open field test[6], show less exploratory activity, and are generally less active than the *C57BL/10J* strain. Caspari[7] noted a difference in total brain serotonin between these two strains (serotonin and 5-HT will hereafter be used interchangeably). My later work[8] indicated that these two strains were significantly differentiated by the 5-HT content of a dissected portion of brain consisting of pons, mesencephalon, and diencephalon, whereas the norepinephrine content of this dissected portion was the same for both strains. Specifically, the *BALB/cJ* mice had 1·34 μg of serotonin/g of dissected brain tissue and the *C57BL/10J* 1·07 μg/g ($P = < 0·01$) while the values for norepinephrine were 0·73 μg/g and 0·70 μg/g respectively ($P = > 0·25$). This article presents further work as to the nature of this strain difference in serotonin-levels.

All mice used in these experiments were from the Jackson Memorial Laboratories in Bar Harbor, Maine, all were males, and were 8–11 weeks of age at the time of death. All experiments were designed so that a given mouse from one strain was always paired with a mouse from the opposite strain which had been reared and housed under similar conditions and was of course of the same age. The method used for 5-HT determinations was basically that of Bogdanski[9] with the modifications suggested by Kuntzman[10] being used for microanalytical procedures. The mice were decapitated without premedication. The dissected portion of brain referred to frequently in this article was prepared as follows. The medulla, cerebellum, cerebral lobes, all tissue anterior to the optic chiasm and lateral to the optic tracts were dissected away and the portion left thus consisted of pons, mesencephalon, and diencephalon.

Although there was no reason to suspect that the mice from the Jackson Laboratories were behaviourally different from those previously reported, the two strains were tested, with the sample consisting of fifteen mice from each group, as to 'fear' response using a modified Hall open field apparatus[8]. It was found that following one two-minute trial in the field 95 per cent of the *BALB/cJ*'s and 25 per cent of the *C57BL/10J*'s had defæcated whereas the respective incidences of urination was 62 per cent and 0 per cent. Another group was tested by measuring the number of 5 in. × 5 in. squares crossed by the mouse while in the 'open field'. Here twenty mice from each strain were used. The mean number of 5 in. × 5 in. squares crossed was 32·4 for the *BALB/cJ*'s and 80·8 for the *C57BL/10J*'s. This difference is statistically significant ($P = < 0·01$). These results agree favourably with those of other investigators and suggest that the mice presently being obtained from the Jackson Laboratories have retained the earlier noted behavioural differences.

Table 1

	Strain *BALB/cJ*	Significance of difference	Strain *C57BL/10J*
Serotonin in total brain ± S.E.	0·807 ± 0·013 μg/g (*N* = 9 determinations on a total of 50 brains)	N.S.	0·783 ± 0·018 μg/g (*N* = 6 determinations on a total of 20 brains)
Mean weight of total brain ± *S.E.*	474·63 ± 0·0067 mg (*N* = 50 brains)	*P* = < 0·05	452·42 ± 0·0066 mg (*N* = 20 brains)
Serotonin in dissected portion of brain ± *S.E.* (ref. 8)	1·34 ± 0·046 μg/g (*N* = 19 brains and 19 determinations)	*P* = < 0·01	1·07 ± 0·037 μg/g (*N* = 19 brains and 19 determinations)
Mean weight of dissected portion of brain ± *S.E.* (ref. 8)	146·4 ± 1·56 mg (*N* = 45 dissections)	*P* = < 0·01	140·7 ± 1·60 m (*N* = 45 dissections)
Total* serotonin in portion of brain remaining after dissection ± *S.E.*	0·1578 ± 0·0033 μg (*N* = 4 determinations on 8 brains)	N.S.	0·1535 ± 0·0061 μg (*N* = 4 determinations on 8 brains)

* Serotonin values are expressed as total amount present rather than as μg/g because those structures rich in serotonin in this portion of brain constitute a relatively small fraction of the total weight.

Serotonin values (see Table 1) for total, that is, non-dissected, brain were found not to differ significantly for the two strains. This is in contradiction to the earlier noted finding of significant differences in 5-HT levels using a dissected portion of brain[8]. This disparity immediately suggested that the earlier observed difference is not a function of a general increase in serotonin in all those structures known to be rich in this amine but rather that the greater serotonin noted in the *BALB/cJ* strain is to be found specifically in that portion of the brain which had been dissected out. Experimental evidence supporting this was found when determinations of the serotonin present in that portion of the brain remaining following the formerly described dissection indicated no strain difference (Table 1). From this it is concluded that the earlier noted difference in serotonin content for the two strains is not generalized but rather is found only in that portion of brain consisting of pons, mesencephalon, and diencephalon. Further investigations may be of use in more accurate localization as to specific structures which may account for the difference found.

In order further to understand the biochemistry of the noted difference in the two strains a potent monoamine oxidase inhibitor, 'Parnate', was injected intraperitoneally at 5 mg/kg. 1 h and 5 h after injections, the animals were killed and serotonin determined on the earlier described dissected portion of brain. For the 1-h experiment five animals from each strain and for the 5-h experiment ten animals from each strain were used. Results obtained are presented in Fig. 1. Although the differences between the two strains are still significantly different at the 1-h and 5-h point, it is noted that the characteristic 125–130 per cent greater serotonin-levels found in the *BALB/cJ* animals has decreased to 115–116 per cent at the 1-h and 5-h points. These results suggest that the observed differences between the two strains cannot be explained solely on the basis of a differential in the rate of transfer of 5-hydroxytryptophan to brain nor to some impairment of decarboxylation of this precursor of serotonin. (The presence of equal amounts of norepinephrine found in earlier work[8] also suggests that the serotonin difference is not due to different activities of 5-hydroxytryptophan decarboxylase as this enzyme seems to be the same as Dopa decarboxylase[10].)

These findings are consistent with the possibility that the *C57BL/10J* strain has more or more active monoamine oxidase and that if 'Parnate' completely inhibits all the monoamine oxidase, there is a slightly more rapid increase of serotonin. However, the fact that a significant difference persists throughout the entire 5-h period suggests that a difference in monoamine oxidase levels or activity could be only a partial explanation. Because of the foregoing, it is concluded that it is possible but unlikely that the observed differences can be explained on the basis of differences in production or catabolism of serotonin.

Although the prime purpose of the injection of 'Parnate' was the examination of changes in serotonin-levels, an observed difference in behaviour between the two strains was noted. Following the drug administration the *BALB/cJ* mice were periodically hyperactive; they assumed an 'alert' stance with the head up when not moving about; and they were most sensitive to any stimuli during periods of non-movement. This behaviour reached its maximum 30–45 min after injection and continued for the 2 h during which they were observed. In contrast the *C57BL/10J* mice while alert remained huddled in the corner of the cage with their heads down and showed only moderate sensitivity to stimuli. At the end of 2 h the differences were still noted but the behaviour of the *C57BL/10J* mice was more like that of the *BALB/cJ* mice, that is, with time the behaviour of the two strains became nearer to being equal. These effects were noted both for animals housed in groups of five following the injections and to a slightly lesser degree when housed singly.

It is well known that serotonin exists in the brain in at least two forms, a bound and a free, and that reserpine

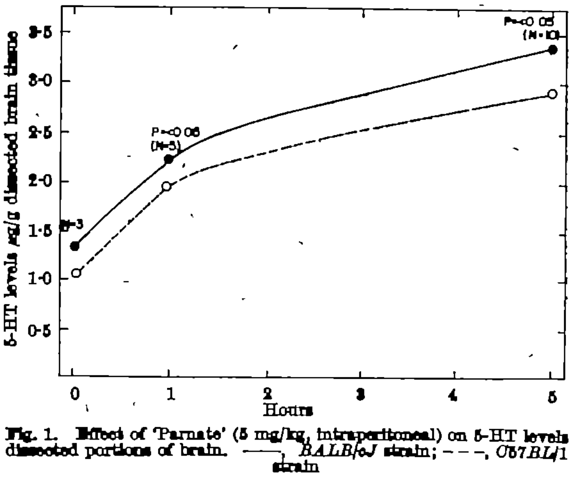

Fig. 1. Effect of 'Parnate' (5 mg/kg, intraperitoneal) on 5-HT levels in dissected portions of brain. ——, *BALB/cJ* strain; – – –, *C57BL/10J* strain

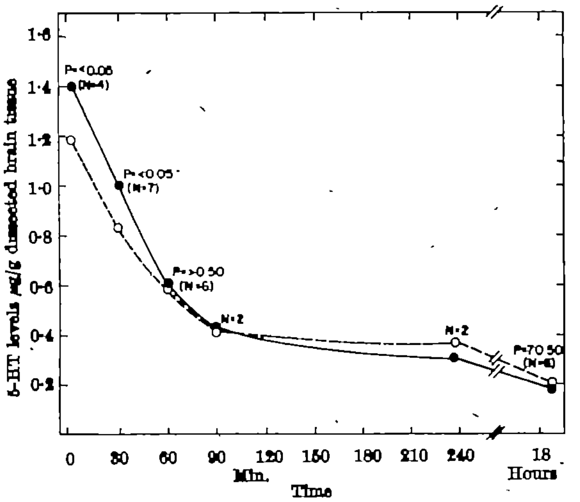

Fig. 2. Rate of serotonin depletion in dissected portion of brain following reserpine injection (2·5 mg/k body-wt. intraperitoneal) ——, *BALB/cJ* strain; – – –, *C57BL/10J* strain

acts on the binding or transport mechanism in some way so as to effect a release of 5-HT and norepinephrine. Furthermore, Potter *et al.*[11] as well as Weil-Malherbe *et al.*[12] have suggested that for norepinephrine the bound form is divided into two components, an easily releasable, and a more tightly bound. It was felt that it would be of some interest to compare 5-HT depletion curves for the two strains following an intraperitoneal injection of 2·5 mg reserpine/kg body-wt. These curves are presented in Fig. 2. Note that by 60 min the two strains have similar values for 5-HT, suggesting a more rapid release in the *BALB/cJ* strain. Parenthetically, these curves offer further evidence that the lower 5-HT levels in the *C57BL/10J* strain are not due to a relative deficit of monoamine oxidase in the *BALB/cJ* strain, for if this were the case the rate of depletion of 5-HT in the *C57BL/10J* would be expected to be greater than for the *BALB/cJ*. These curves do suggest, however, that there may be a difference in the binding mechanisms for the two strains and that this may in some way be responsible for the differential in 5-HT levels found between the two groups. The paucity of basic knowledge about binding and release mechanisms, and cellular partitions, is such that opposite hypotheses could be erected from the data presented. For example, if one assumes three cellular locations for 5-HT as does Brodie[13], that is, a granular, a lipid membrane bound area, and the portion of the cell having free monoamine oxidase, then one could explain the higher 5-HT in the *BALB/cJ* strain by postulating that there is *in vivo* a more rapid release of 5-HT from the granular fraction in the *BALB/cJ* strain with a consequent increase in 5-HT in the portion of the cell having the lipid membrane. Thus, measuring 5-HT in a homogenate would indicate more 5-HT in the *BALB/cJ* even though the granular fraction was less.

On the other hand, an alternative hypothesis is that the 5-HT *in vivo* is released less rapidly from the granular fraction in the *BALB/cJ* strain and thus the observed differential. This hypothesis would hold whether one uses Brodie's concept or the simpler one of a granular and non-granular fraction. This concept might also explain the closer approximation of 5-HT levels after 'Parnate' administration.

It is felt that the use of these inbred strains of mice for further clarification of the found neurochemical differences and their possible relationship to well-established behavioural differences is particularly appealing because the differences are naturalistic, that is, they occur without introducing non-physiological conditions which heretofore have been necessary in order to produce chemical and behavioural changes.

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SUCCESSFUL DEVELOPMENT OF CHICK EMBRYOS NOURISHED BY YOLK-SAC PERFUSION WITH CALCIUM-LOW MEDIA

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THE abundant supply of yolk and white in the hen's egg hampers direct study of chick-embryo nutrition. In order to remove yolk nutrients and maintain essentially normal relationships between the embryo, its membranes, and the shell, techniques were devised first to replace the yolk of three-day embryos by the injection of salts solution[1], and later to perfuse the yolk sac with various media[2]. By use of an electrosurgical unit, a canal of coagulated white was formed, thus connecting the yolk sac of three-day embryos with the exterior of the shell. Next, metal connecting tubes were attached, and a salts solution was slowly injected upward into the yolk through a hypodermic needle, so that most of the yolk, being heavier, was displaced by the lighter salts solution. An overflow tube was then attached, and fluids were perfused through the yolk sac.

Since the publication of these preliminary studies efforts have been made to obtain normal development, but without success until recently. A few embryos survived more than 100 h when perfused with 15 ml. of fluid every 8 h, starting at 4 days of incubation, but even these were small and had poor extra-embryonic circulation and little allantoic fluid. Despite failure to obtain normal development, it has been possible to study survival time in relation to salt balance, solute concentration, glucose requirement, and casein or egg-white as components of the medium. When the medium lacked either casein or white, the median death-time was about 30 h; when either one was present, median death-time was sometimes more than 100 h. The effects of white were less remarkable, perhaps, than the observation that the presence of yolk in the perfusion medium was not beneficial; on the

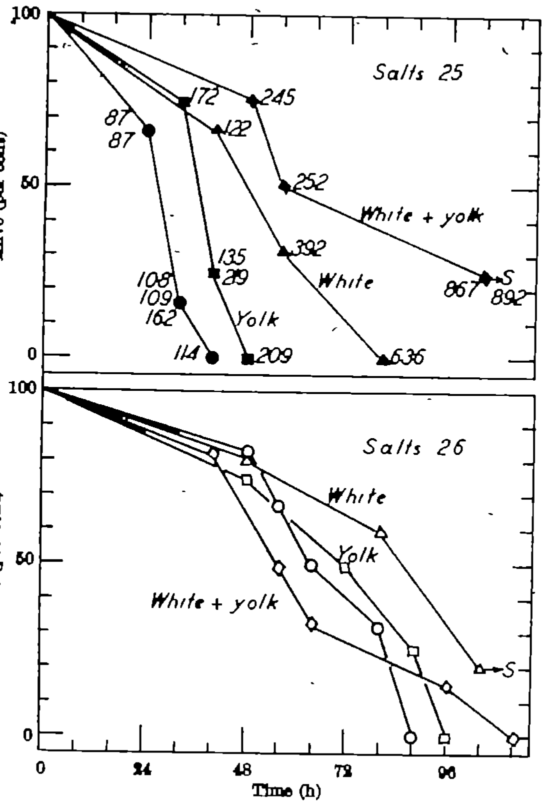

Fig. 1. Survival of embryos perfused with 30 ml. of medium every 8 h, starting after 4 days of incubation. Unmarked curves are for groups perfused with salts 25 or 26 only, compared with yolk (10 ml./l.), white (20 ml./l.), or both. Each curve represents from 3–6 embryos. Embryos marked *S* were killed at 112 h. Upper curves, salts 25. Numbers are the wet weights of embryos that had died. Lower curves, salts 26. The embryo weights are given in Fig. 2

contrary, embryos receiving a medium containing 12 ml. of yolk per litre died earlier than those receiving salts alone. In contrast to the results obtained during perfusion were the findings that embryos left undisturbed after most, but not all, of the yolk had been displaced by the salts solutions survived well and grew essentially normally for several days. After thorough removal of yolk at 4 days, embryo size at 8 or 9 days was found to be influenced by the amount of yolk returned to the yolk sac[3].

The removal and replacement system was then utilized in studies on glucose, magnesium and calcium in relation to survival and development, and to concentrations of these nutrients and of sodium and potassium, in various fluids. Survival was improved by reduction of the calcium chloride level of the salts solution from 200 mg/l., a concentration commonly used in cell and organ culture media, to 20 mg/l. In these investigations, 2 ml. of yolk (containing 0·15 m.equiv. calcium) were injected into the yolk sac; thus, the total concentrations in the 25 ml. volume of yolk sac were increased by the additions of yolk to 9·7 m.equiv./l. from the original level of 3·7. When the calcium-level of the salts solution was 0·37 m.equiv./l., the addition of 2 ml. of yolk raised the calcium-level to 6·4 m.equiv./l. From these and other experiments yet to be published, it appears that the major problem in previous perfusion studies may have been that excess calcium limited survival.

The working hypothesis of the present investigation was as follows: Perfusion removes components of the white which have entered the yolk sac. These soluble materials have, in addition to their value as nutrients, a greater ability to bind calcium than have yolk components. When the embryo is subjected to a level of available calcium in excess of its tolerance, it succumbs. The effective level of calcium in the perfusion medium is related to calcium-levels of yolk, white and the salt solution, and to the calcium-binding capacity of components of yolk and white.

This hypothesis was tested by two experiments in which the effects of yolk and white supplements were studied in relation to the calcium-level of the medium, using embryo survival and weight as criteria. The embryos were prepared for the experiments in essentially the same manner as before[3]. The salts solutions contained the following: sodium chloride, 4·46 g; potassium chloride, 1·70 g; magnesium chloride $(6H_2O)$, 0·34 g; magnesium sulphate $(7H_2O)$, 0·20 g; sodium dihydrogen phosphate (H_2O), 0·28 g; glucose, 5·00 g; sodium bicarbonate, 1·1 g; erythromycin, 0·095 g (as glucoheptonate); dihydrostreptomycin, 0·66 g (as sulphate); and deionized water to make 1 l. of solution. In addition, calcium chloride was present at 0·2 g/l. (salts 25), or 0·02 g/l. (salts 26).

After a total incubation time of 4 days, the canal of each egg was cleared of yolk by a few ml. of fluid, an overflow tube was attached, and the yolk sac was connected through a hypodermic needle to a reservoir from which 30 ml. of fluid were dispensed every 8 h. It required approximately 10 min for each of two 15-ml. volumes to flow through the yolk sac. Death times were noted, and the dead embryos were preserved in 10 per cent formalin for study and for determinations of wet weights.

The salts solutions contained more potassium and magnesium, and less sodium, than solutions ordinarily used for cell and organ culture[4]. Salts 25 contained 3·7 m.equiv. calcium/l.; salts 26, 0·37 m.equiv./l. 10 ml. of fresh yolk contain 0·75 m.equiv. of calcium; 20 ml. of liquid white contain 0·075 m.equiv. (ref. 5).

The results obtained from one experiment are presented in Figs. 1 and 2; the results of the other experiment are consistent with these, but are omitted for brevity. When the basal medium contained the higher level of calcium (Fig. 1, upper curves), death occurred quickly in both the absence and the presence of yolk, but the addition either of white or of both yolk and white prolonged survival. Comparison of the upper and lower curves of Fig. 1 shows that reduction of the calcium-level from 3·7 to 0·37 m.equiv./l. improved survival in the absence of the supplements (unmarked curves) or in the presence of

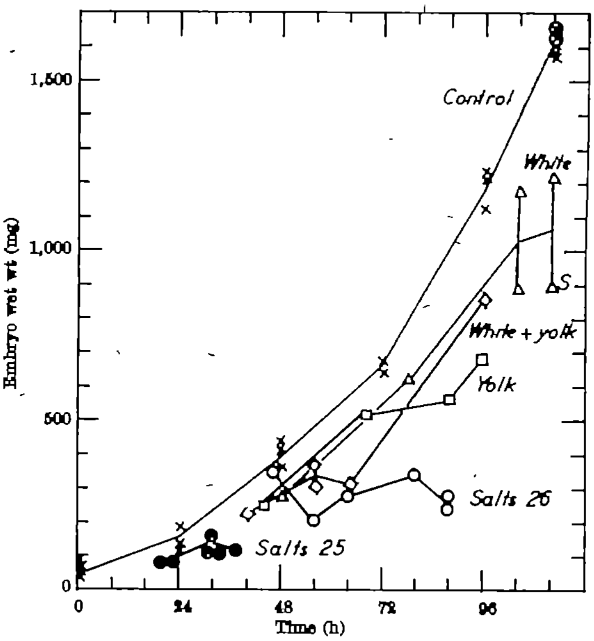

Fig. 2. Wet weights of embryos in relation to time of death. All embryos except controls were perfused with 30 ml. of medium every 8 h. Embryos marked by solid circles were perfused with salts 25 alone; all others received salts 26, plus supplements as shown. Points marked by x in circles were from embryos which had been subjected to the preparative steps, but had not had their yolk displaced

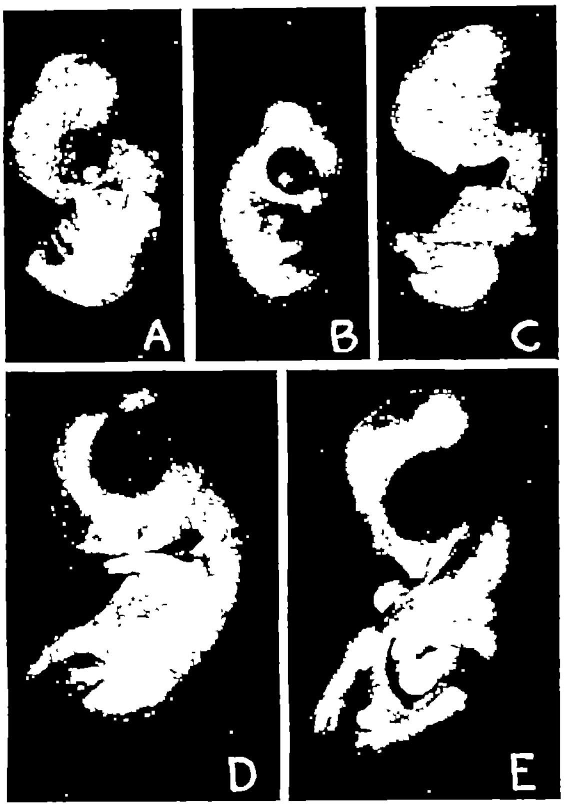

Fig. 3. Effects of media on embryo growth and developmental stage (ref. 6). Embryos *A*, *B*, and *C* died between 78 and 88 h: *A*, salts 26 plus yolk, 560 mg, stage 30; *B*, salts 26 only (no white or yolk), 270 mg, stage 27, *C*, salts 26 plus white, 600 mg, stage 30. Embryos *D* and *E* were examined at 104 h: *D*, salts 26 plus white, 1,190 mg, stage 33; *E*, control embryo, killed, 1,330 mg, stage 34. Embryos *B* and *C* were normal, *A*, *D*, and *E* had beak abnormalities. *D* was slightly edematous

yolk. When white was present, the effect was less marked, and when both yolk and white were present, there was no effect.

The relation of embryo weight to time of death is shown in Fig. 2, from which it is clear that the unsupplemented salts 26 solution permitted only poor growth. This result is in contrast to those with supplemented media: when yolk and white were added to the medium, embryos which died after 80 h weighed twice as much as those perfused with salts alone.

The small embryos were retarded in development, but showed no gross abnormalities. Two embryos perfused with salts 26 supplemented with white were found at autopsy to be edematous, which accounts for their greater weight. In contrast to previous perfusion experiments, the extra-embryonic circulation and allantoic sac volumes were essentially normal in those embryos perfused with salts 26. Some of the embryos which died after 80 h exhibited parrot-beak abnormalities or shortened mandibles, but there was no evidence that any of the treatments was specifically responsible for the abnormalities. When the egg-tooth was present, it was calcified normally.

The control embryos were obtained from eggs of the same group, prepared in the same way as the experimental ones, except that yolk removal was incomplete because of rupture of the canal lining during the first displacement process. Previous trials had shown that such embryos grow normally during this period of incubation. Photographs illustrating some of the size differences in relation to medium content and perfusion time are presented in Fig. 3.

From the results of previous experiments using the replacement system, in which the yolk sac was thoroughly flushed out at 4 days, it was expected that embryos perfused with solutions containing white but no yolk would be smaller than those receiving both white and yolk. In the experiment described here, however, such a thorough washing was not performed, because of the expected effectiveness of subsequent periodic perfusions. The poor growth obtained with salts 26 alone indicated that residual yolk had been effectively removed. When white was present in the medium, however, growth was almost normal. Two possible explanations for this observation are: (1) when white was present in the medium, removal of vestiges of yolk clinging to the entodermal lining of the yolk sac may have been impaired; or (2) when white was present, yolk materials in entodermal cells may have been more effectively utilized than when white was absent.

Questions concerning the relative effectiveness of white and yolk as supplements, the concentrations required, and their possible replacement by other protein sources or by amino-acids in defined media await further investigation. The results reported here show that it is possible to obtain almost normal growth and development through the eighth day of incubation by perfusing through the yolk sac of 4-day chick embryos a salts and glucose solution, supplemented with white and yolk. Compared with the media commonly used for cell and organ culture[4] this medium is characterized by a low sodium-to-potassium ratio and by a very low level of calcium. It is concluded that the proposed hypothesis, namely, that components of the white enter the yolk sac and protect the embryo by combining with calcium from the yolk or salts solution, is still tenable.

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SERUM TRANSFERRINS IN CHIMPANZEES

Serum Transferrins in the Orange Park Chimpanzee Colony classified by the Boyer and Young Scheme

BOYER and Young[1] observed seven phenotypes of transferrin on analysing the sera of 25 chimpanzees for iron-binding components by vertical starch-gel electrophoresis[2] and autoradiography[3]. Each serum (pretreated with iron-59) contained after the starch-gel separation either one or two heavily labelled zones of iron-binding protein and 1–3 additional bands of more weakly labelled protein (Fig. 2). In naming these phenotypes it was assumed that if an animal was homozygotic for transferrin only one heavily labelled band would be present; but, if heterozygotic, two such bands would be present. Thus the seven observed phenotypes were designated: AA, AC, AD, BC, CC, CD and DD, the A transferrin having the highest electrophoretic mobility and the D transferrin having the lowest electrophoretic mobility. Buettner-Janusch[4] also used vertical starch-gel electrophoresis and autoradiography to identify transferrin differences in chimpanzees. He analysed 41 sera, 20 obtained originally from animals in the chimpanzee colony of the Yerkes Laboratories of Primate Biology at Orange Park, Florida. In agreement with the findings of Boyer and Young[1], Buettner-Janusch[4] reported that there were multiple bands of transferrin and a pronounced polymorphism of this protein in chimpanzees. However, he did not consider it wise to attempt to distinguish by the system of nomenclature heavily labelled bands from more weakly labelled bands. Nor did his phenotypes correspond in the relative positioning of components to the Boyer and Young phenotypes. In contrast to Buettner-Janusch, Parker and Bearn[5], who analysed 67 sera from animals in the same Orange Park chimpanzee colony, were able to classify the transferrin phenotypes of these sera according to the Boyer and Young scheme. Their paper described the presence of the AC, BC, CC, and CD phenotypes in the Orange Park colony.

Here we present the results of our own analyses performed on 122 sera drawn at various times from 77 of the Orange Park chimpanzees. We thank Dr. Arthur J. Riopelle (director of the Yerkes Laboratories of Primate Biology), who provided us with the sera from these animals. Almost all samples were analysed by two-dimensional starch-gel electrophoresis[6] in the *tris* discontinuous buffer system[7], and the principal transferrin components in several representative sera were identified by immunological examination of the separated β-proteins with unispecific chicken antiserum to human transferrin. The method of immunological examination was that previously utilized to identify transferrin components in the two-dimensional starch-gel electrophoresis patterns of rhesus monkey sera[8]. In addition, the iron binding patterns of 70 sera from 60 of the chimpanzees were determined on samples pretreated with iron-59 by comparative one-dimensional starch-gel electrophoresis and autoradiography.

It was observed in both sets of analyses that the transferrin fraction of chimpanzee serum is comprised predominantly of either one or two electrophoretically distinct molecular species. Furthermore, a consistent pattern of minor transferrin components was demonstrated, the variations of which in the chimpanzee sera were related to the variations of the major transferrin components. In accordance with the scheme of Boyer and Young these major components could be labelled in order of decreasing electrophoretic mobility: A, B, C and D. By two-dimensional starch-gel electrophoresis in the discontinuous buffer system, C and D could usually be distinguished from each other and could always be distinguished from A and B, but A and B could not be readily distinguished from each other. Indeed, even by the method of vertical starch-gel electrophoresis in borate buffer, with its much longer period of separation, repeat analyses were necessary clearly to resolve A from B. (The 70 sera pretreated with iron-59 were first analysed by the more rapid horizontal starch-gel electrophoresis in the *tris* discontinuous buffer system. Then the samples having either A or B transferrin were analysed by vertical starch-gel electrophoresis in borate buffer.) The significance for our study of the method of two-dimensional starch-gel immunoelectrophoresis was that it permitted us to visualize clearly the major transferrin components of chimpanzee serum, since by the iron-binding method (as pointed out in the report of Buettner-Janusch[4]) "the possibility occurs that the degree to which the transferrins are saturated *in vivo* before the blood samples were taken plays a part in the extent to which each band will take up iron-59 *in vitro*". Indeed, cord blood obtained from a new-born chimpanzee (Ball No. 195) failed to show any transferrin by the iron-binding method, but was readily typed as CD by two-dimensional starch-gel immunoelectrophoresis.

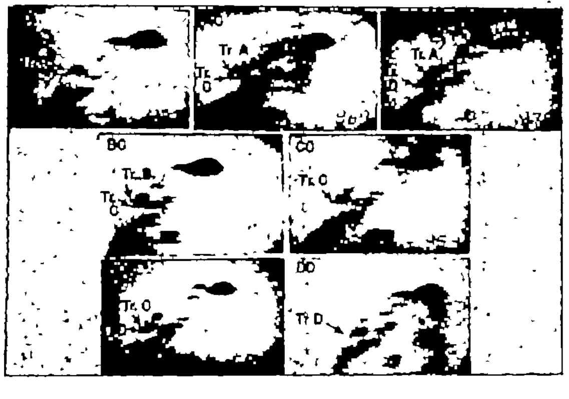

Fig. 1. Two-dimensional starch-gel electrophoretic patterns of chimpanzee sera showing seven phenotypes of transferrin. The distinction between the *A* and *B* transferrins, not evident in the patterns, was established by vertical starch-gel electrophoresis in borate buffer of samples pretreated with iron-59 and by autoradiography

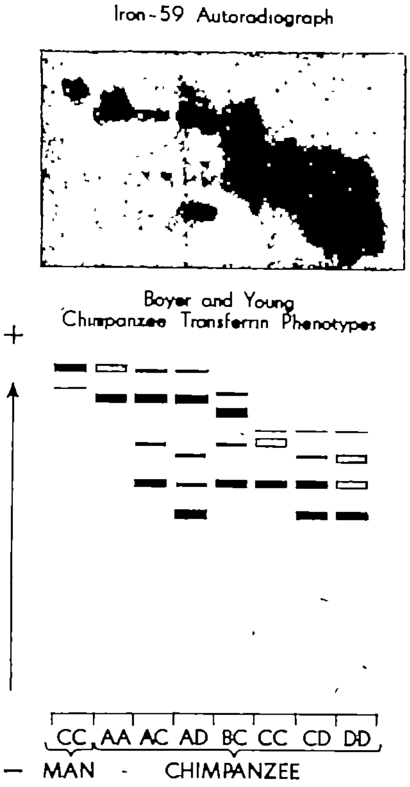

Fig. 2. The photograph presents the results of a vertical starch-gel electrophoresis experiment which compares the transferrins of Orange Park chimpanzees, representing seven phenotypes, with the transferrin of normal human serum (far left). The transferrin bands are indicated by white dots. The diagram below is a representation of the seven chimpanzee transferrin phenotypes discovered by Boyer and Young (ref. 1). The similarity in the relative positioning of major and minor transferrin components can be noted on comparing the photograph with the diagram for each of the seven phenotypes

The results of our analyses are given in Figs. 1 and 2 and Table 1. As can be noted, seven distinct phenotypes of serum transferrin were found in the Orange Park chimpanzee colony. Furthermore, as demonstrated in Fig. 2, these seven phenotypes showed the same relative positioning of minor transferrin bands to major bands as the phenotypes described by Boyer and Young[1]. Thus even though chimpanzee sera were not exchanged with these investigators, we were convinced that our seven phenotypes were the same as theirs. Accordingly, the same system of nomenclature can be used for describing the transferrin phenotypes of the Orange Park chimpanzees.

In recording the distribution of these phenotypes (Table 1) we divided the animals into those which were born in the jungle (birth-date unknown) and those which

Table 1. DISTRIBUTION OF TRANSFERRIN PHENOTYPES IN CHIMPANZEES

Phenotypes	Orange Park Colony Inbred	Orange Park Colony Jungle born	Boyer and Young group
AA	0	1	1
AC	3	2	3
AD	1	1	5
BC	3	2	4
CC	35	11	9
CD	16	0	1
DD	0	2	2
Total	58	19	25

were born in the colony, the latter being an inbred group of chimpanzees. CC was the most common of the phenotypes in both these groups and also in the chimpanzees analysed by Boyer and Young. However, phenotype CD, which appears to be rather rare in jungle born chimpanzees (putting our data and that of Boyer and Young together), was the next most common phenotype in the chimpanzees born at Orange Park. An original breeder female in the colony (Wendy No. 4) has been typed as CD, which accounts in part for the rather frequent occurrence of the CD phenotype in the inbred group. It should be emphasized that as larger numbers of chimpanzees are surveyed new phenotypes and new molecular forms of transferrin in addition to the seven phenotypes observed by Boyer and Young[1] and ourselves might well be discovered. Nevertheless, the use of a nomenclature which in effect describes the transferrin phenotypes of chimpanzees in terms of an allelic series of genes is not inappropriate as the following communication demonstrates.

We thank Dr. Melvin R. Sikov of the Department of Radiology, Wayne State University College of Medicine, for his advice on the radioactive iron-binding analyses and for generously permitting us to use his facilities.

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Inheritance of Serum Transferrins in Chimpanzees

By reviewing the familial relationships of chimpanzees born in the Orange Park Colony, the transferrin phenotypes of which were classified by the Boyer and Young scheme (preceding communication), it was possible to evaluate the genetic basis for the inheritance of these transferrins. The results are recorded in Table 1. To summarize the findings: of the twelve offspring of CC × CD matings, six were CC and six were CD. There were four offspring of AC × CC matings; three were CC and one was AC. The two offspring of BC × CC were BC and CC. The one offspring of CD × CD was CD, and the offspring of the CC × CC mating was CC. Thus without exception each offspring always had one major transferrin identical in type to one held by the mother and one major transferrin identical in type to one held by the father. Furthermore, there were fourteen offspring, each one having a transferrin phenotype which could be compared with the transferrin phenotype of only one of the parents; again, one major transferrin of the offspring was always identical in type to a major transferrin of the parent. Thus, as in the case of human transferrins[8] and rhesus monkey transferrins, these data support the hypothesis that a single autosomal multi-allelic series of genes without dominance controls the transferrin polymorphism of chimpanzees.

Table 1. TRANSFERRIN PHENOTYPES OF PARENTS AND OFFSPRING IN THE ORANGE PARK CHIMPANZEE COLONY

Name and No. of animal Mother (No.)	Father (No.)	Offspring (No.)	Transferrin phenotype M	F	O
Wendy 4	Bokar 5	Web 79	CD	CC	CD
Alpha 28	Hal 43	Halpha 176	CC	CD	CD
Bula 48	Hal 43	Baxter 117	CC	CD	CC
Bula 48	Hal 43	Pix 157	CC	CD	CD
Bula 48	Hal 43	Hew 171	CC	CD	CD
Bula 48	Hal 43	Ball 195	CC	CD	CD
Bula 48	Jent 71	Bent 165	CC	CD	CC
Helene 62	Hal 43	Alec 139	CC	CD	CC
Soda 12	Hal 43	Lad 119	CC	CD	CC
Soda 12	Hal 43	Sadie 154	CC	CD	CD
Soda 12	Hal 43	Sally 78	CC	CD	CC
Fanny 102	Jent 71	Norma 166	CC	CD	CC
Gamma 58	Bokar 5	Margo 160	AC	CC	CC
Gamma 58	Bokar 5	Garbo 168	AC	CC	CC
Pati 42	Bokar 5	Beti 180	AC	CC	AC
Pati 42	Bokar 5	Portia 144	AC	CC	CC
Debi	Frans 103	Dena 186	BC	CC	BC
Debi	Frans 103	Oban 181	BC	CC	CC
Wendy 4	Jent 71	Went 143	CD	CD	CD
Fanny 102	Frans 103	Francy 224	CC	CC	CC
Wendy 4	(Jack)	Jenny 90	CD		CD
Wendy 4	(Jack)	Jed 67	CD		CD
Alpha 28	(Frank)	Alf 59	CC		CC
Alpha 28	(Frank)	Flora 100	CC		CC
Alpha 28	(Frank)	Alan 107	CC		CC
Helene 62	(Dick)	Dehn 95	CC		CD
Soda 12	(Frank)	Frans 103	CC		CC
(Fifi)	Bokar 5	Art 57		CC	CC
(Vert)	Bokar 5	Verb 97		CC	CC
(Vert)	Bokar 5	Kathy 146		CC	CC
(Coma)	Hal 43	Malcom 141		CD	CC
(Coma)	Hal 43	Polly 178		CD	CC
(Vert)	Hal 43	Val 169		CD	CC
(Banka)	Web 79	Wenka 170		CD	CD

Brackets denotes that the animal was no longer available for drawing serum due to death.

There would be four genes in the allelic series controlling respectively transferrins A, B, C and D. The minor transferrin bands would be related to the major bands, and their presence along with these major bands would be controlled by the same allelic system at a single locus. The appearance of the seven chimpanzee phenotypes (Fig. 2 in the preceding communication) suggests that transferrins A, B and C each have one minor component migrating directly in advance of the major component, whereas transferrin D has two components migrating in advance of the major component, the slower of these more rapidly migrating bands occurring in the same position of the major component of transferrin C. Another genetic hypothesis to account for multiple transferrin bands in chimpanzees presumed to be homozygotic for the protein is that genes at more than one locus interact to control the molecular specificities of transferrin, with the observed phenotypes resulting from an allelic series at only one of the gene loci.

Our results by either genetic hypothesis support the assumption that CC is a homozygous phenotype and that AC, BC and CD are heterozygous phenotypes. As yet we have no information on the inheritance of phenotypes AA, AD and DD. Thus the assumption that the phenotypes designated DD and AA are homozygous in character still rests on very tenuous grounds. Clearly the genetic basis for the transferrin differences in chimpanzees cannot be thoroughly evaluated until a more extensive programme of breeding chimpanzees with these various transferrin phenotypes is carried out.

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INCORPORATION OF AMINO-ACID BY ROOT NODULES OF *GALEGA OFFICINALIS*

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AMONG plant tissues, root nodules have two unique features: they contain hæmoglobin[1] and fix nitrogen[2]. These characteristics in their turn imply that nodules can synthesize both hæmoglobin and the nitrogen-fixing enzymes, and hence are suitable organs for the examination of specific protein synthesis.

Root nodules of *Galega officinalis* (Linn.) are robust structures of up to 200 mg fresh weight and can be detached easily from the plant in a metabolically active condition[3]. These nodules contain hæmoglobins of the types normally found in plant tissues[3]. Previous work has shown that the nodules contain amino-acid-activating enzymes[4], and this article outlines experiments which show that intact detached nodules incorporate amino-acids into the water-soluble proteins, including hæmoglobin.

In order to obtain nodules which were actively fixing nitrogen and synthesizing hæmoglobin, a system of 'regenerating nodules' was devised. Young plants growing in the field were uprooted, the majority of nodules were removed from the roots, and the de-nodulated plants were replanted in coarse pumice. These plants were periodically irrigated with a nutrient solution containing: calcium chloride, 0·0045 M; potassium sulphate, 0·00225 M; sodium chloride, 0·0005 M; magnesium sulphate, 0·002 M; potassium dihydrogen phosphate, 0·001 M; dipotassium hydrogen phosphate, 0·001 M; ferric versenate, 0·5 ml./l. of a saturated solution; and 1 ml./l. of a trace element mixture. Within two weeks many new nodules were appearing on the roots. These new nodules incorporated amino-acids four times as fast as mature nodules, and were used in all the experiments described here.

Incorporation of amino-acid was examined with intact nodules bathed with 0·1 M potassium phosphate buffer *p*H 7·1 containing an excess of valine-1-^{14}C with a specific radioactivity of 2,000 counts/min/mg. This suspension was shaken in air by 120 reciprocal 1 in. stroke/min at room temperature. Valine was chosen as marker for protein synthesis because: it contributes mainly to protein valine[5,6]; proteins[7] and nodule hæmoglobin[8] are relatively rich in valine; nodules contain an active valine-activating enzyme[4]; and since the endogenous 'amino-acid pool' contains only traces of valine, the added radioactive valine is diluted very little. At intervals, samples of the incubating nodules were taken, washed with the phosphate buffer containing 5 per cent (w/v) valine-^{12}C, and then crushed with a glass rod in the phosphate buffer. When all the nodules were crushed, the homogenate was centrifuged at 25,000*g* for 15 min. The supernatant solution, containing water-soluble proteins and essentially all the hæmoglobin, was fractionated by boiling with an equal volume of 10 per cent (w/v) trichloroacetic acid. This precipitate (= total water-soluble protein) and the sediment from centrifugation (= sedimentable protein) were washed with 5 per cent (w/v) trichloroacetic acid containing valine-^{12}C. The washed precipitates were dissolved in ammonia solution, plated on planchettes 19 mm in diameter in the presence of cetyl-trimethyl-ammonium-bromide and dried under an infra-red lamp. These infinitely thin samples were monitored for at least 1,000 counts with a Geiger–Müller tube. The background count rate was of the order of 10 counts/min.

The results (Fig. 1) show that valine is incorporated into both sediment and water-soluble fractions, and suggest that both water-soluble and sedimentable proteins are being formed. Also, since the ratio of the radioactivity incorporated into each fraction remains constant, it would seem that there are a constant number of synthetic sites.

Whole nodules contain both bacterial and plant components, and the sediment after centrifugation contained most of the water-insoluble plant material, namely, cell walls and membranes, and the bacteria. The effect of inhibitors on the incorporation of amino-acids was investigated with the view of discriminating between incorporation by plant and incorporation by bacteria, a subsidiary aim being to be able to halt nitrogen assimilation between fixation and protein and thence move the equilibrium reactions in favour of the early products of fixation.

A series of inhibitors was screened with the results shown in Table 1. These results show that although inhibitors of energy production inhibit synthesis of both water-soluble and sedimentable proteins, chloramphenicol (an inhibitor of bacterial protein synthesis[9]) discriminates between these two syntheses. This result is consistent with the postulate that incorporation into the sedimentable protein is mainly by the bacteria, and in further support of this it was shown that 50 μg/ml. chloramphenicol completely inhibited the incorporation of valine-^{14}C by a suspension of the *Rhizobium* sp. from the *Galega officinalis* nodules.

In further experiments nodules were exposed to valine-^{14}C and the water-soluble proteins fractionated by electrophoresis on starch gel[8]. The gel was scanned for hæmoglobin[8] and cut into slices which were dried and monitored for carbon-14. The strip of the gel carrying the hæmoglobin contained between 30 and 47 counts/min. During electrophoresis free valine remained within 5 mm of the

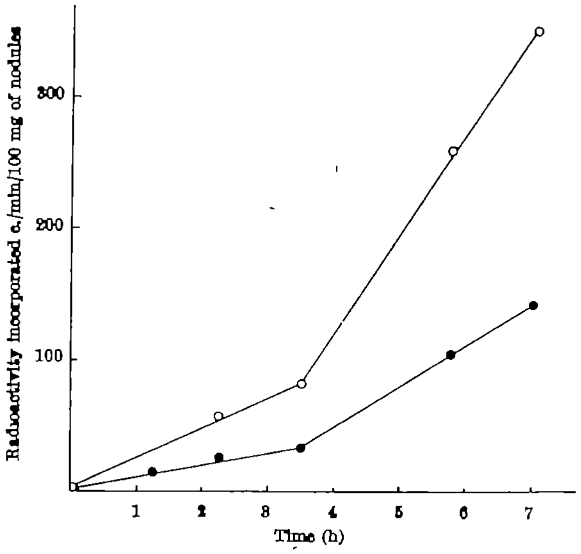

Fig. 1. Time course of incorporation of valine-^{14}C into the water-soluble and sedimentable proteins of intact nodules. The upper curve (open circles) is the incorporation into the sedimentable proteins, the lower curve (solid circles) the incorporation into the water-soluble proteins

Table 1. INHIBITIONS OF INCORPORATION OF AMINO-ACID BY INTACT ROOT NODULES

Inhibitor	Concentration	Counts/min valine-^{14}C incorporated into Water-soluble proteins	Counts/min valine-^{14}C incorporated into Water-insoluble proteins
None	—	34	84
Sodium azide	10^{-4} M	3	7
Sodium arsenite	10^{-4} M	1	2
Potassium cyanide	10^{-3} M	2	8
Chloramphenicol	50 mgm/ml.	32	27

Samples of nodules were fractionated after 3 h exposure to valine-^{14}C, and the results are expressed as counts/min/100 mg of nodules. Individual groups of nodules varied in fresh weight from 78–112 mg.

origin while hæmoglobin moved 70 mm; and electrophoresis of mixtures of valine and hæmoglobin gave a clear separation. These experiments suggest that detached nodules are able to synthesize hæmoglobin.

In the final series of experiments, nodules were crushed in the 0·1 M phosphate buffer pH 7·1 and the resultant homogenate clarified by centrifugation at 15,000*g* for 10 min. The supernatant fluid was carefully pipetted from the residue, supplemented with adenosine triphosphate, magnesium and sodium, then assayed for ability to incorporate valine-^{14}C. After incubation for 3 h the extract was fractionated with ammonium sulphate. The precipitate between 58 and 75 per cent saturation was collected, washed with 75 per cent saturated ammonium sulphate containing valine-^{12}C and then monitored for carbon-14. In a series of four experiments the precipitate contained between 12 and 27 counts/min, and a typical rationale is shown in Table 2. These results suggest that the extracts of the nodules are able to synthesize hæmoglobin. Considering the low specific radioactivity of the valine used even the low level of incorporation observed here indicates that the net synthesis of hæmoglobin under these conditions is considerable.

Table 2. INCORPORATION OF AMINO-ACID BY EXTRACT OF ROOT NODULES

Incorporation system	Counts/min in 58–75% ammonium sulphate precipitate
Complete	12
No adenosine triphosphate	7
Boiled extract	1
Complete + ribonuclease	3

An extract from 1 g of nodules was fractionated after 1 h exposure to valine-^{14}C.

Preliminary examination of this extract by plating samples on a mannitol–yeast extract medium showed that it was free of the *Rhizobium* sp. which inhabits the nodules of *Galega officinalis*. The extract contained amino-acid activating enzymes, soluble ribonucleic acid, ribonucleic acid sedimented by 105,000*g* for 2 h, and probably some mitochondria from the plant tissue.

Further experiments are in progress to extend the generality of these findings to nodules of *Lotus uliginosus* and *Trifolium* spp., and to determine in reconstructed systems the specific roles of bacterial and plant components in nodule hæmoglobin synthesis.

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LEVELS OF NITRATE REDUCTASE ACTIVITY IN INBRED LINES AND F_1 HYBRIDS IN MAIZE

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THE enzyme nitrate reductase is of major importance in the nitrogen metabolism of maize[1-4]. Evidence from earlier work in our laboratory[1,2] suggested that it may be significantly involved in grain-yielding capacity, and also established[1] that the activity of nitrate reductase is drastically reduced by short-term artificial shading, at the level of 80–90 per cent reduction in incident light. These findings, when considered in connexion with McIlrath and Earley's demonstration[5] that similar shading at a critical period in ear formation greatly reduces grain yield, indicate that nitrate reductase activity-levels may serve as an index of protein-producing potential, which in turn may have an important influence on ear development and grain production.

These observations on nitrate reductase assumed additional significance when considered in relation to investigations of possible physiological bases for heterosis in maize, which were begun in our laboratory in 1954. As originally proposed, the intent was to determine whether the enhanced growth rate of hybrid plants is dependent on quantitatively greater amounts of certain enzymes, or qualitatively greater enzyme efficiency. Initial work[6] with triosephosphate dehydrogenase, extracted from acetone powders of seeds and seedlings, indicated that the hybrid *WF*9 × *M*14 exceeded both its parental inbred lines in the quantity of this enzyme per unit of protein or per gram of dry weight of seed or seedling. The same conclusion was reached for aldolase[6], maltase and amount of mitochondrial fractions[7]. Assay of partially purified triosephosphate dehydrogenase extracts indicated the presence of significantly more enzyme (V_{max}) in the hybrid, but suggested that the enzymes were qualitatively (Km) the same as in the inbreds.

Thus, our work with nitrate reductase logically raised the questions: (*a*) are there genetically controlled differences in nitrate reductase activity in maize; (*b*) are such differences involved in heterotic phenomena? Studies[1,2] conducted in 1958 and 1959 showed that the F_1 hybrid *Hy*2 × *Oh*7 had a higher level of nitrate reductase activity in leaf tissue than did the F_1 hybrid *WF*9 × *C*103. These findings were considered particularly interesting because *Hy*2 × *Oh*7 has repeatedly been shown[8-11] to have significantly higher grain-producing capacity than *WF*9 × *C*103 under high plant populations and other stress conditions. Preliminary studies in 1958 and 1959 indicated that the inbred parents of these hybrids differed in nitrate reductase activity-levels, with *C*103, *Hy*2 and *Oh*7 being approximately equal in nitrate reductase activity but all significantly higher (two-fold) than *WF*9. Insufficient information was available to test the significance of the differences observed.

In 1960 and 1961 more extensive studies were undertaken to evaluate nitrate reductase activity in a number of agronomically desirable inbred lines of maize, and to compare nitrate reductase activity-levels in inbred lines with those of F_1 hybrid progeny. Forty-seven inbred lines were assayed in 1960 and 12 of the same lines were re-tested in 1961. In 1960, the maize was sown on

May 23 and sampled from June 25 to August 24. The 1961 crop was sown June 1 and sampled at weekly intervals from June 28 through August 24. The entire aerial parts of the plants were assayed on the earlier sampling dates (June 27, 1960, and June 28, 1961). Subsequent samples in both years were taken only from the uppermost leaf of each plant chosen.

Four hybrids—the double-cross 'Illinois 1996' (*Hy*2 × *Oh*7) (*B*14 × *C*103) and three F_1 hybrids, *WF*9 × *C*103, *WF*9 × *Oh*7 and *Hy*2 × *Oh*7—were examined in 1960 under conditions comparable with those used for comparisons of the inbred lines. Two of these F_1 hybrids, *Hy*2 × *Oh*7 and *WF*9 × *Oh*7, were examined again in 1961.

Complete details of cultural conditions, sampling techniques and assay methods are being published in other reports[4,12]. Nitrate reductase assays and protein determinations were made according to the methods of Hageman and Flesher[1]. Specific nitrate reductase activity is defined as µm potassium nitrite produced per mg protein per h.

The results (Table 1) clearly indicate the existence of wide differences (up to three-fold) in specific nitrate reductase activity of the various inbred lines tested. Although the 1960 data were taken only from single field plots, without replication, their agreement with replicated data in 1961 for 12 inbred lines was very close (correlation between seasonal means for the two years, $r = +0·84$). Of the 12 lines tested in both years, only two showed significant deviations in specific nitrate reductase activity ranking. One of these inbreds (*R*181) is not considered to be genetically fixed, since it has exhibited considerable phenotypic variability in other characters. The difference in the specific nitrate reductase activity-levels in 1960 and 1961 is attributed to climatological seasonal effects and adds weight to the contention that specific nitrate reductase activity and productivity are closely interrelated. Mean grain yields on comparable university farm plots were 30–40 bushels per acre higher in 1961 than in 1960. The hereditary nature of specific nitrate reductase activity response is evident from the reproducibility of inbred specific nitrate reductase activity, seasonal patterns and relative rankings (Table 1) and from the observed resemblance between inbreds and their F_1 hybrids in specific nitrate reductase activity. Related inbred lines were found[4] to resemble each other in level and seasonal patterns of specific nitrate reductase activity.

Comparisons of seasonal specific nitrate reductase activity patterns (Fig. 1) or seasonal mean values (Table 1) gave no evidence of heterotic effects on specific nitrate reductase activity-levels in the material studied. With the exception of two early samplings in 1960, specific nitrate reductase activity in F_1 hybrids always was found to be intermediate between the specific nitrate reductase activity-levels of the respective parental inbred lines.

Table 1. COMPARISON OF THE NITRATE REDUCTASE ACTIVITY OF TWELVE INBRED LINES OF MAIZE GROWN UNDER FIELD CONDITIONS IN 1960 AND 1961 (Seasonal means)

Inbred or hybrid	Nitrate reductase activity (µm KNO$_2$/mg protein/h) 1960	1961*
*Oh*43	0·16	0·28
*H*49	0·23	0·34
*R*177	0·21	0·34
*WF*9	0·26	0·41
*R*168	0·42	0·55
*A*545	0·35	0·61
*Hy*2	0·35	0·70
*Oh*7	0·51	0·70
*M*14	0·51	0·78
CI.21*E*	0·48	0·82
*R*151	0·58	0·83
*R*181	0·37	0·94
*C*103	0·38	—
*B*14	0·17	—
Illinois 1996 (*Hy*2 × *Oh*7) (*C*103 × *B*14)	0·41	0·64
*Hy*2 × *Oh*7	0·35	0·69
*WF*9 × *Oh*7	0·34	0·49
*WF*9 × *C*103	0·31	0·46

* Duncan's multiple range test (for 1961 inbred data only) requires values of 0·27 and 0·30 for shortest significant range when 2 and 5 to 12 means, respectively, are to be compared.

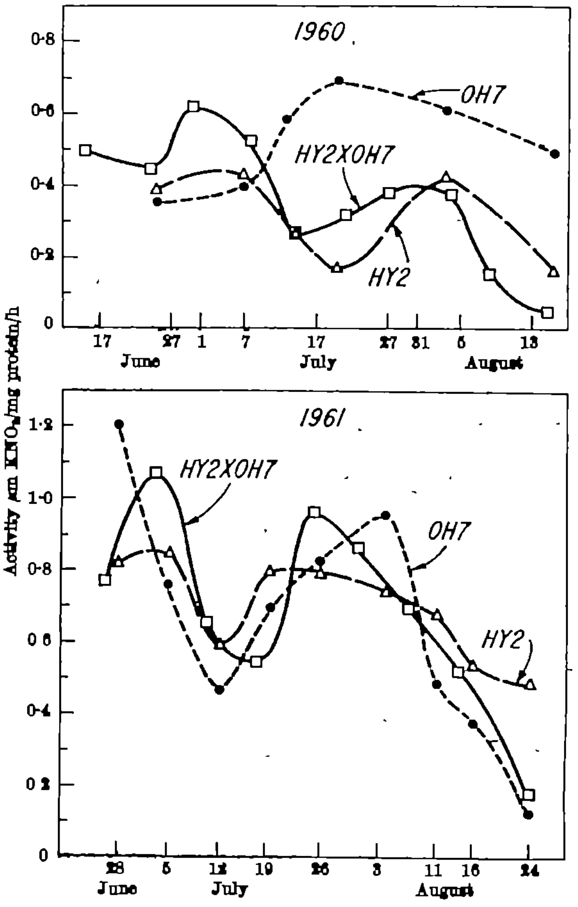

Fig. 1. Comparison of the seasonal pattern of nitrate reductase activity of the F_1 hybrid *Hy*2 × *Oh*7 with that of the parental inbreds in 1960 and 1961

Frequently, the F_1 specific nitrate reductase activity values were more nearly parallel to those of the lower parent inbreds than to those of the higher parent.

Variations in seasonal specific nitrate reductase activity patterns of certain inbred lines and hybrids were apparent when specific nitrate reductase activity values for the different sampling dates were plotted, and also are reflected in a significant (0·05 level) interaction between inbred line specific nitrate reductase activity values and sampling dates. In general, the data indicated that early-season specific nitrate reductase activity-levels were relatively low when whole plants were sampled. The specific nitrate reductase activity values were high for the initial leaf-tissue samples but then declined sharply to minimal levels 42–50 days after sowing, increasing rapidly again to peak activity at flowering time. Activity of the enzyme decreased markedly in all material sampled after flowering had been completed.

The observed early-season low levels of specific nitrate reductase activity almost certainly reflect differences in enzyme activity in the tissues sampled, since whole plants were used in these assays. Nitrate reductase activity is known[13] to be high in leaves and low in stems; thus, the inclusion of variable amounts of stem material in the early samples undoubtedly introduced a bias toward lower specific nitrate reductase activity in these data. With this exception, the observed specific nitrate reductase activity patterns in most inbred lines and hybrids studied closely parallel the cyclic pattern of auxin content in maize plants, reported by Laibach and Meyer[14]. Auxin (indolyl-3-acetic acid) and specific nitrate reductase activity have been shown[9,15] to vary diurnally. This apparent relation between specific nitrate reductase activity and auxin activity is so supported by recent evidence obtained in our laboratory (Beevers, L., unpublished), indicating that specific nitrate reductase activity in maize plants is increased by spray applications of 2,4-D (dichlorophenoxyacetate).

Interesting parallels also were noted between specific nitrate reductase activity seasonal patterns presented in detail elsewhere[12] and established information[16] on time-relationships in development of certain of the inbred lines studied. For example, inbred *M*14, an early-flowering line, showed an earlier date of increase, a more rapid rate of increase, and a slower decline in nitrate reductase activity than most other inbred lines tested. Inbred 38-11, a late-flowering line which has been shown[16] to exhibit an unusually long developmental period between tassel initiation and anthesis, had a high level of specific nitrate reductase activity in 4-week seedlings. This was followed by a sharp decrease in specific nitrate reductase activity and a long lag period before enzyme activity again increased at flowering time. Inbred *K*4, a slow-starting, very late-flowering line under Illinois conditions, showed a different seasonal specific nitrate reductase activity pattern, in that the initially high specific nitrate reductase activity-level declined sharply in early July and showed no return to high levels as the season progressed.

Probably the most significant information to be derived from this work and our previous studies of other enzyme systems is the indication of quantitative differences in enzyme activity between various inbred lines of maize, and between certain inbred lines and their F_1 hybrids. Moreover, our findings with all enzyme systems studied indicate either that the hybrids are intermediate between their parents in enzyme activity-levels (specific nitrate reductase activity) or else that superiority of hybrids over parental lines in enzyme activity resides in the presence of greater amounts of enzymes (triosephosphate dehydrogenase), rather than in the presence of different or more efficient enzyme systems. These findings appear to differ from those of Schwartz[17], who has proposed that heterosis may result from the formation of enzyme dimers or polymers in hybrid plants. Schwartz's work, based on starch-gel column electrophoretic separation of basic proteins, which were identified as esterases by a non-specific histochemical stain, does not indicate the presence of more 'esterase' activity in the hybrid material than in the parent lines.

More extensive and detailed studies are planned to gain information on the genetic control of nitrate reductase activity in maize, and to determine more specifically the role of this enzyme activity in conditioning grain-yielding ability in the crop. It would appear that such investigations, as well as similar work with other enzymes or enzyme systems, might also be expected to yield valuable information on possible physiological bases for heterosis in maize hybrids.

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PHOTOKINETIC FUNCTION OF APHID ANTENNÆ

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JOHNSON[1] reported that alate alienicolæ of *Aphis fabae* Scop. deprived of their antennæ could not be stimulated to fly as readily as intact aphids, when both were denied a foothold. Nevertheless, because the antennaless aphids took off from an unspecified surface as readily as intact ones, he inferred that aphid antennæ did not have the kinetic function shown by Wigglesworth and Gillett[2] for the antennæ of another hemipteran, *Rhodnius prolixus* Stål. He concluded that aphid antennæ served instead to control flight, on the grounds that, on take-off, the aphids failed to fly up out of the black-walled lower part of an open-topped box toward the light, unless their own, or an artificial, antennal flagellum was attached to the third antennal segment and freely movable so as to stimulate Johnston's organ in the second segment.

When aphids of the same form of the same species were deprived of their antennæ and released under the illuminated central roof window of a large black-walled flight chamber[3], they flew without any apparent loss of control. But, instead of flying persistently up toward the central light like intact aphids, they flew away almost immediately toward the dark walls or dark peripheral roof. This photonegative behaviour would have looked like uncontrolled flight if observed at the bottom of a box when photopositive behaviour was expected. In fact it resembled the behaviour of intact aphids after a long flight, when locomotor excitability is low[3]. Thus it seemed that the antennæ might have a kinetic function and experiments were undertaken to test this hypothesis by the simpler method of measuring the readiness of fresh *A. fabae* alatæ to take off from a host leaf, after various treatments of the antennæ. The hypothetical kinetic effect could very well depend on mechanical stimulation of the antennæ, as suspected in *Rhodnius*. But in view of the very pronounced photokinetic responsiveness of fresh winged aphids it was decided to examine first the possibility that the antennæ might contribute to that, as auxiliary light-sensitive organs.

The aphids for these tests were collected singly with a fine camel-hair brush as soon as they took off for the first time from their rearing plants and landed on a glass plate standing 45 cm away, in front of two 40-W, 2-ft. *MCF/U* fluorescent tubes. Each aphid was placed at once on filter paper in the bottom of a metal dish embedded in ice, which kept the aphid immobilized while both its antennæ were treated in one of the following ways:

(*A*) *Brushed*, left intact but stroked gently with the brush for about 45 sec, the period needed for the other treatments.

(*B*) *Clear-coated* from the tip of the last to the base of the third segment with a film of cod liver oil, by sleeving several times inside the tip of glass capillary containing the oil.

(*C*) *Clear-coated, weighted.* Since the black coating (*D*) was somewhat heavier than the clear coating (*B*), this treatment, giving a clear coating that was heavier than

D, was also included, using the same oil thickened to a 'sludge' with 'Vaseline' and applied in the same way.

(*D*) *Black-coated*, exactly as for *B* except that the oil now contained a dense suspension of pure carbon black.

(*E*) *Amputated* with fine-pointed forceps somewhere along the third segment. Treatments *B*, *C* and *D* were arrived at after many trials of other materials applied by brush or capillary, none of which gave such easy, controllable, uniform and apparently harmless cover without subsequent loss of mobility of the antennæ.

Immediately after its treatment each aphid was transferred with the brush to the upper surface of a fully expanded leaf of *Vicia faba* with the cut petiole in water. This was exposed to the same light intensity as the rearing plants, from another ventilated *MCF/U* lamp 25 cm away behind an upright sheet of 'Perspex'. The aphid promptly resumed activity on the leaf and the time elapsing before it took off toward the light was recorded. It was re-collected as it alighted on the 'Perspex' and replaced on the leaf, and this was repeated until it had taken off five times or had settled down on the leaf for 1 h or more. The collection, treatment and testing were all carried out in the same dark room kept at about 15° C.

Table 1 shows that the aphids with intact (*A*) or clear-coated (*B*) antennæ invariably took off in less than 6 min and frequently in less than 1 min; and the aphids with heavily weighted antennæ (*C*) were almost equally quick to take off. But a large majority of the take-offs by the aphids with black-coated (*D*) antennæ were delayed more than 6 min. The time to take off was very erratic in this lot *D*, but every individual stayed for more than 15 min before at least one of its take-offs, whereas none of the individuals in the control lots *A*, *B* and *C* ever did so. Table 1 in fact underestimates the arresting effect of blackening the antennæ. Only 73 instead of the intended 100 take-offs could be timed after this treatment because twelve of the aphids settled down on their leaves for 1–4 h and had to be abandoned before they had been given five tests. Amputation of most of the antennal flagella (*E*) also delayed taking off clearly but erratically, although not so much as the black coating did.

These results were confirmed when the experiment was repeated with 25 aphids per treatment and some technical improvements. The temperature was raised to 24° C to prevent over-long settling. Amputation was made more thorough and uniform by removal of the entire third antennal segment in every case. A major uncontrolled variable in the first experiment was the handling of the aphids with the brush, which sometimes produced struggling and speedy take-off. With more care and much practice this effect was virtually eliminated. The result was to unify the behaviour of the aphids with black-coated and amputated antennæ, and to bring out a clear difference between them and the three control lots at the first take-off, as seen in Table 2.

Table 1. PERCENTAGE FREQUENCY DISTRIBUTION OF TIMES TO TAKE-OFF FROM A HOST LEAF AFTER VARIOUS TREATMENTS OF THE ANTENNÆ

Antennal treatment	Aphids treated	Total take-offs timed	Time to take-off: 0–59″	1′–2′59″	3′–5′59″	6′–11′59″	12′–23′59″	24′–47′59″	48′–
(*A*) Brushed	20	100	46	42	12	0	0	0	0
(*B*) Clear-coated	20	100	38	48	14	0	0	0	0
(*C*) Clear-coated, weighted	15	72	38	45	15	1	0	0	0
(*D*) Black-coated	20	73	1	9	19	11	15	12	31
(*E*) Amputated	15	64	11	18	22	9	16	6	17

Table 2. FREQUENCY DISTRIBUTION OF TIMES TO FIRST TAKE-OFF FROM A HOST LEAF AFTER VARIOUS TREATMENTS OF THE ANTENNÆ (Second experiment)

Antennal treatment	Time to take off: 0 – 59′	1′ – 2′ 59″	3′ – 5′ 59″	6′ – 11′ 59″	12′ – 23′ 59″	24′ – 47′ 59″	48′ –
(*A*) Brushed	12	8	2	4	3	0	0
(*B*) Clear-coated	7	9	4	5	0	0	0
(*C*) Clear-coated, weighted	2	14	4	3	2	0	0
(*D*) Black-coated	1	0	3	0	5	7	9
(*E*) Amputated	1	1	1	1	4	12	5

That the akinetic effect of the coating of carbon black in cod liver oil was not peculiar to this mixture was evident from preliminary experiments in which a clear watery paste of 'Polycell' (a mixture of water-soluble cellulose ethers) was used in place of cod liver oil, and indian ink in place of pure carbon black. These coatings were physically less satisfactory but the results were the same: aphids with black-coated antennæ were slower to take off than aphids with clear-coated or intact antennæ.

It seems difficult to avoid the conclusion that the antennæ of these aphids bear photoreceptors of some kind which function as 'stimulatory organs'[3] (and perhaps in other ways also). The third and fourth segments of the antennæ of winged (but not wingless) aphids bear numerous placoid sensilla, once dubbed 'rhinaria' from their presumed olfactory function[4]. Slifer and Sekhon[5] could find no morphological support for the presumed olfactory function of similar sensilla on the antennal flagellum of the honey-bee and, among other possibilities, suggested, 'improbable as it may seem', that these organs might be sensitive to light. The aphids' photokinetic response certainly does not depend on the antennæ alone. The flight-delaying effect of darkening or removing the antennæ was readily counteracted by increasing the light intensity, as well as by other types of kinetic stimulation.

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NON-ENZYMATIC BROWNING REACTIONS: CONSIDERATION OF SUGAR STABILITY

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THE properties of the aldose sugars have proved intriguing and in the absence of full conformational data it is difficult to predict fully their behaviour.

In a recent study, Burton, McWeeny and Biltcliffe[1] showed that the addition of sulphites modified the reaction sequences in glucose/glycine solutions. This work showed that sulphite produced a more pronounced retardation in chromophore development when added initially (at $t = 0$) than when added at a later stage. Similar experiments to determine whether mannose would produce a chromophore more rapidly than glucose in aqueous solutions 1·0–2·5 M with respect to glucose and 1·0–1·5 M with respect to glycine showed that if the glucose in the system described in our previous publication was replaced in whole or in part by mannose, then the development of chromophores proceeded at a faster rate, and one which increased with increasing mannose content.

Thus we found that mannose gives chromophoric material at a faster rate than glucose, and at first we were inclined to believe that this was due to the *cis* hydroxyls on C_2 and C_3. A comparison showed that galactose reacted with amino-acids at a much greater rate than did mannose, with a more rapid production of unsaturated compounds prior to chromophore development. The aldose/glycine reaction appeared to depend initially on the conformational stability of the aldose molecule as a whole. On this basis one would expect the pentoses to be less stable than the hexoses, and it was apparent both from our own results and those of others that the aldopentoses reacted with glycine and amino compounds more rapidly than did aldohexoses to give chromophores.

A search of the literature revealed that on the question of sugar stability, Cantor and Peniston[2] found by polarographic studies that the wave-heights obtained in the aldohexose series were in the ascending order glucose, mannose and galactose, thus paralleling our observed rate of initial chromophore development, and that there appeared to be a correlation both with mutarotation velocity and the quantity of supposedly free aldehydic sugars as found by the cyanide addition experiments of Lippich[3].

Moreover, in the pentose series much greater polarographic wave-heights were recorded, and, unlike the hexoses, no pentose showed linear increase of current with increasing *p*H, at any concentration.

Wiesner[4] claimed that the height of the wave depended on the rate of non-reducible aldose–reducible aldose reaction. Delahay and Strassner[5] confirmed that the wave-heights were in the order: arabinose–xylose–galactose–mannose–glucose and stated that in aldoses the bond between the oxygen and hydrogen of the hydroxyl attached to carbon C_1 is slightly ionic. Consequently, the bond-strength is weakened as *p*H increases. Likewise, the rate of ring sugar aldehyde transformation increases rapidly with *p*H.

Tobi[6] found that whereas the aldoses gave no colour with Schiff's reagent, they did so provided that the sulphur dioxide level was sufficiently reduced, and Stepachenko[7], following up this work, found that the degree of colour obtained from hexoses was in the ascending order: glucose, mannose, galactose. The pentoses gave stronger colours than the hexoses and the order of colour there was arabinose, xylose, both pentoses giving stronger colours than the hexoses. The Tobi–Schiff's colour obtained varied in a notable way with concentration, being markedly greater with increasing aldose concentration. The reason given for this was that in dilute solution any free aldose form would be markedly hydrated. A consideration of the figures published by Downer[8] and by Ingram[9], in which the quantity of sulphur dioxide bound in a non-iodine-titratable, Monier–Williams recoverable manner were determined, leads us to believe that the same factor may be involved.

This effect of concentration appears to be significant in another way, for Lewin[10] has found that whereas some products of sugar/amino reactions are common to both dilute and concentrated solutions, a number of ninhydrin positive compounds are found only in highly concentrated solutions, while Leuck[11] claimed that sulphur dioxide could assist in the self-polymerization of glucose in the dry state.

Considering the sugars on the basis of Stewart models, it appeared that the stability of the sugars in the pyranose form depended on the degree of electronic balance between the two halves of the sugar, and on this consideration the sugars would be expected to have the order of stability as surmised from Lippich's 'instantaneous cyanide' addition, Cantor's polarographic current measurements, and the degree of Tobi–Schiff colour obtained.

We have now found that the same order holds true for initial colour development in aldose/amino-acid systems[12] and in those of the aldose/amino and aldose/organic-acid systems which we have examined, over the range 0·2–2·5 M, and it appears therefore that one factor in the speed of development of initial chromophores is the actual conformational stability of the sugar.

In the presence of sulphur dioxide or sulphites, the initial staes in colour development are markedly retarded in all these cases.

The effect of phosphates is to decrease the initial stability of the aldose sugar. In the presence of phosphates the polarographic wave height is increased, and both we and others (cf. Ellis[13]) have found that the rate of initial colour development is increased as a result for aldose/amino reactions in solution. This effect of phosphate did not occur in parallel lecithin/amino browning reactions (Burton and McWeeny, to be published), phosphate showing a very slight retarding action in this system, which is significant.

The role of ketose sugars in non-enzymatic browning has been largely obscured by the fact that aldoses are regarded as the main reducing sugars, and the ease of measurement of the latter has led to attempted correlation between the reducing sugar figures and browning observed. Ketoses in general give greater polarographic waves than the corresponding aldoses and in browning caused by ketose/amino reactions the initial chromophoric development is greater than for aldoses. The same is true for the rate of initial browning of ketoses as compared with aldoses in the presence of organic acids even in the solid state. In both cases sulphur dioxide has an early retardative effect on browning. We think that the later development of the more highly coloured products which sometimes appear in the case of the aldoses may be related to the character of the intermediates produced.

In general, one may say that the observed order of initial chromophore development in aldose/amino-acid systems follows the pattern shown by the polarographic results. Colour development increases rapidly above *p*H 7, but is much reduced with increasing acidity, at least down to *p*H 4. Ribose appears to have some special interest. Cantor found that ribose gave a *p*H minimum at around *p*H 6·8. In aldose/amino reactions, we have found ribose to be much less stable than xylose at concentrations 0·2–2·5 M, which is what one would expect, Stepachenko and Cantor results giving xylose the most stable character among the pentoses. In arsenate buffer a corresponding *p*H minimum effect for initial colour development was observed for the ribose/glycine reaction at 0·2–0·5 M concentration. In phosphate buffer, although a similar *p*H minimum effect was observed, the degree of initial coloration appeared to be reversed, discoloration occurring more rapidly at *p*H 6 than at 7·4. It is noticeable that ribose itself was originally held to be responsible for browning reactions in fish muscle, though now it is thought that ribose- and hexose-phosphates are responsible[14].

That the stability of the sugar present in natural products has a marked effect in the development of chromophores is indicated by the fact that when reducing sugars are removed by fermentation and replaced by glucose, brown colours take longer to develop. Under these conditions, too, replacement of the aldose sugar with fructose has been found to cause more rapid colour development than with glucose[15].

Later browning may well depend on a number of factors, but here again the initial hydroxyl configuration of the sugar seems to be of importance in some browning reactions. The speed of dehydration and the intermediates and complexes which form will naturally depend to some extent on this factor.

Anet[16] isolated the *cis* and *trans* forms of 3,4 dideoxy 3,4 unsaturated hexosone from aldose/glycine systems. The *cis* form dehydrates very easily, the reaction in 0·03 N acetic acid being half completed in 12 min. The *trans* form gives a half reaction time of 8 h, the same as for 3-deoxy-D-erythrohexosone. It would appear that 3-deoxy-D-threohexosone gives a very much greater yield of the *cis* unsaturated hexosone since a comparison of the rate of formation of hydroxymethylfurfural in 2 N acetic acid at 100° C showed that 3-deoxy-D-erythrohexosone (formed from glucose via di-D-fructose glycine) gave a 45 per cent yield in 2 h whereas the *threo* isomer (formed from galactose via di-D-tagatose glycine) gave an 80 per cent yield.

Thus the initial configuration of the aldose has an important bearing on the rate of production of potential chromophore-developing compounds.

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IMMUNOLOGICAL ASPECTS OF PARASITISM

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DURING evolution of many host/parasite relationships an intimate association of the parasitic species is developed with the host. In these cases survival of the parasitic species is largely dependent on persistence within, and as a consequence, tolerance by, the host. For this type of relationship, where survival of the parasitic species is dependent on a long-term, intra-mural association with the host, the immunological response of the host should not be regarded simply as a mechanism which may cause elimination of the parasitic infection, but rather as an environment which has a profound effect on evolution of the host/parasite relationship.

The role of the immunological environment in the partially immune host population as an intense selective pressure for antigenic variants of bacteria and viruses has been suggested by many workers. Burnet[1] termed this process in nature "immunological drift" and the proposed natural phenomenon is strongly supported by laboratory observations. In particular, studies on the selection of antigenic variants of the apparently highly-mutable influenza virus in the presence of immune sera in eggs and in partially immune mice are well documented (for review, see Edney[2]). Laboratory studies on the immunological selection of bacterial variants are also sufficiently well known as to require no further elaboration. The epizootic spread of a bacterial or virus infection may occur when the invading organism can multiply in individuals of the host population to transmissible levels before each member of the population can in turn elaborate an effective immunological response. When, however, a significant proportion of the surviving host population has elaborated an immune response this immunity may limit further spread of the epidemic strain of bacterium or virus and favour antigenic variants. It is important to note, however, that in this situation where the immune response is effective retrospectively in individuals of the host population, then any random mutation of antigenic characters associated with the stimulation of resistance will favour survival of the pathogenic organism.

With the metazoan endoparasite, however, it is necessary for the organism to reside in intimate contact with the host until termination of the reproductive phase of its life-cycle, that is, usually for a period extending beyond the minimum time required for the host to express an effective immune response. In this situation, where survival of the host/parasite relationship depends on persistence of the parasite within individual members of the host population for immunologically significant periods, the selective pressure provided by the contemporary immune response is likely to be more precise and may favour the survival of only those variants of the metazoan parasite which display a sufficiently reduced antigenic disparity with the host.

This process of immunological selection of genetic variants whereby a parasite may become less antigenically foreign to the host may be based on either simple loss of an appropriate antigenic character, or on acquisition of the antigenic character of the host, or on both.

The concept that there is a minimum or threshold level of antigenic information necessary for stimulation of an immunological response is both implicit in present theories on the mechanism of the immune response and compatible with observations on the nature of immunity. The factors which may determine the mean threshold level in any host/parasite relationship are: (1) the degree of antigenic disparity between host and parasite; (2) the rate of flow of antigenic information.

Potentially the degree of antigenic disparity may be dependent on evolutionary selection of genetic components of both host and parasite. Assuming the same rate of genetic mutation of host and parasite, it is likely that the relative fecundity of the interactants will play a major part in determining the contribution each may make to the evolution of this antigenic aspect of the relationship. As the fecundity of the parasite is much greater (and generation time is usually shorter) than that of the host, the component of antigenic disparity which may be due to the rate of genetic change of the antigenic character of the host population is likely to be unimportant. If, then, the threshold level for antigenic stimulation of an effective immunological response shows no great systematic drift during evolution of the relationship, antigenic disparity and flow rate should be inversely related. As flow rate is determined by the size of the parasitic burden it follows that reduction of antigenic disparity will result in subliminal tolerance of an increased population of the parasite. The term 'subliminal tolerance' is used here to describe a state of relative immunological unresponsiveness. However, the threshold level of antigenic information is, by definition, sensitizing, and consequently one may anticipate that the host will develop an acquired resistance to additional worm infections. Indeed, the 'self-cure' phenomenon[3] which is observed under field conditions in some host/parasite relationships may be due to exaggerated expression of an immunological response in a presensitized host. This response may be viewed as the extreme case of the general phenomenon postulated here. If the sensitized host is suddenly confronted by a relatively large parasitic infection then a gross antigenic insult beyond the threshold level of the immunological system may be provided. Some, or all, of both the incoming and established parasites are then eliminated. After an appropriate interval of time one might expect that the original threshold level will again prevail, and the subliminal worm burden acquired once more during subsequent grazing on infested pastures.

These theories regarding immunological aspects of parasitism are an extension of those advanced to explain some of the phenomena of transplantation immunity. The antigenic relationship between implanted tissue and host is a crucial factor which determines the nature of the homograft response. The greater the degree of histo-incompatibility between donor and host, the more vigorous is the immunological response developed following transplantation. When antigenic disparity is reduced to a minimum as, for example, in the case of tissue transfer between individuals of co-isogenic strains of mice, or when sex-linked histo-incompatibility provides the only basis for immunological rejection of tissues transplanted between individuals of the same homozygous strain, then prolonged survival of the tissue implants is observed. In a discussion of the nature of the homograft response when 'near compatibility' of host and donor is obtained, Woodruff[4] quotes the report of Longmire, Stone, Daniel and Goon[5], who observed the prolonged survival of a skin homograft on a human patient. A second graft obtained from the same donor was completely destroyed within 15 days, and only then, 6 weeks after grafting, did the first graft show signs of reaction. Apparently the immune response of the host was sensitized by the first graft and the 'subliminal' status of this graft was then disturbed by the excess antigenic stimulus provided by the second graft. Similarly, Dineen and Szenberg[6,7] have observed subliminal regeneration of donor epithelial elements in skin homotransplants following the acute phase of the homograft response. They observed that regeneration was not completed as normal hair did not occur in the regenerating tissues. It seems likely, therefore, that the regenerating epithelial cells were brought under immunological control at this stage because the threshold level of antigenic information had again been reached.

If evolution of the host/parasite relationship is characterized by subliminal tolerance of parasite burdens of increasing magnitude then it seems likely that such a trend could only occur if there were, at the same time, selection for attenuation of pathogenicity of the parasite. During this evolutionary process pathogenic activities of the parasite may reach the irreducible minimum due to competition of the parasite with its host for factors which satisfy common physiological and nutritional requirements, and effects which are related but incidental to these factors, for example, anaemia, where the oxygen requirements of the parasite (hookworm, *Ancylostoma caninum*) or the nutritional requirements (*Haemonchus contortus*) are satisfied by blood-sucking. It is important to stress that the evolutionary trend towards reduction of antigenic disparity and attenuation of pathogenesis relate to the population dynamics of parasitism. Great variation about the mean population response to parasitic infections will be observed in individual hosts under field conditions. As the host population is not homozygous for antigenic character, variations in degree of antigenic disparity will be observed among individual relationships between host and parasite. Thus the parasite burdens supported by individual members of the host population will vary greatly in magnitude, and their pathogenic effects may be expected to range from subclinical to lethal. Evolutionary attenuation of pathogenicity is expected to be associated with an increase in the mean worm burden of a host population. Those members of the host population which show extreme levels of antigenic disparity with the parasite may only support a parasite burden so small that the effects of infection are subclinical. In these individuals a state of immunity or premunition, depending on the persistence of a latent infection, is established.

The role of the immunological response as an environment for selection of genetic variants during evolution of the host/parasite relationship is the central theme of this discussion. As a result of this process it is postulated that many antigenic characters of the parasite which stimulate the immune mechanism of the host species are modified during evolution of the relationship. However, one must bear in mind that immunological selection will only affect those antigenic characters which stimulate responses influencing the survival or, in the precise evolutionary sense, 'fitness' of the parasitic species. Many characters, though antigenic, are, in this sense, immunologically impotent and therefore irrelevant to immunological aspects of the host/parasite relationship. Consequently, there will be no immunological selective pressure for modification of such characters which are likely, therefore, to remain highly antigenic notwithstanding evolution of the relationship. Circumstantial support for this argument is provided by the frequently reported failure to observe a correlation between humoral antibody and resistance to infection in many parasitic infections.

So long as infection with the parasite is potentially able to produce significant pathogenic effects then survival of the relationship will be favoured by some degree of relevant antigenic disparity. The effect of such disparity will be to confine pathogenicity within tolerable limits by control of the size of parasitic burdens. It is concluded, therefore, that the role of the immunological response in the 'adapted' host/parasite relationship is to control the parasitic burden rather than to cause complete elimination of the infection. A rational approach to the problem of prophylactic immunization in, for example, helminth diseases of domestic livestock, may be formulated on this basis. Accepting that the immunological response in the evolved host/parasite relationship may only limit the magnitude of the parasite burden, then the most appropriate approach to immunization is first to determine whether the worm burden subliminally tolerated to the primary threshold level of antigenic information is greater than the burden tolerated at the secondary, presensitized level. If a difference is found and this is significant in terms of pathogenic effect, then the aim of vaccination should be to modify the relationship in a manner such that: (1) the first natural infection will be associated with the smaller worm burden permitted by the immunological response of the sensitized host, while (2) the pathogenic effects usually observed when young animals are exposed for the first time to dense larval populations on pasture are avoided.

A similar view was recently expressed by Taylor[8] in a discussion of worm control in ruminants by pasture management.

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TURN-OVER AND DISTRIBUTION OF WATER IN DESERT CAMELS, SHEEP, CATTLE AND KANGAROOS

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THE desert hardiness of the camel has impressed and intrigued observers for more than 2,300 years[1]. Schmidt-Nielsen[2] and Gauthier-Pilters[3] have examined many aspects of the water handling of these ruminants; but the mechanism of resistance to dehydration, particularly the part taken by alimentary water reserves under hot conditions, is difficult to measure and had been an elusive problem. We have estimated water content and water turn-over using tritium oxide in *Camelus dromedarius* and compared them with *Bos taurus* (Shorthorn) and *Macropus rufus* (red kangaroo) at Alice Springs, Central Australia, in further efforts to determine the dynamics of dehydration. These findings were compared with those on tropical Merino sheep studied earlier in similar conditions[4].

In late summer, air temperatures reached 45° C and daily maxima averaged 41° C with wet-bulb maximum temperatures between 20° and 25° C. Adult camels at large on the almost bare gibbous plain fed mainly on trees (*Acacia* and *Casuarina*). Those deprived of water were offered lucerne hay. The animals were weighed daily and body-water distribution was determined on 7 camels by *T* 1824 for plasma, thiocyanide space (SCN) for extracellular and $^{3}H_2O$ for total body-water. Tritium equilibration requires 8 h or more in camels. Measurements were made also on 4 Shorthorn cattle living on the same territory as the camels at the end of 4 years drought, and on 4 red kangaroos in an enclosure, partly sheltered from the sun.

Among the camels feeding at large, water content ranged from 650 to 755 ml./kg. The non-lactating adult females were used in sweat and urine estimations and were watered each second day. Their water turn-over in the heat was about half that of Merino sheep[4] and one-third that of Shorthorn cattle (Fig. 1). The shaded kangaroos turned over 88 ml./kg/24 h, 44 per cent more water than camels in the sun. Lactating camels with calves 1 year old used 82–104 ml./kg/24 h; but this amount was still less than that used by non-lactating sheep (100–130 ml./kg/24 h). The camel conserves water by economical sweating for evaporative cooling (up to 280 ml./m²/h) which wastes less energy and water than the more expensive respiratory cooling of sheep. Camel sweat contains four times more potassium than sodium and is high in bicarbonate, which results in a sweat pH of 8·2–8·5. Sheep sweat is similar except that concentration of potassium is up to 20 times greater than sodium[5]; but the camel sweats a ten-times greater volume per unit area at 40° C than the Merino. Sweat is produced under the influence of adrenaline injected locally or intravenously in camels, as in sheep and cattle, but noradrenaline is inactive on sweat glands. Camels also pass small volumes or urine when on dry feed (2–3 ml./kg/24 h compared with 8–10 ml./kg/24 h in sheep) but the highest camel urine osmolality observed is 3·17 osmols/l. compared with the 3·8 osmols/l. found in dehydrated sheep[6]. Vasopressin

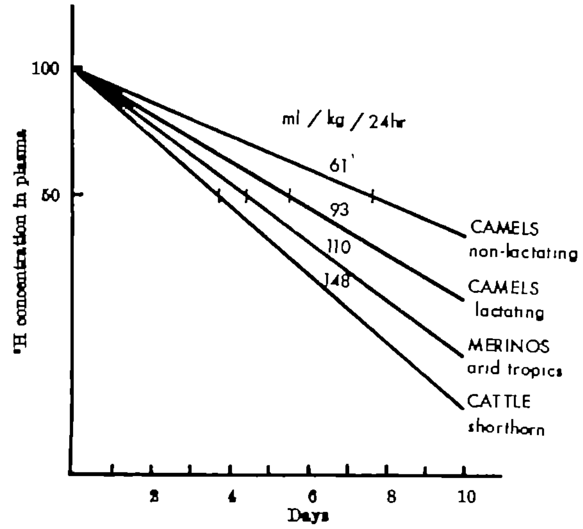

Fig. 1. Tritium oxide turn-over in grazing ruminants exposed during summer to average maximum daily temperatures of 41° C. The sheep were at lat. 21° S. and the other animals at 24° S. Two of the camels were lactating, but none of the other animals was giving milk. Ingested water, food and metabolic water are integrated by this method. Cattle used more than twice as much water daily as camels of the same weight

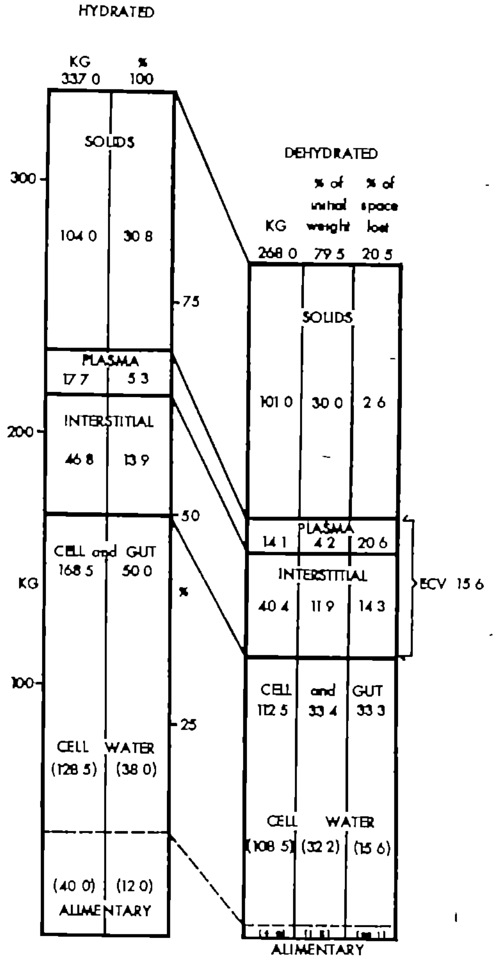

Fig. 2. Average body composition of 3 adult camels before and after 9 days without water in the sun at Alice Springs, with daily maxima of 41° C. The loss of extracellular water was less proportionally than the 20 5 per cent weight loss, but alimentary and cell water fell by 33 3 per cent. It was estimated that the alimentary tract yielded more than 50 per cent of the water

in a dose of 2 mU./kg/h intravenously increases the excretion of water and electrolytes in camels, as in sheep[7] so that the low urine flow of camels is as yet unexplained.

When deprived of water in the sun at mean maximum temperatures of 41° C (Table 1) three camels lost weight at rates of only 19–23 g/kg/24 h because of small losses of sweat, urine, respiratory and fæcal water[3], and lowered metabolism. This was half the rate of weight change observed in Merinos at the same temperature. The differential loss of fluids is, however, a point of great interest (Fig. 2). Whereas the average body-weight of camels fell by 20·5 per cent in 9 days, the SCN space was reduced by only 15·6 per cent. Of the weight lost the interstitial fluid yielded 9·3 per cent and the plasma 5·2 per cent. The cells and gut contributed water to the extent of 81·2 per cent of the loss in weight, in contrast with Merino sheep, which provided only 46·2 per cent of the weight lost as water from these sources, in similar environmental circumstances[4]. Measurements on sheep[4] indicate that differential losses of water occur between spaces and that at relatively slow rates of water loss (30 ml./kg/24 h) the sheep depletes the interstitial fluid faster than plasma, but during rapid dehydration relatively more water is drawn from the cell and gut space. In camels the slow rate of loss in weight during exposure to high temperature (41° C) allows adjustments of fluid spaces which resemble those of sheep at lower temperatures (32° C).

	Camels*	Merino sheep*
Days without water mean max. temp. 41° C.	9	5
Rate of dehydration, ml./kg/24 h	22	47
Weight lost (% body-weight)	20·3	23·5
% from SCN space	14·5	50·4
% from cells and gut	81·2	46·4

* Mean of 3 in each case

The question of whether the cells and alimentary fluids share equally in providing water is hard to answer directly because gut water is not readily estimated. It is known, however, that in camels rumen contents may comprise an average of 110 g/kg (ref. 7) and the alimentary tract may hold up to 180 g/kg, of which more than 80 per cent is water. During dehydration the passage of gut fluids was reduced to about 5 per cent of the initial flow in a fistulated camel studied by Stepankina and Tashenov[8]. The abdomen also of a dehydrated camel has a highly concave contour, as though little contents remain in the gut. These observations suggest that the alimentary tract may provide much of the water lost.

If it be assumed that the camel begins dehydration with 120 g water/kg in the alimentary tract and that at the end of 9 days without water there is approximate osmotic equilibrium between cells and interstitial fluid, it would then be likely that 52·2 per cent of the weight lost was provided by the gut fluids and 29 per cent by cell water, if both extracellular and cellular water spaces fell by 15·6 per cent. In this sense, the alimentary reserve would not be pure water but a more or less isotonic solution. By using the water component of gut fluids for cooling, and by excreting electrolytes in urine and sweat, the circulating volume would be preserved. In man and sheep the extracellular water takes most of the strain of rapid dehydration, and circulation is likely to be correspondingly unstable, so that men in the sun without water at 41° C succumb in 1–2 days, and tropical Merino sheep in 6 days after losing half the extracellular fluid. Camels survive probably more than 15 days in such conditions while sustaining, like sheep, over 30 per cent of loss of body-weight.

No major difference between the electrophoretic and ultracentrifuge pictures of sheep and camel plasmas was found, so that the differential fluid losses from extracellular and alimentary compartments appear to result from peculiarities of permeability of vascular and intestinal membranes, and the rate of dehydration modifies the amount of transfer from the different compartments.

We thank Prof. K. Schmidt-Nielsen for making it possible to undertake these measurements as part of investigations supported by grant No. G–17619 from the U.S. National Science Foundation. The Northern Territory Administration, Animal Industry Division (Mr. J. Whittem and Mr. A. Newsome) kindly made animals and laboratory space available.

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A DIFFERENCE BETWEEN REAL AND APPARENT VISUAL MOVEMENT

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A NUMBER of workers have confirmed the fact that detectability of the first of two briefly presented flashes of light varies inversely with the luminance of the second and the temporal separation between them. The results of many experiments on this topic have recently been summarized[1]. Extending these investigations on asynchronous stationary visual stimuli to the case of a moving masking stimulus has produced complementary data[2]. The results in the latter case were that the detectability of a brief fixed target stimulus flashed in the path of a moving line varied with the luminance and position of the latter. The brighter the moving line and the closer it was to the target, the less detectable was the latter.

These results provide the opportunity to investigate some properties of a related kind of visual perception, that of apparent (beta) movement. This illusion of movement occurs when two neighbouring stimulus objects of proper luminance are alternated in place at an appropriate rate. When two lines such as *A* and *B* of Fig. 1 are presented, each for 50 msec and a proper pause intervenes between the offset of each and the onset of the other, the observer sees a single line moving smoothly and continuously between *A* and *B*. The perception is referred to as 'optimal movement'[3]. The apparent movement of the line is a compelling illusion; and many investigators have reported that, for given rates of apparent displacement, the perceptions of real and apparent movement are indistinguishable one from the other. Since a number of these authors have been led by the phenomenal identity of the two perceptions to infer that the underlying mechanisms are similarly identical, the question arises whether a line in apparent movement will affect detectability of a target in the way described here for real movement.

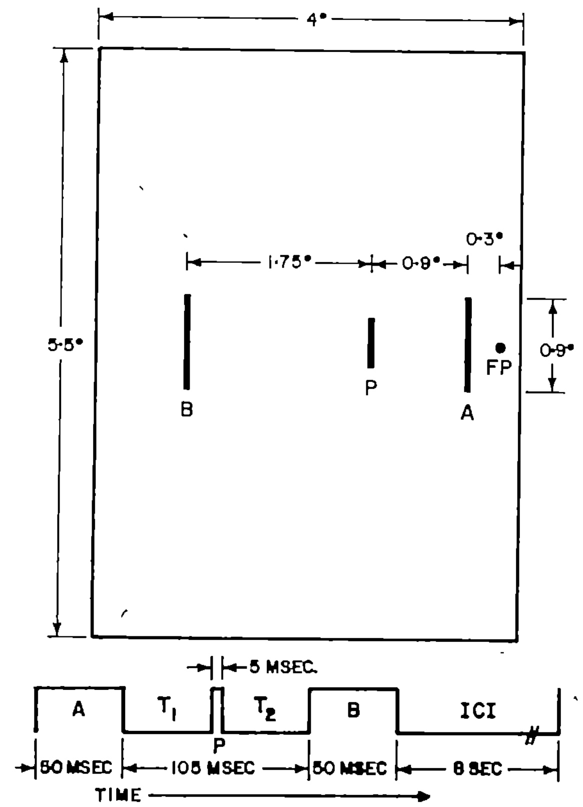

Fig. 1. Spatial arrangement of the alternated forms. *A* and *B* are the termini of apparent movement; *P*, the target or probe flashed in the path of movement; *FP*, the fixation point. The time diagram shows one sequence of presentations

To answer the question, it is necessary to specify the 'position' of the line in apparent movement during its traverse. This was done by fractionating the temporal interval between *A* and *B* that yielded optimal movement, and presenting the small line *P* for 5 msec at various times between the offset of *A* and the onset of *B*. One sequence of presentations is shown in the time diagram of Fig. 1. Let T_1 be the time between the offset of *A* and the onset of *P*, and T_2 the time between the offset of *P* and the onset of *B*. In the experiments, the sum of $(T_1 + P + T_2)$ was 105 msec, while the particular values of T_1 and T_2 were varied. For example, when T_1 was 5 msec, T_2 was 95 msec; when T_1 was 25 msec, T_2 was 75 msec; and so on. In this way, the 5 msec long *P* was made to appear when the line seen as moving between *A* and *B* had proceeded through various portions of its path.

The luminance of the lines *A* and *B* was 3 foot-Lamberts, while the luminance of *P* varied from 0·09 to 0·3 foot-Lamberts for each of three subjects. The latter value was established by finding for each subject the luminance of *P* when it was presented alone that enabled him to detect it with probability of 0·90+. The stimuli appeared in a 4 × 5·5° field of 0·002 foot-Lamberts luminance. Viewing distance was 75 cm and observations were made monocularly with a dark-adapted eye from within a light-tight booth through a 3-mm artificial pupil. A minute red light (< 2 min visual angle) provided a fixation point. Presentations were made at the rate of one every 8·2 sec, and a warning bell sounded 2 sec before the onset of a presentation.

The results were collected by presenting the subject with identical sets of stimulus conditions a large number of times in various mixed random orders and having him signal each time whether or not he had detected *P*. The relative frequency of detections, that is, the empirical probability of detection, is the measure used on the data. Each plotted point, thus, is the result of 80 or 100 observations for a given pair of T_1–T_2 values.

In addition to these conditions, the precaution was taken of presenting catch-trials: on approximately 16 per cent of all presentations, *P* was absent. The 'false alarm' rate in these trials was found always to be less than 10 per cent for all subjects, and will not be further considered.

The results are presented in Fig. 2, each curve showing the results for a single subject. The abscissa plots the time after the offset of *A*, and the ordinate shows the probability of detecting *P*. The curves show a generalized inhibitory effect: the probability of detecting *P* is always less than 0·90, its minimal values in the absence of *A* and *B*. Within that generalized inhibition, $p(P)$ increases slightly with an increase in T_1 and then decreases, for two subjects, while for the third, $p(P)$ decreases only.

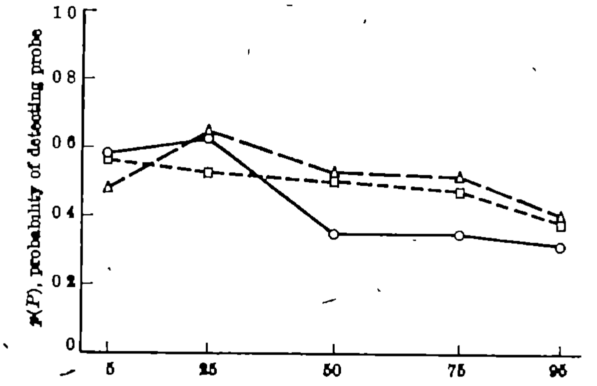

Fig. 2. Probability of detecting the target, *P*, in the path of apparent movement. The 5 msec long target appeared at a variable interval after the offset of *A*, that interval shown on the abscissa. Subjects *D* (○), *K* (△) and *P* (□)

As is shown in Fig. 1, *P* was placed approximately one-third of the distance between *A* and *B*, so that $T_1 = 25$ msec is the position closest to 'overlap' of the apparently moving line and the target. It is at this position, in Fig. 2, that detectability of *P* is highest for two subjects. Thus, while Fig. 2 shows that detectability of *P* varies with the 'position' of the apparently moving line, the form of this variation is exactly opposite that found with the line in real movement, cited here[3]. If the apparently moving line had acted on *P* in the same way as a physically moving line, the curves of Fig. 2 would be U-shaped: *P* would be maximally detectable when the apparently moving line was at the beginning or at the end of its traverse, and would be least detectable in the region $T_1 = 25$ msec. The curves clearly are not of this shape.

One major difference characterizes the stimulus conditions required for the perceptions of real and apparent movement: no image moves across the retina in the latter case. Apparently, then, the image of an object on the retina is required if the detectability of a contiguous object is to be affected. It may be inferred then that the phenomenal identity of the two perceptions notwithstanding, real movement and apparent movement partake of different processes, since their effects are different. The illusion of movement seems to be constructed by the observer out of the information provided by the order and timing of the flickered stimuli. The fragility of this construction will be described in another report.

The experimental work was performed in the U.S. Naval Medical Research Laboratory. Preparation of this report was supported by National Science Foundation grant *G*-16486 to the Center for Cognitive Studies, Harvard University.

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LETTERS TO THE EDITOR

ASTRONOMY

Distribution and Origin of Lunar Craters

THE communication by McCall[1] leads me to make some further comments. It is certainly true that the non-random distribution of the large craters is a serious and probably fatal objection to the impact hypothesis so far as the major formations are concerned, though no doubt many small meteoric craters exist. The Mare Crisium seems to be nothing more than an exceptionally large crater-like formation, and is a member of the great western chain which also includes Petavius and Langrenus; in which case it seems that the other circular-type maria are basically similar. Moreover, in the case where one crater breaks into another, the wall of the broken formation is perfectly preserved up to the point of junction; Thebit is a good case. This could not be so were the second, younger crater produced by a violent impact. I directed attention to this in 1956[2], and the same point has since been made independently by Kopal and others.

It seems, however, that this argument can also be used against the idea of violently explosive vulcanism, and it may be argued that the process of crater formation was relatively gentle and prolonged. In making this suggestion[3], I had in mind an uplift and subsidence process which, if repeated several times with interior remelting, would result in the production of a terraced crater.

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I HAVE elsewhere[1] expressed agreement with Mr. Moore's view that the lack of shaking-down effects provides an important clue to the origin of lunar craters, and at the same time expressed my own view that the structure of the crater Porter is inexplicable in terms of explosive evisceration, a process which I have never favoured, though simple dish-craters in maria could be produced by a bubble-burst through a thin plastic veneer. The fact that craters appear to have been the product of slow and gentle processes does not, in my view, invalidate my hypothesis. Certainly the large craters stem from subsidence, and, as on Earth, the initiating upwards push may not necessarily have been explosive in every case. The blisters seen in some craters probably reflect gentle pressures without break-out. It remains possible that many of the large cirques were initiated by point-focus explosion, forming axial vents and confocal fractures, not craters. This seems most likely in the case of early structures, since, once fractured, the retaining crust would allow slow gas leakage rather than explosive discharge. It is interesting to note that at least one carbonatite complex reveals some evidence of a recessive stage involving cauldron subsidence[2]. Moore's suggestion of gentle uplift and subsidence, repeated several times, fits in well with patterns of history in terrestrial volcanic foci, but, as on Earth, one must expect no uniformity of detailed history, and I do not think point-focus explosion can be ruled out, entirely, because of the objection made.

One of the most potent arguments for a volcanic origin of lunar craters is the progressive decrease in size with age. A large crater never shows evidence of developing on the same focus as an earlier smaller structure. This is the essential pattern of history displayed by terrestrial calderas: I do not know of a single recorded case where a secondary caldera has not been of diminished magnitude compared with its predecessor. I would be interested to know if other geologists can record such a case.

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PHYSICS

Re-entrant Motion in Special Relativity

THE argument by Dr. T. E. Phipps[1], in which he claims to show that the velocity at the edge of a rotating disk is not ωr and that ωr can be greater than c, contains a simple fallacy from the point of view of relativity theory.

The fallacy lies in the assumption that if the radius of the rotating disk is r then the "rest length" circumference of the disk will be $2\pi r$. This relation is true only when the disk as a whole is at rest in the inertial frame S. Of course, the radius of the disk when it is stationary in S will in general be different from its value when rotating. In the actual rotation considered an element of the boundary which has rest length δx^* in S^* suffers the Lorentz contraction when witnessed by the observer in S. Thus the observer in S ascribes to the circumference the total length $\Sigma\delta x = 2\pi r$, which is appropriate to the Euclidean geometry in his frame; on the other hand, the aggregate of measurements by observers in the local frames S^* on the boundary will be $\Sigma\delta x^* = 2\pi r/(1-v^2/c^2)^{\frac{1}{2}}$, albeit the disk dwellers would also ascribe a total length r to the radius. The non-Euclidean character of the geometry in accelerating frames of reference was inferred in this way by Einstein long ago and led him to adopt the Riemannian metric in general relativity.

Subject to this correction the rest of Phipps's calculations are now seen to be consistent with $|v| = |\omega r| < c$. Thus his equation (2) should read $2\pi r/T = -v$ and since, as Phipps agrees, $T = 2\pi/\omega$, (3) then becomes $v = -\omega r$ and because of the appearance of v in the factor $\sqrt{1-v^2/c^2}$ we can conclude as usual that $|\omega r| < c$.

Thus there is no question, as Dr. Phipps goes on to suggest, that the much discussed identical twins age equally and that both "stay young". The experiment of Hay *et al.*[2], discussed by Sherwin[3] to whom Phipps refers, is actually in reasonable agreement with the expected age discrepancy proportional to the factor $\sqrt{1-v^2/c^2}$.

Note added in proof. Since this communication was submitted letters from Dr. R. H. Boyer and Dr. D. B. Lichtenberg, pointing out the error in Dr. Phipps's argument that I have indicated above, have appeared in *Nature* together with Dr. Phipps's reply[4]. Nothing that has been said in these communications alters my view of the correctness of the interpretation of relativity theory given here.

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Observation of Mixing at Optical Frequencies in a *p–n* Junction

MIXING of light corresponding to adjacent axial standing wave modes of a ruby laser has been effected in a silicon *p–n* junction. The junction was prepared by grinding down the side of an *S.T.C. RS–28A* power rectifier diode to expose the junction. After protecting the metal casing with wax, the junction was treated with *CP*–6 etch, washed, dried and coated with distrine cement.

The reverse current after this treatment was 15 μamp at 100 V.

Light from the ruby laser was focused on to the junction, reverse biased at 108 V. The diode was situated in the centre conductor of a coaxial line feeding directly into a Rhode and Schwarz type *USVD* microwave receiver the output of which was displayed on an oscilloscope triggered at the beginning of the laser pumping flash.

Microwave output was observed in a narrow band at 1,635 Mc/s as a spiky trace on the oscilloscope. This frequency corresponds to the difference between neighbouring axial modes in the ruby rod 5 cm long. The power in a single spike in a 1 Mc/s band at the centre frequency was about 1 μW at the 50 ohms input to the receiver.

Output from the diode was also found at 3,270 Mc/s, which is the beat frequency associated with a pair of next-nearest neighbour axial modes.

Electron-hole pairs are generated in the semiconductor at a rate proportional to the intensity of the incident radiation so the device acts as a square law detector. The carriers generated in the depletion layer are swept out by the high field in a time short compared with a period of the beat frequency of the incident light from the laser, and it is this transit time in the depletion layer rather than minority carrier lifetime which limits the high-frequency performance of the mixer.

Inaba and Siegman[1] have reported mixing in a reverse biased *p–i–n* junction photodiode. The use of a *p–n* junction may offer an advantage at higher frequencies, since the carrier transit time across the depletion layer can be very short.

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Phenomena occurring at Electrically Stressed Metallic Surfaces in Long Gaps in Vacuum

Many hypotheses have been put forward attempting to explain the initiation of electrical breakdown between metallic electrodes insulated by high vacuum[1]. Of these, several depend on the removal of a loosely attached aggregate of material (a clump) from one of the electrodes[2,3]. Attempts to prove the existence of clumps in a vacuum gap have already been reviewed[4].

The main objections to a clump-type mechanism have been that the electrostatic forces acting prior to breakdown appeared to be insufficient to pull off clumps[5] and that loose aggregates would not exist on smoothly polished clean electrodes.

During an investigation of the breakdown mechanism in long vacuum gaps, smooth uniform-field commercial copper electrodes and 1/50 negative impulse voltages were used, and the resultant markings at the electrode surfaces due to single breakdowns were examined[6]. In several cases 'clover leaf' patterns, which were indicative of the removal of material from the actual electrode metal, were found on the anode surface. At the corresponding cathode surfaces damage was found similar to that caused by the impact of particles[7].

In the case shown in Figs. 1 and 2 the electrodes had been polished to a 1μ diamond paste finish, the gap was 2 cm and the peak of the applied voltage wave-form causing breakdown was 73 kV. The strain marks at the anode and the impact bands at the cathode can be clearly seen. The embedded particles in the cathode are almost certainly those torn out from the anode as the distance between the centres of the two sets of markings is identical, being 2×10^{-3} cm. These particular clumps did not cause the breakdown because damage caused by the arc was found elsewhere on the electrodes, and had the arc arisen in this area the markings would have been destroyed due to the heating by the arc. This then is direct evidence that clumps can arise due to the tearing out of material rather than the removal of loosely attached aggregates, even though the forces acting at the surface are many magnitudes below the ultimate tensile strength of the material. The mean electrostatic stress acting on the surface in the present case was about 10^{3} dyn/cm².

Carbon replicas were taken from a thermally etched surface of the same grade of commercial copper which had been heated to 1,000° C in vacuum in a hot-stage microscope. Apart from general etching effects, these revealed the presence of dendritic inclusions, a typical example being shown in Fig. 3. The close resemblance in shape to the previously mentioned anode markings seems significant. Comparison of the diameters of the markings and the inclusions from photographs showed that the relative sizes were within an order of magnitude. The larger diameter of the anode markings may be attributed to the

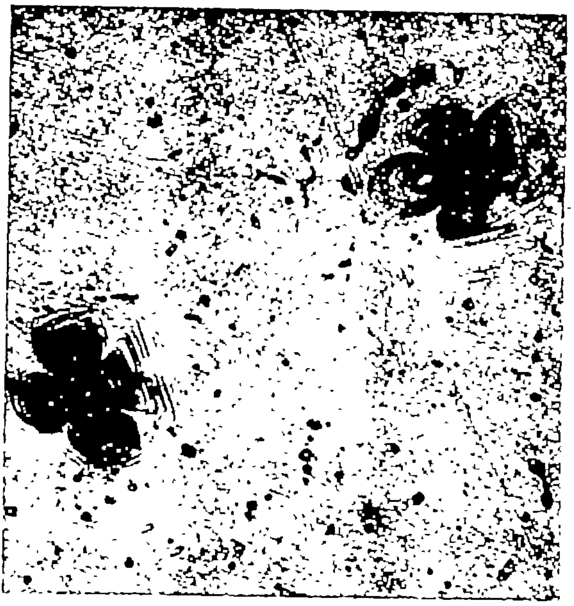

Fig 1. Photomicrograph showing 'clover leaf' patterns on copper anode (× c. 244)

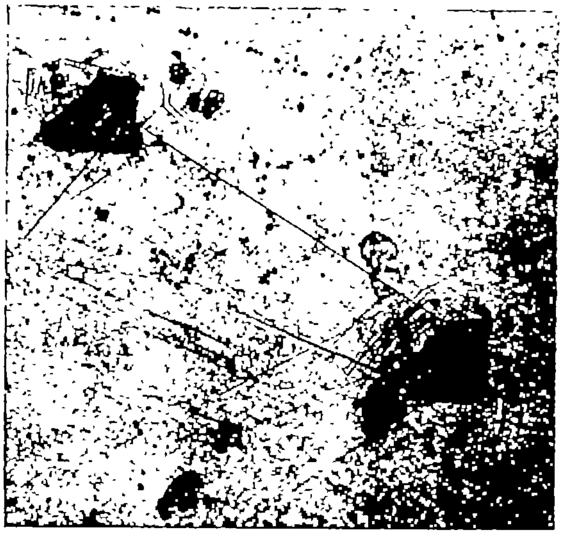

Fig 2. Photomicrograph showing impact damage on copper cathode (× c. 225)

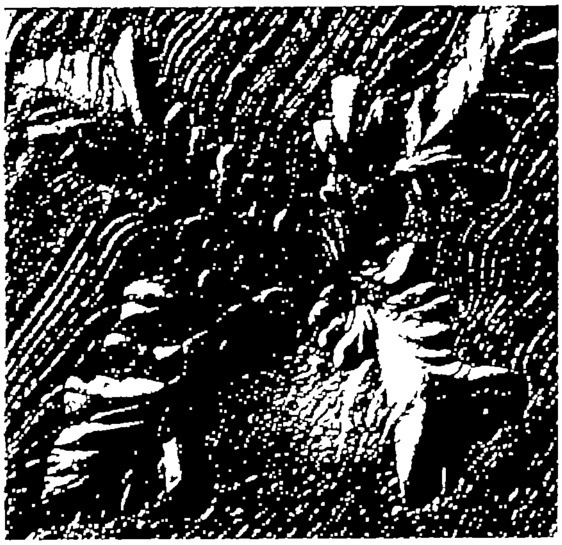

Fig. 3. Electron micrograph of inclusion in commercial copper (× c. 4,000)

deformation accompanying removal of the inclusions. The electrostatic stress required to remove such inclusions would be considerably less than the ultimate tensile strength of the metal itself due to the low cohesive forces usually existing between inclusion-metal interfaces. This is generally about 10³ dyn/cm, the average surface tension for most metals.

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Interaction of Amino-Acids with Riboflavin

IN two recent papers it has been shown[1,2] that amino-acids, both aromatic and non-aromatic, interact with the electron acceptors oxygen and chloranil (tetrachloroquinone) in a very similar manner to the aliphatic amines. It is thought that $n-\pi$ charge transfer complexes are formed, the donated electron being one of the lone-pair electrons localized on the nitrogen atom in the amino group.

Isenberg and Szent-Györgyi[3] have investigated the interaction of amino-acids with another quinone, riboflavin-5′-phosphate, and have come to the conclusion that only tryptophan reacts with this salt, forming a $\pi-\pi$ charge-transfer complex. They showed that adding tryptophan to riboflavin-5′-phosphate decreased the optical density of the quinone salt and caused a new absorption band to appear at about 510 nm. Weber[4] has investigated purine–riboflavin complexes and has shown that the absorption spectrum of riboflavin is weakened on adding purine but a slight increase occurs in the long wave-length tail.

Studies have been made of spectra of mixtures of riboflavin and amino-acid in aqueous buffer. It was observed that adding a 10^{-2} M amino-acid solution to 10^{-4} M riboflavin solution in aqueous *p*H 7 buffer caused certain changes in the absorption spectrum of the riboflavin. These changes were observed as the difference spectra of riboflavin and riboflavin plus amino-acid. The difference spectra appeared as a negative mirror image of the riboflavin absorption in the region of 340–500 nm. The difference spectra also give a positive peak at about 300 nm and in some cases a very faint positive peak at 500 nm. There is an isobestic point at about 330 nm. These effects were found to be quite similar for the aromatic amino-acids, tryptophan, phenylalanine and tyrosine and the non-aromatic amino-acids, glycine, alanine and leucine. Typical spectra are shown in Fig. 1. It was found that the riboflavin solutions were not stable, but that over a period of a day or so, the spectrum of the riboflavin changed from that of the oxidized form to that of the reduced form[5]. It was found that the reduction of riboflavin occurred quite rapidly in aqueous *p*H 9 buffer. The difference spectra between riboflavin and riboflavin plus amino-acid in aqueous *p*H 9 buffer were found to be positive with an isobestic point in the region of 330 nm as shown in Fig. 2. All the amino-acids behaved in a similar manner with riboflavin in aqueous *p*H 9 buffer.

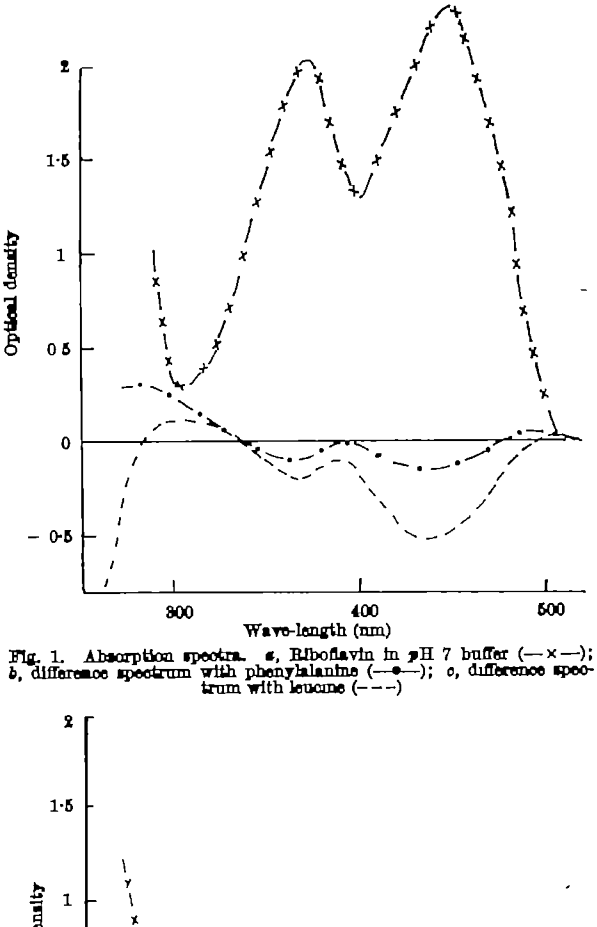

Fig. 1. Absorption spectra. *a*, Riboflavin in *p*H 7 buffer (—×—); *b*, difference spectrum with phenylalanine (—●—); *c*, difference spectrum with leucine (– – –)

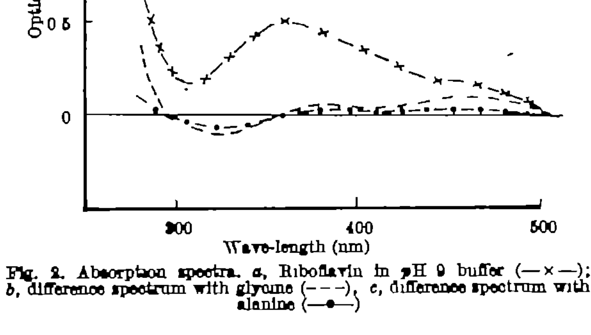

Fig. 2. Absorption spectra. *a*, Riboflavin in *p*H 9 buffer (—×—); *b*, difference spectrum with glycine (– – –), *c*, difference spectrum with alanine (—●—)

Beinert[5] has investigated the spectrum of riboflavin as it goes from the oxidized to the reduced form. The absorption spectrum of oxidized riboflavin has peaks at 375 and 445 nm whereas the reduced riboflavin only has a peak at 251 nm and has less absorption than the oxidized form. There is an isobestic point in the spectrum at 330 nm. Beinert has concluded that the spectrum of riboflavin is due to the sum of three components, the oxidized form, the reduced form, and an intermediate form, the semiquinone.

The difference spectra observed in amino-acid–riboflavin mixtures at different pH's can best be explained by the stabilization of the semiquinone form of riboflavin by the amino-acid. Isenberg and Szent-Györgyi[3] have suggested that the tryptophan riboflavin-5′-phosphate interaction can be explained by the tryptophan forming a $\pi - \pi$ charge-transfer complex with the riboflavin-5′-phosphate thus:

$$\text{tryptophan} + \text{riboflavin-5}'\text{-phosphate} \rightleftharpoons \left(\begin{array}{c}\text{oxidized tryptophan–semiquinone}\\ \text{riboflavin-5}'\text{-phosphate}\end{array}\right)\text{complex}$$

Such a scheme could well describe the interaction of the amino-acids with riboflavin, except that the donated electron reducing the riboflavin should be a lone-pair (n) electron, probably from the amino-group. There appears no reason why only tryptophan should interact with riboflavin-5′-phosphate to give a $\pi - \pi$ complex, whereas the amino-acids as a whole react with other quinones such as chloranil and riboflavin in such a manner that an n-electron is transferred from the amino-acid to the quinone.

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GEOPHYSICS

Formation of Mountains by Radial Contraction of the Earth

MODERN ideas on the origin of the planets suggest that the Earth began as a cool solid body, and on this basis the development of a liquid core, of gradually increasing size, would be ascribed to subsequent radioactive heating.

The elastic constants of the liquid core and of the solid mantle for the actual Earth are known, and these imply that the core-material, although liquid, is more compressible than the mantle-material at the pressures prevailing deep within the Earth. Making use of the known values, the properties of a linear series of Earth-models of gradually increasing core-mass can be investigated, beginning with an entirely solid Earth. Calculations show that the radius decreases as the core-mass increases, and that an initial solid Earth would have outer radius 1·043 times the present value R_E, while allowance for the lower uncompressed density of the material of the outer layers would increase this figure to 1·056 R_E. This result would mean an initial circumferential length more than 2,000 km greater than the present value, and an initial surface-area exceeding by nearly 60 million square kilometres the present area of the Earth.

The importance of such a contraction of the Earth as a whole to the problem of the formation of mountains is evident, since it would gradually build up shearing stresses in the outer layers that the materials there would eventually be unable to withstand, and periods of folding and thrusting would have to occur intermittently to relieve the stresses. The mechanism appears to be fully capable of providing the seemingly enormous amounts of contraction that have long been considered necessary by geologists to explain the actual mountain systems on Earth.

The mathematical theory and detailed calculations leading to these conclusions are in course of publication.

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GEOCHEMISTRY

Low Concentration of Tritium in Iron Meteorites

A SERIOUS geochemical problem that is not yet solved is the quantity of tritium found in iron meteorites. Although all other isotopes produced by cosmic rays in both iron and stone meteorites are in close agreement with expected values estimated by cross-section measurements using large accelerators[1], tritium is either absent or at least an order of magnitude less than expected in iron meteorites (for example, ref. 2, and the references listed in that article). The tritium content of stone meteorites is not abnormal.

Two explanations have been proposed for the low concentration of tritium in iron meteorites. It has been suggested that the chemical treatment methods are inefficient and the tritium is not extracted from the iron. This, however, seems difficult to believe since the two different chemical methods used[2,3] are both reliable and completely attack the iron. Furthermore, the hydrogen gas added as carrier and dissolved in the iron is always recovered, quantitatively.

The other explanation proposed is that tritium diffuses out of iron meteorites. However, direct diffusion measurements of tritium in iron do not agree with this and the ratio of helium-3 to helium-4 in iron meteorites is in close agreement with that predicted from accelerator measurements[4]. This requires that the tritium formed in the meteorites during the flights in space must have remained in the meteorites and decayed to helium-3. Another suggestion is that a special mechanism permits the tritium to diffuse out of the iron meteorites only after they have reached the Earth. At present, there is no plausible theory that would predict this.

Further evidence supporting the diffusion loss hypothesis is the observation of what is called a diffusion of tritium out of iron acceleration targets[5,6]. However, an examination of the experiments reveals that diffusion was not directly observed, but merely the phenomenon that the longer the iron targets remained untreated after the irradiations, the less tritium was extracted. It is noted that this is not observed in other metal targets.

Since neither of the two aforementioned hypotheses seems reasonable, the tritium content of iron meteorites is now an important mystery for science and, therefore, all sorts of possibilities should be checked. I suggest here the possibility that a completely new phenomenon is being observed. That is, that tritium dissolved in iron, possibly denuded of its electrons and held in a strong magnetic field, no longer decays with its ordinary 12-years half-life, but much more rapidly, perhaps with a half-life of the order of several days or even less. It seems that both a combination of a nucleus without an ordinary electron cloud and the presence of a magnetic field would be necessary.

An experiment is possible to test this proposition. A stack of iron foils irradiated in an accelerator or otherwise concentrated with dissolved tritium could be submitted, after various lengths of time, to helium-3 analyses. Presumably, if the tritium formed in the iron is decaying with an abnormally short half-life, a progressive build-up of helium-3 in the target foils would be observed which would be analogous to the decreasing amount of tritium that could be extracted.

It is realized that the suggestion of a half-life change is contrary to the at present well-established theory of β-decay, but the low concentration of tritium in iron meteorites is so great a puzzle it appears possible that a new effect is acting on the tritium.

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Rates of Calcium Carbonate Deposition by Deep-Sea Organisms, Molluscs and the Coral-Algae Association

WHILE completing reports on the marine geochemistry of strontium and on calcium carbonate sedimentation in the Atlantic Ocean, I found that sufficient information was available to make an estimate of the calcium carbonate accumulation rates in the sea by deep-sea and littoral processes. The major agents of calcium carbonate accumulation in the littoral areas are presumably molluscs and the coral–algae association.

In considering the alkaline-earths, calcium and strontium, it is evident that, if the sea is in the steady state (an assumption which seems reasonable at least from the Tertiary), then a material balance equation may be written for each element in the following manner:

$$\text{calcium: } R_M + R_C + R_D = R_S$$
$$\text{strontium: } 1{,}500R_M + 8{,}000R_C + 2{,}500R_D = 2{,}500R_S$$

where R_M, R_C, and R_D are the rates of molluscan, coral–algae, and deep-sea calcium carbonate accumulation respectively and R_S is the rate of supply of calcium, as calcium carbonate, from streams (corrected for cycled calcium from the sea). In the strontium equation the numerical coefficients represent the strontium concentrations (in parts per million) in molluscs[1], corals and algae and 'inorganic aragonite' associated with them[2], deep-sea carbonate sediments[3], and streams[4].

Because of the observed identical values of the strontium–calcium ratios in deep-sea carbonates and streams, these equations can be easily solved for the R_M/R_C ratio. This solution indicates that calcium carbonate is put into the littoral sediments five and a half times more by molluscs than by the coral–algae association. The reasonableness of this ratio is, of course, contingent on the magnitudes of the littoral and deep-sea accumulation rates being similar.

It is possible to calculate the total accumulation rate in the deep-sea if we know the average calcium carbonate concentration of the deep-sea sediments of the major oceanic areas and the corresponding clay accumulation rates, using the relation:

$$\text{Rate calcium carbonate accumulation} = \text{rate clay accumulation} \times (\%\ \text{calcium carbonate}/100 - \%\ \text{calcium carbonate})$$

The mean calcium carbonate concentrations of sediments for both the Atlantic and Pacific Oceans are derived from the average concentration in sediments at different ocean depths[5] and the percentage of each depth in the two oceans[6] (Table 1).

Table 1. CALCULATION OF TOTAL RATES OF CALCIUM CARBONATE ACCUMULATION IN THE OCEANS

Depth (m)	Atlantic % area	Atlantic Average % $CaCO_3$	Atlantic Weighted contribution	Pacific % area	Pacific Average % $CaCO_3$	Pacific Weighted contribution
0–200	5·6	80	4·49	1·7	78	1·33
200–1,000	4·0	80	3·20	2·2	78	1·72
1,000–2,000	3·6	80	2·88	3·4	78	2·67
2,000–3,000	7·6	70	5·32	5·0	78	3·90
3,000–4,000	19·4	70	13·58	19·1	74	14·13
4,000–5,000	32·4	63	20·40	37·7	35	13·20
5,000–6,000	26·6	20	5·32	28·8	3	0·87
6,000–7,000	0·8	5	0·04	1·8	0	—
> 7,000	—	—	—	0·3	0	—
Average % $CaCO_3$			55·23			37·82
Total area (× 10^6 km^2)			82·441			165·246

Using the palaeontological index for the 11,000-year break between the last glacial and post-glacial deposits observed in a number of Atlantic carbonate-rich cores[7], I have been able to calculate clay accumulation rates in post-glacial time for the seven Atlantic cores listed in Table 2. The post-glacial rates of accumulation determined by assuming that the tops of the cores represent an age of 3,000 years is preferred because many radiocarbon-dated core tops indicate that this is the most common age to expect. Most probably this old age is due to loss of the sediment tops by the sampling procedure. The beginning of the last major glacial series (Wurm ?) has been estimated at 73,000 years B.P.[8] and the average glacial rates are calculated on this basis.

Table 2. RATES OF CLAY ACCUMULATION IN THE ATLANTIC DEEP-SEA (G/CM^2 1,000 YEARS)

Lamont No.	Post-glacial rate *A*	Post-glacial rate *B*	Glacial rate *C*
*A*164–44	0·11	0·15	0·45
*V*9–27	0·13	0·18	1·02
*SP*8–4	0·14	0·19	0·33
*A*180–39	0·15	0·21	0·35
*R*5–60	0·24	0·33	0·43
*A*180–74	0·22	0·22	0·66
*A*180–76	0·25	0·34	0·54
Average	0·18	0·23	0·54

A, Assuming top of core not missing; $\Delta t_{\text{post-glacial}} = 11{,}000$ years.
B, Assuming top of core missing; $\Delta t_{\text{post-glacial}} = 8{,}000$ years.
C, Assuming beginning of last glacial sequence is 73,000 B.P.; $\Delta t_{\text{glacial}} = 62{,}000$ years.

The Pacific Ocean apparently has a clay accumulation rate which is one-third or less of the Atlantic rate[8] and on this premise we can calculate the Pacific carbonate accumulation rate. With the paucity of data on the Indian Ocean at present, I will assume that the total calcium carbonate accumulation rate in the Indian Ocean is the same as in the Pacific.

The amount of uncycled calcium brought to the sea by streams is 62 × 10^{14} g calcium/1,000 years, or 154 × 10^{14} g calcium carbonate/1,000 years, using Clarke's estimates[9]. Knowing the supply of calcium to the sea by streams and the amount removed in the deep-sea, we can arrive at the removal rate of calcium by the littoral processes.

Having determined the ratio of molluscan to coral accumulation rates, we can now distribute the calcium carbonate supplied by streams among the three major modes of accumulation in the oceans. These are listed in Table 3 for various rates of clay accumulation. *A*, *B*, and *C* correspond to the three accumulation rates under the same headings in Table 2. *D* is calculated for a model[10] of the oceans which implies that though the Atlantic clay accumulation rate is sensitive to the climatic events of the Pleistocene, the Pacific is relatively insensitive so that for the past 11,000 years the maximum post-glacial rate (*B*) is used to calculate the Atlantic carbonate contribution and

Table 3. MATERIAL BALANCE FOR CALCIUM CARBONATE SUPPLY AND REMOVAL IN THE SEA (IN UNITS OF 10^{14} G $CaCO_3$/1,000 YEARS)

		Stream supply	Deep-sea	Molluscs	Coral–algae
A	Minimum post-glacial rate	154	30	106	18
B	Maximum post-glacial rate	154	39	97	18
C	Average glacial rate	154	91	53	10
D	Atlantic at maximum post-glacial rate and Pacific and Indian at 1/3 Atlantic glacial rate	154	60	80	14

one-third the Atlantic glacial rate (*C*) is used for the Pacific contribution from which the Indian Ocean contribution is then derived.

As our calculations of material balance are based on post-glacial data from the Atlantic cores and present-day stream supply of calcium and strontium, we are constrained to consider only estimates *B* and *D* seriously. This limits our range of accumulation rates of calcium carbonate (in units of 10^{16} g calcium carbonate/1,000 years) to: deep-sea, 39–60; molluscs, 80–97; and the coral–algae assemblage, 14–18.

From these calculations, several conclusions may be drawn:

(1) Of the calcium carbonate supplied to the sea by streams, the percentage deposited by deep-sea processes is between 25 per cent and 39 per cent. The major role of littoral processes in removing calcium from the sea at the present time implies that only small changes in the calcium carbonate accumulation in littoral areas are necessary before the Mesozoic when deep-sea carbonate accumulation may have been trivial. Indeed, if the present time represents a high supply of calcium to the sea compared with the past, as a consequence of extensive limestone exposure and erosion, no unusual requirements are necessary for carbonate deposition in the past.

(2) Because of the major role of littoral deposition it is not possible to decide whether the higher-than-present calcium carbonate deposition rate observed in the Atlantic Ocean during glacial times[8] was due to greater run-off or a higher ratio of the deep-sea to littoral accumulation rate.

(3) Coral reefs represent only 10 per cent of the total calcium carbonate deposition at present. Variations in their rate of formation will not seriously affect the total calcium budget of the sea though it might be reflected in the strontium budget.

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RADIOCHEMISTRY

Separation of Radioiodine from Tellurium by Means of Column Chromatography

THIS communication describes some experimental work carried out concerning the separation of iodine-131 from tellurium-131 by means of column chromatography. The method can also be applied to the separation of ^{132}I (half-life, 2·3 h) from ^{132}Te (half-life, 77 h) which is obtained as a fission product following neutron bombardment of uranium.

The nuclide ^{131}I (half-life, 8·04 days) is produced by the bombardment of natural tellurium with neutrons according to the nuclear reaction: $^{130}\mathrm{Te}(n,\gamma)^{131}\mathrm{Te} \xrightarrow{\beta^-} \mathrm{I}^{131}$. The mother product, ^{131}Te, decays with a half-life of 1·25 days to form ^{131}I. Other isotopes produced in the bombardment are ^{129}I from the reaction: $^{128}\mathrm{Te}(n,\gamma)^{129}\mathrm{Te} \xrightarrow{\beta^-} {}^{129}\mathrm{I}$ (half-life = 1·7 × 10^7 years), and also ^{129}Sb (half-life = 4·6 h), the origin of which is not clearly known, but is thought to be due to an (*n,p*) reaction. Neither of these isotopes presents any difficulty so far as the separation is concerned.

In the present case the target material took the form of telluric acid and was irradiated as the solid for a two-day period in a neutron flux of 10^{13} n/cm^2/sec, in the *Astra* reactor at Seibersdorf near Vienna.

The column was prepared in the following manner: A sheet of glass paper, of type Schleicher and Schull No. 6, was cut into small sections and treated for about 10 min in a mixture of 10 per cent sodium fluoride, 5 per cent hydrofluoric acid and weak hydrochloric acid in the ratio 1 : 1 : 1. This treatment etched the paper surface making it rougher. The liquid was then poured off and the paper washed with distilled water until all trace of the acid wash had been removed. Finally, the paper was soaked in absolute alcohol and packed into the column. The column-packing material could also consist of glass wool or glass powder.

The neutron irradiated telluric acid was dissolved in 1–2 ml. of distilled water and poured on to the top of the column bed. In this way about one-fourth of the absorbent material becomes saturated with the telluric acid solution. Allowing absolute alcohol to flow through the column precipitates the telluric acid in a finely distributed form while simultaneously eluting radioactive iodine.

Both analytical and nuclear spectroscopic studies have shown that the separated iodine is completely free from other contaminants. We would therefore like to point out that the described method has the advantage of being suitable for use where high levels of activity are to be processed since none of the components involved is likely to be effected by radiation damage. The method is, therefore, especially useful for the separation and production of ^{131}I and ^{132}I for medical purposes.

We thank Prof. B. Karlik for her interest in this work and Prof. Nils Ryde of the Department of Physics, Gothenburg, for the necessary leave of absence granted to one of us (W. P.). The same author thanks the Swedish Council of Atomic Research for financial support. We also thank the Bundeskanzleramt der Republik Österreich, Sektion IV, Verstaatlichte Betriebe, for its financial support.

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Rapid Non-destructive Analysis for Gold in Copper–Gold Mixtures by Neutron Activation

IT is known that 7·3-sec gold-197m is formed by neutron irradiation of natural gold and that it emits 0·279-MeV γ-ray. A preliminary experiment was made by Anders[1] using neutrons at a flux of 2 × 10^8 n/cm^2/sec, where only relatively few counts of γ-rays were collected for samples containing 15 mg of gold.

In our method a neutron flux of 3 × 10^{11} n/cm^2/sec is used. A sample is placed in a nuclear reactor for 4·5 sec, cooled for 5 sec, counted for 5 sec, again cooled for 1 sec and counted for 5 sec. The cadmium ratio at the irradiation position is about 3·1. The counter used is a 2-in.

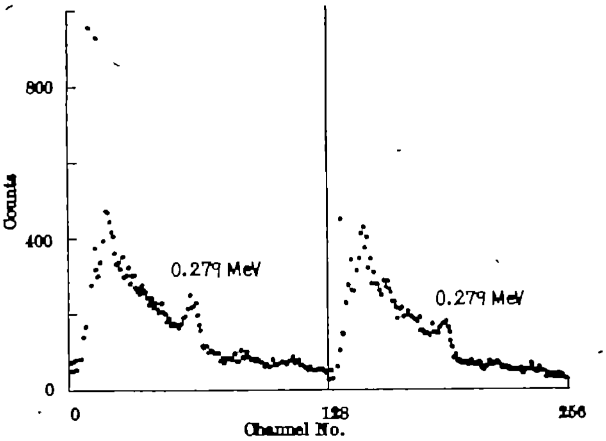

Fig. 1. γ-Ray spectrogram. Sample: 11·95 mg of a mixture of gold (9·4 per cent) and copper (90 6 per cent), both of which are in pieces

thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser. During the counting operation the sample is placed 1 cm above the crystal together with its container. An aluminium disk of 2,150-mg/cm^2 thick is used as an absorber for β-rays from the sample.

An example of successive spectra thus obtained is shown in Fig. 1. From both γ-ray energy and half-life, gold is identified on the basis of the fact that, in the half-life range of 1–10^2 sec, there is no other nuclide having nearly the same γ-energy as gold-197^m. From Fig. 1, which represents the spectra of a sample of 9·4 per cent gold content, it is deduced that one-third of this concentration would produce an adequate size of photo-peak. The lower limit of the coefficient of variation of single analyses can be deduced to be about 5 per cent in comparison with a similar method[2] of the determination of ytterbium.

Though the neutron-absorption cross-section of gold is rather high, the self-shielding effect is negligible for 12-mg sample used in this work. The effect can be estimated, by use of experimental data[3], at less than 5 per cent if the amount of gold in a sample is less than 0·06 g. (Larger amounts can be applied if the sample is not a massive block, or if gold is dispersed in copper.)

We thank Prof. Shoji Makishima for helpful suggestions, Dr. Fumio Aoki and Dr. Toshio Nakai for their interest, and Mr. Shigeru Yamagishi for assistance.

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Rapid Microdetermination of Indium by Neutron Activation

IT is known that 2·5-sec indium-116mm (ref. 1) is formed by neutron irradiation of natural indium and that it emits 0·15-MeV γ-ray. The size of the photopeak due to this γ-ray, obtained immediately after irradiation, can be estimated by the use of the data presented in a γ-ray spectra catalogue[2]. The peak size per 1 μg of indium is as large as that of silver when they are irradiated respectively for a period equal to the half-life of the nuclide formed, that is, 2·5 sec for indium and 24 sec for silver. Since silver is the most sensitive[3,4] in γ-ray counting after neutron irradiation, it is deduced that indium is also very sensitive.

In our experiment, varying amounts (0·01 ~ 10 g) of a sample were placed by turns in the nuclear reactor *JRR*–1 for 2·5 sec to be irradiated with neutrons at a flux of 3 × 10^{11} neutrons per cm^2/sec, cooled for 5 sec, counted for 4 sec, again cooled for 1 sec and counted for 4 sec. The counter used is a 2-in. thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser. During the counting operation the sample was placed at 1 cm above the crystal together with its container. An aluminium-disk of 2,150-mg/cm^2 thick was used as an absorber for β-rays from the sample.

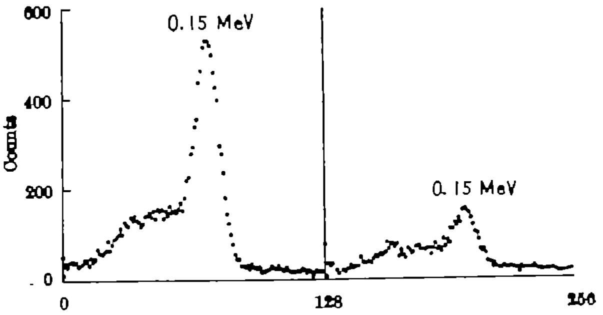

Fig. 1. γ-Ray spectrogram. Sample, 43·5 mg of zinc blende

A typical spectrogram thus obtained is shown in Fig. 1. From such successive spectra as Fig. 1 the half-life of the nuclide formed can be roughly determined. From both γ-ray energy and half-life, indium can be identified on the basis of the fact that, except indium-116mm, there is no nuclide, formed on pile-neutron irradiation, which emits a γ-ray of about 0·15 MeV and has a half-life of 1 ~ 10 sec. The amount of indium in the sample can be determined by the comparative method as usual. The results of analyses are shown in Table 1.

Table 1. RESULTS OF ANALYSES FOR INDIUM

Sample	Sample weight used (mg)	Indium found (p.p.m.)
A (zinc blende powder)	43·5	1 3 × 10^2
B (thallium slab)	3,715	3·6 × 10^{-1}
C (zinc grains)	6,019	< 3 × 10^{-3}

The coefficient of variation of single analyses can be deduced (from a similar experiment[5] in which erbium was determined by use of the formation of 2·5-sec erbium-167m) to be about 10 per cent if the activity-level is suitable to the analyser used and interferences are negligible.

We thank Prof. Shoji Makishima for helpful suggestions, Dr. Fumio Aoki and Dr. Toshio Nakai for advice, and Mr. Shigeru Yamagishi for assistance.

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Some Products of the γ-Radiolysis of Liquid Acetone

BY irradiation of air-free liquid acetone the radicals H˙, $\dot{C}H_3$, $CH_3\dot{C}O$ and $CH_3CO\dot{C}H_2$ are formed[1–3]. Besides the known gaseous products[1] (methane, hydrogen, carbon monoxide and ethane), a mixture of oxygen containing liquid products arising from the radical–radical and radical–excited acetone reactions is formed.

I have studied this mixture partly by paper chromatography (carbonyl compounds as 2,4-dinitrophenylhydrazones (DNPH), hydroxyl compounds as 3,5-dinitrobenzoates (DNB) and acids as ammonium salts), partly by thin layer chromatography on alumina. Separated products were identified by comparison with authentic specimens, by their R_F values and by detection.

Acetone (reagent grade) was purified according to Timmermans and Gillo[4], dried over calcium chloride and potassium carbonate, distilled on a 40 *TP* column in an atmosphere of nitrogen and degassed in a 10 ml. 'Sial' cell at $-196°$ C on the vacuum line until a static pressure of about 10^{-5} mm mercury was obtained and finally sealed off. The irradiation was carried out at room temperature using γ-rays from a cobalt-60 source[5] with a dose rate of 0·5–1·2 × 10^{17} eV/ml. min. The Fricke dosimeter was used ($G_{Fe^{3+}} = 15{\cdot}6$) and total doses of about $10^{20} - 10^{21}$ eV/ml. were attained.

For paper chromatography of DNPH and DNB, sheets of Whatman paper No. 3 were impregnated with a 25 per cent ether solution of dimethylformamide and the mixture hexane/benzene acetone/dimethylformamide/water (75 : 25 : 10 : 10 : 0·5, v/v) was used as developing solvent; after having been reduced with stannous chloride the DNB were detected with *p*-dimethylaminobenzaldehyde[6]. Ammonium salts of acids were analysed as usual[6]. Thin layer chromatography on alumina grade III–IV (Brockmann) was carried out in a mixture of hexane/acetone (4 : 1, v/v), the products being detected by iodine vapour or by spraying with ammoniacal solution of silver nitrate at 140° C.

By the use of paper chromatography we have found these products in form of DNPH : formaldehyde, acetaldehyde, biacetyl, acetonylacetone, diacetone alcohol and traces of two other substances, probably methyl ethyl ketone and methyl isobutyl ketone; DNPH of an unknown substance with R_F value 0·61 was also found. In the form of DNB we have found methanol, ethanol, isopropyl alcohol, diacetone alcohol, pinacol, acetyl acetone in enol-form and several unidentified spots with R_F values 0·11, 0·20, 0·31, 0·37, 0·45 and 0·91. Some of them might be by similar R_F values mono-DNB and di-DNB of glycol, 1,2-propanediol and 2,3-butanediol. Only acetic and oxalic acids have been found on chromatographing the ammonium salts.

Acetyl acetone, acetonylacetone, diacetone alcohol, pinacol and acetic acid were identified also in free form by thin layer chromatography on alumina. In some of the samples biacetyl was found, too. In addition to these, spots of unknown compounds with R_F values 0·21, 0·50, 0·55 and 0·82 were found. The first of them exhibits strong fluorescence in ultra-violet light; the last is probably phoron. The other (R_F values, 0·50 and 0·55) can be revealed by spraying with ammoniacal solution of silver nitrate at 140° C; therefore it might be some hydroxy compounds.

According to our chromatographic analysis we are able to estimate that acetonylacetone and isopropyl alcohol are the main liquid products of irradiated acetone so far. Obtained results are also corroborated by infra-red spectra. The details of these experiments as well as related studies and the supposed mechanism of the γ-radiolysis of liquid acetone will be reported at length elsewhere[7].

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CHEMISTRY

A Mathematical Basis for the Solvent-Reversal Technique of Paper Chromatography

THE applications and limitations of one- and two-dimensional paper chromatographic techniques have been well documented[1]. There remain, however, problems in the separation of certain compounds which cannot be resolved satisfactorily by either of these conventional approaches. Weiss and Rossi[2] recently described a chromatographic method which enables separation of several catecholamines on a single paper strip.

Briefly, the method consists of developing the chromatogram with one solvent system, by the ascending or descending technique, drying the paper, and then redeveloping with a second solvent system, again by the ascending or descending technique. However, the second solvent is run in a direction opposite (180°) to the first solvent.

The solvent-reversal method, by the proper choice of solvent systems, will permit certain separations which could not be achieved by conventional procedures. This may be accomplished by selecting two solvent systems in which the mobilities of the compounds which are most difficult to separate are in reverse order (that is, compound *A*, R_F with solvent 1 > R_F with solvent 2; compound *B*, R_F with solvent 1 < R_F with solvent 2). Migration of the compounds behind the original starting line is prevented by designating as the first solvent system that in which the compounds have, on the average, higher R_F values.

With reference to Fig. 1, we define the quantities 'net R_F' and 'solvent distance ratio' as follows:

$$\text{net } R_F = R_N = \frac{D_3 - D_4}{D_1} \qquad (1)$$

$$\text{solvent distance ratio} = X = \frac{D_2}{D_1} \qquad (2)$$

(When the second solvent front does not cross the original starting line, D_2 is negative, and X is negative.)

The following relationships are then evident:

$$R_{F1} = \frac{D_3}{D_1} \qquad (3)$$

$$R_{F2} = \frac{D_4}{D_3 + D_2} \qquad (4)$$

Substitute in equation 1 for D_3 (from equation 3) and re-arrange to obtain:

$$D_4 = R_{F1}D_1 - R_N D_1 \qquad (5)$$

From (2), (3) and (4), it follows that:

$$D_4 = R_{F2}(R_{F1}D_1 + XD_1) \qquad (6)$$

Equating (5) and (6), eliminating D_1, re-arranging and simplifying, the final equation, in its most useful form, is obtained:

$$R_N = R_{F1}(1 - R_{F2}) - R_{F2}X \qquad (7)$$

This relationship (equation 7) is a linear equation relating the variables R_N and X; slope $= -R_{F2}$, and intercept $= R_{F1}(1 - R_{F2})$. A plot of R_N versus X for a given compound having known R_F values for two solvent systems will yield a straight line from which R_N at any value of X may be determined by inspection. If this procedure is followed for two or more compounds, a series of straight lines is obtained and a value of X may be determined which permits separation of the compounds.

As an illustration of the method, plots were made for several compounds (Fig. 2) and checked experimentally by using the solvent-reversal technique to achieve separation. In the studies reported in Table 1, all R_F values in individual solvent systems were determined in our ble 1. Adsorption of Oxygen (Press 0·4–0·2 mm) on Nickel Oxide at 20° C.

sorption	Pretreatment	Volume adsorbed (c.c. g^{-1}) in 2 min No cold trap	Cold trap
1	Outgas 550° C 16 h	0·0185	0·0125 ± 0·0023*
2	Outgas 20° C 5 h	0·0380	0·0062 ± 0·0006*
3	Outgas 20° C 16 h	0·0255	
4	Outgas 20° C 16 h	0·0125	

* S.D.

Calculated monolayer coverage for oxygen = 3·4 c.c. g^{-1}.

mercury there was a fast initial adsorption of oxygen, mplete within 2 min, followed by a slow adsorption.

In another series in which no cold trap was used, the mple was outgassed for 64 h at room temperature after e initial oxygen saturation, and the uptake in the second dsorption experiment was then as high as 0·0600 c.c. g^{-1}. ualitatively similar results were obtained using a different ickel oxide preparation of surface area 5·9 m^2/g also erived from the nitrate. The following conclusions ere reached:

(1) After outgassing at 550° C in the presence of mercury apour, the adsorption at 20° C is rather higher than usual.

(2) Further outgassing at 20° C, still in the presence of ercury vapour, leads to an enhanced adsorption which creases with the time of pumping and may exceed the itial uptake by a factor of three.

(3) The volume adsorbed diminishes with successive utgassings and adsorptions at room temperature.

Control experiments in a greaseless system confirmed hat the effect was caused by mercury vapour and not by topcock grease, the presence of which in a vacuum system id not influence oxygen adsorption results.

able 2. Adsorption of Oxygen (Press 0·4–0·2 mm) on Nickel Oxide at 20° C

dsorption	Pretreatment	Volume adsorbed (c.c. g^{-1}) in 2 min
1	Outgas 550° C 1 h in absence Hg vapour	0·0117
2	Outgas 20° C 23 h in absence Hg vapour	0·0091
3	Outgas 20° C 20 h in presence Hg vapour	0·0225
4	Outgas 20° C 5 h in absence Hg vapour	0·0084
5	Outgas 550° C 16 h in absence Hg vapour	0·0156
6	Outgas 20° C ½ h in absence Hg vapour	0·0106
7	Outgas 20° C ½ h in absence Hg vapour followed by residence in closed system for 5 h in presence of mercury vapour	0·0187

A further series of experiments was carried out in hich the adsorption of oxygen was measured on a single pecimen before and after exposure to mercury Table 2). A different vacuum system was tilized.

Adsorption 4 indicates that prior exposure to ercury vapour does not affect the magnitude of he reversible adsorption (that is, oxygen which an be desorbed at 20°) since the value observed 0·0084 c.c. g^{-1}) agrees with that for a mercury-ree sample (Table 1). Thus, oxygen absorbed at ercury centres is irreversibly bonded at 20° C, in greement with the decline in oxygen uptake after uccessive outgassings at 20° C (Table 1). Adsorption demonstrates that the enhanced adsorption effect still ersists if, instead of outgassing, the sample is simply llowed to stand *in vacuo* in a closed system containing ercury vapour. The reduced magnitude of the mercury ffect noted in Table 2 compared with Table 1 may be scribed to a difference in geometry of the two vacuum ystems used; this influences the diffusion rate of mercury apour to the specimen. Direct confirmation that chemiorption of mercury vapour occurred was obtained y admitting the vapour to a part of the apparaus containing a Pirani gauge, isolating the merury source and then expanding the vapour to the ickel oxide; the pressure fell as adsorption proeeded. Finally, in order to determine whether ercury atoms gave rise to fresh oxygen adsorpion sites through interacting with the oxide urface itself or with oxygen adsorbed in the reliminary adsorption, the sample was first outgassed at 50° C. in the absence of mercury vapour, and then further vacuated for 16 h at 20° C, while exposed to mercury apour, without an intermediate adsorption. Again the subsequent oxygen uptake was large, indicating that mercury interacts with the outgassed surface to provide extra sites for oxygen adsorption.

Mercury vapour can then be regarded as a 'promoter' rather than a 'poison' for oxygen adsorption on nickel oxide. The mechanism of this effect has not been established, and any discussion is necessarily speculative. Associated experiments with neutron-irradiated nickel oxide[5] have established the importance of metal excess centres for oxygen chemisorption, and it is now suggested that adsorbed mercury may act as such centres. In both cases there is an increase in irreversible adsorption, while reversible adsorption scarcely changes. In the gas phase, mercury atoms are well known as scavengers for oxygen atoms in chain reactions. The decline in oxygen uptake after successive outgassings at 20° C and the low surface coverages involved (Table 1) indicate that the sites on the nickel oxide surface which may adsorb and activate mercury atoms are restricted in numbers. These results emphasize the importance of avoiding mercury contamination in chemisorption investigations on oxides.

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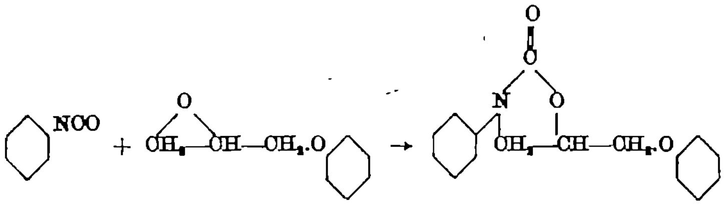
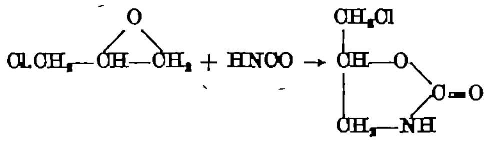
Reaction of Phenyl Isocyanate with 2,3-Epoxy Propyl Phenyl Ether (Phenyl Glycidyl Ether)

Reaction of phenyl isocyanate and 2,3-epoxy propyl phenyl ether gives a one-to-one addition compound, and there is evidence to suggest this is 3 phenyl 5 phenoxy methyl 2 oxazolidone. The reaction is believed to be:

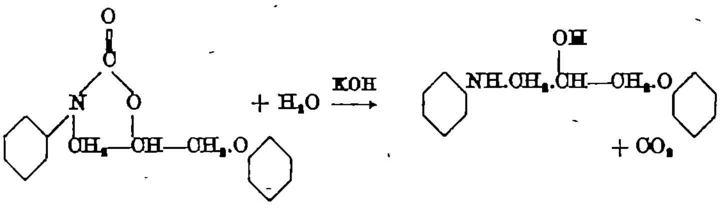


This is similar to a reaction reported by A. Thompson[1] between acidulated potassium cyanate and epichlorhydrin.



Evidence for the foregoing scheme is:

(1) Reaction with alcoholic potash yielded a compound in agreement with the reaction:



The product of this reaction gave a yellow oil on reaction with nitrous acid, suggesting a secondary amine.

(2) There was no reaction of the reaction product with nitrous acid. The nitrogen of this compound is thus

not present in the form of a primary or secondary amine.

(3) The product of the reaction was broken down by heating with concentrated hydrochloric acid in a sealed tube at 200° C to give an oil, which was shown to be a chlorine containing compound. This is in agreement with the concept of acid hydrolysis of the oxazolidone ring.

It was also noted that the material did not yield an oxime on reaction with hydroxylamine hydrochloride.

Phenyl isocyanate (11·6 g) was refluxed with phenyl glycidyl ether (15 g) for 6 h at 170° C. On cooling, a dark solid mass was obtained which was extracted with chloroform (300 ml.). After decolorization with charcoal and reduction in volume by distillation a white material was obtained (10·1 g). Complete evaporation of the chloroform left a reddish-brown tar (6·2 g). The white material was recrystallized from chloroform to give needles of m.p. 134°–135°.

$C_{16}H_{15}NO_3$ required: C, 71·32 per cent: H, 5·61 per cent; N, 5·20 per cent; obtained: C, 71·44 per cent; H, 5·76 per cent; N, 5·16 per cent.

1 g of the above material was refluxed wiih 40 ml. of a solution of 80 per cent ethanolic potash for 2 h. The ethanol was removed by fractional distillation leaving a small amount of water behind, which was extracted with ether. Ether was removed by evaporation and left a yellow oil, which crystallized to a pale yellow solid (0·57 g), m.p. 46°–48° C. On reaction with nitrous acid this gave a yellow oil, suggesting it was a secondary amine. Required for 1 phenyl-amino 2 hydroxy propyl phenyl ether, $C_{15}H_{17}NO_2$: C, 74·11 per cent; H, 7·03 per cent; N, 5·75 per cent; obtained: C, 72·73 per cent; H, 7·14 per cent; N, 6·06 per cent. This material is reported by Fourneau and Poulenc[2], but attempts to repeat their work gave a material the analysis of which did not agree with theory. No analyses are quoted in their paper.

0·5 g of the original substance was sealed in a tube with approx. 0·5 ml. of concentrated hydrochloric acid and the tube was heated in an oil bath for 15 min at 170° C and for 1½ h at 200° C. After cooling the tube was broken under water and washed with water. The tube broke in a manner suggesting it was under pressure at room temperature. The water was extracted with ether and the ether extract evaporated down, to yield a small quantity of a dark oil. The Lassaigne test indicated the presence of chlorine in this material.

I thank the Department of Materials for the facilities and materials with which the work was done.

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BIOCHEMISTRY

Molecular Deficiency of Glucose-6-Phosphate Dehydrogenase in Primaquine Sensitivity

GENETICALLY determined deficiencies in activity of human glucose-6-phosphate dehydrogenase (G–6–PD) occur among various ethnic groups. The deficiency affecting 8–12 per cent of American Negro males is known as primaquine sensitivity and is incompletely expressed: affected persons have 7–15 per cent of the normal activity of erythrocytic G–6–PD and an even higher proportion of activity of this enzyme in some other tissues[1,2]. In view of similarities in qualitative characteristics between the partially purified G–6–PD of affected Negroes and that of normal persons, one of us has questioned the possibility that sensitive Negroes have a G–6–PD that is impaired in its catalytic efficiency (turnover number)[3]. A comparative examination of more extensively purified G–6–PD's seems to offer new evidence on this subject.

Glock and McLean assay[4] for G–6–PD permitted identification of day-old pints of enzyme-deficient blood provided by Negro males at the University Medical Center Blood Bank. On starch-gel electrophoresis[5], that G–6–PD of each pint migrated slightly faster than that of normal Caucasians, as has been noted previously[6,7]. The enzyme from each pint of blood from control, G–6–PD-active males migrated at the same rate as the G–6–PD prevalent in Caucasians. The enzyme was purified by a method previously described[8]. Five pints of deficient blood provided 0·31 mg (protein) of a preparation with a specific activity[8] of 9·1 units/mg, representing 16,000-fold purification and a yield of 2 per cent. From five pints of normal blood 0·55 mg of a preparation was obtained with specific activity of 94 units/mg. This represented 17,000-fold purification and a yield of 3 per cent. When subjected to starch-gel electrophoresis and staining with amido-schwartz, 33 μg (3·1 units) of normal preparation appeared predominantly as a single band. It had a position identical to the location of activity of the G–6–PD, as indicated in Fig. 1 by the 1·23 : 1 distance from the origin of the activity relative to hæmoglobin A. This position of activity relative to hæmoglobin also has been observed with more than 200 normal hæmolysates previously studied in this laboratory. In contrast, 98 μg (0·89 unit) of sensitive enzyme did not migrate predominantly as one band of protein (Fig. 1, channel 3). An arrow indicates the position of sensitive enzyme expected from the location of activity in channel 3a and in the gel pattern of 30 previously studied hæmolysates from sensitive persons. Elutions from serial sections of gel from another channel, containing 0·52 unit of sensitive enzyme, revealed the relative location of activity to be identical to that of the smaller amount of enzyme in channel 3a. The total activity of sensitive enzyme added to channel 3 was almost one-third that of the normal enzyme in channel 1. About a ten-fold difference in specific activity exists between hæmolysates of normal subjects and sensitive Negroes. If this difference were due largely to a difference in turnover number, the arrowed position in channel 3 should have shown a band of protein staining several times more intensely than that of the normal enzyme.

Titration of the enzymes with reduced triphosphopyridine nucleotide (TPNH) offers additional evidence against a ten-fold difference in turnover numbers. TPNH fluoresces when excited with light of 360 mμ wave-length. The fluorescence of TPNH bound to human G–6–PD is

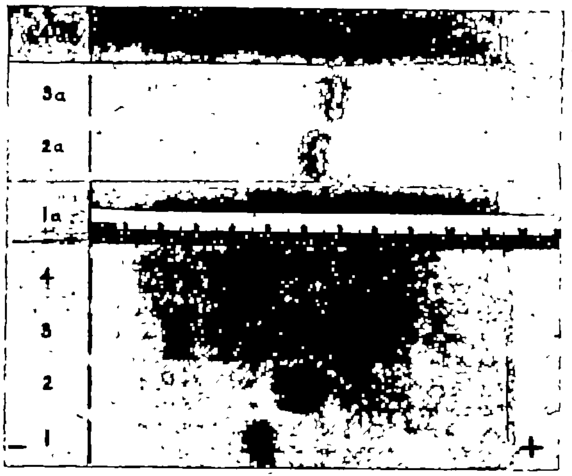

Fig. 1. Location of human hæmoglobin and G-6-PD on starch gel after vertical electrophoresis. First gel. 1 and 4, 37 μg of hæmoglobin; 2, 33 μg (3 1 units) of G-6-PD from normal males; 3, 98 μg (0·89 unit) of G-6-PD from primaquine-sensitive males. Second gel: 1a and 4a, 31 μg of hæmoglobin, 2a, 0·01 μg (0·97 × 10⁻³ units) of G-6-PD from normal males; 3a, 0·11 μg (1·02 × 10⁻³ units) of G-6-PD from primaquine-sensitive males. Channels 2a and 3a were stained for activity of G-6-PD. All other channels were stained for protein with amido-schwartz. Relative distance in the second gel should be corrected for shrinkage of gel in amido-schwartz solvent, resulting in the slightly greater length of channels 2a, 3a

Table 1. TITRATION OF MUTANT AND NORMAL G-6-PD WITH TPNH

Added	Fluor.	Added	Fluor.	Added	Fluor.
nitial	34·2	Initial	6·5	Initial	8·5
G-6-P 20	40·6	G-6-P 10	68·0		
TPN		TPN		TPNH	
·021	47·3	0·107	84·5	0·206	44·0
·042	51·0	0·214	99·7	0·309	57·5
·064	55·0	0·321	108	0·412	71·5
·118	61·7	0·428	117	0·618	84·2
·171	66·0	0·535	126	0·824	101
·278	77·6	0·642	135	1·03	116
·492	94·5	0·856	152	1·54	146
·706	111			2·06	184
		Estimate of total TPNH bound			
0·088		0·44		0·35	

Fluor. represents fluorescence emitted at 460 mμ wave-length by 0·1 ml. of sample excited by light of 360 mμ wave-length (ref. 8). Fluorescence is corrected for change in volume caused by the (5 μl.) additions. G-6-P, TPN and TPNH are expressed as total millimicromoles added. The end-point of each titration is taken from the intersection of the initial slope with a straight line drawn through the final readings (refs. 8 and 9).

Sensitive: 0·35 mg (0·384 unit) of G-6-PD with a specific activity of 1·1 units/mg. Normal: 0·045 mg (2·96 units) of G-6-PD with a specific activity of 66·0 units/mg.

approximately 2·7 times more intense than that of free TPNH[8]. Serial addition of small amounts of TPNH (or TPN in the presence of excess G–6–P) to normal enzyme previously stripped of TPN disclosed that normal G–6–PD binds approximately 0·14 × 10^{-8} moles of TPNH per unit of activity[8]. The results in Table 1 indicate that the sensitive enzyme binds 0·23 × 10^{-8} moles of TPNH per unit. In an earlier experiment, apparently irreversible loss in activity of the sensitive enzyme occurred during preliminary removal of TPN by the usual washings[8] with 2·7 M ammonium sulphate and dialysis against 0·05 M *tris* (hydrochloric acid), *p*H 8·0. The number of washings and duration of dialysis were halved. Consequently, TPN was not completely stripped from the normal preparation simultaneously studied (Table 1). Nevertheless, a ratio of 0·15 × 10^{-8} moles of TPNH bound per unit of activity can be computed for this normal preparation by assuming that the rise in fluorescence after G–6–P represents bound TPNH with 2·7 times the fluorescence of free TPNH. A similar ratio (0·12 × 10^{-8} moles per unit) is obtained by titrating the normal preparation with TPNH in the absence of G–6–P.

Activities of the sensitive enzyme following dialysis, purification, or mixing of purified sensitive G–6–PD with sensitive hæmolysates, offer no evidence of a reversible inhibitor of G–6–PD in sensitive hæmolysates. We therefore conclude that the deficiency of activity in erythrocytes of sensitive Negroes is due principally to a deficiency in number of molecules of G–6–PD. These findings do not permit a distinction between a deficiency due to increased destruction[10] or due to impaired synthesis of the active enzyme.

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Effects of Colchicine and Colchemid on Synthesis of Deoxyribonucleic Acid in the Skin of the Guinea Pig's Ear *in vitro*

COLCHICINE and colchemid have been used extensively *in vivo* and *in vitro* as a means of halting cells at metaphase in order to make the counting of mitotic figures easier. It is generally assumed that both have a fairly specific action in this respect, although colchicine is considered to be more toxic to the whole animal than colchemid. Experiments were performed to determine whether colchicine and colchemid, at the concentrations normally used, had any direct and immediate action on the initiation and rate of DNA synthesis in guinea pig epidermal cells as measured by tritiated thymidine and autoradiography, since any such effect would alter the number of cells collected at metaphase.

Slices of skin were taken from the dorsa of the ears of albino guinea pigs, and were floated on 1 ml. of a medium of Hanks's *B.S.S.* and horse serum in the proportion of 3 to 2, containing tritiated thymidine 0·3 μc./ml. (specific activity 360 μc./μm). Skin slices from the same animal were incubated for 1–4 h in an atmosphere of 5 per cent carbon dioxide in oxygen on this medium and the medium containing either colchicine or colchemid at concentrations of 4–12 μg/ml. Autoradiographs were afterwards made of sections prepared from the skin slices, with Kodak *AR* 10 stripping film, and exposed for 3–7 days. They were developed and stained through the film with Harris's hæmatoxylin and basic fuchsin[1,2].

It was found (Table 1) that the colchicine-treated samples showed a significantly lower proportion of labelled epidermal cells even after incubation for 1 h, the mean per cent reduction being 13·2 ± 2·4. The colchemid-treated samples were not significantly different from the controls. When the skin slice was incubated in the colchicine for 2 h and the thymidine added for a further 2 h, the depression was 14 ± 0·9 per cent. When the colchemid was similarly used, there was no depression. Some experiments with colchemid which had been stored in solution at 4° C for some three months suggested that under these conditions it caused slight inhibition. Increasing the concentration of colchicine from 4 μg/ml. to 12 μg/ml. increased the percentage depression to 24 per cent in 1 h. With 12 μg/ml. colchemid, there was 10 per cent depression at 1 h.

Table 1. EFFECTS OF COLCHICINE AND COLCHEMID 4 μG/ML. ON THE PROPORTION OF CELLS IN DNA SYNTHESIS DURING 1 H *in vitro*

Colchicine				Colchemid			
Animal No.	Percentage of positive cells Control	Percentage of positive cells Colchicine	Depression (%)	Animal No.	Percentage of positive cells Control	Percentage of positive cells Colchemid	Depression (%)
1	10·6	8·8	17	9	7·7	8·2	−6
2	10·9	8·8	19	10	6·9	7·0	−1
3	10·8	10·8	0	11	7·5	8·0	−7
4	10·6	8·3	22	12	6·0	5·5	8
5	10·9	8·8	19	13	5·6	5·2	7
6	9·2	8·0	13	14	5·4	5·2	4
7	9·0	7·9	12	15	5·3	5·0	6
8	8·1	7·8	4	16	5·1	4·7	8

Grain counts over 300 labelled cells per section indicated that colchicine caused a significant mean depression of 14·5 ± 2·2 per cent during 4 h. The reduction in grain count caused by colchemid was not significant (6·5 ± 0·48 per cent).

The appreciable drop in the number of cells engaged in synthesis of DNA as a result of incubation for 1 h with colchicine may represent cells being arrested in the *S* period with an incomplete replication, or failure of cells newly arrived at this stage to start synthesis. The depression of grain counts per positive cell indicates that the rate of synthesis is slowed so that even if the normal DNA complement were to be reached the number of cells arriving at metaphase per unit time would be less. Since these two effects are cumulative it follows that the counting of metaphases after colchicine 'block' is very likely to give an underestimate of mitotic activity. Colchemid would appear to suffer less from these disadvantages, but nevertheless before using it for this purpose it would be wise to exclude any such effects under the actual conditions of the experiment.

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A Comparison of the Plasma Proteins of C_3H Strain Mice, found by Cellulose-Acetate and Starch-Gel Electrophoresis

THIS experiment described here gives a comparison of the plasma protein patterns found with two electrophoretic techniques—those using cellulose-acetate and starch-gel as supporting media. The plasma used was that obtained from adult C_3H strain mice.

The zone electrophoresis method of Kohn[1] for cellulose acetate was used with the buffer modifications recently described by Aronsson and Grönwall[2]. The dimensions of the acetate strips were 18 × 5 cm. A voltage gradient of 6 V/cm was applied across the strip and a running time of 20 h was allowed. Staining of the strips was effected with lissamine green. A ribbon of acetate was cut from the main acetate strip, fixed immediately at 110° C for 10 min and afterwards stained in the dye for 10 min. This enabled localization of the protein bands on the remaining parent strip to be made. The full remaining pattern was then sectioned into three segments, each containing one of the three main fractions. Each segment was cut into six small ribbons which could then be inserted into the same slot of a starch-gel plate. The technique for starch-gel electrophoresis described by Smithies[3], with the slightly modified discontinuous buffer system of Poulik[4], was usec The electrophoretic run was made in a borate buffer of *p*I 8·6 at 6 V/cm for 15 h. The starch-plate had the dimen sions of 27 × 18 × 0·6 cm. Staining was made witl amido black 10 *B*.

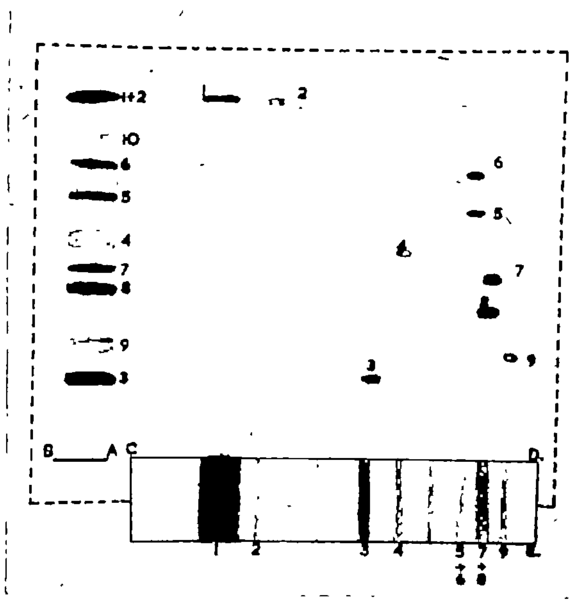

Fig. 1. Diagrammatic representation of two-dimensional electrophoresis in cellulose-acetate and starch-gel media. *AB*, Origin of control C_3H serum in the starch-gel, *CD*, origin of acetate ribbon in the starch-gel; *DE*, origin of original C_3H serum on acetate ribbon. Fractions: 1, albumin; 2, α_1-globulin *B*, 3, $S\alpha_2$-globulin; 4, β-globulin *A*; 5, fast haptoglobin group; 6, $F\alpha_2$-globulin, 7, β-globulin *B*, 8, β-globulin *C*; 9, slow haptoglobin group, 10, an α_1-globulin which has not been located on the acetate

Fig. 2. Two-dimensional pattern of C_3H serum in cellulose-acetate and starch-gel media. The photograph was taken with transmitted light, thus giving a greater resolution of the fainter fractions. This method gave, however, a diffuse picture of the control serum and so a normal C_3H serum pattern, taken with direct light, is interposed on the right of the figure. *AB*, Origin of normal C_3H serum; *CD*, origins of acetate ribbon and control serum in the starch-gel. Fractions: 1, albumin; 2, α_1-globulin *B*; 3, $S\alpha_2$-globulin; 4, β-globulin *A*; 5, fast haptoglobulin group; 6, $F\alpha_2$-globulin; 7, β-globulin *B*; 8, β-globulin *C*; 9, slow haptoglobulin group; 10, an α_1-globulin which has not been located on the acetate

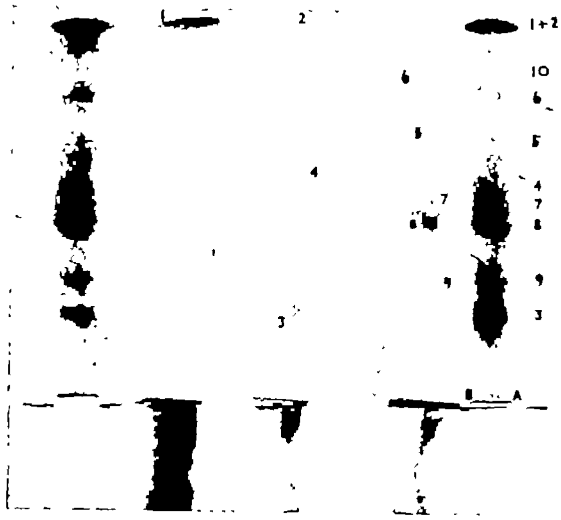

Fig. 3. Two-dimensional pattern of C_3H serum in cellulose-acetate and starch-gel media. The acetate strip can be seen to have been sectioned into three, each section being inserted into the starch at a different point. *AB* is the origin of the control serum in the starch-gel. Fractions: 1, albumin; 2, α_1-globulin *B*; 3, $S\alpha_2$-globulin; 4, β-globulin *A*; 5, fast haptoglobin group; 6, $F\alpha_2$-globulin; 7, β-globulin *B*; 8, β-globulin *C*; 9, slow haptoglobulin group; 10, an α_1-globulin which has not been located on the acetate

A comparison of the plasma protein pattern of th acetate with that of the starch was made possible by thi two-dimensional system. The comparison was based o the proteins having the same mobilities off the acetat strip into the starch as they have off the filter-paper whic was used in application of the control plasma. Fron Figs. 1, 2 and 3, the identity of the acetate fractions ca be made out, and their sequence compared with that o the starch. The most striking difference is the reversa in positions of the $S\alpha_2$-globulin (fraction 3) and th β-globulin complex (fractions 4, 7, and 8). The $S\alpha_2$ i shown to form the second main fraction on the acetate while on the starch it is the slowest-moving fraction, apar possibly from the γ-globulins, for which no evidence ha een obtained. The $F\alpha_2$ is identified with acetate fraction , which is located in the same acetate band as fraction 6, ut forms the more anterior portion of the band. Fractions and 9 are interpreted as haptoglobulins, but this still mains subject to question. Fraction 9 might also be terpreted as an α_2–β type globulin, similar to that escribed by Poulik and Smithies[5], for human serum. raction 2, which has the same mobility as the main lbumin band, is identified as an α_1-globulin. The fact at it has exactly the same mobility as the albumin ould suggest it to be similar in nature to the α_1-globulin of Poulik and Smithies.

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Effect of Linoleic Acid on the Deposition of Dietary-saturated Fatty Acids in Chicken Tissues and Eggs

KAUNITZ *et al.* have postulated that linoleate functions 1 the regulation of fatty acid composition of depot fat by acilitating the laying down of depot fat more representaive of the diet[1]. In confirmation of their report, we have bserved that the depot fat of chickens fed a diet with 5 er cent laurate and 4 per cent myristate contained igher proportions of these fatty acids when linoleate was dded to the diet[2]. However, we could not adequately neasure the effect of linoleate on the proportion of laurate nd myristate in other, non-adipose tissues. In the work escribed here 12 per cent methyl laurate was included in a urified diet (diet *C*-21, Table 1) with and without a ource of linoleate. The diets were fed to 7-day-old chicks naintained previously on a low fat diet. At several timentervals the chickens were fasted overnight, killed, and he liver and heart analysed for fatty acids using gas hromatography[3].

Table 1. EXPERIMENTAL DIETS

Ingredient	*C*-21 (%)	*C*-20–*G*-5 (%)
Casein (vitamin-free)	21·0	20·0
Gelatin	—	5·0
Mineral mixture*	5·4	5·4
Limestone	—	2·5
Cellulose	1·0	5·0
L-Glutamic acid	5·0	—
L-Arginine-HCl	0·6	—
Methionine hydroxy analogue (MHA)†	0·2	—
Vitamin mixture‡	0·5	0·5
Vitamin A concentrate (50,000 I.U./g)	0·05	0·05
Vitamin D concentrate (7,500 I.U./g)	0·02	0·10
Choline chloride	0·20	0·25
Procaine penicillin (50%)	0·002	—
d-α-Tocopherol acetate	—	0·004
Santoquin§	0·015	0·015
Glucose (cerelose)	to 100	to 100

* See Fisher and Johnson (ref. 4) for composition.
† Registered trade-mark of the Monsanto Chemical Co. for calcium *dl*-2-ydroxy, methyl thiobutyrate.
‡ See Machlin and Gordon (ref. 5) for composition.
§ Registered trade-mark of the Monsanto Chemical Co. for 6-ethoxy, 2-dehydro, 2,2,4-trimethylquinoline (an anti-oxidant).

In a second experiment, White Leghorn hens were fed , purified basal diet (diet *C*-20–*G*-5, Table 1). Two levels f methyl myristate were added with and without a ource of linoleic acid. Eggs were collected after 112 days n this régime and analysed for fatty acids. Five or six eplicates per analysis were conducted.

The results given in Table 2 demonstrate that the liver nd heart of chickens fed diets containing linoleic acid ontained a higher proportion of laurate than chicks fed inoleate-free diets. Dietary linoleate resulted in an ncreased proportion of myristate in eggs laid by hens fed nyristate. One would expect an increased proportion of laurate and/or myristate in tissue lipids if addition of linoleic acid resulted in: (1) increased absorption of dietary laurate and myristate from the intestine; (2) decreased oxidation of these fatty acids; (3) altered membrane permeability in ovary, liver, or heart cells resulting in greater uptake of laurate and myristate from the blood.

Table 2. EFFECT OF DIETARY LINOLEIC ACID ON THE DEPOSITION OF SHORT CHAIN FATTY ACIDS IN TISSUES

Tissue	Duration of feeding (days)		Dietary linoleic acid* 0%	1·5%
		% of diet (*C*–21)	Lauric acid % of tissue fat†	
Liver	4	11·4	0·3 ± 0·2	0·9 ± 0·5
Liver	30	11·4	1·2 ± 0·2	3·8 ± 0·8
Heart	12	11·4	2·0 ± 0·5	5·0 ± 1·9
		% of diet (*C*-20–*G*-5)	Myristic acid % of egg fat	
Egg	112	0·9	2·2 ± 0·3	5·1 ± 1·8
Egg	112	14·1	10·9 ± 0·5	26·0 ± 3·2

* 2·0 per cent safflower was the dietary source of linoleic acid, 12 per cent methyl laurate the source of lauric acid and 1·0 and 15·0 per cent methyl myristate the dietary source of myristic acid.
† Average ± *S.D.* The addition of linoleic acid resulted in a significant ($P < 0·01$ by *t* test) increase in deposition of lauric or myristic acid in all comparisons.

Mead *et al.*[6] have reported that essential fatty acid deficient mice oxidize stearate at a higher rate and incorporate less stearate in their tissue lipid than mice fed a source of essential fatty acids. More work is necessary before the biochemical mechanism underlying the change in saturated fatty acid composition following ingestion of linoleate can be defined.

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Distribution of Radioactive Sulphur after Oral Administration to the Rat of a Water-insoluble Fraction of Sulphur-35-labelled Thermal Amino-Acid Copolymer

THE preparation of amino-acid copolymer labelled with sulphur-35 by thermal polymerization of hydrolysates of yeast (*Torula utilis*) incubated with $^{35}SO_4$ has already been described by us[1]. The polymerization resulted in at least two different types of copolymers. One part of the preparation was absolutely insoluble in distilled water, while the other part could be dissolved in water. Both fractions showed protein-positive colour reactions and radioactivity.

By chromatography on ion exchange columns ^{35}S-activity could be observed at the positions of cysteic acid (sulphate) and methionine. The radioactivity of the cystine peak was very low. Another radioactive component could be eluted from the 150 cm 'Dowex 50' column by 0·2 N sodium hydroxide. This component could also be detected in hydrolysates of ^{35}S-labelled *T. utilis*[2].

We ourselves have reported recently on a feeding trial using the soluble fraction of proteinoid. In further experiments the water-insoluble fraction of proteinoid was collected and administered to a rat. The male rat had a weight of 85 g at the beginning of the experiment. The animal was kept in an air-tight cage in which a separate collection of urine and fæces was possible. The air passed through the cage by suction was filtered through traps to remove gaseous metabolites from the air.

The rat received food and water *ad libitum*. The diet consisted of 86 per cent maize, 10 per cent skimmed milk powder, 3 per cent mineral mixture *U.S.P. XIV* and 1 per cent of a vitamin mixture essential for rats.

The water-insoluble proteinoid fraction was suspended in 0·2 N sodium phosphate buffer *p*H 6·8. Twice daily 0·5-ml. portions of this suspension were applied to the rat by tube. The experiment was continued for 28 days. During this time the rat showed normal behaviour; the increase in weight was 90·5 g. 24 h after administration of the last ^{35}S-proteinoid portion the animal was decapitated. The macroscopic inspection and the histological examination indicated no abnormal change in organs or tissues.

The components labelled with sulphur-35 in blood, liver, kidneys and brain were separated by 20 per cent trichloroacetic acid into a protein-containing fraction and a non-protein fraction. Each of these fractions, as well as the rest of the tissues and the excrements, were hydrolysed with 6 N hydrochloric acid and the activity of sulphur-35 was determined in the manner already described[1]. Moreover, hydrolysates of urine and fæces were chromatographed on ion exchange columns and the activity of sulphur-35 of the effluent was registered continuously[3]. The specific activity of other tissue hydrolysates was too low for chromatography.

The results of the determination of activity of sulphur-35 are summarized in Tables 1 and 2.

Table 1. DISTRIBUTION OF ACTIVITY OF SULPHUR-35

(Percentage of recovered activity)

	In hydrolysate	In hydrolysate residue	Total
Body	1·14	1·44	2·58
Intestinal tract content	0·21	0·22	0·43
Fæces	12·05	76·45	88·50
Urine	7·73	0·63	8·36
Air			0·13

Table 2. DISTRIBUTION OF ACTIVITY OF SULPHUR-35 IN RAT BODY

(Percentage of recovered activity of the body)

	Non-protein fraction	Protein fraction	Total
Blood	8·21	28·68	36·89
Liver	7·99	1·05	9·04
Kidneys	1·93	1·70	3·63
Brain	5·87	0·81	6·68
Skin and hair	—	—	13·56
Intestinal tract	—	—	3·79
Rest of body	—	—	26·41

Table 1 shows that the main part of the administered sulphur-35 activity is excreted in the fæces. When the sulphur-35 activity of the stomach-intestine content is added to the radioactivity of the fæces the results indicate that about 89 per cent of the radioactive material was not absorbed, while about 61 per cent of the water-soluble fraction was absorbed[1]. Most of the absorbed radioactivity of the insoluble proteinoid fraction was excreted in the urine, so that 2·58 per cent only of the total activity was retained while 34·35 per cent of the radioactive sulphur could be found in the body in the case of the soluble fraction[1].

These results indicate that the water-insoluble fraction can be utilized essentially less by the rat than the soluble fraction.

Table 2 shows that most of the radioactivity in the body was found in the blood. The liver contained the highest amount of radioactivity of the organs analysed. When sulphur-35 content is calculated on the basis of the weights of the organs, the brain yielded the highest rate of radioactivity. While the protein fraction of the blood showed more radioactivity than the protein-free fraction all the other organs indicated reversed proportions.

Chromatography of hydrolysates of fæces showed the same elution pattern of radioactivity as the proteinoid administered. The chromatograms of urine, however, showed only one radioactive peak, situated directly behind the 'hold up volume' of the columns containing a mixture of ninhydrin-positive components, probably cysteic acid, other acid sulphur-components and sulphates. Consequently the absorbed part of ^{35}S-proteinoid fraction applied must have been included in the intermediat metabolism.

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Spectrophotometric Micro-Estimation of some Simple Plant Amines

VERY little is known about the mode of origin of th simple amines in higher plants. Stein von Kamienski[1] an Guggenheim[2] have reviewed the biosynthesis of amines i plants. Work in this laboratory[3] has indicated tha *N*-methyl ethanolamine may be a precursor of methyl amine in *Mercurialis perennis* L. Alternatively methyl amine may arise by decarboxylation of glycine. Evidence for the occurrence of amino-acid decarboxylases i plants is scanty[4–7], but recent work by Simon[8] has show the presence of a valine decarboxylase in the spadix o *Arum maculatum* L.

1-fluoro-2,4-dinitrobenzene (FDNB) has been used as quantitative reagent for the determination of histamine[9,1 and *d*-deoxyephedrine[11]. This reagent has been used i studies on the ion-exchange chromatography of some o the simpler plant amines. The method used was substantially as reported by McIntire *et al.*[11] with the followin modifications. Aliquot volumes of 1 ml. of each amin could be dealt with provided that 0·1 ml. of the FDN reagent was used, followed by 1 ml. of sodium bicarbonate The resulting 2,4-dinitrophenylamines were extracted int 10 ml. of *sym*-tetrachloroethane, followed by a furthe portion of 10 ml. of the solvent to remove turbidity. Th optical densities were determined in a Unicam *S.P.* 500 using the tungsten 1 amp, 1-cm silica cells, slit-width 0·04–0·09.

Table 1

Amine	λ_{max}(mμ)	μmole at λ_{max} E 1 cm
Ammonia	335	0·112
Methylamine	352·5	0·832
Ethylamine	352·5	0·902
Ethanolamine	352·5	0·839
Isoamylamine	352·5	0·863
Isobutylamine	352·5	0·859
β-Phenyl ethylamine	352·5	0·771
Dimethylamine	377·5	0·924
Diethylamine	385	0·774
N-Methyl ethanolamine	377·5	0·845
Putrescine	355	1·121
Cadaverine	355	1·451
Dimethyl ethanolamine	—	—
Trimethylamine	—	—
Choline	—	—
Betaine	—	—

Table 1 shows the absorption maxima of the simple amines which have been reported in higher plants[1,2,12–14]. The optical densities given result from the 2,4-dinitrophenyl derivatives of µmolar quantities of the amines in a total 20 ml. of solvent.

The 2,4-dinitrophenyl derivatives of the following amino-acids: glycine, L-valine, L-alanine, L-lysine, L-leucine, DL-serine, L-threonine, DL-glutamic acid, L-arginine, DL-methionine, L-proline, L-histidine, L-ornithine, in quantities of 100 µg, were insoluble in *sym*-tetrachloroethane and therefore gave no absorption at 352·5 mµ. Similar quantities of DL-phenylalanine and L-tyrosine, however, gave absorbancies of 0·012 and 0·070 respectively.

This method makes possible the direct determination of nine production in the presence of amino-acids and there- re possesses advantages over previously reported ethods[15,16]. A further advantage of the method is that is possible to estimate methylamine and *N*-methyl hanolamine simultaneously in mixtures of the two by termining the optical densities at the respective absorp- on maxima and substituting in the following equations:

$$\text{Methylamine} = \frac{(0{\cdot}080 \times E^{377\cdot5}) - (0{\cdot}112 \times E^{352\cdot5})}{-0{\cdot}00186}\,\mu\text{g}$$

$$\text{-methyl ethanolamine} = \frac{(0{\cdot}268 \times E^{377\cdot5}) - (0{\cdot}142 \times E^{352\cdot5})}{0{\cdot}00186}\,\mu\text{g}$$

ixtures of different known concentrations of the two mines were examined by this method. Results from ten ich mixtures gave only 2·5 per cent error for each of the wo amines.

The method has been particularly useful in studies on he ion-exchange chromatography of plant amines, since was found that, using the method of Blau[17] with columns f 'Amberlite *CG*-50' and 'Permutit *ZK*-226', the peaks f methylamine and *N*-methyl ethanolamine showed close roximity under certain conditions. Furthermore, the nethod is being used in studies of the metabolism of these wo compounds in *Mercurialis perennis* L., *Nicotiana ibacum* L., and *Elodea canadensis* Michx.

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Enhancement of the Effect of Prednisolone by 2-Methyl-1,4-naphthoquinone

IN conjunction with the microbiological transformation of steroids, small ring compounds were found to inhibit the breakdown of sterane structure. Since this phenomenon was encountered in the case of both fungi and bacteria[1–4], the idea presented itself that this biochemical observation might be of more general validity. We therefore undertook to investigate the influence of 2-methyl-1,4-naphthoquinone (MNQ), found to be the most efficient by ourselves, on the effect of prednisolone in various pharmacological tests.

In these studies we used three methods: the thymus involution and liver glycogen tests, and the antiphlogistic action, generally used for the pharmacological evaluation of glucocorticoids, with the modifications devised by ourselves[5].

The experiments consisted of administering prednisolone itself in two doses in a ratio of 1 : 3, and then studying how far the effect of the smaller dose of prednisolone could be enhanced by the aid of simultaneously applied MNQ. Untreated animals and groups given only MNQ were used as controls. In all the tests, both prednisolone and MNQ were injected subcutaneously; in case of concurrent use, from separate syringes at different sites.

The results of tests for thymus atrophy are given in Table 1. Thymus atrophy was more marked with animals given small doses of prednisolone (P) and MNQ than with those given three-fold amounts of prednisolone alone; hence the effect was intensified to more than three-fold. It may furthermore be seen that in itself MNQ was completely without effect.

Table 1

No.	No. of animals	Treatment	Thymus (mg %)
1	12	0·9 mg P	134
2	12	0·3 mg P	170
3	12	0·3 mg P + 0·06 mg MNQ	116
4	12	0·06 mg MNQ	225
5	12	control	222
		$P_{2-3} < 0{\cdot}001$	
		$P_{1-3} > 0{\cdot}05$	

Table 2

No.	No. of animals	Treatment	Liver glycogen (mg %)
1	7	0·45 mg P	1,660
2	7	0·15 mg P	259
3	7	0·15 mg P + 0·0075 mg MNQ	198
4	7	0·15 mg P + 0·075 mg MNQ	1,840
		$P_{2-4} < 0{\cdot}01$	
		$P_{1-4} > 0{\cdot}2$	

Table 3

No.	No. of animals	Treatment	Paw volume (mm³)
1	40	2·4 mg P	305
2	40	0·8 mg P	350
3	40	0·8 mg P + 0·2 mg MNQ	240
4	40	0·2 mg MNQ	422
5	40	control	431
		$P_{1-3} < 0{\cdot}01$	
		$P_{2-3} < 0{\cdot}001$	

The liver glycogen tests were undertaken with two doses of MNQ in an attempt to enhance the effect of prednisolone. As shown in Table 2, the smaller dose was completely ineffective, whereas the larger dose of MNQ, similarly to the results of the thymus tests, increased the effect of the smaller dose of prednisolone to such an extent as to cause the formation of larger deposits of liver glycogen than did three-fold doses of prednisolone alone.

Table 3 presents the results obtained by tests for antiphlogistic action. As demonstrated by our findings, MNQ alone practically failed to exert any influence on œdema due to dextran. On the other hand, when added to prednisolone, MNQ strongly intensified the effect of the latter; administered simultaneously with MNQ, 0·8 mg/kg of prednisolone inhibited the development of œdema induced by dextran much more efficiently than did 2·4 mg of prednisolone in itself.

According to our results, the efficiency of prednisolone is significantly intensified by concurrently administered MNQ. Attempts to elucidate the mechanism responsible for this action are now being made.

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Isolation of Lampterol, an Antitumour Substance from *Lampteromyces Japonicus*

AN intensive screening of about 600 mushrooms for antitumour substances[1] revealed that *Lampteromyces japonicus*, a bioluminescent mushroom which grows on rotten beech in October, possessed high activity. The mushroom is also known for its toxicity, which has occasionally caused fatal accidents due to its similarity in appearance to edible mushrooms. Isolation of the toxic principle in a semi-pure state was reported by Nakai in 1958 [2].

We have been carrying out isolations of the antitumour and toxic principles, respectively, by different methods, the former being checked by the cell–agar-plate[3] method and the effect on Ehrlich mouse ascitic tumour, and the latter by injection into ordinary mice. However, at the final stages of fractionation it became clear that the two activities were due to the same substance. A preliminary account of the extraction of the crystalline substance, which we wish to designate 'lampterol', is given here. A total of one ton of the fresh mushroom was collected in Yamagata (Mogami), Aomori (Towada), and Shizuoka (Fuji) Prefectures, and these were: (1) boiled for 10 min in water; (2) steamed for 20 min; (3) or soaked in methanol, at the site of collection in order to prevent the marked loss in activity which was encountered with dried mushrooms.

Processing of the initial material treated by method (1) was as follows. The boiling-water extract (10 kg) from a batch of 150 kg of mushrooms was concentrated *in vacuo* to 500 ml., and the residual syrup was treated with a three-fold amount (volume) of methanol to remove mannitol. The methanol filtrate was concentrated at 50°, the residue (190 g) was made into a 500 ml. aqueous solution, and this was defatted by several extractions with petroleum ether. The aqueous layer was extracted repeatedly (eight times) with the same volume of butyl acetate, and the combined butyl acetate extract was concentrated *in vacuo* to give 12·34 g of residue. This residue was mixed with 5·0 g of 'Sephadex *G*–25', placed on top of a 2·5 × 10 cm 'Sephadex' column, and eluted with 1 l. water. The combined eluate was passed through an 'Amberlite *CG*–45' column (2·5 × 20 cm) to remove acids (mostly succinic acid), and the eluate was concentrated *in vacuo* below 50° to yield 2·54 g of dried residue. The residue was chromatographed on a 1·8 × 21 cm silicic acid partition column, using water-saturated ether as the solvent. The eluate was again submitted to silicic acid partition chromatography and eluted with chloroform–ether mixtures (1 : 0, 1 : 1, 1 : 4, 0 : 1).

The activity was found in the chloroform–ether (1 : 4) eluate, which when dried up *in vacuo* gave 500 mg of a yellow gum. Crystallization of the gum from methanol and finally from benzene afforded, after some difficulty, crystalline 'lampterol', m.p. 124°–127°; the yield from the gum was about 20 per cent; found: C, 67·47; H, 7·39. $C_{15}H_{20}O_4$ (the molecular formula was based on analyses and nuclear magnetic resonance spectra of 'lampterol' and derivatives); requires: C, 68·16; H, 7·68 per cent. λ_{max}^{MeOH} 235, 320 mμ (log ε 4·10, 3·54); ν_{max}^{KBr} 3,300 (broad), 1,698, 1,660, 1,602 cm^{-1}. The acetate was easily prepared by treating the gum with pyridine and acetic anhydride, m.p. 102°; found: C, 65·74, H, 7·17. $C_{19}H_{24}O_6$ requires: C, 65·50; H, 6·94 per cent. λ_{max}^{MeOH} 227, 245 infl., 313 mμ (log ε 4·08, 4·02, 3·54); ν_{max}^{KBr} 3,540, 1,740 shoulder, 1,731, 1,698, 1,666, 1,603 cm^{-1}; NMR, two acetate peaks at τ 8·02 and 8·03 in carbon tetrachloride. Passage of 'lampterol' through an alumina column converted it into 'isolampterol', m.p. 179°–180°; found: C, 67·62; H, 7·28. $C_{15}H_{20}O_4$ requires: C, 68·18; H, 7·63 per cent. λ_{max}^{MeOH} 252 mμ (log ε 4·31); ν_{max}^{KBr} 3,400 (broad); 1,697, 1,645, cm^{-1}.

The activity of 'lampterol' was 120 γ/kg as measured b the effect on Ehrlich mouse ascitic tumours, and th toxicity was 5 mg/kg on intraperitoneal injection int ordinary mice. More detailed animal tests are bein carried out.

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PHYSIOLOGY

Progestational Activity of 4-Hydroxy-17-Alpha-Acetoxyprogesterone

RECENT biological research in the field of the gestagens has been directed at finding out new compounds having oral progestational activity but lacking virilizing effects on fœtuses.

Since dioxyprogesterone (4-hydroxy-17-α-acetoxyprogesterone) has such properties, we summarize here such properties of this new derivative.

Progestational activity was determined by evaluation of the endometrial proliferation according to McPhail[1] in immature female rabbits primed with œstrogens and treated subcutaneously or orally with the different compounds.

In Table 1 are presented the relative potencies of the tested steroids, calculated according to Gaddum[2] at two dosage-levels.

Table 1. PROGESTATIONAL ACTIVITY OF DIOXYPROGESTERONE

Steroid	Route	Relative potency
Progesterone	s.c.	1
Dioxyprogesterone	s.c.	4·73
17-α-Acetoxyprogesterone	os	1
Dioxyprogesterone	os	5·50
17-α-Ethynil-19-nortestosterone	os	8·00
9-β,10-α-pregna-4,6-dien-17-α-ol-3,20-dione 17 acetate	os	< 0·3
17-α-Allyl-17-β-hydroxy-oestr-4-ene	os	2·18
17-α-Acetoxyprogesterone-3-cyclopentyl-enol-ether	os	2·07
6-α-Methyl-17-α-acetoxy-progesterone (MAP)	os	51·28

Progesterone is assumed as standard for the subcutaneous treatments and 17-α-acetoxyprogesterone for the oral administrations.

As is shown in Table 1, dioxyprogesterone has a good subcutaneous and oral progestational activity; orally it is less active than MAP, but definitely more active than other compounds recently described. Furthermore, dioxyprogesterone induces endometrial proliferation in rabbits after local intrauterine application of 20 μg. The progestational activity of dioxyprogesterone is also shown by the increase of uterine carbonic anhydrase activity in immature female rabbits primed with œstrogens, according to Lutwak-Mann and Adams[3]: at a total dose of 0·5 mg, it ncreases carbonic-anhydrase activity to 68·8 U/g and at the total dose of 1 mg to 94·7 U/g (activity of control animals: 13·7 U/g).

At 1–2 mg/day subcutaneously injected dioxyprogesterone shows a deciduomatogenic activity in ovariectomized rats[4] of the same order of that of progesterone.

Dioxyprogesterone maintains pregnancy in ovariectomized mice at a subcutaneous dose of 5 mg/day (97 per cent foetuses alive at the end of pregnancy) and in ovariectomized rabbits at the oral daily dose of 10 mg (63·5 per cent of foetuses alive).

While most progestins virilize foetuses, dioxyprogesterone does not influence the ano-genital distance or causes morphological changes in the secondary sexual characters of female and male rats born from mothers treated from the 15th to the 20th day of pregnancy with doses up to 50 mg day subcutaneous or *per os*. Dioxyprogesterone presents no androgenic or myotrophic properties both by oral or subcutaneous route[5]. It does not increase the weight of the uterus of young immature female mice. Its anti-oestrogenic activity[6] is about 0·89 times that of progesterone and its anti-gonadotrophic effect[7] is only 0·08 times that of testosterone propionate (subcutaneous administration).

Up to a dose of 5 mg/day both by oral and subcutaneous route, dioxy-progesterone does not alter the oestral cycle n normal female rats.

It does not possess any corticoid effects (survival of adrenalectomized rats, liver glycogen deposition, anti-inflammatory properties, sodium-retaining activity).

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Effect of Posterior Pituitary Polypeptides on the Flow of Urine after Injection in Lateral Ventricle of the Brain of a Cat

Previous observations in this laboratory have ndicated that the injection of catecholamines and certain posterior pituitary polypeptides into the lateral ventricle of the cat is associated with characteristic and drug-specific pressor–depressor responses of the peripheral circulation[1]. During the course of these earlier experiments, it was noted that the intraventricular introduction of posterior pituitary polypeptides was followed by a prompt increase of flow in urine. Other observers have reported that the peripheral administration of various posterior pituitary preparations may, on occasion, be attended by a rise of urine flow consequent to a solute diuresis[2,3]. The present experiments were designed to define certain aspects of the 'diuretic' effect of these drugs during their central administration.

Using standard renal clearance techniques, acceptable experiments were obtained in 30 healthy cats maintained on normal diet of cat chow. Appropriate local anaesthetic techniques were used in place of general anaesthesia. After curarization of the animal, respiration was maintained with a positive-pressure respirator through a tracheotomy tube. After surgical exposure of the urethra, an indwelling Foley catheter was placed in the bladder under direct vision in order to secure complete bladder emptying. A constant infusion of creatinine via the femoral vein was maintained after the initial dose. When creatinine equilibration occurred, control clearance periods of 5–20 min were obtained. Control rates of flow of urine were differed by changing the rate of infusion of creatinine. When the flow of urine was stabilized at a constant rate, the drugs were injected into the left lateral ventricle via a 21 tapered needle inserted under stereotaxic control. Sandoz *L*8 vasopressin (10–15 units), Sandoz *PLV*2 vasopressin (0·5–1·0 unit), surgical pituitrin (1·0–3·5 units), oxytocin (0·5–1·5 units), and hypertensin Ciba (0·005 mg) were used and after drug injection additional clearance periods were obtained.

Blood samples were drawn at the mid-point of every third clearance period for the determination of creatinine, osmolality, sodium, potassium and chloride. Similar measurements were performed on each urine sample. Blood pressure and pulse were recorded at appropriate intervals throughout the study. Respirations were constant at the rate of 24/min. Calculations of creatinine clearance, osmolar clearance, and either free-water clearance or T_cH_2O were made for all clearance periods.

The *L*8 vasopressin was associated with a significant rise of urine flow and no change was noted with *PLV*2, surgical pituitrin, oxytocin and hypertensin. It appears that this effect is most evident after administration of the *L*8 vasopressin at low rates of urine flow. In most studies, the increased urine flow was accompanied by an increased osmolar clearance which could be attributed to an increased excretion of sodium. Since filtration-rate and plasma sodium usually remained unchanged or decreased slightly, the observed solute diuresis may be tentatively attributed to a diminished tubular re-absorption of sodium. This effect could not be prevented by prior trans-section of the spinal cord at the *C*-1 level. Peripheral administration of the same amounts of *L*8 vasopressin under similar conditions of study was followed by the usual antidiuretic response unassociated with changes of electrolyte excretion.

Additional studies are still in progress to evaluate the exact nature of this *L*8 vasopressin 'diuretic' effect more conclusively. Tentatively, the present findings suggest that the central administration of this drug produces renal effects which are dissimilar to those obtained after the peripheral administration of identical amounts of drug. Although the stimulus may be mediated via the nervous system, further studies are needed to evaluate the relative roles of neural and humoral factors to its occurrence.

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Relation between Calcium Ions and the Action of Serotonin on a Fundus Strip of the Rat

According to Gaddum[1] and Vane[2] serotonin added to the medium in which smooth muscle is suspended causes muscular contraction. The mechanism of the action, however, is not understood. In the mechanical response of isolated muscle, changes in the membrane permeability and ionic movements are supposed to be involved. Some observations support the view that ionic movements across the cell membrane establish a binding chain between events which occur in the cell membrane and the contractile system of the muscle fibres[3,4].

It was found that binding of calcium ions by sodium ethylenediamine tetraacetic acid (NaEDTA) (Na_2H_2-EDTA·$2H_2O$) may lead to blocking of the action of serotonin on the isolated rat uterus. It is believed that serotonin acts by carrying calcium ions across the cell membrane of the smooth muscle fibre[5].

We have therefore examined the effect of calcium ions on the action of serotonin in the isolated stomach strip of the rat[4]. The fundus strip was immersed at 37° C in a bath containing 6–7 ml. Tyrode solution with 0·1 per cent glucose. A rapid stream of pure oxygen was bubbled through the bathing fluid. Contractions of the muscle strip were recorded by isotonic lever on a kymograph. Test compounds (serotonin, acetylcholine, potassium chloride, NaEDTA, CaEDTA) were added in a small volume to the bathing solution while it was in contact with the tissue. NaEDTA was neutralized to *p*H 7 before adding by 2 N sodium hydroxide. The contraction was allowed to develop for 90 sec, then the bath was washed out and a weight was added to the lever so that it came to the former baseline immediately. After 30 sec the extra weight was removed and the muscle was allowed to rest for a further 2 min. The following contractions were examined in 4-min cycles.

The effects of NaEDTA (3 mg/7 ml. and 6 mg/6 ml.) were similar to those found on the rat uterus[5]. NaEDTA added to the solution 1 or 2 min before the addition of serotonin, according to the concentration used, decreased or fully inhibited the activity of serotonin (0·03 μg 5-HT/6 ml.). NaEDTA alone, especially in higher concentration (6 mg/6 ml.). evoked contraction which was very fast (lasting about 20 sec), and which was followed by relaxation although NaEDTA was still present in the solution. The next contraction produced by serotonin was, in the presence of NaEDTA, remarkably smaller. When NaEDTA was washed out (after 2 min action on the muscle) the serotonin activity returned to its former level and there was no irreversible damage of the muscle fibres. It was also possible to remove the blockage of serotonin contractions (0·1 μg/7 ml.) caused by NaEDTA (6 mg/7 ml.) by adding calcium ions (3·6 mg $CaCl_2$/7 ml.) to the bathing solution. Calcium not only removed the blockage, but also strongly potentiated the sensitivity of the muscle toward serotonin.

This action of NaEDTA cannot be explained by isolation of the calcium ions present in the bath fluid only. This view is supported by the failure to obtain large changes in serotonin activity in the modified Tyrode solution where calcium ions were omitted. NaEDTA also blocked under these conditions the response of the muscle to serotonin. It thus seems probable that the action of NaEDTA may be associated with its direct effect on calcium present in the cell membrane.

The relation of calcium ions to the action of serotonin was confirmed in further experiments on the effects of CaEDTA. This salt which is not able to isolate calcium ions had no effect on the sensitivity of the fundus strip to serotonin. The dose of CaEDTA tested was 4·1 mg/7 ml. of the Tyrode solution. Response of the fundus strip preparation to serotonin under various experimental conditions is qualitatively presented in Table 1 (blockage of serotonin activity indicated by minus sign).

Table 1

Suspension fluid	Test compounds		
	Control	NaEDTA	CaEDTA
Normal Tyrode solution	+	−	+
Tyrode solution without calcium ions	+	−	

Further results were in agreement with Woolley[5] that the action of NaEDTA is not a specific one. NaEDTA also blocked the response of the stomach preparation to acetylcholine and potassium.

According to these observations the close relation between calcium and serotonin activity has been confirmed. It seems, however, that the mode of serotonin action cannot be interpreted by getting the calcium across the cell membrane only as was suggested by Woolley[5] working on the isolated rat uterus.

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Antagonistic Action of Urokinase to Bradykinin- and Plasminogen-induced Capillary Permeability

Previously we reported observations on the inhibitory action of fibrinolysin and streptokinase, as well as of other proteolytic enzymes, on capillary permeability induced by different mediators in rats and guinea pigs[1,2]. These findings led to investigations on urokinase and plasminogen. We used the blueing reaction, a semi-quantitative test, in these animals and found in control studies that urokinase did not induce capillary permeability. The failure of plasmin to induce capillary permeability was already published by Bhoola, Calle and Schachter[3], and we could confirm this both with streptokinase and chloroform activated fibrinolysin (plasmin). We have now found that urokinase behaves in a similar manner. In control experiments using buffered plasminogen, we found that it produced the opposite effect to that obtained with fibrinolysin, streptokinase and urokinase, respectively.

Guinea pigs and rats under sodium pentobarbital anaesthesia were used. The skin on the ventral side was closely shaved for intradermal injection of 0·02 ml. volumes with No. 30 gauge needle. Intravenous injection of Evans blue was used for the blueing reaction followed by intradermal injection of bradykinin or plasminogen and/or the intravenous injection of urokinase with subsequent intradermal testing. The diameter and intensity of the blue lesion at the site of injection, due to the dye from the leaking vessels, was recorded from its first occurrence until it attained its largest size. In charting the findings, we utilized a method similar to that used by Miles[4]. The mean values are derived from a minimum number of 8 lesions in not less than 4 animals.

Plasminogen prepared by Dr. D. L. Kline was buffered at *p*H 7·1 with 1-lysine hydrochloride, while the preparation made by Dr. W. Baumgarten contained phosphate buffer at *p*H 7·4. Both lysine and phosphate buffers, when used intradermally as controls, resulted either in a trace of blueing or in none at all. All our findings gave similar results in guinea pigs and rats.

The inhibitory action on capillary permeability induced by plasminogen was also examined in the capillary bed of the cremaster muscle of the rat, a preparation recently developed by Majno and Palade[5]. This qualitative test was used to see whether an entirely different capillary bed would yield similar results. This is the case.

In comparing Figs. 1 and 2, it can readily be seen that plasminogen is as effective in inducing capillary permeability as is bradykinin. The quantities used for the data presented in Figs. 1 and 2 are 0·2 mg plasminogen and 2 μg bradykinin, respectively. By reducing the amount of bradykinin to 0·2 μg its capillary permeability inducing effect corresponded to that produced by 0·025 mg. The inhibitory effect of urokinase was undiminished when the plasminogen or bradykinin was combined with 100

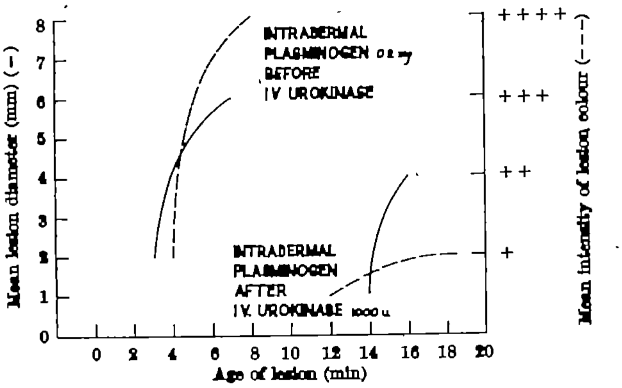

Fig. 1

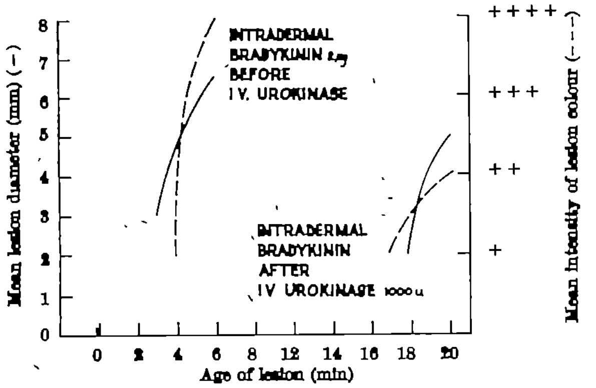

Fig. 2

Ploug units or equally following intravenous injection of 1,000 Ploug units. Figs. 1 and 2 follow the course of the actual experimental results.

Table 1 compiles our findings in guinea pigs and rats in which capillary permeability is induced with bradykinin and plasminogen before and after intravenous administration of urokinase. The differences between the maximum points of the graphs of all experiments described here are tabulated in an effort to establish certain patterns of response. Urokinase inhibits markedly the optimal development of lesions regarding the time, size and intensity induced by bradykinin or plasminogen with or without the mixture of urokinase in both species. In comparing the mean time of occurrence, bradykinin appears to be more inhibited than plasminogen, particularly in the guinea pig. Any change in comparing the mean diameter and intensity of colour in capillary permeability induced by bradykinin and plasminogen in the two species does not appear to be significant.

Table 1. Bluing Reaction induced with Bradykinin and Plasminogen in Guinea Pigs and Rats, before and after Administration of 1,000 Ploug Units of Urokinase per 100 g Weight

Intradermal agent	Animal	Differentials of optimally developed blue lesion		
		Mean time of occurrence (mm)	Mean diameter (mm)	Mean intensity of colour (deg.)
Bradykinin, 2 μg	Rat	14	1	+ + +
	Guinea pig	14	2	+ +
Plasminogen, 0·2 mg	Rat	11	2	+ +
	Guinea pig	9	2	+ + +
Bradykinin, 2 μg + urokinase, 100 u.	Rat	16	2	+ +
	Guinea pig	13	2	+ +
Plasminogen, 0·2 mg + urokinase, 100 u.	Rat	10	3	+ +
	Guinea pig	7	2	+ +

Comparisons are also shown in Table 1 between results secured with intradermally administered mixtures of urokinase with bradykinin or plasminogen, respectively. The mean time of occurrence is again higher with bradykinin than with plasminogen. The mean diameter and intensity of colour do not exhibit significant differences with the two substances.

The capillary permeability inducing action of plasminogen remains obscure. It may be due to contamination with bradykinin or similar mediators, or it may be due to a direct or indirect action of the plasminogen itself or some breakdown product. These considerations are at present under experimental study.

The inhibitory action of urokinase on capillary permeability is explained by the concept of Copley on the endothelial fibrin film modified recently[1,2] to include the basement membrane on the basis of electron microscopic observations by Majno and Palade[5]. Copley contends that a special kind of fibrin of sub-microscopic dimensions is a physiological constituent of the microstructure of the capillary wall, functioning as a vascular cement which coats the endothelial cells and is contained also in the basement membrane. In the capillary wall, fibrin deposition is controlled homeostatically by fibrinolysis which appears to act against mediators inducing capillary permeability.

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HÆMATOLOGY

Anti-Js^b, the Expected Antithetical Antibody of the Sutter Blood Group System

The serum of Mrs. Y. M., a 34-year-old Memphis negress, was found to be incompatible with all donors cross-matched when she was admitted with vaginal hæmorrhage and hypofibrinogenæmia complicating the missed abortion of her eleventh pregnancy. After having five normal live births, her sixth pregnancy resulted in a macerated stillborn at eight months; the last five were missed abortions at four or five months, and hypofibrinogenæmia occurred with four of these. In the management of these pregnancies she was given 16 pints of blood between 1955 and 1957. No evidence of any transfusion reactions could be found in her hospital records.

The antibodies in her serum reacted strongly by the antiglobulin technique with all 20 cells of a highly selected panel of donors (Milwaukee Blood Center), but not with the patient's own cells. The reactions were less intense by Löw's papain method[1] and were not demonstrable by other commonly used procedures. The antiglobulin titre was 1:256.

Testing of the patient's red cells and subsequent family studies revealed her blood groups to be $O,MsMS^u$, P_1+, cDe, Lu^bLu^b, k^bk^b, Le (a−b+), Fy (a−b−), Jk^aJk^b, Di (a−), Js (a+). It was noted that the reaction with anti-Js^a was very strong and that all her four living children (the first had died in infancy) were Js (a+). At this point it was thought that the antibody might well be anti-Js^b. For this reason the serum was tested with the Js (a+), S^uS^u red cells of two sisters whom we had earlier believed to be homozygous for Js^a because dosage studies were suggestive and family investigations showed all 10 of their children to be Js (a+). Their red cells were found to be compatible with Mrs. Y. M.'s serum. Dr. R. Sanger and Dr. R. R. Race[2] kindly tested our patient's serum against the red cells of eight Js (a+) children from two Js (a+) × Js (a+) matings and found two of them to be negative or Js^aJs^a.

The identity of the antibody as anti-Js^b was further established by population studies. Eight of 705 Memphis negroes and none of 105 from Milwaukee were found compatible with the patient's serum. One of the Memphis donors gave weak reactions and since his red cells are the only ones possessing the Rh factor C we suspect the presence of anti-C in a titre of 1 : 16 by the antiglobulin test. It has not yet been possible to establish the identity of this second antibody. The specimens of one of the Memphis donors did not arrive in suitable condition for testing, but the other seven all reacted strongly with anti-Js^a. The probability of selecting seven consecutive Js (a+) negroes by chance would be 1 in 78,125. The incidence of eight non-reactors among the 810 negroes tested is equivalent to a proportion of 0·0099, which agrees well with Giblett's[3,4] estimate of 0·0106 for Seattle negroes ($\chi^2 = 0{\cdot}04$, $0{\cdot}9 > P > 0{\cdot}8$). No Js (b−) individuals have been found among 820 Caucasians tested.

We thank Drs. E. R. Giblett, R. E. Rosenfield and E. E. Muirhead for supplies of anti-Js^a serum.

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Production of Specific Hæmagglutinins in Pigs after receiving Skin Homografts

SEVERAL workers have reported recently on the relationship of blood group differences to rejection of homografts. Silber *et al.*[1] state that they were unable to demonstrate any apparent relationship of rejection of graft to blood group compatibility in turkeys. On the other hand, Schierman and Nordskog[2] report that the locus of the B blood group system in chickens was involved in histocompatibility in this species. Production of anti-A hæmagglutinins by *CBA* mice after receiving skin homografts from A strain mice was observed by Micklem and Brown[3]. Griffiths and Crikelair[4] found that, in man, the titres of anti-A and anti-B were increased following skin homotransplantation from donors possessing the specific blood group substances.

A preliminary investigation of skin homograft reactions in pigs was carried out using a series of litters of various breeds. The purpose of these experiments was to ascertain whether the length of survival of homografts is affected by genetic differences demonstrable by erythrocyte antigen–antibody reactions. The possibility that epidermal cells carry some of the antigens present on red blood cells was investigated. The red blood cells of siblings were tested against a panel of antisera and the animals were matched in pairs showing one, two, or three blood factor differences, or no known differences. The antisera used for the blood typing of the animals were prepared by iso-immunization, with the exception of anti-A, which is a naturally occurring antibody in the pig. Reciprocal grafts were performed between pairs, and each animal also received an autograft. When an extra pig was available in a litter, an autograft was performed on it, also, to act as an additional control. Full thickness skin grafts, about 1 cm² in area, were made high on the shoulder, just lateral to the mid-line. This site was chosen because it was easily bandaged, and the grafts would not be readily damaged by the pigs rubbing against walls, etc.

In the first litters, surgical and bandaging techniques were not perfected and the autografts, as well as the homografts, were eventually sloughed. In later litters all autografts survived. Animals were bled before grafts were made and at regular intervals thereafter.

The first litter used in the experiment consisted of eight pigs. The homografts were all sloughed between the fourteenth and twentieth days. There was no apparent correlation between immune response and time of the rejection of the homografts.

Table 1 shows the blood group differences between pairs, and the antibodies, produced in response to skin homotransplants, which react with blood group antigens. Pre- and post-graft sera were tested by direct agglutination and antiglobulin methods against a panel of pig red blood cells for antibody specificity.

Five of the pigs produced antibodies and no antibody production was detected in the other three. Three of the recipients lacking the E^a factor received grafts from E^a positive donors. This factor is known to be strongly antigenic and is easily produced by iso-immunization with red blood cells. Two of the recipients, Nos. 486 and 491, produced anti-Ea, but no antibodies were demonstrated in the serum of the third pig, No. 488.

Table 1. IMMUNOLOGICAL RESPONSE OF RECIPIENTS TO BLOOD GROUP ANTIGENS OF SIBLING DONORS AFTER RECIPROCAL SKIN HOMOGRAFTS

(Breed: Large Black/Wessex Cross)

Recipient	Donor	Known red cell antigens present in donor and lacking in recipient	Antibody produced
486 ♀	492 ♂	E^a	E^a
492 ♂	486 ♀	None	None
488 ♂	489 ♀	E^a	None
489 ♀	488 ♂	K^a	Weak anti-L*
490 ♀	495 ♂	None	Anti-L (probably)*
495 ♂	490 ♀	None	Unidentified antibody
491 ♀	493 ♀	E^a K^a R	Ea
493 ♀	491 ♀	None	None

* New antibody—characterized afterwards.

The K^a factor is also known to be strongly antigenic, but neither of the recipients receiving grafts from K^a positive pigs responded to this antigen. No. 489 produced an antibody to a previously unknown antigen, but no anti-Ka. No. 491 produced only anti-Ea in response to a graft from No. 493, whose cells possessed three antigens not present on the recipients' red blood cells; E^a, K^a and R.

Nos. 489, 490 and 495 all produced antibodies in response to the homografts. In the cases of No. 490 and No. 495 there were no known antigenic differences demonstrable by red cell agglutination prior to skin transplantation. However, each produced an antibody after receiving reciprocal skin grafts. In order to identify the antibodies made by these pigs, intravenous inoculations were done, using the whole blood of the donor pig. Unfortunately, pig No. 495 died, but the cells of No. 491, which reacted with the unidentified antibody in the serum of No. 490, were used for inoculating pig No. 490. In both cases a strong antibody was produced which has been designated anti-L. After the transfusion of red blood cells, No. 489 made anti-Ka, to a lower titre, and No. 490 produced a second antibody which has not yet been characterized.

The second and third litters used in these experiments produced no antibodies. The fourth and fifth litters produced some antibodies in response to the skin homografts. In litter No. 4 there were seven pigs. One pig made a very weak antibody, specificity unknown; a second made a weak anti-G; a third, anti-Ea; the remainder failed to produce any demonstrable antibody.

Of the fifth litter, two pigs responded with a weak antibody and two showed no antibody response. In these two litters all autografts survived and hair growth was evident. Grafts were oriented with the hair growth going in the opposite direction to that of the host. By this method it was possible to identify growth of hair from the graft as opposed to that of the host.

To summarize the results, five litters of pigs were used in these skin-grafting experiments comprising a total of thirty-three pigs. Of these, three were used as controls, receiving only autografts. Three pigs died under anaesthesia. The remainder received autografts and homografts. Of the twenty-seven pigs receiving homografts, ten animals from three litters produced antibodies to red blood cell antigens in response to skin homografts from litter mates. Six of these antibodies were identified and four are of unknown specificity.

We thank Dr. A. J. Cawley and Dr. C. K. H. Roe for their assistance with the surgery in these experiments.

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PATHOLOGY

Ontogenesis of Brain Thromboplastin and of Encephalitogenic Substance

THE accidental detection of the low thromboplastic activity[1] of encephalitogene-free vaccines from the brain of suckling rats produced in this laboratory against neuroviral infections[2] led us to a comparative examination of the ontogenetic development of thromboplastin and of the encephalitogenic substance.

The encephalitogenic activity of the cerebral substance was assayed by administering them together with the Freund adjuvant according to a technique described elsewhere[3]. The thromboplastic activity was assayed according to the modified[4] Quick method[5] from the clotting time of recalcified oxalated rabbit plasma on addition for 15 min at 58° of the centrifuged homogenate of the cerebral substance as thromboplastin. In addition thromboplastic activity was estimated from the time of clotting of heparinized plasma (antiheparin test).

A distinct parallelism will be seen in Table 1 in the development of thromboplastic activity and of encephalitogenicity of the albino rat brain in ontogenesis. The absolute values of thromboplastic activity vary from one experiment to another, in particular, according to the kind of plasma sample, and yet the thromboplastic activity of the brain of new-born rats is invariably lower than in adults. Similarly, the non-encephalitogenic brain of embryos and new-born rabbits and mice (not presented in Table 1) exhibits a lower thromboplastic activity than the adult brain. On the other hand, the encephalitogenic spinal cord of the new-born animals shows a high thromboplastic activity.

Table 1. THROMBOPLASTIC ACTIVITY AND ENCEPHALITOGENICITY OF THE ALBINO RAT BRAIN IN ONTOGENESIS

Age of rats (days)	Thromboplastic activity: With oxalated plasma (sec.)	Thromboplastic activity: With heparinized plasma (min)	Encephalitogenicity*
0	90–95	> 15	0/10
1	85–90	> 15	0/10
3	85–90	> 15	0/10
8	90–95	> 15	0/10
10–12	80–85	> 15	0/10
16	65–70	7	0/10
20	55–65	6	1/10
21	55–65	5	3/10
25	45–50	3	nt†
56	40–45	2	7/9
Plasma without cerebral thromboplastin	130–140	> 15	—

* Numerator indicates the No. of guinea pigs with clinical signs of experimental allergic encephalomyelitis; denominator, the No. of inoculated animals.

† Not tested.

The bovine spinal cord successively defatted by organic solvents, namely, acetone, benzene, ether, chloroform, methanol and ethanol, is devoided of any thromboplastic activity although it remains encephalitogenic. The collagen-like encephalitogenic protein prepared according to Roboz *et al.*[6] is likewise devoid of thromboplastic activity. On the other hand, various phospholipids isolated from the cerebral substance or synthesized exhibit the thromboplastic activity[7]. Although these substances are far from identical, namely, thromboplastin-lipid[7] encephalitogenic substance-protein[6], the appearance in the brain of encephalitogenicity coincides with an appreciable increase in its thromboplastic activity. These substances might possibly be constituents of the same macromolecular myelin structures.

In any event, the strict correspondence between encephalitogenicity and thromboplastic activity rendered it possible to use thromboplastic activity as an additional criterion for the allergen-free antirabies vaccine from the brain of rat sucklings. The low thromboplastic activity of this vaccine is by itself useful since the conventional antirabies vaccine of Fermi increases the clotting capacity of human blood.

With regard to the connexion between thromboplastic activity and encephalitogenicity, of interest are Allegretti's[8] recent results concerning encephalitogenicity of the pulmonary tissue which, along with the cerebral tissue, is known to possess the highest thromboplastic activity.

If Allegretti's results are confirmed they might cast new light on the concepts on the pathogenesis of demyelinizing allergic processes. In this case the initiation of such processes does not require any contact of lymphatic organs with the cerebral substance, while lesions of the pulmonary tissue, particularly in tuberculosis, might give rise to the process.

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Tumour Induction in C_3H and Swiss *ICR* Mice by Tissue Culture Preparations of Polyoma Virus

'Plaque-purified' lines of polyoma virus are capable of inducing more than one type of tumour[1] and this property is neither strain- nor species-specific[2,3]. However, mice show a wider variety of tumours than other rodents, and although evidence for some strain specificity in mice has been presented[1], the incidence of parotid gland tumours has exceeded that of any other histological type.

In work on the oncogenic activity of polyoma virus for C_3H mice, the incidence of mammary tumours in this strain was observed to be higher than that previously found in Swiss mice inoculated with polyoma virus. The virus used in these investigations had been propagated in a cell line derived from a C_3H-mouse mammary tumour[4], and, therefore, a comparison of the oncogenic responses of C_3H and Swiss strains of mice to other preparations of virus was of interest.

Polyoma virus used was strain 1956 *8C* (supplied by Dr. Bernice Eddy, National Institutes of Health, Bethesda, Md.) further passed in this laboratory in cultures of either mouse mammary tumour cells[4] or primary cultures of C_3H or Swiss mouse embryo. Tissue cultures used to propagate viral stocks and to assay for virus infectivity were prepared by methods previously described[4].

Mice used were the Swiss *ICR* strain and the high mammary cancer C_3H strain (descended from C_3H/St) obtained from inbred stocks housed apart from inoculated animals. (Both strains were obtained originally from colonies developed by Dr. T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, New York.) Animals were inoculated within 24 h of birth with 0·1–0·2 ml. of virus, deposited into the subcutaneous tissues of the back and head, as described by Stewart[5], and the sexes were separated. They were weaned when about one month old. To exclude mammary tumours occurring spontaneously in the C_3H strain, experimental observations were limited to virgin animals and were terminated 10 months after inoculation. Animals with tumours were killed, and their tissues removed for histological examination. Sera were tested for hæmagglutinin inhibitors to polyoma virus by the technique and criteria of Eddy *et al.*[6].

C_3H mice showed a higher incidence of tumours and a slightly shorter latent period of tumour development than *ICR* mice, with all virus preparations (Table 1).

The stunted growth and loss of hair reported previously for Swiss mice inoculated with potent virus preparations[2] were observed in some litters of both strains, and were more apparent during the first two to three weeks after inoculation. The stunting was not always seen in mice which later developed tumours; but stunted mice that survived weaning frequently developed early tumours.

Since the apparent difference in susceptibility of the two mouse strains could have been accounted for by the presence of antibodies passed to litters by mothers indirectly contaminated with polyoma virus, mice were selected from normal colonies of each strain and bled by cardiac puncture. When individual sera from old male and female breeders were tested for hæmagglutinin inhibitors to polyoma virus, antibody was demonstrated for 33 per cent of the C_3H mice and only 9 per cent of the *ICR* mice, which suggested that the greater resistance to tumour induction of the *ICR* mice was not due to the presence of maternal antibodies.

In the Swiss *ICR* strain used here, the incidence of polyoma virus induction of tumours is somewhat lower than the 80–100 per cent incidence observed in random-bred Swiss *NIH* strain[2,7]. A lower tumour incidence in *ICR* mice was also found by Mirand *et al.*[8] and by Howatson *et al.*[9]. The latter report attributed this finding to less carcinogenic activity in the variant strain of polyoma used; but it could also reflect genetic variation and differences in susceptibility among Swiss strains of mice.

Table 2 summarizes the numbers of neoplasms of various types revealed by histological examination of the tumour-bearing mice of each strain. Only the totals for each strain are tabulated, since the tumours induced by the different virus preparations did not differ significantly in frequency or type. The high incidence of mammary tumours in the C_3H mice with the virus preparations used in this work resembles our earlier observations when using virus propagated in C_3H-mouse mammary tumour cells. Although some tumour-type specificity was found by Dulaney and Goss[10] with cell-free extracts of tumours induced by polyoma virus, our findings lend some support to those of Winocour and Sachs[1], who showed that the probability of initiating a parotid tumour in mice is the same for polyoma virus recovered from mammary and renal tumour cells *in vitro* as for virus of parotid tumour origin.

In the Swiss *ICR* mice studied here, parotid gland tumours occurred more frequently than any other neoplasm. This has also been reported for $(AKR \times C_3Hf)F_1$ and other strains of Swiss mice[2,7]. However, in the C_3H mice, parotid and mammary gland tumours occurred with equal frequency, except that the frequency of mammary gland tumours was slightly higher in females. Although mammary cancer in C_3H mice has heretofore been limited to females, in the absence of œstrogenic stimulation, and

Table 1. Tumour Incidence in C_3H and Swiss *ICR* Mice postnatally inoculated with Polyoma Virus

Virus pool	Infectivity titre*	Mouse strain	Sex	No. mice surviving for evaluation	No. mice with tumours after 5 mo.	8 mo.	10 mo.	Mice with tumours (per cent)	Average age when tumour appeared (mo.)
71A	$10^{-\text{[illegible]}}$	C_3H	Female	44	15	26	33	75	5 1
			Male	40	4	9	12	30	6 1
		Swiss	F	34	1	3	4	12	5 6
		ICR	M	31	0	0	2	6	8 5
71B	$10^{-\text{[illegible]}}$	C_3H	F	29	16	20	24	83	3·1
			M	27	8	14	17	63	4·7
		Swiss	F	43	9	15	18	42	5·7
		ICR	M	37	4	12	13	35	5·9
71C	$10^{-\text{[illegible]}}$	C_3H	F	17	3	5	5	29	4 6
			M	21	3	5	5	24	4·4
		Swiss	F	40	2	6	6	15	5·0
		ICR	M	30	0	0	0	0	—

* Negative log of $TCID_{50}$/ml.

Table 2. Types and Frequency of Neoplasms observed in Mice inoculated with Polyoma Virus

	Mouse strain	No. mice examined	Parotid gland No.	Parotid gland Per cent	Mammary gland No.	Mammary gland Per cent	Thymus No.	Thymus Per cent	Other* No.	Other* Per cent
Total	C_3H	55	34	62	35	64	11	20	5	9
	Swiss *ICR*	34	24	71	4	12	6	11	17	31
Males only	C_3H	19	14	74	10	53	4	21	0	—
	Swiss *ICR*	10	9	90	1	10	1	10	3	30
Females only	C_3H	36	20	56	26	72	7	19	5	14
	Swiss *ICR*	24	15	63	3	13	5	21	12	50

* Other types: kidney, bone, ovary, subcutaneous lipoma, sarcoma.

limited even in virgin females to those at least 10 months old, in this work 53 per cent of the tumours in the tumour-bearing C_3H males were tumours of the mammary gland. The possibility of a relationship between polyoma virus and the mammary-tumour (Bittner) agent present in this mouse strain was suggested by Sachs *et al.*[9].

Parotid gland tumours were found by Stewart *et al.*[5] in 96 per cent of the Swiss and $(AKR \times C_3Hf)\ F_1$ hybrid mice developing neoplasms induced by postnatal injection of polyoma virus, and such tumours exceeded all other types in incidence and extent of glandular involvement. Swiss mice inoculated *in utero*[7] showed a higher incidence of tumours and a shorter latent period than those inoculated as new-borns, but no marked difference in the frequency of histological types of tumour. Thus the selective site of tumour formation did not appear to depend on the state of cellular differentiation and development at the time of inoculation. It was concluded that the cells of the salivary glands either are more susceptible to tumour induction or had been more consistently exposed to the oncogenic inoculum by the method of inoculation used.

The present work, where C_3H mice inoculated by the same route developed mammary and parotid tumours with equal frequency, suggests that the vulnerability of individual host tissues to the oncogenic action of polyoma virus is a genetic feature of the host, and not entirely dependent on the localization of the inoculum.

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IMMUNOLOGY

Immunological Investigations of Sheep Prolactin

ZONE electrophoresis on starch revealed that purified sheep prolactin contained, according to Cole and Li[1], three, and, according to Ferguson and Wallace[2], four, components. All these components were found to have biological activity when tested by the crop sac test in pigeons[3]. However, Reisfeld *et al.*[3], testing the biological activity of purified components, found one of these components to be a highly active prolactin, whereas the two further components were biologically less active; these authors considered the latter fractions altered the form of the active hormone.

In the work reported here the immunological properties of whole sheep prolactin (*NIH–P–S–3*) and its electrophoretic components were investigated. The immunological methods used have been described previously in detail[4]. On agar-gel electrophoresis sheep prolactin showed three components (Fig. 1). Component 1 corresponded to the mobility between β- and α-globulin, component 2 to α-2-globulin, and component 3 to α-1-globulin. Whereas components 1 and 2 stained strongly, component 3 was less distinct. As already shown by others[5], using starch electrophoresis, the electrophoretic mobility of the individual components of sheep prolactin resembled that of human growth hormone ($HGH{-}A_3$), but the latter hormone stained less intensively (Fig. 1).

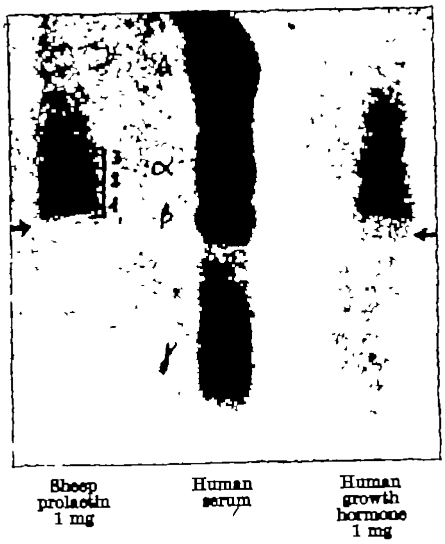

Fig. 1. Agar-gel electrophoresis of sheep prolactin and human growth hormone compared with human serum. Electrophoresis was run for 3½ h at 120 V and 80–90 m.amp. Arrow indicates origin. Stained with amido-black

Antiserum to sheep prolactin was prepared by immunizing rabbits weighing 2 kg in the following manner: 1·5 mg prolactin dissolved in 0·3 ml. sterile and pyrogen-free distilled water and mixed with 0·3 ml. complete Freund's adjuvant (Difco) (the prolactin was added dropwise during constant stirring) was injected intradermally into two foot-pads. Two weeks later the procedure was repeated injecting the two other foot-pads. After a further two weeks, 3·3 mg prolactin in 0·6 ml. water and mixed with an equal volume of complete Freund's adjuvant was injected subcutaneously. Two weeks after this subcutaneous booster, the rabbits were bled and the antiserum

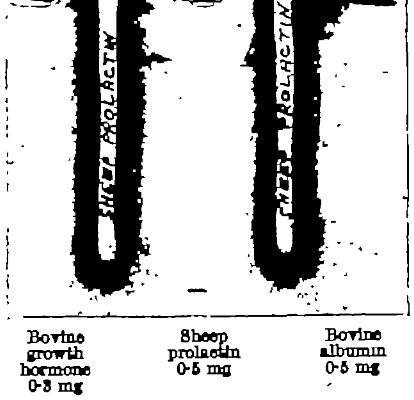
+
SHEEP PROLACTIN ANTISERUM
SHEEP PROLACTIN ANTISERUM
−
Bovine growth hormone 0·3 mg
Sheep prolactin 0·5 mg
Bovine albumin 0·5 mg

Fig. 2. Interaction of antiserum against sheep prolactin with whole sheep prolactin, bovine growth hormone and bovine albumin as determined by agar-gel immunoelectrophoresis. The rectangular wells indicate the origin. Stained with amido-black

titre was 1/2,500. When tested by agar-gel immunoelectrophoresis against its own antiserum, prolactin formed one precipitation arch (Fig. 2); showing that the preparation used was immunologically uniform and free of antigenically potent contaminants. Bovine growth hormone (*NIH–GH–B*$_2$) tested with the antiserum to sheep prolactin formed one precipitation line, corresponding to the level of prolactin. Bovine albumin (Armour Pharm. Co.) did not react with antiserum to sheep prolactin.

The immunological properties of the individual electrophoretic components of sheep prolactin were examined after their elution from the agar gel by a method described previously[6]. When tested by the agar-gel double diffusion test of Ouchterlony, using antiserum against whole sheep prolactin, the individual components formed one precipitation line. These lines were confluent between themselves and with the precipitation line formed by whole sheep prolactin indicating immunological identity. The bovine growth hormone preparation used formed one precipitation line confluent with the precipitation lines of sheep prolactin or its components. Bovine albumin did not react with the antiserum to sheep prolactin. Using the hæmagglutination technique[7] similar results were obtained. There was no interaction between human growth hormone or its electrophoretic components and antiserum to sheep prolactin or between sheep prolactin and antiserum to human growth hormone.

As shown by immunoelectrophoresis the precipitation line between bovine growth hormone and the antiserum to sheep prolactin occurred at the same level with the precipitation given by sheep prolactin, and not in the area of mobility of bovine growth hormone (midway between β- and γ-globulins), we think that the preparation of bovine hormone used was contaminated with prolactin. Our findings would therefore indicate that the electrophoretic mobility of bovine prolactin is identical to that of sheep prolactin. As a further proof for the immunological identity between the two prolactins is the confluency of the precipitation lines obtained by the Ouchterlony agar-gel double diffusion test (Fig. 3). It is noteworthy that sheep and bovine growth hormone have been found to be immunologically identical[8].

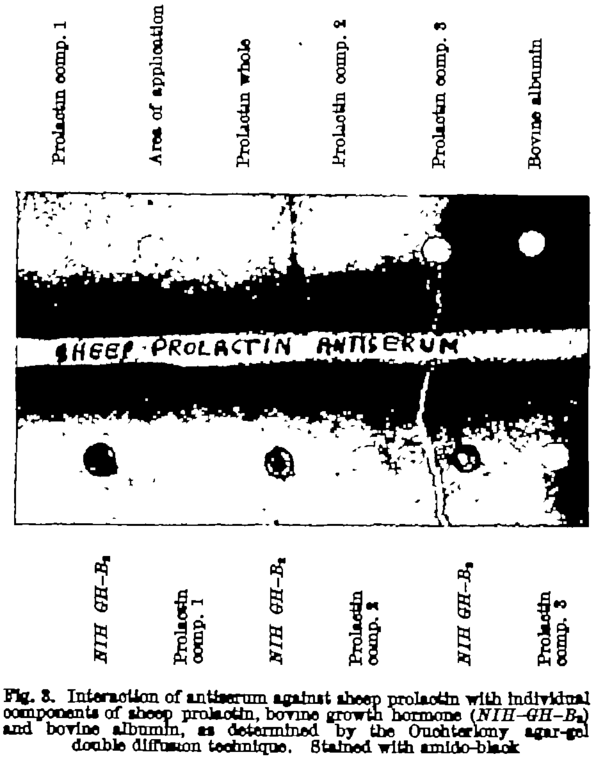

Fig. 3. Interaction of antiserum against sheep prolactin with individual components of sheep prolactin, bovine growth hormone (*NIH–GH–B*$_2$) and bovine albumin, as determined by the Ouchterlony agar-gel double diffusion technique. Stained with amido-black

In conclusion, the present work has shown that sheep prolactin is antigenically potent and that its electrophoretic components, though possibly differing in their biological activity, are immunologically identical. The immunological identity of bovine and ovine prolactins is also discussed.

The sheep prolactin (*NIH–P–S–3*) and bovine growth hormone (*NIH–GH–B–2*) were generously supplied by the Endocrinology Study Section, U.S. National Institutes of Health. The human growth hormone (*HGA–A*$_2$) and its antiserum used in this work were prepared in this laboratory[4].

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Influence of Excess of Antigen on Immunological Tolerance in Radiation Chimæras

THE existence of a specific immunological tolerance towards host tissue antigens in radiation chimæras has been clearly demonstrated with different techniques[1-3]. Van Bekkum has shown that this tolerance is lost when the bone marrow of the chimæra is transferred to irradiated mice which carry antigens not related to the first host. A new tolerance was shown to develop towards the antigens of the new host which were not present in the original bone marrow donor. These findings seem to demonstrate that, for a maintenance of the tolerance, a continuous presence of antigens is required.

In this communication, results are given which show that maintenance of tolerance without excess of antigen is possible if not only bone marrow but also lymphatic cells are transferred. For these experiments long-term allogenic chimæras without clinical signs of secondary disease were chosen. In order to remove the antigen excess from the donor type lymphatic tissues in the tolerant chimæras, the bone marrow together with the lymphatic cells were transferred to lethally irradiated mice isogenic to the original bone marrow donor (Table 1). Chimærism was determined by agglutination of peripheral blood erythrocytes[4]. The cells transferred were tested with cytotoxic antisera[5] to ascertain the origin of the cells used (Table 2). After about 40 days the irradiated animals injected with the chimæric cells were grafted with skin, isogenic with the chimæra host strain, together with skin from a non-related strain. In Table 3 the results with different combinations are presented. In all chimæras the skin of the unrelated strain showed a somewhat prolonged survival which is in accordance with the results of Bridges *et al.*[6]. In 12 of 19 *CBA* mice which received $CBA \rightarrow (CBA \times C_{57}Bl)F_1$ chimæra bone marrow together with lymph node cells, tolerance was maintained during the whole observation period (Exp. No. 2). It could not be assumed that antigen excess was present in these animals. A second transfer of bone marrow and lymph node cells from these tolerant animals resulted in a prolonged rejection of the grafted skin, while one animal at the time of writing still carried a graft which showed no signs of rejection (Tables 1 and 3, Exp. No. 3).

No tolerance was observed in mice injected with lymph-node cells from $CBA \rightarrow C_{57}Bl$ chimæras. The cell transfer

Table 1. TRANSFER OF BONE MARROW, LYMPH NODE AND SPLEEN CELLS THROUGH HYBRID HOST STRAINS*

Exp. No.	Cell donor	First host	Time-interval (days)	Second host	Time-interval (days)	Third host
1	*CBA* →	*CBA*	109	→ *CBA* †		
2	*CBA* →	F_1 ‡	230	→ *CBA* †		
3	*CBA* →	F_1	230	→ *CBA* †	157 and 175	→ *CBA* †

* Hosts were exposed to an LD_{100} of X-rays before transplantation.
† Mice tested for tolerance.
‡ (*CBA* × $C_{57}Bl$)F_1 hybrids.

Table 2. CYTOTOXICITY DETERMINATIONS ON CELL SUSPENSIONS USED FOR THE TRANSFER EXPERIMENTS

		Per cent non-viable cells after incubation with antisera*		
No. exp.	Cell suspension	Anti *CBA*	Anti $C_{57}Bl$	Controls†
	CBA spleen	97	34	30
Exp. 2	$C_{57}Bl$ spleen	37	98	38
	Chimæra spleen	95	37	34
	CBA lymph node cells	99	39	38
Exp. 2	$C_{57}Bl$ lymph node cells	63	100	60
	Chimæra lymph node cells	100	50	46

* Average of duplicate determinations.
† Average of determinations on tubes with antiserum and inactivated complement and on tubes with complement alone.

Table 3. SURVIVAL OF ALLOGENEIC SKIN, GRAFTED ON LETHALLY IRRADIATED *CBA* MICE INJECTED WITH BONE MARROW AND LYMPH NODE CELLS FROM RADIATION CHIMÆRAS

Exp. No.*	Transferred cells	Origin skin graft	Time between cell transfer and grafting (days)	Number of mice grafted	Last graft rejected (days)	Grafts intact at time of writing
1	Bone marrow 2 × 10⁶	*RFC*†	32	5	22	—
		$C_{57}Bl$	32	5	18	—
1	Bone marrow 2 × 10⁶ and lymph node cells 10 × 10⁶	*RFC*	32	5	15	—
		$C_{57}Bl$	32	6	16	—
2	Bone marrow 3 × 10⁶	*RFC*	43	10	18	—
		$C_{57}Bl$	43	14	15	—
2	Bone marrow 3 × 10⁶ and lymph node cells 12 × 10⁶	*RFC*	43	19	24	—
		$C_{57}Bl$	43	19	109	12‡
3	Bone marrow 3 × 10⁶	*RFC*	41	5	21	—
		$C_{57}Bl$	41	4	18	—
3	Bone marrow 3 × 10⁶ and lymph node cells 7 × 10⁶	*RFC*	41	5	17	—
		$C_{57}Bl$	41	8	76	3§ (91 days)

* See Table 1.
† (*CBA* × *RF*)F_1 hybrids.
‡ Between 116 and 134 days after skin grafting nine animals with normal looking skin grafts were killed for cell transfer. Histology revealed that in 3 grafts a low grade lymphocytic infiltration around the hair follicles was present. At the time of writing, 248 days after grafting, with the three remaining animals the grafts are intact but hairless and scaly.
§ One graft is normal with normal hair growth, two grafts are hairless and scaly.

procedure, however, caused a high lethality of the lymphatic cells from these chimæras.

The effect of chimæric spleen cells as compared with the effect of chimæric lymph node cells was difficult to evaluate because a very weak therapeutic effect against irradiation death could be obtained. In the few surviving animals no tolerance was observed. The exact cause of the failure of these chimæric spleen cells, to prevent irradiation death, is not yet determined.

It is concluded that when the lymphatic system of an irradiated allogenic animal is repopulated with cells from normal bone marrow, tolerance may only develop if sufficient antigens are present[1-3]. Transplantation of even a small number of normal lymph node cells always resulted in an immunological graft versus host reaction and a 'killing effect' of the host[6]. Transfer of bone marrow from a tolerant chimæra to an irradiated animal in which the specific antigens are absent results in a loss of this tolerance, and when excess of another antigen is present tolerance to this antigen may develop[3]. If, however, the lymphatic tissue is re-populated by cells derived from the lymph nodes of a tolerant chimæra, the tolerance in a number of cases is maintained without the continuous presence of the specific antigens. A factor which determines the success of the transfer of tolerance is the dose of lymph node cells. Their number should be far in excess of the bone marrow cell dose. A competition to re-populate the lymphatic tissues seems to exist between bone marrow and lymphoid cells.

In view of these results it is postulated that mouse bone marrow is a source of cells with a still-undetermined immunological function. These cells can develop either into immunologically active lymphoid cells, or into tolerant lymphoid cells when an antigen excess is present. On the other hand, the lymph node cells, which already have an immunological function, do not become tolerant in the presence of antigen excess but remain immunologically active. However, lymphoid cells, once tolerant, pass this tolerant state on to the daughter cells, so no antigen excess is needed to maintain this tolerance. A gradual replacement of tolerant cells in the lymphatic tissues by non-tolerant cells from the bone marrow may be responsible for the gradual loss of tolerance observed in some of these animals.

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Immunization against Ehrlich's Ascites Carcinoma with Streptomycin Complexes from Tumour Cells

IT is well known that immunity to transplantable tumours can be produced by pre-treatment of animals with X-irradiated tumour cells[1] or with fractions of tumour cells[2]. Although such experimental observations have little or no direct application to the problem of human cancer[3] there may be some value in recording devices or methods for the preparation of antigenic fractions from transplantable tumour cells. This communication reports the observation that the complexes, which precipitate when extracts of osmotic-disrupted Ehrlich's ascites carcinoma are treated with dihydrostreptomycin, are antigenic.

The antigenic complexes were prepared in the following manner. Packed cells (20 ml.) were obtained by centrifugation (800*g*) of 110 ml. of pooled ascites exudate of 7-day tumour from 21 Swiss albino mice. The cells were washed with 80 ml. of cold water and then re-suspended in 250 ml. of water. After 2 h at 2° C. the suspension was centrifuged (8,000*g*) and the clear supernatant fluid was treated with dihydrostreptomycin to give a final concentration of 1,000 units per ml. The resulting precipitate was collected by centrifugation, and resuspended in 110 ml. of saline to give fraction 1. Fraction 2 was obtained by a second water extraction of the cell residue, followed by precipitation with dihydrostreptomycin and suspension in saline as described for fraction 1. The course of immunization consisted of intraperitoneal injections of 0·2, 0·4 and 0·8 ml. at successive 5-day intervals. One week after the last injection, all mice were challenged by intraperitoneal injections of fresh ascites exudate.

In several experiments, conducted in this manner, the controls invariably showed 100 per cent mortality within 1–3 weeks, whereas one or more treated animals survived. The number of treated animals which survived appeared to be related to the amount of challenging dose. In one

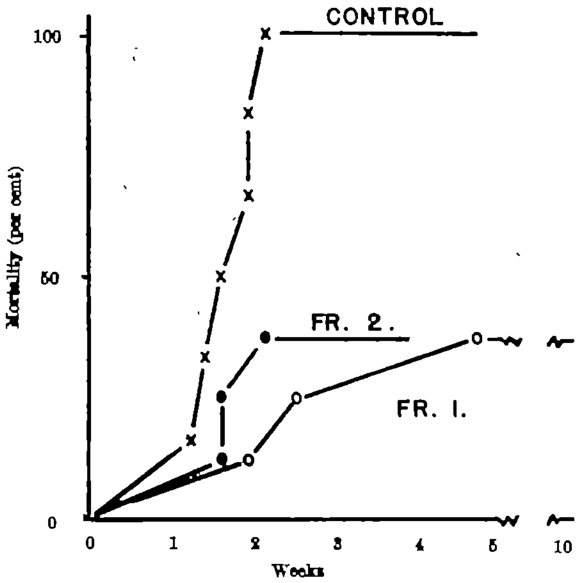

Fig. 1. Percentage mortality of mice receiving 0·2 ml. of a 1/100 dilution of 7-day tumour exudate. ×, Non-immunized controls (6 mice); ●, pre-treated with dihydrostreptomycin-complex, fraction 2 (8 mice); ○, pre-treated with dihydrostreptomycin-complex, fraction 1 (8 mice)

experiment in which all mice were challenged with 0·2 ml. of a 1/100 dilution in saline of fresh ascites exudate from a 7-day tumour, more than 50 per cent of treated mice survived for more than 10 weeks (Fig. 1).

Although the chemical nature of the cell material precipitable by streptomycin (or dihydrostreptomycin) is not known in detail, the work of Cohen[4] permits one to speculate that these fractions would be rich in nucleoprotein.

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RADIOBIOLOGY

Retention in the Skeleton of Radiostrontium as Influenced by Tetracycline

SEVERAL recent investigations have directed attention to the influence of tetracycline on the metabolic behaviour of radiostrontium. The results, however, are ambiguous. In contrast to Nakatsuka *et al.*[1], who were not able to observe any significant effect, it was claimed by Ogawa *et al.*[2] that tetracycline administered immediately after radiostrontium injection prevents its skeletal deposition and enhances its urinary excretion. This effect was thought to be due to the chelating properties of the compound. Positive results were also obtained by Richards *et al.*[3]. Repeated daily doses of tetracycline—given 4 days prior until 24 days after the administration of radiostrontium—did not affect the initial deposition of radiostrontium, but rather accelerated its elimination from the bone. The treatment schedule used by the foregoing authors, however, does not permit deciding whether the pre-treatment, or post-treatment, or both, were responsible for the effect.

In order to elucidate this question, albino rats and mice, injected with tracer amounts of carrier-free $^{85}SrNO_3$, were treated with tetracycline hydrochloride (by courtesy of Farbenfabriken Bayer AG, Leverkusen) in different ways. The dosage of tetracycline was the same as that used by Richards *et al.*; it proved, however, to be toxic, and several animals died in the course of the experiment. Both femurs were ashed at 600° C and their activity assayed by use of a sodium iodide (Tl)-scintillation crystal. The strontium-85 content of the whole skeleton was assumed to equal the ten-fold activity of both femurs.

The results (Table 1) show that neither post-treatment nor the simultaneous administration of tetracycline gives rise to a reduced skeletal retention of strontium-85. On the contrary, one experimental series appears to indicate that the elimination of strontium-85 from the bone is inhibited. A significantly reduced deposition was achieved by tetracycline only if given prior to injection of strontium-85.

Table 1. STRONTIUM-85 CONTENT OF THE SKELETON (MEAN AVERAGE ± *S.E.*)

Treatment (mg tetracycline/kg)		No. of rats (*R*) or mice (*M*)	Average body-wt. (g)	Day of death	^{85}Sr-dose (per cent)	*P*
	Control	6 *R*	179	2	53·6 ± 1·05	
53	Simultaneously	6 *R*	188	2	50·9 ± 1·34	0·14
	Control	6 *R*	164	3	46·2 ± 1·06	
150	4 days prior	5 *R*	140	3	34·7 ± 3·12	0·006
	Control	10 *R*	169	13	32·7 ± 1·09	
100	10 days after*	11 *R*	162	13	31·1 ± 1·02	0·3
	Control	7 *M*	29	12	30·4 ± 1·42	
150	5 days prior	6 *M*	23	12	21·5 ± 1·53	0·002
150	10 days after*	4 *M*	29	12	35·4 ± 1·88	0·06

* Treatment was started 24 h after injection of strontium-85.

These findings make it unlikely that the effectiveness of tetracycline can be attributed to the chelation of radiostrontium. On the other hand, taking into account the pronounced affinity of tetracycline to bone tissue[4-7] (which is thought to be due to the formation of calcium chelates *in situ*[7]), we may tentatively assume that this reaction leads to an impairment of the mechanism(s) responsible for the retention of radiostrontium by bone. As to the mode of action, final conclusions can be drawn only if more detailed investigations on the effectiveness of different tetracyclines, dose dependence, time and duration of treatment, are made available. Such experiments are now under way and will be published elsewhere.

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Effect of Thyroid Irradiation on the Release of Labelled Protein-bound Iodine in Rats

THE level of protein-bound iodine in the serum of rats was found to undergo significant changes within 24 h after their thyroids were irradiated internally by iodine-131 at total doses above 150,000 rads[1]. In subsequent studies, it was found that labelled thyroglobulin is released from the thyroid on internal irradiation with doses above 250,000 rads[2] and also at 130,000 rads[3]. No effect was, however, observed after the administration of 100 μc. iodine-131, which deliver to the thyroid a dose of about 15,000 rads[2,3]. Release of radioactivity from thyroids of dogs on external irradiation with 21,000 rads was also reported[4]. In experiments performed on animals so far, there is no evidence for an increase in the level of protein-bound iodine or in the release of thyroglobulin at doses lower than 20,000 rads. On the other hand, certain experimental results were reported which may indicate the release of thyroglobulin on irradiation even at lower

Table 1. PERCENTAGE PROTEIN-BOUND IODINE IN SERUM OF RATS AFTER INTERNAL AND EXTERNAL-LOCAL IRRADIATION OF THYROID GLANDS
(Protein-bound ^{132}I determined 1 h after intraperitoneal injection)

	Source of radiation	Dose (rads)	Dose (µc.)	Accumulated dose (rads)	Time after first irradiation (h)	PB^{132}I/PB^{132}I+^{132}I 1 h after administration of ^{132}I (×100)
1.	Int. ^{132}I	200		200	1	3.5
2.	,, ,,	8,000	450	8,000	24	23
3.	,, ,,	6,000	350	14,000	48	19
4.	,, ,,	4,000	240	18,000	72	18
5.	,, ,,	3,000	180	21,000	96	19
6.	Int. ^{132}I	< 20		20	1	4.2
7.	,, ,,	1,000	60	1,000	24	14
8.	,, ,,	700	40	1,700	48	18.2
9.	Ext. ^{90}Sr–^{90}Y	1,600		1,000	1	4.2
10.	Int. ^{132}I	1,000	60	2,600	24	14.5
11.	,, ,,	700	40	3,300	48	19.2
12.	Int. ^{132}I	< 20		20	1	2.6 (0.5*)
13.	Ext. ^{90}Sr–^{90}Y	120		120	24	5.7 (2.8*)
14.	,, ,, ,,	200		200	24	4.8 (4.0*)
15.	,, ,, ,,	400		400	24	7.1 (34.0*)
16.	,, ,, ,,	1,600		1,600	24	14.5 (59.0*)
17.	Int. ^{132}I	700	40	2,300	48	15.0
18.	Int. ^{132}I	< 20		100	1	4.8
19.	Int. ^{131}I	50	0.5	50	24	7.5 (38†)
20.	,, ,, ,,	150	1.5	150	24	8.5 (40†)
21.	,, ,, ,,	500	5.0	500	24	9.0 (43†)

*Per cent butanol non-extractable activity in the protein-bound iodine. †PB^{131}I/PB^{131}I+^{131}I 24 h after administration of iodine-131.

doses. It was shown by bioassay that the thyroxine content of thyroids of mice diminished after doses of total body irradiation as low as 50 rads[5]. A slight increase in the labelled protein-bound iodine was observed in rats at total body irradiation of 450 rads 48 h after irradiation[6]; this effect was, however, interpreted as being due to an enhanced turn-over. On the other hand, it was stated in another investigation[7] that the overall turn-over of the thyroid decreases on irradiation.

In clinical investigations, it was established that thyroglobulin is released into circulation as a result of therapeutic doses of internal irradiation[8]; the doses involved were of the order of 10,000 rads. In a recent clinical investigation, there are indications that some thyroglobulin is released at much lower doses (about 1,000 rads) and it was suggested that the extent of thyroglobulin release is not necessarily proportional to the dose of radiation[9]. It may be concluded that little information is available on the radiobiological behaviour of the thyroid gland on absorption of doses lower than 10,000 rads.

It was of interest to determine whether any increase in the rate of production of labelled protein-bound iodine occurs after local irradiation at doses lower than those cited here. It was possible to carry out this examination by the administration of repeated doses of iodine-132 and determining the protein-bound ^{132}I/protein-bound ^{132}I + iodine-132 at intervals. When the determinations were carried out at 24-h intervals, it was possible to determine the protein-bound ^{132}I level without interference of the labelled protein-bound iodine formed before. Next, it was possible to use a strontium-90 – yttrium-90 source for local external irradiation and iodine-131 as an internal source of radiation, and to determine by aids of iodine-132 the protein-bound ^{132}I level in serum. The iodine-131 present in the organism does not interfere with the radioassay of iodine-132 when carried out with a scintillation counter provided with an appropriate energy discrimination.

The experiments were carried out on groups of rats (5 rats per group). The rats were injected intraperitoneally 'carrier-free' iodide-131 or iodide-132 as necessary (iodine-132 was separated from an alumina column loaded with tellurium-132). One hour after injection of the iodide, blood samples were drawn from the tail. The serum was separated and the protein-bound iodine was precipitated by trichloroacetic acid. The iodide was then precipitated by silver nitrate and both protein-bound iodine and free iodine were radioassayed consecutively in a well-type scintillation counter. The butanol non-extractable fraction in the protein-bound iodine was determined by the conventional method, counting the protein-bound iodine first and the residual activity, after consecutive extractions with water-saturated *n*-butanol, thereafter. Wherever necessary, the measured activity was corrected for the decay of iodine-132. When the experiment was performed on rats containing iodine-131, care was taken to adjust the energy discrimination of the counting system so as to cut off any iodine-131 activity. The doses absorbed from iodine-131 and iodine-132 accumulated in the thyroid were calculated using the formulæ of Feller *et al.*[1]. External irradiations were carried out with a strontium-90–yttrium-90 source plated on a silver foil (8 × 8 mm); the foil was attached to the rats' necks by an adhesive tape for a pre-determined time. The source was calibrated by low-geometry β-counting and the absorbed dose rate at 5-mm depth was calculated to be 40 rads/h.

The experimental results presented in Table 1 show the protein-bound iodine-level in rats at different time-intervals after absorption of different doses of radiation. In the first two groups of experiments, iodine-132 was used both as an internal source of radiation and as an indicator of the extent of production of labelled protein-bound iodine within 1 h of injection. The fourth group of experiments summarizes the effect of external irradiation, whereas in the fifth group the thyroids were internally irradiated with an iodine-131 at a much lower dose rate than that of the external irradiations. It may be seen that previously intact animals form in 1 h about 3–5 per cent protein-bound iodine (Exps. 1, 6, 9, 12, 18) in the range of 20–2,000 rads, irrespective of the immediate dose absorbed (cf. Exps. 6, 12, 18 with Exps. 1 and 9). When examined 24 h after initial irradiation, a sharp rise in the labelled protein-bound iodine production rate is observed (Exps. 2, 7, 10). When the same rats were examined at 48, 72 and 96 h after irradiation, and the accumulated dose increased up to 21,000 rads (Exp. 5), there was no further increase in the rate of protein-bound iodine production. An almost analogous behaviour is observed at lower accumulated doses up to 3,000 rads (Exps. 7, 8; 10, 11; 16, 17).

At much lower doses (less than 1,000 rads), the increase in the rate of protein-bound iodine production depends on the administered dose; a similar effect is obtained whether the dose is given externally at a high dose rate (40 rads/min) or internally (less than 5 rads/h) (Exps. 13–16, 19–21). The extent of protein-bound ^{131}I production in 24 h, which was examined simultaneously with the protein-bound ^{132}I produced within 1 h, showed also a slight increase with absorbed dose (Exps. 19–21); this increase, however, is much less conspicuous, because a major part of the protein-bound ^{131}I present in blood at

24 h has been formed during the first hours after administration, that is, before any radiological changes have occurred.

The lowest dose of radiation which still was found to affect the rate of labelled protein-bound iodine production is less than 5 rads (Exp. 19; 50 rads is the accumulated dose over 24 h, but it should be remembered that the effects of radiation manifest themselves only a few hours after irradiation). This is of the order of magnitude of doses delivered to patients when determining the rate of formation of protein-bound iodine.

In one series of experiments (group 4) the butanol non-extractable fraction of the protein-bound iodine has been determined, and it was found to increase rapidly with the increase of absorbed dose (Exps. 12–16). This has been previously observed, but at one hundred-fold doses[8,9]. The question remains whether this observation is true also of the human thyroid; a clinical investigation has been undertaken in this laboratory to examine this possibility.

It may be concluded that a certain radiological damage is caused to the thyroid gland of rats at relatively low doses of radiation, of the same order of magnitude delivered by diagnostic tracer doses. This damage manifests itself by an increased production rate of protein-bound iodine which is of abnormal composition, containing substantial amounts of a butanol non-extractable component.

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Genetic Effects of Strontium-90 Injected into Male Mice

IN estimations of the genetic risks from the fall-out after bomb-tests little attention has been paid to strontium-90. This is in fact remarkable since Mazia[1], as early as 1954, suggested that bivalent metallic ions are bound in the chromosomes. Steffensen and LaChance[2] have shown that strontium-90 is incorporated into chromosomes in *Lilium* and *Habrobracon*. Therefore it is desirable to examine the possible genetic consequences. As a first step to study the effects of strontium-90 on the chromosomes we have analysed the rate of intra-uterine death among offspring to male mice, which received intraperitoneal injections of strontium-90. Strontium could cause chromosome breaks in spermatozoa and spermatids due to the radiation emitted from the strontium circulating in the blood and the lymph. Furthermore, strontium-ions could be incorporated into the chromosomes and could there, also through the disintegration process, cause chromosome breaks which afterwards cause the death of a zygote.

In order to distinguish between these two processes, which are not mutually exclusive, we also examined the effects of injections with caesium-137, which within the animal emits most of its radiation energy through β-radiation, as does strontium-90. If at first the time that the isotopes are in the circulatory system is ignored one would expect about similar effects from caesium-137 and strontium-90. Since about half the amount of strontium-90 injected is incorporated into the bones within a rather short time, the dose delivered from the isotopes in blood and lymph would probably be reasonably higher for caesium-137 than for strontium-90. Hence, one would *a priori* expect a stronger effect of caesium-137 than from strontium-90 on the rate of intra-uterine deaths.

Table 1. EFFECT ON INTRA-UTERINE DEATH AFTER INJECTION OF CAESIUM-137 AND STRONTIUM-90 IN 4 AND 5 WEEKS RESPECTIVELY AFTER INJECTION

Treatment		No. of Dead	No. of Implants	% dead
^{137}Cs	18 μc.	216	2,161	10·0*
^{137}Cs	38 μc.	55	703	7·82
NaCl		188	2,078	9·05
^{90}Sr	18 μc.	987	8,051	12·26†
NaCl		690	8,848	7·80

* Caesium-137 18 μc. versus NaCl $\chi^2 = 1·1$; d.f. 1
† Strontium-90 versus NaCl $\chi^2 = 91·4$; d.f. 1

In these experiments animals from inbred *CBA* strain were used. The males were injected intraperitoneally with either 18 μc. caesium-137 or strontium-90. In one small series some males were given 38 μc. caesium-137. Each male was mated to three females within 1 h after the injection. Every 7th day the females were replaced by new virgins. After the mating period the females were stored until the 19th day following the start of mating and were then killed and the uterine content examined. Here only the results from the first 5 matings are presented. In the case of caesium-137 only 4 matings were performed. In all cases there were simultaneous controls where the males were given saline injections.

The results are given in Table 1 wherein the data are combined for all mating periods. From Table 1 it is evident that the injections with caesium-137 had no demonstrable effect as regards intra-uterine death. On the other hand, the material in the strontium series shows a marked increase compared with its control. Separation of the material into mating periods is presented graphically in Fig. 1 for caesium-137 and Fig. 2 for strontium-90. This reveals in both cases a peak in the 3rd week after

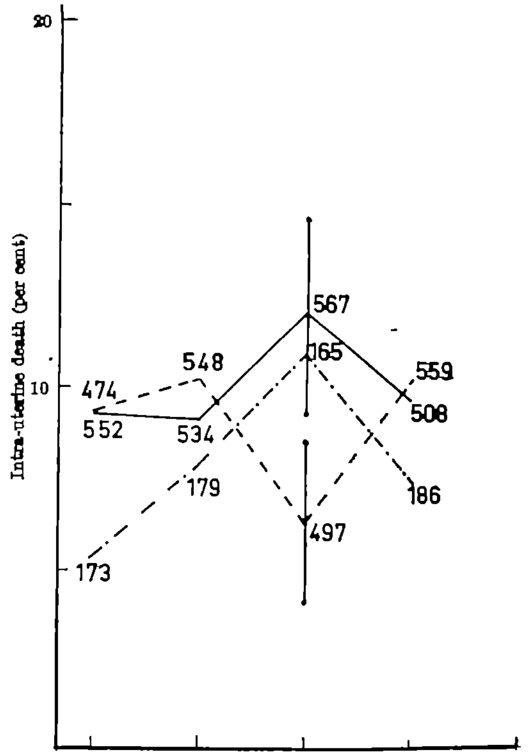

Fig. 1. Intra-uterine death in weeks after injection of caesium-137. Number of implants are given on the graphs. —, 18 μc. caesium; —·—, 38 μc. caesium; - - -, sodium chloride

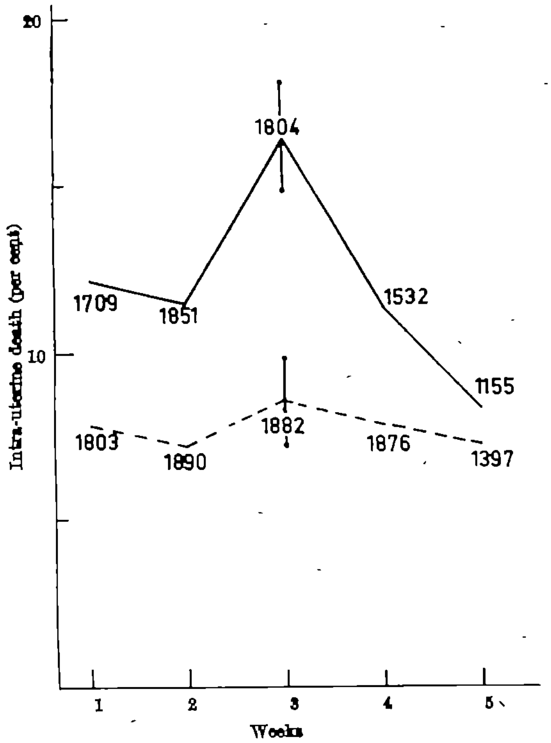

Fig. 2. Intra-uterine death in weeks after injection of strontium-90. Number of implants are given on the graphs. ——, 18 μc. strontium; – – –, sodium chloride

injection. This is similar to the results from X-ray experiments[3] when the peak also appears in the third week after treatment. It is supposed that this corresponds to the spermatid stage, which in *Drosophila*, too, is a very sensitive stage[4].

The aforementioned hypothesis about equal or stronger effect of injected caesium-137 compared with strontium-90 is not supported by the results presented here; rather the reverse. The effect of strontium-90 is much stronger than that of caesium-137. This could be explained if strontium-90, in agreement with Mazia's idea about divalent metallic ions, were incorporated into the chromosomes and, hence, could more easily cause chromosome breaks.

Since sperm used as early as the first week after the injection had a markedly increased rate of intra-uterine death, one would suppose that strontium-ions could penetrate the sperm and exchange with calcium-ions. If the process can go in that direction it may also revert. When the concentration of strontium in blood decreases due to incorporation of this element into bones and due to excretion in urine and faeces the strontium-ions in chromosomes could be replaced by calcium-ions.

In favour of this tentative hypothesis are the results from the fourth and the fifth weeks. During these weeks germ cells are used which at the time of the injection were in meiosis or prior to meiosis; in other words, stages where the chromosomes to be included in sperm are synthesized. If strontium-90 were incorporated stably in the chromosomes one would expect that during spermatid and spermatozoon stages disintegration would cause chromosome breaks that would cause zygotic death. The results show rather that during these periods the rate of intra-uterine death in the strontium-90 series is approaching that of the control.

If the incorporation of strontium-90 into the chromosomes were for a short time only, chromosome breaks induced in meiotic and pre-meiotic stages would be eliminated before being included in sperm. Hence, one would not expect any considerable effect of strontium-90 in sperm from weeks 4 and 5 after injection.

In order to examine further the effects of strontium-90 we have started one experiment with injections into females and in another we shall analyse the possible occurrence of recessive lethals in spermatogonia after injections of strontium-90.

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Protein Nutrition and Radiation Damage in Mice

In the case of protein nutrition and irradiation, a few experiments have been reported but are not all in agreement. In the work reported here, experiments were performed to test the influence of protein content in the diet on the survival rate of γ-ray irradiated mice. The effect of protein was examined both before and after irradiation.

The experimental animals were young *d–d* colony male mice. Some animals were fed the basal standard diet (including 18 per cent of casein, 69 per cent of α-starch, 6 per cent of corn and liver oil, 1 per cent of Macoollum's salt mixture, 1 per cent of vitamin mixture and 2 per cent yeast powder), and others were fed the protein-deficient diet (casein was replaced with α-starch) for two or three weeks before radiation. Diets and water were given *ad libitum*. At the end of the period they were exposed to a single dose of 1,000 r. of whole-body γ-irradiation. The nutritional conditions afterwards were kept the same as before radiation. The control groups without irradiation served as references. The survival rate for two weeks after irradiation is given in Table 1.

Table 1. Effect of Protein Diet before Irradiation on the Survival Rate of Mice

Group No.	Diet groups Protein content of diet (%)	γ-ray radiation	No. of animals before irradiation	No. of animals survived two weeks after irradiation
1	6	+	25	0
2	6	—	24	24
3	18	+	25	3
4	18	−	25	25

In other separate experiments the effects of protein diets after radiation were examined. Mice were fed and irradiated as already described. Directly after irradiation, the diets were switched from low protein to standard and vice versa. The results are given in Table 2. Those of the control groups without change of diet as well as without irradiation are also shown in Table 2.

Table 2. Effect of Protein Diet after Irradiation on the Survival Rate of Mice

Group No.	γ-ray irradiation	Diet groups Protein content of diet before radiation (%)	Protein content of diet after radiation (%)	No. of animals before irradiation	No. of animals survived three weeks after irradiation
1	−	6	6	11	10
2	−	6	18	12	11
3	−	18	18	18	18
4	+	6	6	20	4
5	+	6	18	20	11
6	+	18	18	20	11

A favourable effect of protein enrichment after irradiation was demonstrated.

In the next series of experiments, the optimum amount of protein necessary after irradiation was studied. Experimental conditions were the same as described here.

Table 3. OPTIMUM AMOUNT OF PROTEIN NECESSARY FOR THE RECOVERY FROM RADIATION DAMAGE

		Diet groups			
Group No.	γ-ray irradiation	Protein content of diet before irradiation (%)	Protein content of diet after irradiation	No. of animals before irradiation	No. of animals survived two weeks after irradiation
1	–	6	6	10	9
2	–	6	12	10	10
3	–	6	18	9	7
4	–	6	24	9	7
5	–	6	30	15	7
6	+	6	6	20	7
7	+	6	12	20	8
8	+	6	18	20	14
9	+	6	24	19	4
10	+	6	30	19	4
11	+	18	6	20	5

The results are given in Table 3. The 6–8 group showed the most favourable effects.

From these results it may be concluded that protein diet after, as well as before, irradiation had a pronounced influence on the mortality of whole-body irradiated mice, and that excessive supplement of protein after radiation produced unfavourable effects. Jennings[1] reported high susceptibility of protein-depleted rats to whole-body X-irradiation. His results were essentially in accordance with ours. Smith[2] observed little effect of protein reduction or enrichment after radiation, which was contrary to our results.

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BIOLOGY

Occurrence of *Discocotyle sagittata* on Sea Trout

Discocotyle sagittata Leuckart is a well-known monogenetic trematode found on the gills of European salmonids[1–3]. So far as I am aware it has previously only been found on hosts caught in fresh water, and it is therefore of interest to record it from sea trout (*Salmo trutta* L.) caught in the sea.

During 1953–61 inclusive, sea trout caught by beach seine in Port Erin bay were examined for their ectoparasites. The gills of each fish were removed soon after capture and examined under a binocular microscope. The numbers infested with *Discocotyle* are given in Table 1.

Table 1. NUMBERS OF SEA TROUT INFESTED WITH *Discocotyle sagittata*

Sea trout examined		No. infested
Males	30	6 (20·0%)
Females	132	32 (24·2%)
Total	162	38 (23·5%)

The number of worms varied between 1 and 14 per infested fish with a mean of 4·2. Out of 151 worms, 117 (77·5 per cent) were on the first gill arch, 15 (9·9 per cent) on the second, 11 (7·3 per cent) on the third and 8 (5·3 per cent) on the fourth gill arch. Compared with the data given by Llewellyn and Owen[4] for *Discocotyle* on trout from fresh water, the percentage infestation is much lower, although the mean number of parasites per infested fish is much the same. Furthermore, at Port Erin a much higher proportion of parasites was found on the first gill arch.

There are insufficient data to distinguish definit seasonal fluctuations in the incidence of *Discocotyle*. Se trout were caught in all months of the year with a peak i spring, and although *Discocotyle* was recorded in al months except December–February inclusive, its absenc at that time may have been due to low fishing effort Specimens of *Discocotyle* were usually producing egg whenever they were found. The eggs appeared normal but attempts to hatch them in salt water or fresh wate were unsuccessful.

As it has now been found on hosts in both marine an fresh waters, it would appear that *Discocotyle* possesse osmoregulatory powers. It is also possible that we ar dealing with two closely related species, but I could fin no differences in whole mounts of *Discocotyle* from se trout caught in the sea and brown trout from fresh water Furthermore, Frankland[5] notes an instance of a rainbo trout which had been gradually acclimatized to sea water and which was found to harbour healthy specimens o *Discocotyle sagittata*.

Some simple experiments showed that *Discocotyle* i capable of osmoregulation. *Discocotyle* from sea trou survived for at least 48 h after direct transfer from se ($S = 34$ parts per thousand) to fresh water. In the revers case, however, a period of acclimatization may be necessary, for specimens of *Discocotyle* from fresh water brown trout did not survive transfer to full sea water, although they would tolerate 50 per cent sea water for at least 48 h.

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Daily Age Markings on the Shell of Cuttlefishes

MUCH information concerning the growth of the shell in mollusca is available, and this indicates the close relation between growth of shell and physiological periodicity of the living body[1].

As was pointed out by Appellöf[2], Naef[3] and Yasuda[4], the formation of the stripe pattern on the shell of cuttlefishes shows a set periodicity. Yagi[5], who followed the growth of the shell stripe pattern of *Sepia esculenta* Hoyle, contends that, although the shell stripe pattern increases with the age, a double resultant sigmoid growth-curve holds (with age as the basis) good to this case, that is, that the periodicity of formation of shell stripe pattern is about 2 days at the stage immediately after hatching, 1·5 days at the most rapid growth stage and about 9 days at the latter stages of growth with less formative force. This gives an average of about 3·5 days throughout the whole period of growth. However, since Yagi's results are based on the assumption that the hatching date is July 1 for *Sepia esculenta* and that the environmental conditions are the same for all individuals, certain errors become involved.

Immediately after hatching, the cuttlefish has a frail shell. On the dorsum of the shell, there are observed a distinct Wurstlamellen, and, in the striated area of the ventrum of the shell, there are as many stripe-lines as Wurstlamellen are formed. The number of the shell stripe-lines is 7–8 for *Sepia officinalis*[2] just hatched, 6–8 (7 average) for *Sepia esculenta*, 7–9 (7·8 average) for the *Sepia subaculeata*, and 7–8 (8 average) for *Sepiella maindroni* (Table 1). These numbers of stripe-lines increase with growth.

The periodicity of formation of the stripe-pattern of the shell of *Sepia esculenta* is affected by nutritive and environment conditions such as deficiency in feed, lower salinity of the surrounding sea-water, lower oxygen content, lower

Table 1. Relation between the Day-Age and the Number of Stripe-lines of the Shell in the Cuttlefish

Species	No. of specimens	Days after hatching	Shell-length (mm)		No. of stripe-line	
			Range	Mean	Range	Mean
Sepia esculenta	12	0	3·59–5·04	4·23	6–8	7·0
	12	18–19	9·4–12·4	11·1	16–20	18·6
	1	23	14·0	14·0	23	23·0
	5	24	14·0–16·0	14·7	24–24	24·0
	3	26	15·5–18·0	17·2	25–27	26·0
	11	29–30	18·0–24·0	19·9	29–31	29·7
	5	32–33	21·2–24·2	23·2	32–33	32·8
	2	35	21·0–21·3	21·2	34–36	35·0
	2	37–40	22·6–24·2	24·8	38–39	38·3
Sepia subaculeata	16	0	5·11–7·59	5·80	7–9	7·8
	8	27–29	17·4–24·2	21·7	27–30	28·3
	2	31	24·1–26·1	25·1	31–31	31·0
	2	32–33	24·2–24·6	24·4	32–33	32·5
	19	54–63	42·0–60·0	49·8	54–64	59·1
	2	66–75	56·5–64·0	60·3	68–71	69·5
	6	109–114	124–155	142·4	108–112	110·0
Sepiella maindroni	10	0	3·04–3·17	3·14	7–8	8·0
	29	21–27	12·0–16·2	14·2	8* + 21–28	8* + 25
	1	40–45	33·0	33·0	8* + 43	8* + 43
	1	48	35·8	35·8	8* + 48	8* + 48
	3	50–52	51·2–56·0	53·5	8* + 52–54	8* + 53·3
	3	54	45·2–46·2	45·4	8* + 54–54	8* + 54
	18	94–100	68·5–86·5	78·7	?† + 88–98	?† + 92·1
	8	127–133	94·0–106·0	97·8	?† + 90–100	?† + 95·5

* The original mean number of stripe-line of the shell in the newly hatched *Sepiella maindroni*.

† Since the rear end of the shell was broken, the number of stripe-lines could not be counted exactly.

temperature of the water, etc. Table 1 shows the relation between the day-age and the number of stripe-lines for three species of cuttlefishes (*Sepia esculenta* Hoyle, *Sepia subaculeata* Sasaki and *Sepiella maindroni* de Rochebrune) reared under sufficient feeding and environmental conditions at a water temperature of 19°–30° C. As with *Sepia esculenta* and *Sepia subaculeata*, the number of stripe-lines, including those observable at the time of hatching, approximates well to age in days; in *Sepiella maindroni*, the number is equal to the age in days plus the lines at the time of having been hatched. In other words, it can be said that the shell stripe-lines are formed at the rate of one each day. Between *Sepia esculenta* or *Sepia subaculeata* and *Sepiella maindroni*, however, there is a difference in periodicity of stripe-line formation: the *Sepiella maindroni* is seen to start forming its stripe-lines at the rate of one per day from the very date of hatching, whereas the other two species are presumed to be dull in their stripe-line formation at the earlier period after hatching. Noteworthy in this connexion is that the resistance against fasting of *Sepiella maindroni* is 6–7 days, whereas it is 10–11 days in *Sepia esculenta* and 12–15 days in *Sepia subaculeata*.

Table 1 gives results obtained under sufficient nutritive and environmental conditions at a water temperature of 19°–30° C. When amount of food is insufficient or feeding does not go smoothly owing to unfavourable changes in the sea-water for rearing, the formation of stripe-pattern becomes disturbed and the periodicity of one stripe-line per day is no longer exact. With insufficient feeding especially, the stripe-lines either do not appear or are indistinct with a reduced distance between each. The locular index for this sort of shell is likely to be decreasing. The shell locular index of the cuttlefishes is one of the most significant characteristics for diagnosis of species[4], so it must be recognized that this characteristic within a somewhat wider range, depending on conditions for growth, varies.

The fact that the number of stripe-lines for *Sepiella maindroni* which were reared for 127–133 days or until October 31 averages + 95·5 (Table 1) is thought to be ascribable to the lowering of the water temperature to below 19° C during mid-October and afterwards, that is, to the consequent disturbance of the periodicity of formation of the stripe-line.

The shell stripe-line of cuttlefishes is therefore shown to be formed one per day as a rule under good conditions of nourishment, but this does not hold in cases where the nutritive and environment conditions become disturbed so that physiological periodicity is upset.

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Rearing of Cuttlefishes and Squids

We have succeeded (through improving the feed during the earlier stages after hatching) in rearing three species of the cuttlefish (*Sepia esculenta* Hoyle, *Sepia subaculeata* Sasaki and *Sepiella maindroni* de Rochebrune) and two species of the squid (*Sepioteuthis lessoniana* Lesson and *Euprymna beryii* Sasaki)[1]. The main points to note in rearing these animals are as follows:

Great care should be taken of eggs over a fairly long period until hatching. The vital point in the care of eggs is always to arrange that they are in contact with fresh-water, thus preventing the lowering in salinity of the storage sea-water.

If the eggs are exposed to too much light, diatoms or green algae tend to adhere to their surface, thus affecting adversely the development of the eggs. Eggs are better kept in the dark.

The five species of the cuttlefish and the squid mentioned here start feeding 16–48 h after hatching.

At the earlier stages, when about 2–10 mm long, considerable motility, though not too much, should be allowed. Suitable food are *Mysis*, larvae of the shrimp and other small crustacea.

A glass water tank is best for rearing. Flowing sea-water containing dissolved oxygen at saturation point encourages feeding.

Having grown to 20 mm mantle-length (four species except *Euprymna beryii* achieve a mantle-length of 20 mm in about 30 days after hatching), the animals begin to prey on small-sized shrimps or fishes.

Following these requirements, 80 per cent survival can be expected, and provided that the supply of food is satisfactory even after the animals begin to prey on large-sized food, they may reach adult size 3–4 months after hatching.

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Estimation of Dry-Matter Intake in Wild Herbivores

For some years now, I have been studying nutrition in the wallaby, *Setonix brachyurus*, on Rottnest Island, Western Australia. A technique[1] was developed for estimating the relative abundance of various food items in the diet. To convert these data to absolute quantities, total daily intake of dry matter was required. The methods at present used in estimating dry-matter intake by freely grazing livestock may be classified according as they make use of: (1) pasture productivity assessments; (2) consumption-defæcation ratios; (3) indigestible markers; (4) fæcal nitrogen. For various reasons none of them is applicable to a wild herbivore feeding on natural vegetation.

It seemed axiomatic that the quantity of dry matter ingested each day should be equal on average to that leaving the stomach either by local absorption or by passage into the duodenum. In other words, to obtain the daily rate of ingestion, all that was needed was the daily rate of loss from the stomach. It was believed (wrongly, it was learnt later) that the wallabies fed all night and that their stomachs would consequently be most full at dawn. If a large number of wallabies were caught before sunrise and divided into batches which were dissected at regular intervals throughout the following day, comparison between batches in mean weight of dried stomach contents should reveal the rate of loss from the stomach for all possible weights of contents (Calaby[2] had suggested that the rate of passage in *Setonix* was affected by degree of fill). This would cover the period of fasting, sunrise to late afternoon. For the remainder of the day the loss would be computed by applying to weights of stomach contents observed at various times in the night the rate of loss obtained at those weights in fasting animals.

Seventeen adult wallabies were caught early in the evening of November 16, 1960, and another 11 before midnight, both batches being dissected within an hour of capture. On the following morning, between 3 and 4.30 a.m., a further 55 animals were caught and divided into 5 batches. The stomachs from one batch were immediately dissected out, the remainder at approximately 4-hourly intervals. Stomachs were preserved in a drum of 70 per cent alcohol; their contents were later scraped into enamel dishes, oven-dried at 100° C for 36 h, and weighed. Mean weight of wallabies was 3·05 kg for males, and 2·51 for females.

When the results were graphed, stomach contents against time of dissection, a straight line could be fitted through the means of batches 2–7. This suggested: (1) that feeding had virtually ceased by 11 p.m.; (2) that loss from the stomach was proportional only to time. Both discoveries were contrary to the preconceived ideas with which the investigation was designed. The second, however, greatly simplified the analysis. Instead of having to integrate changing rates of stomach loss through 24 h, all that was required now was the slope of the best fitting line through the means of batches 2–7.

Table 1. ADULT MALE WALLABIES, SHOWING BY BATCHES AVERAGE TIME OF CAPTURE AND DISSECTION AND MEAN DRY WEIGHT OF STOMACH CONTENTS (FEMALE DATA IN BRACKETS)

Batch	No.	Time Caught	Time Killed	Dry weight of stomach contents (g)
1	10 (7)	19.48	20.42	88·8 (71·7)
2	4 (7)	23.17	23.56	95·8 (87·2)
3	9 (4)	03.35	05.41	82·9 (79·3)
4	6 (5)	03.37	09.05	77·1 (74·9)
5	3 (8)	03.47	12.37	73·9 (70·8)
6	5 (4)	03.39	15.54	64·2 (65·5)
7	4 (7)	03.55	20.29	54·2 (58·0)

The line through the means of batches 2–7, weighted for number of animals in batch and fitted by the method of least squares, had an equation of $y = 96{\cdot}1 - 1{\cdot}86\,x$ for males and $y = 87{\cdot}9 - 1{\cdot}39\,x$ for females, where y is the dry weight of stomach contents in grams and x is the time elapsed in hours since the dissection of batch 2. Thus the rate of loss of dry matter from the stomach of adult males fasting for at least 20 h was constant at 1·86 g/h, and in adult females at 1·39 g/h. Although the investigation could not give information on the rate in feeding animals, there is no obvious reason why it should differ from that in fasting wallabies. Material cannot pass through the pylorus until it attains a certain degree of maceration, and it seems unlikely, on reflexion, that the process should be hastened by feeding or other activity. The hourly rates have therefore been extrapolated to give daily rates of loss of dry matter from the stomach of 44·7 g in males and 33·4 g in females.

Strictly, observation of the rate of loss from the stomach yields an estimate of the food required for 'topping up', rather than a prediction of how much would have been eaten in the following evening. Financial assistance was granted by the C.S.I.R.O. and the University of Western Australia.

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Termination of Pregnancy in the Rat with Norethynodrel

CLEAR indications that norethynodrel diminishes the chances of survival of the conceptus have been obtained in the rat[1,2]. It was therefore conceivable that post-coital administration of the steroid could induce sterility in this species. The possibility seemed well worth investigation, as a similar action in humans would appear to offer efficient alternatives to the present contraceptive application of norethynodrel.

Data are presented that demonstrate the effectiveness of norethynodrel in terminating pregnancy in rats before and after nidation.

Rate of Nidation. Adult female white rats detected in procestrus or cestrus from daily vaginal smears were mated with fertile males. Mating was allowed to occur during a 24-h period. Thereupon, the female rats were randomly allocated to treatment groups. Soon after fertilization was presumed to have occurred, and before the sixth day post-coitum when nidation usually takes place in the rat, norethynodrel in sesame oil was administered subcutaneously either by single injection or daily injections for 3 days in the quantities shown in Table 1. The rate of nidation was ascertained at autopsy 10–12 days after mating (all intervals are taken from the initial day of mating). With the aid of a dissecting microscope the number of mature corpora lutea was counted. The number of corpora lutea was assumed to be an estimate of the number of ova shed. Thus, the rate of implantation in a group represented the proportion of fœtuses to corpora lutea. In Group III (Table 1) the rate of nidation was based on only four rats because corpora lutea present in the ovaries of one rat had not been counted.

A glance at Table 1 reveals that norethynodrel prevented the occurrence of nidation in Groups I, II and IV. A possibility of delayed implantation was checked by flushing the uteri with saline. No blastocysts were found to be present.

Fœtal Resorption. As in the previous experiment, adult female rats were mated when in procestrus or cestrus. Norethynodrel was administered by gavage in doses of 10 mg per day for 3–5 days as shown in Table 2. Control animals were given 1 ml. of sesame oil for 5 days by gavage. Pregnancy was permitted to go to term, and the frequency and size of litters were recorded. The incidence of resorption was determined from signs of blood in the vagina at the time of treatment and from examination of the uterine contents at autopsy 21–27 days after mating.

Clearly, norethynodrel induced extensive fœtal resorption. The treatment of longer duration proved completely effective in terminating pregnancy. Included in the litter-size data for Group II are at least five stillbirths, on the grounds that they represented failures to resorb. In two rats, stillbirths were associated with delays in parturition of about 4 and 6 days.

These data show that post-coital administration of norethynodrel can suppress fertility in the rat. The

Table 1. EFFECT OF SUBCUTANEOUS ADMINISTRATION OF NORETHYNODREL ON THE RATE OF NIDATION IN RATS

Group	Norethynodrel Dose (mg)	Day from mating	No. of rats	No. pregnant	Mean No. corpora lutea ± *S.E.*	Mean No. foetuses ± *S.E.*	Rate of nidation (per cent corpus luteum)
Control	0·5 ml. oil	2	5	4	16·0 ± 1·3	10·8 ± 2·8	67·5
	0·5 ml. oil	3					
	0·5 ml. oil	4					
I	1	2	4	0	14·8 ± 2·3	0	0
	3	3					
	5	4					
II	9	2	4	0	14·5 ± 3·0	0	0
III	0·1	2	5	4	18·3 ± 1·9*	11·2 ± 2·8	86·2*
	0·3	3					
	0·5	4					
IV	0·9	2	5	0	16·6 ± 2·0	0	0

* Based on 4 rats.

Table 2. EFFECT OF ORAL ADMINISTRATION OF NORETHYNODREL ON FOETAL RESORPTION IN RATS

Group	Norethynodrel Daily dose (mg)	Days from mating	No. of rats	Incidence of resorption	No. of litters	Mean litter size ± *S.E.*
Control	1 ml. oil	9–13	5	0	4	8·2 ± 2·3
I	10	9–13	5	5	0	0
II	10	10–12	5	5	3	2·4 ± 1·4*

* Includes stillborn; less than control, $P < 0·01$.

treatment proved to be most effective before implantation; 0·9 mg norethynodrel given subcutaneously on the second day post-coitum completely abolished pregnancy, while daily 10 mg doses by gavage from day 10–12 allowed some fœtal development to proceed unimpaired, though litter size was very significantly depressed. Hence, previous reports[3,4] that in ovariectomized rabbits as much as 2 mg per day of norethynodrel post-coitum fails to induce implantation are most probably explained by its potent inhibitory action on this phase of pregnancy, rather than a simple lack of progestational activity. It is known that nidation of the rabbit blastocyst can be prevented by administration of the steroid prior to coitus[4].

How norethynodrel terminates pregnancy is a pressing question. There is some indication that the steroid exerts an effect during tubal passage of the zygote. From Table 1 it can be seen that 0·9 mg norethynodrel completely suppressed fertility when given on the second day post-coitum, but when 0·5 mg of the same total dose was given on day 4 (Group III), by which time the rat blastocyst is usually in the uterus, the treatment was ineffective. It is possible that norethynodrel may interfere with tubal transport, but its ovacidal effects[4] clearly suggest a direct effect on the fertilized egg. These considerations minimize the importance of any influence norethynodrel may have on the uterus. However, the failure of large doses of norethynodrel to induce deciduomata in rats[3,4] together with its œstrogenic activity may lead one to suspect the steroid of an antidecidual property that could impede nidation. A disturbance of the developmental synchronization between the endometrium and blastocyst by the steroid may also contribute to preventing implantation[5]. The induction of fœtal resorption is probably the result of a specific action by norethynodrel; certainly it would not seem to result from an unmodified œstrogenic action by the steroid as the equivalent of 2·5 mg/kg/day œstrone (that is, 10 mg norethynodrel to 200-g rats, with norethynodrel possessing 5 per cent of the œstrogenic activity of œstrone) for 3 days was required to reduce mean litter size to 2·4. However, it is known that 0·4 mg œstrone/kg on day 10 can produce such a response[6]. If Sammelwitz *et al.*[7] are correct in asserting the hypophysis of the rat during pregnancy is responsible for only a single release of luteotrophin at the time of mating, then obviously a mediated effect via the hypophysis, similar to that involved in blocking ovulation with norethynodrel, cannot be invoked to explain the termination of pregnancy. Moreover, the steroid seems to have no direct influence on the ovary[4,5]. Consequently, the present effects on the conceptus appear to result from a direct action of norethynodrel or a metabolite. It should be pointed out that these observations are in no way incongruous with norethynodrel exerting an inhibitory effect on ovulation.

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Armillaria mellea: an Ozonophilic Basidiomycete

OZONE is a strong oxidizing agent and in very low concentrations is generally toxic to both micro-organisms and higher plants and animals. Davis[1] and others[2] have examined the bactericidal effects of ozone by growing *E. coli* in ozonated water solutions and found that 30 p.p.m./vol. caused 99 per cent lethality in 1 min or less, while 1 p.p.m./vol./8 h-day is considered toxic to man[3]. It was therefore surprising to find that *Armillaria mellea*, a luminescent basidiomycete grown in culture, had its light emission stimulated by high ozone concentrations and that it survived long ozone exposures without apparent lasting ill-effect.

I used both a commercial negative ion generator which produces ozone by ultra-violet light and a Wellsbach *T*-23 ozonator with either an air or an oxygen source. The concentrations were determined iodometrically[1,4] and by using a Wellsbach ozone meter with good correlation between the two methods. Changes in the light emission were used as an index of the respiration[5]. The general physiology of the organism and the culture and photometric methods have been previously described[5]. For these experiments the ozone was drawn through a flowmeter and into a glass experimental chamber with a light-tight metal jacket. Between the ozone source and the flow meter there was a by-pass system for sampling and calibration. Since there is a drop of approximately 50 per cent in ozone concentration across the jar which encloses the sample, the values given are those at the entrance of the experimental chamber. Concentrations were varied by changing both the source output and the rate of flow. The 7–10 day old cultures in small Petri dishes were mounted upright against the glass wall of the jar and the light emission was monitored continuously through an aperture in the metal shield. It was also determined that the uninoculated agar on which *A. mellea* was grown does not absorb or decompose ozone at a faster rate than an actively growing culture.

At all concentrations tested (75–500 p.p.m./vol.) there was an initial and usually transient rise in the light emission intensity (Fig. 1). At 75 p.p.m. it took 3 h for this peak to be reached while at 500 p.p.m. it took 3 min. At concentrations of 200–500 p.p.m. it took 3–6 h for this gain to be lost and the light-level to return to the

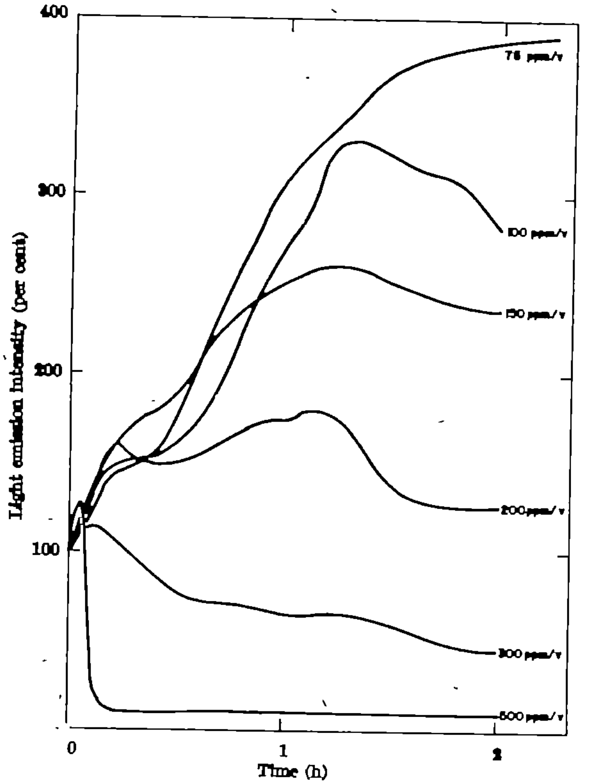

Fig. 1. Changes in light emission intensity of *Armillaria mellea* during exposure to various concentrations of ozone

original steady state. A 3-h exposure at all concentrations did not kill any of the samples. When removed from the ozone atmosphere all recovered to light emission levels at least equivalent to the pre-exposure value (100 per cent) within 3 days. When *A. mellea* was grown in an ozone atmosphere of 100 p.p.m. for 5 days the light intensity declined about 50 per cent in the first 24 h. After 5 days under these conditions the light-level was extremely low but measurable. Part of this low intensity is undoubtedly due to drying of the agar.

At concentrations of 200 p.p.m. and higher there was distinct bleaching of the brown melanin-type pigment found in normal hyphæ of *A. mellea*. During recovery there was no reducing of the pigment of exposed cells back to the original brown colour. Hyphæ growing out of the exposed mycelium have the usual brown pigmentation. There is thus a strong possibility that this pigment acts as an ozone protecting substance by preventing the formation of free radicals and peroxides. Ozone damage has usually been attributed to these causes[6,7]. This is particularly likely since we have found that *Panus stipticus*, another luminescent basidiomycete with no pigment, is killed within 10 min in 100 p.p.m. of ozone. Also, young cultures of *A. mellea* which have little or no pigment are much more affected by equivalent concentrations than more mature samples. This unusual ozone protection mechanism is under continued investigation.

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Increased Tolerance of Bean Plants to Soil Drought by means of Growth-retarding Substances

In experiments with *Chrysanthemum morifolium*[1] plants treated with (2-chloroethyl) trimethyl ammonium chloride ('CCC') and *Petunia hybrida*[2] plants treated with 2,4-dichlorobenzyltributyl phosphonium chloride ('Phosfon') it has been observed that treated plants seemed to be less susceptible to water stress than untreated ones. High temperatures following periods of low light intensities caused wilting in control plants, while treated plants remained turgid. These substances are growth-retarding materials[1,2], and some of their physiological activities have been reported[3].

In recent work on drought resistance it was found that it is possible sometimes to increase the tolerance of plants to drought by means of chemical treatment[4]. The foregoing observations prompted us now to include these chemical groups in studies on drought responses of plants, and this report summarizes two typical experiments using 'CCC' and 'Phosfon'.

Experiments were carried out with *Phaseolus vulgaris* L. var. Brittle Wax. Plants were grown in half-gallon cans, containing a loamy-sand soil (field capacity 14·7 per cent, wilting point of sunflowers 4·3 per cent). Ten seedlings were grown in each can. When the first pairs of leaves were fully expanded in most seedlings, 250 ml. of aqueous solutions were applied to each can containing 50 mg 'Phosfon' and 500 mg 'CCC', respectively. Each treatment was replicated 4 times (cans). Routine irrigation of the cans was continued until the expansion of the third leaf, after which the supply of water was stopped.

Five days after the last watering the leaves of the control (untreated) plants started wilting, and the growth of these plants ceased. Nine days afterwards leaves of the untreated plants began to dry and dropped. Most of the control plants were completely desiccated 30 days after the last irrigation. Fig. 1 represents the situation at the time when only a few of the youngest leaves of some of the control plants remained attached at a wilting stage.

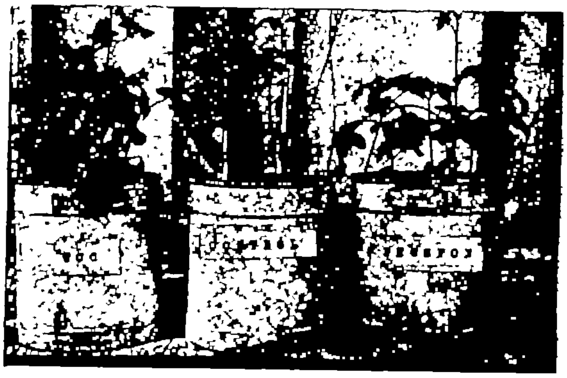

Fig. 1. Effect of 'Phosfon' and 'CCC' on bean plants, 30 days after last irrigation

In contrast, the treated plants remained turgid in these 30 critical days (Fig. 1). They continued growing for 22 days after the last irrigation. A few of the oldest leaves started showing signs of wilting on the twenty-eighth day after irrigation in case of 'CCC'-treated plants. In 'Phosphon'-treated plants some wilting was noticed only after 33 days. Nine days afterwards (42 days after last watering) leaves of the treated plants began to drop and the plants to dry up. At this stage soil moisture was determined in each can and is given in Table 1.

As seen from Table 1, the moisture percentage in the control soil was at the wilting point (4·36 per cent), while in the treated soil it was below wilting point (3·98 and able 1. SOIL MOISTURE OF CONTROL AND TREATED SOILS (42 DAYS AFTER IRRIGATION)

Treatment	Soil moisture (%)
Control	4·36
'Phosfon'	3·98
'CCC'	3 62
L.S.D. 0 05	0·31

3·62 per cent for the 'Phosfon' and 'CCC' treated soil, espectively).

In a second experiment, beans were sown in clay pots ontaining 500 g of soil (19 per cent field capacity, 7 per ent wilting point), 1 plant per pot. After emergence half he pots were watered with an aqueous solution of 'Phos-on' (12 mg/plant/pot). Routine irrigation was followed until the plants reached a stage of two fully expanded eaves, after which they were subjected to three different water régimes. This experiment was designed as a randomized block experiment, including four replicates per reatment, each of which was composed of five plants (pots). The soil moisture was determined by daily weighing of the pots. In the water treatments the plants were irrigated at different levels of soil moisture, as follows: *A*, 95 per cent available water; *B*, 20 per cent available water; *C*, near wilting point.

As the plants of treatment *A* reached a stage of six leaves all the plants were collected and their fresh and dry weights were determined (Table 2). As seen from Table 2, both the fresh and dry weights of 'Phosfon'-treated plants were considerably higher in the 'dry' treatments (*B* and *C*) than the untreated plants.

Table 2. EFFECT OF 'PHOSFON' ON THE GROWTH OF BEAN PLANTS GROWN AT DIFFERENT WATER REGIMES

Watering treatment	Control Fresh weight (g)	Control Dry weight (g)	'Phosfon' Fresh weight (g)	'Phosfon' Dry weight (g)
A) 95% available water	13·76	1·93	13·52	1·94
B) 20% available water	11·31	1·52	13·40	1·85
C) Wilting point	7·93	0·94	12·00	1·60

These results suggest that both 'CCC' and 'Phosfon' evidently increase the drought tolerance of plants.

'Phosfon' was supplied by Virginia-Carolina Chemical Corporation and 'CCC' by the Cyanamid Co., whom we thank.

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ENTOMOLOGY

Fatty Acid Characteristics of Some Insect Taxa

As a prelude to a study of fat metabolism in the parasitic dipteron *Agria affinis* (Fall.) 30 species of insects and 2 of spiders were surveyed for fatty acids by gas chromatography. Because *A. affinis* has peculiar nutritional requirements[1] it was supposed that it had a peculiar fat metabolism which would produce some equally peculiar fat composition.

The survey not only showed that such a peculiarity exists, but also that some broad similarities exist within taxa. Thus, among the 30 species of insects, representing 23 families and 8 orders, the 5 species of Diptera were characterized by a relatively high proportion of palmitoleic acid, a proportion approached by only the single neuropteron examined. Furthermore, among the 9 species of Homoptera, the 5 members of the family-group Aphidoidea had a proportion of myristic acid much greater than any other of the 30 species. No obvious characteristics of the orders were found among the 7 species of Coleoptera and 4 of Lepidoptera. The remaining orders and families were represented by too few species to permit any inferences.

Thus a biochemical property probably characteristic of an order has been shown in Diptera and of a family-group in Aphidoidea. These observations indicate that chromatography of the fatty acids may prove more useful than other techniques used so far in showing biochemical characteristics of insect taxa.

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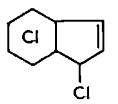
Significance of the Epoxidation of the Isomeric Insecticides Aldrin and Isodrin by the Adult Housefly *in vivo*

PERRY[1], commenting on the latent period between application of heptachlor (I) to susceptible houseflies and onset of signs of poisoning, pointed out that although there is an adequate amount of heptachlor in the insect tissues during this period, onset of signs of poisoning seemed to coincide with the appearance of heptachlor epoxide (II) in the tissues. These observations apply also to aldrin (III) and isodrin (IV)[2].



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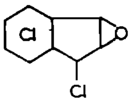

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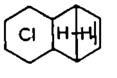

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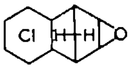

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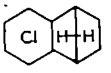
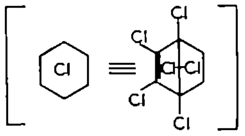


VII or VIII



Winteringham[3] suggested that the remarkable ability of houseflies to survive long periods of anoxia[4] might be exploited to determine the rôle of atmospheric oxygen in epoxidation *in vivo* and its toxicological significance. That epoxidation of III or IV by the adult housefly *in vivo* requires the presence of atmospheric oxygen was demonstrated by the failure to detect the formation of the corresponding epoxides (V or VI) when the insects were dosed and kept in oxygen-free nitrogen. Penetration of the applied insecticides through the cuticle was, however, unaffected by anoxia.

Compounds III, IV, V or VI were applied topically in acetone (2 µl.) to groups of ten susceptible adult female houseflies, *Musca domestica*, at a dose of 1·1 µg per insect. Groups were treated and observed in air at 25° C. Alternatively, groups were similarly treated after immobilization in oxygen-free nitrogen and held in nitrogen at 25° C for 60 min after treatment. These groups were then transferred to fresh air at about 25° C. The times of appearance of signs of poisoning and later knockdown effects are summarized in Table 1. The results show that anoxia has delayed by about 60 min the onset of signs of poisoning in the insects treated with aldrin or isodrin. The interval between appearance of signs of poisoning and complete knockdown was somewhat shorter than that observed in insects kept entirely in air. The knockdown effect in the dieldrin- or endrin-treated groups could, however, be recognized when they recovered from anoxia.

(95 min) and so was not delayed by more than about 15 min; the failure to observe the earlier signs of poisoning suggests that this stage was actually reached during anoxia. Moreover, aldrin- or isodrin-treated insects actually recovered and behaved normally between the times of removal from nitrogen and the times of appearance of first signs of poisoning (that is, between 60 and 133 min). One conceivable explanation was that anoxia had delayed penetration of compounds III and IV through the insect cuticle considerably more than that of compounds V and VI. However, gas-chromatographic analysis of tissue extracts showed that following a dose of 1·1 μg of endrin in air, 0·14 μg of the unchanged insecticide was recovered from the tissues within the hour, and following a dose of 1·1 μg of isodrin in nitrogen, 0·13 μg of unchanged insecticide was recovered after 1 h. This shows that the delays indicated in Table 1 are not due to delayed penetration of the insecticide through the cuticle.

Table 1. APPEARANCE OF SIGNS OF POISONING IN ADULT HOUSEFLIES EXPOSED TO INSECTICIDES IN AIR OR IN OXYGEN-FREE NITROGEN

	Insects dosed and kept in air at 25° C		Insects dosed and kept in nitrogen for 60 min and then observed in air at 25° C	
Compound	Time (min.) after treatment of first positive signs of poisoning, 'flight convulsions', etc.	Time (min) after treatment of 'knockdown' effects	Time (min) after treatment of first positive signs of poisoning, 'flight convulsions', etc.	Time (min) after treatment of 'knockdown' effects
Aldrin (III)	70	120	133	176
Isodrin (IV)	80	135	136	175
Dieldrin (V)	45	78	—	96
Endrin (VI)	45	80	—	90

These results show that aldrin and isodrin possess lower intrinsic toxicities than the corresponding epoxides. The higher polarity of the epoxides (as indicated by their higher R_F values under conditions of reversed-phase paper chromatography) may favour a more rapid penetration of these insecticides to the sites of action *in vivo*, possibly by permitting a more favourable partition between aqueous and lipid phases. Relevant is the observation that isodrin (0·37 μg), injected in 1 μl. of liquid paraffin, produced no signs of poisoning for 5 h in air, while with this dose of endrin signs of poisoning appeared in 2 h. This suggests that the more polar epoxide was released more rapidly into the hæmolymph from the injected oil. The insecticidal properties of aldrin or isodrin seem unlikely to be due entirely to epoxidation since the related dihydro-compounds (VII or VIII), which do not undergo epoxidation *in vivo*, are moderately toxic on their own account.

These experiments are connected with an investigation supported in part by a grant from the World Health Organization.

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Probable Mechanism regulating Water Economy of *Rhodnius prolixus*

FIFTH-STAGE larvæ of *Rhodnius prolixus* decapitated immediately (10–20 min) after feeding have been shown[1] to excrete less urine than normal controls. These results apparently contradict the report of Maddrell that "ligating the fed insect anterior to the ganglionic mass has no effect on urine production".

To decide between these alternatives the amount of urine produced by three groups of animals was measured: (a) with all the abdominal nerves (originating at the large fused ganglionic mass situated in the mesothorax) sectioned (the leg nerves were not sectioned); (*b*) with th connectives between the prothoracic ganglion and th mesothoracic fused ganglionic mass sectioned (the animals lose control over the two pairs of posterior legs (*c*) controls operated without neurotomy.

20 h after the operation all animals were fed wit oxalic (1 part per thousand) blood; their ani wer previously plugged to avoid loss of excreted urine. 3–5 after feeding they were dissected; the success of the opera tion was verified and the amount of urine produced we determined. The weight of the rectal contents we measured by difference with non-fed controls.

The values given in Table 1 confirm the earlier result obtained by decapitation in showing inactivity of th Malpighian tubes in all operated animals if operation i performed before feeding.

Table 1

	Weight of rectum (mg)		Weight of rectum contents (mg)	Weight animals (mg)
Non-fed controls	3	(*n* = 5)*		40
Fed controls	14	(*n* = 12)	11	197
Group (*a*)	4·8	(*n* = 18)	1·8	155
Group (*b*)	4·9	(*n* = 7)	1·9	250

* *n* = No. of animals

The absence of diuretic hormone in operated animal (judged from the amount of urine accumulated in th rectum) may be attributed, according to preliminary observations, as follows: In animals of group (*a*), to th interruption of the neurosecretory pathway caused by sectioning of the axons of the neurosecretory cells (responsible for the production of the diuretic hormone) in the fused ganglionic mass (the absence of neurosecretion in these cells resembles that described by Scharrer[3] after section of the n.c. allatum). In animals of group (*b*), to the interruption of the central nervous efference (efference which is due, probably, to dilatation of the gut[4]).

Furthermore, unpublished results show in normal animals the existence of a peripheral inhibition of central origin which is exerted on the abdominal wall and which can be demonstrated on sectioning the abdominal nerves on one side. This inhibition, probably related to that found by Dethier[5] in *Phormia*, could serve as a feed-back mechanism (via the hydrostatic pressure in the gut) for the regulation of the time of ingestion in *Rhodnius*.

The apparent contradiction with Maddrell's results may be due to the fact that already during ingestion there is liberation of some diuretic hormone since urine production starts in the first 15 min from the beginning of feeding.

It remains to be decided whether in the input of the regulating circuit there operate signals of abdomen volume apart from those signals which arrive through the prothoracic-mesothoracic connective.

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Mating Behaviour of *Anopheles stephensi*

THE successful eradication of screw worm fly from Curaçao and from the southern United States has created a great interest in the possibility of insect eradication by release of sterile males[1]. Even where complete eradication cannot be achieved, the method can still be used for limiting the population density of harmful species to a level where it ceases to be of importance. If this can be done for a certain length of time in the case of vectors of diseases, it is possible that the disease-cycle may be completely interrupted and the disease itself eradicated.

With this in view, we examined the possibility of the radication' of *Anopheles stephensi*, the main vector of alaria in southern Iran. This species has shown a marked sistance to DDT, BHC and dieldrin[2], thereby making alaria eradication by the use of residual insecticides lmost impossible. The use of sterile males, we thought, ay yield encouraging results and seemed feasible ecause: (1) they can be easily reared in the laboratory;) the males and females can be easily separated in the upal stage by the mechanical separating device of Fay nd Morlan[3]; (3) the males are completely harmless; (4) e males are powerful fliers—a flight-range of up to 4·3 m has been recorded (Quraishi *et al.*, 1962, data npublished)—and form big swarms for mating; (5) the ngevity of the male seems to be quite comparable with hat of the female[4].

The mating behaviour of the female was examined first. abelling' the male with phosphorus-32 was found very onvenient for such investigations. This was done by earing the third and fourth instar larvæ in water containing 10–20 μc. phosphorus-32/l. The emerging male osquitoes were counted in the top shelf of an Ericsson caling unit type 1221*C*. Their counts varied from 2,500 o 38,000 c.p.m. The mosquitoes were then dissected and heir reproductive organs counted. Table 1 summarizes he counts of males and their reproductive organs.

Table 1

Males (c.p.m.)	Reproductive organs (c.p.m.)
2,500—4,000	75—147
4,000—6,000	120—230
6,000—8,000	197—308
11,000—13,000	327—373
30,000—38,000	541—741

Males giving a count of 7,000–13,000 were chosen for his experiment and were kept with virgin females and emales which had oviposited once. Similar females were ept with 'unlabelled' males. After 24 h the spermathecæ f all the females were dissected. The spermathecæ of emales (both virgin and those which had completed one onotrophic cycle) kept with 'labelled' males showed a ount of about 1½–2 times the background. Background n this case was 12 c.p.m. The counts of the spermathecæ aried from 17·5 c.p.m. to 25 c.p.m.

The spermathecæ of the females kept with normal males id not show any increase in count rate above the background.

This clearly indicates that female *Anopheles stephensi* ates more than once and that mating can be investigated asily by the use of 'active' males.

We are carrying on further investigations on the easibility of using this method.

We thank Mr. H. A. C. McKay and Dr. M. L. Smith, ormer director and present director, respectively, of the ENTO Institute of Nuclear Science, for help and advice, nd Dr. Ch. M. H. Mofidi for providing the adults and arvæ of *Anopheles stephensi*.

Note. After having completed this communication, we ead with interest the article, "Chemical Sterilization of osquitoes", by Dr. Donald E. Weidhaas[5], and feel that elease of sterile males coupled with spraying structures ith 'Tepa' (provided the cost is reasonable and no health azards are involved) may provide an answer for the eradication[1] of the species.

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MICROBIOLOGY

Identification of Early Labelled Products of CO_2-Fixation by Hydrogen Bacteria accumulating Poly-β-hydroxybutyric Acid

THE effect of the reductive pentose phosphate cycle in hydrogen oxidizing bacteria has already been established[1,2]; the detection of early labelled organic acids[3] and of an unknown compound, situated beyond the area of phosphate esters on chromatograms[4], led to the assumption that other carbon dioxide fixation reactions might occur under certain conditions.

Since poly-β-hydroxybutyrate (PHBA) is synthesized from carbon dioxide and water in hydrogen-oxidizing bacteria[5], it became possible to investigate the fixation of carbon dioxide into this storage material by *Hydrogenomonas spec. 16* under controlled physiological conditions and excluding all side-reactions.

The incorporation of $^{14}CO_2$ into different cell fractions was investigated with autotrophically grown, washed cells, suspended in a dilute, nitrogen-free mineral buffer solution. After the incorporation time of 20 sec (30° C), 84 per cent of the totally fixed radioactive carbon was found in the ethanol-soluble extracts. About an equal amount of radioactivity of this ethanol extract (11 per cent) was observed in both the steam-volatile and the ether-soluble fractions, while the bulk of radiocarbon (73 per cent) was contained in the extract after removal of the fractions mentioned here (in P-esters, amino-acids and sugars).

The most intensively labelled compounds of the steam-volatile and ether-soluble acids of a 1-min experiment were identified by paper-co-chromatography and by conversion into and separation of the corresponding hydroxamic acids: acetate, lactate, pyruvate, succinate, malate, fumarate, β-hydroxybutyrate, citrate, glutamate and α-alanine. Lactate evidently was an artefact, formed from dihydroxyacetone phosphate, when the extracts were alkalized before concentrating them *in vacuo*.

Organic acids, amino-acids, sugars and phosphate esters have been separated by two-dimensional paper chromatography (Schleicher and Schüll 2043 *a*, washed paper) using the solvent system *n*-propanol–ammonia–water (6 : 3 : 1 by vol.) and *sec* butanol–formic acid–water (6 : 1 : 2 by vol.) according to Metzner[6]. Labelled products of carbon dioxide-fixation were identified by R_F-values or positon constants, by co-chromatography with reference substances, and by comparing extracts of *Hydrogenomonas 16* labelled with carbon-14 and phosphorus-32 with extracts of *Chlorella pyrenoidosa* labelled with carbon-14. In *Hydrogenomonas 16* phosphoglyceric acid and hexose monophosphates dominated; ribose-5-phosphate, dihydroxyacetone phosphate, nucleotides and phosphoglycolic acid were also found to be labelled. *Chlorella* showed considerably more activity in phosphoenolpyruvate, triose phosphates and hexose diphosphate.

When ^{14}C-acetate was given instead of $^{14}CO_2$ under storing conditions, the P-esters were not labelled, but β-hydroxybutyric acid and some other organic acids were found to be radioactive.

However, in autotrophically grown, washed cells under air incubated with $^{14}CO_2$ in the presence of succinate for 8 sec, the radioactivity first appeared in P-esters with a pattern typical for the Calvin cycle. Therefore we have to assume that even under heterotrophic conditions C-autotrophically grown cells assimilate limited amounts of carbon dioxide via the Calvin cycle.

When washed cells grown on succinate were exposed to $^{14}CO_2$ under oxy-hydrogen atmosphere for 9 sec, phosphoglyceric acid, hexose mono- and di-phosphates, and aspartic acid were very intensively labelled. The same cells under air, exposed to $^{14}CO_2$ in the presence of succinate, incorporated $^{14}CO_2$ mainly into malate, succinate and citrate, and, to a limited extent still, into phosphate ester compounds. These observations show that even cells grown heterotrophically on succinate, but which were derived from a 0·1 per cent autotrophically grown inoculum, contain a carbon dioxide fixation system identical with or similar to the reductive pentose phosphate cycle.

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Effects of Incorporation of Iododeoxyuridine on *Escherichia coli*

A variety of base analogues which are incorporated into deoxyribonucleic acid (DNA) affect the growth of mammalian cells, bacteria, plant and animal viruses. It has been shown[1,2] that thymine substitution by halogenated analogues is higher in thymine-less than in wild bacterial strains. To increase the incorporation in wild-strains, inhibitors of thymidylic acid synthesis were used (aminopterin or sulphanilamide). The substitution of the natural base by the brominated analogue produces some physiological and genetical effects. It was demonstrated[3] that bacteria labelled with bromouracil are highly sensitive to ultra-violet irradiation. In some cases the replacement of thymine in DNA produced a significant increase of the frequency of mutations at specific locations of phages T_4 or T_2 (refs. 4 and 5) or in *Salmonella typhimurium* at the tryptophan *D* locus[6]. In contrast to the brominated analogues, the iodinated analogues of thymine were not thoroughly examined until now.

To enhance the incorporation of iododeoxyuridine (IUdR) into a wild-strain of *Escherichia coli* I used fluorodeoxyuridine (FUdR), a specific inhibitor of thymidylate synthetase. Bacteria propagated in nutrient broth in log-phase were transferred into a minimal medium supplemented with 200 μg/ml. IUdR and 1 μg/ml. FUdR and grown for 3 h. In the presence of the fluorinated analogue, 43 per cent of thymine was substituted by IUdR, while without FUdR only 18 per cent. Bacteria containing IUdR showed an increased sensitivity to ultra-violet light. This sensitization of micro-organisms labelled with IUdR to ultra-violet light was significantly lower than that of bacteria containing bromodeoxyuridine.

The resulting radiosensitization, expressed as ultra-violet dose ratio for identical survivals of normal and analogue-labelled cells (dose reduction ratio), amounted to as much as 2·2 (43 mol per cent IUdR substitution) or 3·4 (46 mol per cent BUdR substitution). At the same time the rates of mutation were estimated in the system from streptomycin dependent to streptomycin independent. In bacteria containing a large amount of IUdR (about 50 per cent) the frequency of mutations increased 20 times, whereas in micro-organisms containing 15–20 per cent of the analogue the number of mutants was increased only three times. After ultra-violet irradiation, the frequency of mutation increased significantly, but the rate of the increase was higher in the control culture than in that containing IUdR. After irradiation with a dose of 2,000 ergs/mm^2 the number of mutants per survivals in the normal culture was even higher than in IUdR-labelled bacteria. This was presumably connected with the selection by ultra-violet light of IUdR labelled bacterial mutants. DNA isolated from bacteria containing IUdR showed a broadening of the profile of hyperchromasis after heating in comparison with the normal DNA.

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Utilization of Amino-Acids during Metabolism in *Escherichia coli*

While engaged in the examination of metabolism and hydrolysis by soil bacteria of benzoic acid derivatives, amino-acids, and acylated amino-acids[1-3], we have shown that there was a significant difference in strains of soil bacteria in their capability of metabolizing organic substances of different structures. We have now examined the metabolic activities of twenty-five strains of *Escherichia coli* standard *O* group on the following nineteen amino-acids to be used as a sole source of carbon: L-glutamic and DL-aspartic acids, DL-alanine, DL-serine, glycine, DL-valine, DL-leucine, L-isoleucine, DL-phenylalanine, L-tryptophan, DL-threonine, L-lysine, DL-methionine, L-cystine, L-arginine, L-histidine, L-tyrosine, L-proline, L-hydroxyproline.

The constituents of the culture medium used were: ammonium chloride, 0·1 gm; dipotassium hydrogen phosphate, 0·1 gm; crystalline magnesium sulphate, 0·05 gm; 1 per cent crystalline calcium chloride, 2 drops; 1 per cent crystalline ferric chloride, 1 drop; DL-amino-acid to be tested, 0·2 gm (L-amino-acid, 0·1 gm; L-cystine and L-tyrosine, 0·02 gm); distilled water, 100 c.c.; *p*H 7·4–7·6 (adjusted with 10 per cent sodium hydroxide).

Special attention may here be directed to the following points: (1) All 25 strains grew luxuriantly in the synthetic medium containing alanine, aspartic and glutamic acids or proline as the sole source of carbon at 37° within four days. This cultivation experiment was repeated three times in succession. (2) Of 25 strains, 19 were able to utilize serine as well as alanine. (3) The following 14 amino-acids could not or only just be utilized by any of 25 strains at 37° in four days: glycine, valine, leucine, isoleucine, phenylalanine, tryptophan, threonine, lysine, methionine, cystine, arginine, histidine, tyrosine, hydroxyproline. (4) The response of *E. coli* to histidine is worth noting from the point of view of phylogeny for Enterobacteriaceae when considered together with the fact that some strains of *Klebsiella*, *Cloaca*, *Citrobacter*, *Hafnia* and *Proteus* could utilize histidine as a source of carbon in our preliminary experiments. (5) 8 essential amino-acids such as tryptophan, lysine and methionine could not be utilized by *E. coli*.

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VIROLOGY

Guanidine Conditioned Infectivity of Ribonucleic Acid extracted from a Strain of Guanidine-dependent Polio-1-Virus

We have previously shown that polio-1-virus, when ropagated in HeLa cells in the presence of guanidine ydrochloride, can become resistant to guanidine, and, fter a number of additional transfers, will become lependent on guanidine[1-3].

The work recorded here attempts to evaluate: (*a*) the nfectivity of RNA extracted from either guanidine-ensitive or guanidine-dependent strains of polio-1-virus; *b*) the influence of guanidine on the infectivity of such ₹NA preparations.

The RNA was prepared essentially according to the nethod of Gierer and Schramm[4], except that the extracion was performed at 56° C. The infectivity of such reparations was tested on cells pretreated with hypertonic aline solutions. Virus preparations, as well as viral ibonucleic acid, were prepared as follows: three-day culures of HeLa cells were infected with 10^{8} cytopathogenic mits (CPU) of either guanidine-sensitive polio virus or irus which had become guanidine-dependent by 77 transers in the presence of 200 μg guanidine hydrochloride per nl. After incubation at 37° C for 2 h, 10 ml. of maintennce medium[5] were added to each flask. In some cases, the naintenance medium also contained 200 μg guanidine ıydrochloride per ml. The cultures were incubated at 7° C until cytopathogenic lesions were clearly evident. They were then submitted to three cycles of freezing at − 30° C, and thawing at + 20° C. Each preparation was livided into two portions. One portion was centrifuged at ,000 r.p.m. for 5 min, and the supernatant utilized for the letermination of complete virus by CPU titration. The econd portion was shaken for 10 min in a 56° C water bath vith an equal volume of phenol (Merck; peroxide free, aturated with water at 4° C). The mixture was briefly ooled in ice and centrifuged. The upper layer was xtracted 5 times with peroxide-free ether, and stored at − 30° C in small open Petri dishes.

HeLa cell monolayers were pretreated prior to infection y successive washes with 0·3 M sodium chloride, and hen 0·6 M sodium chloride. Immediately before infecion, the RNA preparations were thawed, diluted into volumes of 1·2 M sodium chloride, and incubated for 3 nin at room temperature in the presence or absence of μg RNase per ml. (RNase: BDH, Chemical Division, Poole, England). 1 ml. of decimal dilutions in 1·2 M odium chloride were used to infect the pretreated cell ayers. After 20 min at room temperature, unabsorbed RNA was removed with 2 washes of 0·6 M sodium chloride; 0 ml. of maintenance medium (minus serum) were added. The cultures were scored for cytopathogenic lesions after days at 37° C. All the cultures were disrupted by 3 ycles of freezing and thawing as before, and cell débris removed by centrifugation. Virus production in each of the cultures was assayed by determining the CPU in each of the supernatants. As shown in Table 1, RNA extracted from guanidine-sensitive polio virus was able to infect cell cultures and produce whole virus, as shown by the appearance of CPU. Guanidine hydrochloride inhibited the ability of this RNA to produce whole virus. On the other hand, RNA obtained from guanidine-dependent virus induces whole guanidine-dependent virus only when guanidine is present in the maintenance medium. The lack of infectivity of phenol extracts treated with RNase confirms the RNA nature of the infective materials.

Table 1

Inocula	CPU produced in three days by HeLa cells cultured in absence of guanidine	CPU produced in three days by HeLa cells cultured in presence of guanidine hydrochloride (200 μg/ml.)
Polio 1 guanidine-sensible	10^{6}	10^{5}
dem + RNase	10^{5}	10^{6}
RNA from polio-1 guanidine-sensitive; maintenance medium without guanidine	10^{5}	<10
dem ; maintenance medium with guanidine hydrochloride 100 μg/ml.	<10	<10
dem ; in presence of RNase; maintenance medium without guanidine	<10	<10
Polio 1 guanidine dependent	$<10^{4}$	10^{6}
dem + RNase	$<10^{4}$	10^{6}
RNA from polio 1 guanidine dependent; maintenance medium without guanidine	<10	<10
dem ; maintenance medium with guanidine hydrochloride 100 μg/ml.	<10	10^{5}
dem ; in presence of RNase; maintenance medium with guanidine hydrochloride 100 μg/ml.	<10	<10

These experiments clearly show that the guanidine-dependence of polio virus is a character assumed by viral RNA.

Experiments with guanidine labelled with carbon-14 are in progress to ascertain the possibility whether or not guanidine-dependent viruses incorporate guanidine.

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Immunological Response of an Elasmobranch to Human Influenza Virus

Investigations here concerning virus infections of marine animals and of their cells in tissue culture indicated the need for an understanding of the immunological responses of such animals. To our knowledge there are no published reports on antibody production in marine fish (elasmobranchs or teleosts), although such investigations have been reported for fresh-water fish[1-4]. This paper represents a preliminary report on the production of viral antibodies in sharks.

Three female 10–15 lb. lemon sharks, *Negaprion brevirostris*, were injected with the *PR* 8 strain of influenza *A* virus (chick allantoic fluid, hæmagglutinin titre of 6,400 units/ml.) according to the schedules seen in Table 1. The results of hæmagglutination inhibition tests with sera from these animals, performed at room temperature (approximately 27° C) and using guinea pig red blood cells, are also shown in Table 1. They clearly indicate that sharks are capable of forming antibodies to a mammalian virus. Heating these immune sera to 56° C for 30 min failed to reduce the hæmagglutination inhibition activity. Both normal and immune shark sera contained hæmagglutinins for guinea pig red blood cells as well as non-specific inhibitors in low concentration for influenza *A*–2 (Asian strain) and influenza *B* (Lee strain). The former could be removed by adsorption with guinea pig red blood cells and the latter by adsorption with kaolin without reducing the immune hæmagglutination inhibition titre to *PR*8 virus. Immune shark sera also neutralized *PR*8 virus infectivity for chick embryos, as seen in the corresponding part of Table 1. However, complement fixation tests with *PR*8 antigen indicated that shark sera did not fix guinea pig complement. Indirect complement fixation tests have not yet been done.

These investigations indicate the elasmobranchs can form specific heat-stable antibodies to influenza virus. Work is at present in progress to determine the effects of

Table 1. SEROLOGICAL TESTS ON SERA OF SHARKS IMMUNIZED WITH INFLUENZA A-*PR8* VIRUS

Animal No.	Time, dosage and route* of injection	Date of blood collection (days)	Serum haem-agglutination inhibition titre	Neutralization index at serum dilution 1:10	1:20	1:80
3	0 days 8 × 0·5 ml. S.C.	0	< 1:10	1	—	—
	10 days 4 × 0·5 ml. S.C.	10	1:40			
	19 days 4 × 0·5 ml. S.C.	19	1:80			
	+8 ml. I.P.	43	1:640	> 300	> 300	> 300
5	0 days 2 ml. I.P.	0	—			
	10 days 2 × 0·5 ml. S.C. +8 ml. I.P.	10	< 1:20			
	21 days 5 ml. I.P.	33	1:80			
8	0 days 2 ml. I.P.	0	< 1:10			
	10 days 2 × 0·5 ml. S.C. +8 ml. I.P.	10	1:10			
	21 days 5 ml. I.P.	33	1:40			

* S.C. = subcutaneous; I.P. = intraperitoneal.
— = Not tested.

different routes, dosages and schedules of injection of *PR*8 as well as other antigens. In addition to serological investigations, the present work includes chemical characterization of shark antibodies.

Part of this work was carried out at the Lerner Marine Laboratory, Bimini, British West Indies. We wish to thank Dr. Robert F. Mathewson and his staff for their help. The work was supported by a contract from the Office of Naval Research, U.S. Navy.

Note added in proof. Recent results indicate that the lemon shark is capable of producing hæmagglutination-inhibition titres of from 1 : 5,000 to 1 : 40,000 under certain conditions of dosage and route of injection. This work will be published elsewhere.

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An Anti-Virus Action of Azaserine-Thioguanine *in vivo*

RECENT work on the tumour-inhibiting action of 6-thioguanine (TG) has suggested strongly that growth inhibition is correlated with the incorporation of TG into the tumour nucleic acids, and particularly into DNA. Pre-treatment of tumour-bearing mice with azaserine (AS), an inhibitor of *de novo* purine synthesis, greatly enhanced the inhibitory action of TG, and this was associated with an increased incorporation of TG into the tumour nucleic acids[1,2].

From these results it seemed possible that TG, with or without AS, might have anti-virus activity, and some preliminary observations are reported in this communication. As a test system it was decided to use the virus dis covered by Riley *et al.*[3], which causes an elevation of plasm lactic dehydrogenase (LDH) activity by a factor of abou 6-fold in infected mice. These authors showed that th virus was present in many varieties of transplantabl mouse tumours and that it was readily transmitted b inoculation of small amounts of blood or plasma take from tumour-bearing mice. The inoculated animals ma in turn be used as sources of virus. These observation have been confirmed and extended by many investigators[4]

One important reason for using the Riley virus-plasm LDH system in the present work was the readiness wit which virus activity can be assayed by using the rise i plasma LDH activity which occurs after the inoculation o an infective dose. After inoculation, Riley virus builds u very rapidly in the plasma after a short lag phase. Rowso and Adams[5] showed that the plasma of mice inoculate with about 10^4 infective doses contained little virus 5 h afterwards, but that during the next few hours the plasm virus titre rose to about 10^8 infective doses/ml.

Experimentally, groups of four mice were inoculated with 0·01 ml. of plasma taken from a mouse which had been infected four weeks previously with Riley virus. Fifteen minutes before the inoculation one group of mice was injected intraperitoneally with 10 mg/kg AS. One hour after inoculation this group was injected with 10 mg/kg TG, repeated at hourly intervals for 6 h, and finally with two similar doses of TG at 90-min intervals. Blood (0·03 ml.) was withdrawn from the tails of the control and treated mice at intervals, and the blood from each group pooled, diluted in heparin saline, and centrifuged. Serial dilutions of plasma were then injected into groups of three mice. After seven days their LDH activity was determined[4] and the results are given in Table 1 as high (infected) or low (normal). Five hours after infection the plasma of both groups of mice contained less than 10^2 infective doses/ml. After a further 2 h the titre of the untreated group had risen to approximately 10^5 infective doses/ml. of plasma, while that of the treated group was between 10^3 and 10^4 infective doses/ml. Eleven hours after infection the titre of the untreated group had risen to between 10^6 and 10^7 infective doses/ml. while that of the treated mice remained at around 10^4 infective doses/ml. Twenty-four hours after infection the titre of both groups was at least 10^{11} infective doses/ml., which is considerably higher than the maximum titre obtained by Rowson and Adams[5]. This may be due to the use of a different strain of mice, or a higher initial infective dose.

The results show that over the period during which the injections were given there was a large reduction in plasma virus titre in the treated animals. By analogy with the mechanism of the inhibition of tumour growth resulting from treatment with an AS–TG combination it seems reasonable to suggest that TG is being incorporated into the virus nucleic acid to produce a non-infective molecule. Further experiments are in progress to test this hypothesis.

The nine doses of 10 mg/kg TG given to the mice during the course of treatment caused a loss in body-weight of about 5 per cent, which was regained rapidly, and the mice appeared healthy and active at all times.

Table 1. RILEY VIRUS CONTENT OF SERUM FROM NORMAL (N) AND AS–TG TREATED (TG) MICE AFTER INFECTION WITH 0·01 ML. OF PLASMA FROM A PREVIOUSLY INFECTED MOUSE

Ml. of plasma injected	Time of withdrawal of blood samples (hours after infection) 5 N	5 TG	7 N	7 TG	11 N	11 TG	24 N	24 TG
10^{-11}							+++	+−−
10^{-10}							+++	+++
10^{-10}							+++	+++
10^{-9}							+++	+++
10^{-8}					−−−	−−−		
10^{-7}			−−−	−−−	−−−	−−−		
10^{-6}			−−−	−−−	+++	−−−		
10^{-5}			++−	−−−	+++	−−−		
10^{-4}	−−−	−−−	+++	−−−	+++	++−		
10^{-3}	−−−	−−−	+++	+++	+++	+++		
10^{-2}	−−−	−−−	+++	+++				

Blood was withdrawn at intervals and serial dilutions of plasma were inoculated into groups of three mice. The plasma LDH activity of these animals was determined seven days after inoculation. LDH activities in the individual mice are given as normal (−) (non-infected) or raised (+) (infected).

Very recently a report has appeared suggesting that ioguanosine reduces by a factor of 10 the infective Rous rcoma virus in a chick embryo tissue culture[6].

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SOIL SCIENCE

Reaction between Monocalcium Phosphate and Calcium Carbonate

WHEN an acid phosphate is applied to a soil containing alcium carbonate or mixed with liming material a reaction rill occur leading to the formation of more basic phoshates such as dicalcium phosphate, octocalcium phoshate and hydroxyapatite.

In order to investigate what phosphate may initially be ormed and at what rate when triple superphosphate eacts with calcium carbonate, the following simple xperiments were conducted.

In the first experiment, which was performed at room emperature, a 5-cm layer of dry calcium carbonate was laced in a glass tube and a 2·5-cm layer of monocalcium hosphate (analytical grade) was placed on top. Air aturated with water vapour was then passed down the olumn for 10 days. During this experiment the layer of alcium phosphate was seen to absorb moisture and a hard –10-mm thick cake was formed at the top of the calcium arbonate column. After separation of this cake from the alcium carbonate column it was found (by X-ray diffracion analysis) to consist mainly of dicalcium phosphate lihydrate (about 80 per cent) and some calcite.

The experiment was repeated with commercial granular riple superphosphate (49 per cent phosphorus pentoxide, article size 2–4 mm) instead of pure monocalcium phoshate and the result was similar to that with the pure hosphate.

able 1. RATE OF REACTION BETWEEN DRY TRIPLE SUPERPHOSPHATE (TSP) ND DRY CALCIUM CARBONATE WHEN ABSORBING WATER FROM MOIST AIR

Amount of TSP used (g)	ml. N/10 Ba(OH)$_2$ consumed per g triple superphosphate			
	1 day	2 days	3 days	8 days
0·0477	38·2	62·0	69·7	74·3
0·0808	40·0	64·8	68·3	70·1
0·0992	38·6	60·6	63·1	66·0
fean	38·9	62·2	67·0	70·1
		Theoretical: - 69·0		

In a second experiment the evolution of carbon dioxide esulting from the following reaction between monocalcium hosphate and calcium carbonate $Ca(H_2PO_4)_2 + CaCO_3 + H_2O \rightarrow 2CaHPO_4.2H_2O + CO_2$ was measured as a function f time, using Cavett micro-diffusion flasks[1]. The small essels were half-filled with dry calcium carbonate and a nown weight of dry triple superphosphate (49 per cent hosphorus pentoxide, particle size 2–4 mm) was placed on he surface of the calcium carbonate. 5 ml. N/10 barium hydroxide was measured into each of the flasks, which were kept at laboratory temperature, and the unused barium hydroxide was titrated with N/10 hydrochloric acid at intervals. A blank with no phosphate was included.

The results of this experiment, expressed as ml. N/10 barium hydroxide used per 1 g triple superphosphate, are given in Table 1 and indicate that the initial reaction leading to the formation of dicalcium phosphate terminated within 3–4 days.

The finding of the first experiment supports the assumption of Lehr *et al.*[2] that the monocalcium phosphate in fertilizer granules can absorb sufficient water from the vapour phase for complete dissolution. The formation of dicalcium phosphate dihydrate by reaction between calcium carbonate and the acidic solution from monocalcium phosphate is also in agreement with investigations at Tennessee Valley Authority[2,3]. The length of the period required to complete the reaction (Table 1) corresponds well to the time needed by tablets of monocalcium phosphate to absorb enough water from saturated air for complete hydrolysis[2].

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Soil Nitrogen Accretion under Different Covers in a Tropical Rain-Forest Environment

IN the course of an investigation[1] of soil fertility-levels in plots on the Faculty of Agriculture farm at University College, Ibadan, total (Kjeldahl) nitrogen accumulation in the upper 16 in. of soil under five different covers over a three-year period was measured.

The plots (not replicated) were laid down at the end of 1953 on a site cleared from secondary bush, as a pilot experiment to examine changes in soil microflora and physical and chemical properties. Most of the surface soil layer, high in organic matter, was removed in the process of clearing and levelling. The cover treatments were: (1) stargrass (*Cynodon plectostachyus*); (2) no vegetation; (3) a mulch of speargrass (*Imperata cylindrica*) renewed about once a month in the wet season; (4) tropical kudzu (*Pueraria phaseoloides*); (5) regenerated bush. With the exception of the mulched plot no plant material was removed from, or added to, the plots at any time. Annual rainfall at the site was as follows: 59·6 in. (1957); 39·2 in. (1958); 45·1 in. (1959). Losses as a result of leaching and gains in rainwater are not known, but published figures indicate that the net gains or losses due to these factors are likely to be small compared with the differences reported in this communication. Under tropical conditions losses by leaching may be 15–35 lb.[2] and additions in rainwater and dust 4–50 lb.[3] nitrogen per acre per annum.

Soil samples were taken to a depth of 16 in. in January 1957 and December 1959; the two sampling sites on each plot were 4–5 ft. apart.

Table 1 shows the total nitrogen contents of these samples. In converting nitrogen percentages to lb./acre it has been assumed that an acre-6 in. of soil weighs two million lb. Differences between the values for the 1957 and 1959 samples give a measure of accumulation or loss of nitrogen over a period of about three years.

Striking differences are apparent in the legume and bush plots, increases of 770 lb. and 640 lb. nitrogen per acre per annum respectively having occurred in the 0–16 in. layer. In the case of the legume, which was well-nodulated, most of this increase may be attributed to symbiotic nitrogen fixation. This average annual increase in soil nitrogen was two or three times that usually anticipated under either tropical or temperate legumes, although in New Zealand

Table 1. KJELDAHL NITROGEN AND CARBON : NITROGEN RATIOS OF SOIL UNDER FIVE DIFFERENT COVER TREATMENTS AT IBADAN, NIGERIA

	Cover														
Soil depth (in.)	Bare			Mulch			Stargrass			Tropical kudzu			Bush		
	Jan. 1957	Dec. 1959	Difference	Jan. 1957	Dec. 1959	Difference	Jan. 1957	Dec. 1959	Difference	Jan. 1957	Dec. 1959	Difference	Jan. 1957	Dec. 1959	Difference
							Nitrogen, 10 lb./acre								
0–4	96	123	+27	127	164	+37	198	235	+37	150	283	+133	211	341	+130
4–8	67	76	+9	113	123	+10	91	152	+61	76	100	+24	82	112	+30
8–12	71	66	−5	84	84	0	88	65	−23	53	87	+34	57	91	+34
12–16	72	73	+1	71	74	+3	73	55	−18	45	85	+40	76	75	−1
0–16	306	338	+32	395	445	+50	450	507	+57	324	555	+231	426	619	+193
							Carbon : nitrogen ratio								
0–16	7·4	7·1		7·8	8·3		8·3	8·4		9·8	8·1		8·5	8·6	

symbiotic fixation of 600 lb. nitrogen per acre per annum has been reported[4]. It is perhaps significant also that the annual nitrogen fixation by certified New Zealand white clover ranges from 400–500 lb. per acre in the warmer northern areas to 200–250 lb. per acre in the colder south[5], suggesting that temperature is an important factor in the rate at which fixation occurs.

The increase in soil nitrogen in the bush plot was somewhat lower than that in the legume plot. If, however, nitrogen stored in the vegetation is taken into account, the annual increase in nitrogen in the soil–bush system is probably greater than that in the soil–legume system since an equilibrium amount of green vegetative matter would have been reached by the legume before 1957 while the bush would not reach this stage until at least five years[6] had elapsed. The large gains of nitrogen under bush are something of an enigma, particularly as there appeared to be no legume species present in this vegetation. The possibility of non-legumes being symbionts in nitrogen fixation or of free-living nitrogen-fixing micro-organisms in the phyllosphere[7] making an appreciable contribution cannot be dismissed. It is more probable, however, that the greatest nitrogen-fixing activity, presumably by free-living micro-organisms, was in the litter and surface soil layers where very rapid turnover of organic matter occurs.

In the absence of a statistical estimate of error too much emphasis should not be placed on the smaller gains of nitrogen reported for the remaining three treatments. On the mulched plot, of course, nitrogen has been added in the mulch and this is reflected in higher values for the 1959 samples. The apparent accumulation of nitrogen in the 0–16 in. layer of the bare plot, amounting to 110 lb. per acre per annum, may or may not be real. Most of the increase occurred in the surface layer and the possibility of non-symbiotic fixation by blue-green algae cannot be excluded. These are commonly observed in the tropical rain-forest region on bare soil surfaces when the latter are moistened (cf. Lawson[8]). Fuller *et al.*[9] have reported that soil crusts containing blue-green algae showed increases in nitrogen of up to 0·04 per cent in four weeks. Fixation of this magnitude could account for increases of the order mentioned here.

The grass plot showed an increase in nitrogen in the 0–16 in. layer amounting to 80 lb. per acre per annum more than that in the bare plot. Non-symbiotic fixation has very likely made a contribution in this instance as it has been shown in a greenhouse experiment with a similar soil that the presence of grass stimulated nitrogen fixation[10]. It should be noted, however, that the upper 0–8 in. layer showed an increase of 980 lb. nitrogen per acre over the three-year period while the 8–16 in. layer showed a decrease of 410 lb. per acre. This suggests that some, at least, of the nitrogen accumulation in the surface soil layers under the grass was due to the redistribution of nitrogen in the profile. Obviously care must be taken in the interpretation of nitrogen increases in soils under grass if only the upper horizons have been sampled.

It is probable that the same redistribution mechanism was operating in the other plots carrying living vegetation as no nitrogen accumulated in the 12–16 in. layer of the bush plot. In the legume plot this appears to have been masked by symbiotic nitrogen fixation, nitrogen being added down to the 16 in. depth by excretion or death of roots.

A factor which may be involved in the vegetated plots is the release of fixed ammonia in the lower parts of the profile and its transfer to the surface via vegetation. This suggestion is purely speculative as little is known of the quantities of fixed ammonia present in tropical soils, or its significance as far as plant growth is concerned. An appreciable proportion (14–78 per cent) of the total nitrogen in some British Caribbean soils has been reported to consist of fixed ammonium nitrogen[11], and it is known that enormous quantities of nitrogen are present in igneous rocks[12]. Under tropical conditions, in particular, where weathering frequently extends to a considerable depth, the release of this nitrogen may be important in plant nutrition.

Over the 0–16 in. depth little change in the carbon : nitrogen ratios occurred during the three-year period between samplings, with the possible exception of the tropical kudzu plot where the ratio declined from 9·8 to 8·1 (cf. the experiments of Chapman *et al.*[13] with temperate legumes in lysimeters). The other plots showed differences of 0·5 or less. In general, therefore, it appears that net carbon fixation and net nitrogen fixation occurred at approximately the same rate. This has been observed previously by other workers[12–16].

The experiment described here was of a preliminary nature only and any conclusions are of necessity tentative, but the data do suggest that the following processes were involved to a significant degree in the nitrogen economy of the latosol under consideration: symbiotic and non-symbiotic fixation; redistribution by vegetation of nitrogen within the profile; and, perhaps, the release of fixed ammonia by weathering.

It is intended that this experiment be followed by similar experiments, involving larger plots, adequate replication, periodic sampling over a long time, and repetition at a number of locations in different ecological zones within Nigeria.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, January 21

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at p.m.—Mr. Peter Salway: "Exploring the Roman Fenland".

Institution of Electrical Engineers, Electronics Division (at voy Place, London, W.C.2), at 5.30 p.m.—Dr. P. C. Clemmow: "Magtohydrodynamic Waves in the Ionosphere".

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. B. E. Bunce: rying of Electroplated Parts".

Royal Institute of Chemistry, London Section (at Enfield Technical llege, Queensway, Ponders End), at 7 p.m.—Dr. A. G. Maddock: "Actinide emistry".

Tuesday, January 22

University of London (in the Anatomy Theatre, University College, wer Street, London, W.C.1), at 1.15 p.m.—Dr. A. D. Blest: "Moths d Predators".*

University of London (at the Imperial College of Science and Techlogy, London, S.W.7), at 5.30 p.m.—Prof. A. G. Gaydon: "Application Spectroscopy to the Study of High-Temperature Gases" (Inaugural cture).

University of London (at the London School of Hygiene and Tropical edicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—of. K. R. Hill: "Hepatic Veno-Occlusive Disease".* (Fourth of twelve ctures on "The Scientific Basis of Medicine" organized by the British stgraduate Medical Federation.)

Society of Chemical Industry, Plastics and Polymer Group (at Belgrave Square, London, S.W.1) at 6.30 p.m.—Prof. H. Batzer (Basle): ycloaliphatic Epoxides, Synthesis and Properties".

Institute of Biology (at the Royal Entomological Society, 41 Queen's ate, London, S.W.7), at 7.15 p.m.—Mr. W. H. Dowdeswell: "Research in hools".

Wednesday, January 23

University of London (at the Postgraduate Medical School of London, oane Road, London, W.12), at 2 p.m.—Dr. K. L. Goldsmith: "Antidies to Platelets and Leucocytes".*

Institution of Electrical Engineers, Power Division (at Savoy ace, London, W.C.2), at 5.30 p.m.—Mr. T. B. Burnett and Mr. G. W. aham: "Transductor-Assisted Tap Changing on a 50 c/s Locomotive with miconductor Rectifier".

British Institution of Radio Engineers, Computer Group (at the ondon School of Hygiene and Tropical Medicine, Keppel Street, Gower reet, London, W.C.1), at 6 p.m.—Symposium on "Tunnel Diodes as orage Elements".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westinster, London, S.W.1), at 6 p.m.—Prof. M. Yoshiki: "The Construction d Fabrication of Large Tankers in Japan" (Thomas Lowe Gray Lecture).

Society of Chemical Industry, Food Group (joint meeting with the emical Engineering Group, at 14 Belgrave Square, London, S.W.1), at 15 p.m.—Meeting on "Heat Transfer in Food Processing".

Royal Institute of Chemistry, London Section (joint meeting with e Slough College Scientific Society, at Slough College of Further ducation, William Street, Slough), at 7.30 p.m.—Prof. Wilson Baker, R.S.: "Non-benzenoid Aromatic Hydrocarbons".

Thursday, January 24

University of London (in the Anatomy Theatre, University College, ower Street, London, W.C.1), at 1.15 p.m.—Mr. A. P. Cavendish: "Philophy as Ritual".*

Linnean Society of London (at Burlington House, Piccadilly, London, .1), at 5 p.m.—Dr. C. G. Johnson: "Insect Migration, Dispersal, and the rial Plankton"; Mrs. Maureen E. Lacey: "The Discovery of *Pithomyces artarum* in Britain"; Dr. R. R. Davies: "Studies on the Microbiology City Atmospheres".

University of London (at Westfield College, Kidderpore Avenue, ndon, N.W.3), at 5.15 p.m.—Prof. E. H. Bellamy: "Elementary Partles" (Inaugural Lecture).*

University of London (at the London School of Hygiene and Tropical edicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—r. J. C. F. Poole: "Structural Aspects of Thrombosis".* (Fifth of twelve ctures on "The Scientific Basis of Medicine" organized by the British stgraduate Medical Federation.)

Institution of Mechanical Engineers, Hydraulic Plant and achinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), 6 p.m.—Discussion on "Hydraulic Engineering and the Common Market".

Friday, January 25

Royal Astronomical Society (at Burlington House, Piccadilly, London, .1), at 4.15 p.m.—Geophysical Discussion on "Long Sea Waves". Chairan: Prof. J. Proudman, F.R.S.

University of London (at the Royal Holloway College, Englefield reen, Surrey), at 8.15 p.m.—Sir Richard van der Riet Woolley, F.R.S.: The Size of the Galaxy".

Royal Institute of Chemistry, London Section (joint meeting with e Cambridge University Chemical Society, at the University Chemical aboratory, Lensfield Road, Cambridge), at 8.30 p.m.—Mr. D. G. Ohlman: The Developing Activities of the Royal Institute of Chemistry".

Saturday, January 26

Systematics Association (in the Department of Botany, The Univerty, Edgbaston, Birmingham), at 2.15 p.m.—Meeting on "The Place of axonomy in the Teaching of Biology".

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. G. de G. Sieveking: "Upper Palaeolithic Cave Painting in Europe".*

Monday, January 28

Plastics Institute, Reinforced Plastics Group (at the Wellcome Building, Euston Road, London, N.W.1), at 4 p.m.—Symposium on "Costing".

Institution of Chemical Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. Ju Chin Chu (Polytechnic Institute, Brooklyn): "The Role of the Chemical Engineer in the Space Age".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Electricity as a Measure of the Rising Standard of Material Prosperity" opened by the President.

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on the Government White Paper "Industrial Training: Government Proposals".

University of London (in the Chemistry Lecture Theatre, Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. D. J. G. Ives: "Some Reflections on Water".* (Inaugural Lecture.)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Chief Inspector (with a first- or second-class honours degree in physics or engineering, and corporate member of an appropriate professional institution) in the Inspectorate of Nuclear Installations of the Ministry of Power, to take charge of sections of the Inspectorate concerned with the safety assessment of nuclear installations, and with special problems in nuclear physics, chemistry, metallurgy, and health physics—The Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5612/63 (January 31).

Lecturer in Physics—The Registrar, King's College (University of London), Strand, London, W.C.2 (January 31).

Senior Lecturer, Lecturer, or Assistant Lecturer in Mathematics—The Registrar, University of Sussex, Stanmer House, Stanmer, Brighton (January 31).

Assistant Lecturers (2) in the Department of Physics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 2).

Lecturer or Assistant Lecturer (one or two posts) in Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (February 2).

Research Demonstrator in the Department of Physics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 2).

Lecturer or Assistant Lecturer (with special interests in vertebrates) in Zoology at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 4).

Assistant Lecturer (with a good degree and preferably teaching experience) in Physics and Mathematics at Bendigo Technical College, Bendigo, Victoria, Australia—Council for Technical Education and Training for Overseas Countries, 12 Lincoln's Inn Fields, London, W.C.2 (February 5).

Lecturer in Biochemistry—The Secretary of University Court, The University, Glasgow (February 7).

Reader (preferably with a higher degree and appropriate professional qualifications) in Fluids and Hydrology in the Department of Civil Engineering—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (February 8).

Lecturer and an Assistant Lecturer in Physics—The Registrar, The University, Sheffield (February 9).

Assistant Lecturer or Lecturer in the Department of Applied Mathematics and Mathematical Physics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 11).

Lecturer (graduate with some research experience, with special interests in soil or rock mechanics) in Civil Engineering—The Secretary, University College, Gower Street, London, W.C.1 (February 14).

Reader in Organic Chemistry at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 14).

Lecturer in the Department of Biological Chemistry—The Secretary, The University, Aberdeen (February 15).

Lecturer or Assistant Lecturer (preferably with morphological and taxonomic interests) in Botany at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 15).

Lecturer or Assistant Lecturer (preferably with the ability to teach either logic or the philosophy of science) in Philosophy—The Registrar, The University, Hull (February 15).

Lecturer/Senior Lecturer in Pure Mathematics at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 15).

Senior Tutor–Demonstrator in Applied Mathematics at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 15).

Official Fellow and Tutor in Pure Mathematics—The Master, St. Catherine's College, Oxford (February 15).

Chair of Animal Science—The Registrar, The University, Nottingham (February 18).

Chair of Zoology in the School of Biological Sciences at King's College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 19).

Chair of Botany in the School of Biological Sciences at King's College—The Academic Registrar, University of London, Senate House, London W.C.1 (February 20).

Chair of Electronic Engineering—The Registrar, The University, Nottingham (February 22).

Lecturer (chiefly concerned with mycology and cytogenetics) in the Department of Botany—The Registrar (Room 22, O.R.B.), The University, Reading (February 23).

Lecturer/Senior Lecturer in Physics at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth

(Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 25).

Reader in Science of Materials at Queen Mary College—The Academic Registrar, The University of London, Senate House, London, W.C.1 (February 25).

Chair of Civil Engineering—The Secretary, The Queen's University, Belfast, N. Ireland (February 28).

College Lecturer in Pure Mathematics—The Master, Emmanuel College, Cambridge (March 1).

Lecturer in Agricultural Chemistry with special reference to Animal Biochemistry and Nutrition—The Secretary, Wye College (University of London, near Ashford, Kent (March 1).

Chair of Veterinary Clinical Studies—The Registrar, The University, Liverpool, quoting Ref. No. CV/233/N (March 2).

Research Fellow for Independent Research—The Assistant Registrar (Room 28, O.R.B.), The University, Reading (March 4).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Civil Engineering or **Physics**—The Registrar, Trinity College, Dublin 2, Republic of Ireland (March 5).

Imperial Chemical Industries Research Fellows in Chemistry or **Physics**—The Registrar, The University, Keele, Staffs (March 5).

Chair of Biology (in the field of genetics) in the School of Biological Sciences, University of Sydney, Australia—The Secretary, Association of the Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 15).

Lecturer (with qualifications and experience in the general field of inorganic, structural and physical chemistry) **in Chemistry** at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 31).

Second Chair of Biochemistry in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

Graduate Research Assistant for the tissue-culture assay of drugs—Dr. O'Sullivan, Courtauld Institute, Middlesex Hospital Medical School, London, W.1.

Graduate Research Assistant, to work on the chemistry of medicinal natural products—Dr. G. B. Francis, Department of Biochemistry, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, E.C.1.

Lecturer (man or woman) **in Geography and Education**—The Principal, Hockerill College, Bishop's Stortford, Herts.

Lecturer or **Assistant Professor** (Ph.D. physicist) **in the Department of Physics**—The Chairman, Department of Physics, Sir George Williams University, Montreal, Quebec, Canada.

Librarian (female, with a science degree and preferably a working knowledge of German and French), for a small scientific library—The Director of Research, Cereals Research Station, Research Association of British Flour-Millers, Old London Road, St. Albans, Herts.

Marine Botanist in the Department of Biology—Head of the Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Physicist (honours graduate) for basic research of considerable human interest concerning the physics of respirable dust production, its measurement and control—The Personnel Officer (X1650), Mining Research Establishment, National Coal Board, Worton Hall, Worton Road, Isleworth, Middlesex.

Plant Breeder (22–45, with a degree in natural science and two years postgraduate training with plant breeding as a major discipline) in the Ministry of Agriculture, Kenya, to work as a member of the Plant Breeding Team in particular on wheat breeding—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. C.1/RC.213/80/04.

Principal Lecturer (well qualified and with sound teaching and research experience) **in Chemistry**—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

Technician in the Department of Zoology—The Assistant Secretary, Westfield College (University of London), Hampstead, London, N.W.3.

Vice-Principal—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref. CST.63/2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

A Short History of The Royal Society Club of Edinburgh, 1820 to 1962. By Douglas Guthrie. Pp. 40 + 5 plates. (Edinburgh: The Royal Society Club, 1962.) [712

Department of Scientific and Industrial Research. Using Paint in Industry. By George Copping. Pp. ii + 18. (London: Department of Scientific and Industrial Research, 1962.) [712

Library Telex Directory: a List of Libraries in the United Kingdom with Access to Telex. Second edition. Pp. 24. (London: The Library Association, 1962.) 8s. (L.A. members, 6s.) [712

Department of Scientific and Industrial Research. Memoirs of the Geological Survey—Scotland. The Geology of the Neighbourhood of Edinburgh. By Dr. H. G. Mitchell and W. Mykura. With contributions by W. Tulloch, J. Knox and Dr. J. R. Earp. Palaeontology by R. B. Wilson and J. D. D. Smith. Third edition. Pp. xii + 159 + 4 plates. (Edinburgh and London: H.M. Stationery Office, 1962.) 35s. net. [712

London County Council. Public Health Department—Scientific Branch. Annual Report of the Scientific Adviser for the year 1961. Pp. 71. (No. 4179.) (London : London County Council, 1962.) 1s. 3d. [712

British Antarctic Survey (formerly Falkland Islands Dependencies Survey). Scientific Reports. No. 36: The Geology of the Loubet Coast, Graham Land. By Dr. D. C. Goldring. Pp. ii + 50 + 5 plates. 18s. net. No. 39: Palaeomagnetic Investigations in the Falkland Islands Dependencies. By Dr. D. J. Blundell. Pp. ii + 24. 9s. net. (London: British Antarctic Survey, 4 Millbank, 1962.) [712

Building Research Station Digest (Second Series). No. 29: Aluminium in Building—1: Properties and Uses. Pp. 6. (London: H.M. Stationery Office, 1962.) 4d. [712

Bulletin of the British Museum (Natural History). Vol. 2, No. 12: The Genus *Epilobium* in the Himalayan Region. By P. H. Raven. Pp. 325–382 + plates 33–39. 30s. Zoology. Vol. 8, No. 10: The Identification of Cephalopod "Beaks" and the Relationship Between Beak Size and Total Body Size. By Malcolm R. Clarke. Pp. 419–480 + plates 13–22. 26s. Vol. 9, No. 4: A Revision of the Lake Victoria *Haplochromis* Species (Pisces, Cichlidae). By P. H. Greenwood. Pp. 139–214 + plate 1. 26s. Vol. 9, No. 5: Note On, and Descriptions of, New Hebridean Land Snails. By Alan Solem Pp. 215–247 + plates 1 and 2. 16s. Vol. 9, No. 6: Observations on th Heteromi, an Order of Teleost Fishes. By N. B. Marshall. Pp. 249–270 8 Vol. 9, No. 7: Darwin's Type Specimens of Varieties of *Balanus amphitrit* By J. P. Harding. Pp. 271–296 + plates 1–10. 20s. (London: Britis Museum (Natural History), 1962.) [71

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Nederlandse Vereniging voor Weer- en Sterrenkunde. Observations o Variable Stars, January–June, 1962—Report No. 2. Pp. 6. (Groningen Kapteyn Astronomical Laboratory, 1962.) [261

Annual Report of the Trustees of the Museum of Applied Arts an Sciences, Sydney, for the year ended 31st December, 1961. Pp. 24. (Sydney Museum of Applied Arts and Sciences, 1962.) [261

United States Department of Commerce: National Bureau of Standard Monograph No. 56: Systems of Electrical Units. By Francis B. Silsbe (Reprinted from the Journal of Research of the National Bureau of Standards—C. Engineering and Instrumentation, Vol. 66C, N.2, April–Jun 1962.) Pp. iii + 42. (Washington, D.C.: Government Printing Office, 1962 30 cents. [261

Food and Agriculture Organization of the United Nations. FAO Fisherie Biology Technical Paper No. 19, Vol. 1: World List of Periodicals fo Aquatic Sciences and Fisheries. Prepared by FAO Fisheries Divisio Biology Branch. Pp. vi + 260. (Rome: Food and Agriculture Organizatio of the United Nations, 1962.) [261

Companhia de Diamantes de Angola (DIAMANG). Servicos Cultura Dundo–Lunda–Angola. Museu do Dundo. Publicacoes Culturais No. 56 Subsidios para o Estudo da Biologia na Lunda—Estudios Diversos (XXII Pp. 154. (Lisboa: Companhia de Diamantes de Angola, 1962.) [261

Australia: Commonwealth Scientific and Industrial Research Organization. Rabbit Control. Pp. 8. Dissolved Gypsum for Irrigation Soils. P 12. Soil and Pasture Research in South-Western Australia. Pp. 80. (Me bourne: Commonwealth Scientific and Industrial Research Organizatio 1962.) [301

South Australia. The Institute of Medical and Veterinary Sciences—Twenty-third Annual Report of the Council, July 1960–June 1961. Pp 74 + 5 plates. (Adelaide: Institute of Medical and Veterinary Science 1962.) [301

Australia: Commonwealth Scientific and Industrial Research Organization. Soil Publication No. 20: The Chemistry and Mineralogy of Laterit Soils in the South-West of Western Australia. By A. G. Turton, N. L Marsh, R. M. McKenzie and M. J. Mulcahy. Pp. 40. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1962.) [301

Federation of Malaya. Annual Report of the Institute for Medica Research for 1960. Pp. vi + 184. (Kuala Lumpur: Institute for Medica Research, 1962.) 2.50 Malayan Dollars. [301

Annals of the New York Academy of Sciences. Vol. 99, Article 3: Fift Tissue Homotransplantation Conference. By John M. Converse, Blair O Rogers and 143 other authors. Pp. 335–942. Vol. 102, Article 1: Automate and Semi-Automated Systems in Clinical Chemistry. By A. Hainline, Jr and 27 other authors. Pp. 1–180. (New York: New York Academy o Sciences, 1962.) [301

Publications de l'Institut National pour l'Étude Agronomique du Congo Série Scientifique, No. 97: l'Altération des Roches et la Formation de Sols au Kivu (République du Congo). Par A. Pécrot, M. C. Gastuche, J Delvigne, L. Vielvoye et J. J. Fripiat. Pp. 90 + 38 Photos. (Bruxelles: Institu National pour l'Étude Agronomique du Congo, 1962.) 150 francs. [301

Institute for Northern Studies, University of Saskatchewan. First Annua Report, 1960–1961. Pp. 11. Second Annual Report, 1961–1962. Pp. 12 (Saskatoon, Canada: Institute for Northern Studies, The University, 196 and 1962.) [301

United States Department of the Interior: Fish and Wildlife Service Bureau of Commercial Fisheries. Fishery Bulletin No. 193: Atlas of th Oceanographic Climate of the Hawaiian Islands Region. By Gunther R Seckel. Pp. iv + 371–428. 30 cents. Fishery Bulletin No. 202: Development of Eggs and Yolk-Sac Larvæ of Yellowfin Menhaden. By John W Reintjes. Pp. iv + 93–102. 15 cents. Fishery Bulletin No. 203: Development and Distribution of the Short Bigeye *Pseudopriacanthus altus* (Gil in the Western North Atlantic. By David K. Caldwell. Pp. iv + 103–15 35 cents. Fishery Bulletin No. 205: Hydrological Characteristics of Tamp Bay Tributaries. By Alexander Dragovich and Billie Z. May. P iv + 163–176. 20 cents. (Washington, D.C.: Government Printing Offic 1962.) [41

United States Department of the Interior: Fish and Wildlife Servic Bureau of Commercial Fisheries. Fishery Bulletin No. 207: Pompano (*Trachinotus* spp.) of South Atlantic Coast of the United States. By Hugh M Fields. Pp. iv + 189–222. 30 cents. Fishery Bulletin No. 208: Effect o Certain Electrical Parameters and Water Resistivities on Mortality o Fingerling Silver Salmon. By John R. Pugh. Pp. iv + 223–234. 15 cent Fishery Bulletin No. 209: Relationships Among North American Salmonidae By George A. Rounsefell. Pp. iv + 235–270. 30 cents. (Washington, D.C. Government Printing Office, 1962.) [41

Fisheries Research Board of Canada. Bulletin No. 136: Osteological Studies on Pacific Salmon of the Genus *Oncorhynchus*. By V. D. Vladykov Pp. v + 172. (Ottawa: Fisheries Research Board of Canada, 1962. 2 dollars. [41

Indian Forest Bulletin (New Series)—Entomology. No. 171 (7): A Lis of Insect Pests of Forest Plants in India and the Adjacent Countrie (Arranged Alphabetically According to the Plant Genera and Species, fo the Use of Forest Officers). Part 8: List of Insect Pests of Plant Genera "P" to "R" (*Paederia* to *Rumex*). By R. N. Mathur and Balwant Singh. Pp. 130 (Delhi: Manager of Publications, 1960.) Rs. 16.00; 25s. [41

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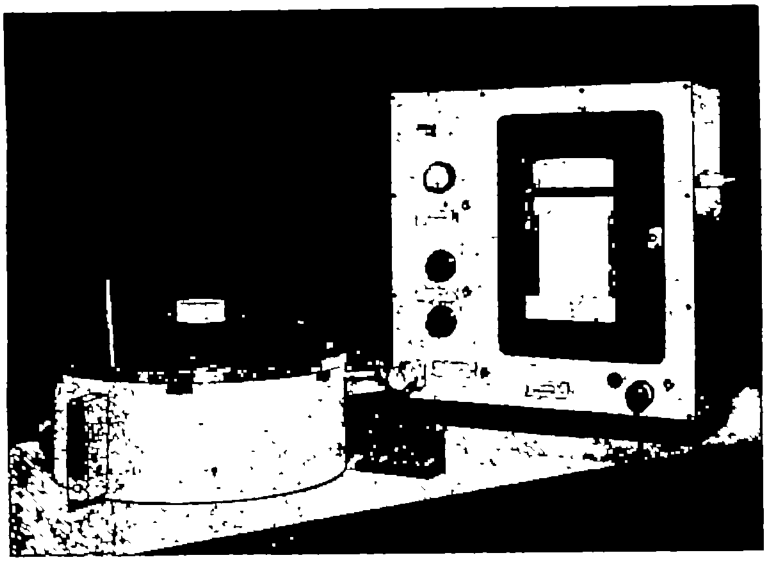

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APPOINTMENTS VACANT

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Completed application forms in triplicate, should reach the above-mentioned address not later than February 15, 1963.

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Dr. John C. Clunie, Technical Adviser to the President, will conduct interviews in the United Kingdom during February, 1963. Please write him c/o British Columbia House, 1-3 Regent Street, London, S.W.1, England, or reply in confidence to.

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CONTROL OF WATER SUPPLY AND CONSERVATION IN GREAT BRITAIN

THE widespread interest aroused by the debate in the House of Lords last February, when the Manchester Corporation Bill proposing to take water for Manchester and south-east Lancashire was rejected, was not entirely due to the amenity aspect and the defeat of an attempt to intrude on a national park. It was also due to the wider issues of water supply generally and the possibilities of alternative sources of supply. The Manchester submission as to the need for extra water supply had been carefully prepared and was fully supported by the evidence of the Committee on the Growing Demand for Water in its first, second and final reports. Since then a technical committee of water engineers has been studying the present and future water demand in the Manchester region and the possible sources of supply, and another group, including representatives of the Lake District and of water users in south-east Lancashire, has been examining the amenity aspect of various projects.

In the meantime, however, the national aspect of the whole problem of water supply and conservation has come to the front, and the particular issues of Manchester and south-east Lancashire, and Ullswater or Windermere seem more likely to be considered, as they should be, in the national context. The Proudman Committee had already presented its final report, in December 1961, when the debate was held, and shortly afterwards, in April 1962, the Government published its proposals in a White Paper, *Water Conservation in England and Wales* (H.M.S.O. Cmnd. 1693). This was debated in the House of Lords on May 23, and later the Government produced its *Water Resources Bill**, which received a first reading in the House of Lords on November 22.

The White Paper sets forth very fairly the background against which the Government had decided that the rising demand for water for domestic, industrial and agricultural use would require action over a wide front and had been led to extend the terms of reference of the Sub-Committee inquiring into the situation. The Sub-Committee's final report convinced the Government that comprehensive action to conserve water resources was urgently required, and, in particular, that a new approach was needed to the whole problem. The Government accepted the recommendation for the establishment of river authorities throughout England and Wales, to be responsible for water conservation and also for the functions now performed by river boards. Moreover, it accepted the need for a central authority distinct from the Ministry of Housing and Local Government, but responsible to the Minister, to collate information about water resources on a national basis and to secure and co-ordinate the promotion by river authorities of major conservation schemes, particularly schemes to transfer water from one river basin to another.

As to the size of the river authorities, the Government agreed with much that the Sub-Committee had to say regarding the advantage of a small and compact body. However, because much of the income of the river authorities, particularly for land drainage functions, will be raised by precept on local authorities, the Government considered that local authorities should be represented and that the proposed membership of 10–15 was too small for this purpose—the White Paper suggests instead 21–31 members. At Ministerial level, however, the Government contemplated a division of responsibility, the Minister of Agriculture, Fisheries and Food being responsible for land drainage and fisheries aspects and the Minister of Housing and Local Government for water conservation and prevention of pollution aspects.

It is to be regretted that the amenity aspect was completely ignored in the White Paper, for inasmuch as the Minister of Housing is responsible for national parks it should be carried out by him. However, the same Minister is to be specifically charged with appointing a small central authority to build up a national picture of water resources and requirements, determine where alternative supplies are best available, advise on capital investment and initiate research. This central authority is to be concerned with water conservation only but, although primarily an advisory body, it will have powers to control the fact-finding activities of the river authorities. Land drainage, prevention of pollution and the administration of fisheries are regarded as local in character and it is considered that the resulting problems can be solved within the individual river basin. The Government accordingly contemplate an authority of not more than six members, mainly, if not wholly, part-time, but comprising a wide range of professional and technical experience and provided with its own expert staff.

When these proposals were discussed in the House of Lords on May 23, Lord Silkin, who had begun by stressing the need for full information as to Britain's natural resources and the prospects of increased consumption, questioned the adequacy of the proposed advisory authority, and, as regards the central authority itself, suggested that the representation of consumers rather than of the local authorities should dominate. This view found wide support in the debate and the advisory character of the proposed authority was also criticized, the preference on balance being for a body with executive powers. The question of amenities was raised by Viscount Colville, Lord Chorley and Lord Walston, who urged that this aspect should be considered from the start and required decisions at a national and not a local level. It was also urged that statutory water suppliers should be more ready to provide non-potable water to industry.

In replying to this debate, the Parliamentary Secretary to the Ministry of Housing and Local Government, Earl Jellicoe, claimed that a consistent national water policy had been pursued since the publication in 1944 of the White Paper, *A National Water Policy*. He pointed out that the present paper showed that the Government fully accepted the view of the Proudman Committee that it should no longer be left to individual initiatives to develop Britain's water resources according to their separate needs. Comprehensive action was urgently required as well as a new approach, and this for the first time was proposed in the White Paper. He also added that it was proposed that conservation should be self-supporting, the large expenditure involved being met from fees and charges from all extractors and users. He suggested that the Government still had an open mind as to the number of authorities, but he strongly defended the view that the new central authority must in the main be advisory.

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* Water Resources Bill. (H.L.) Pp. x+vi+122. (London: H.M.S.O., 1962.) 8s. net.

Earl Jellicoe confirmed that the new central authority would have power of initiation and that its proposed responsibilities were those detailed in the White Paper, but on the question of research, which had been mentioned by several speakers in the debate, he laid particular stress on the part which the central authority might have to play. It would be difficult, he thought, to overstress the importance of research into all aspects of water. As visualized at present, basic research would remain the responsibility of the Hydraulics Research Station, the Water Pollution Research Laboratory at Stevenage, the Meteorological Office, the Geological Survey, the Water Research Association and other existing bodies. However, he pointed out that a special Hydrological Research Unit had recently been established under the wing of the Hydraulics Research Station and suggested that the new authority would be represented on the Co-ordinating Committee on Hydrological Research, and besides commissioning basic research the new authority would stimulate and supervise applied research falling within the province of the new authorities. For these reasons the new central authority would require its own permanent staff and the services of men with a wide range of technical and professional competence.

There is obviously room for some discussion as to the effectiveness of the proposed organization for research. Apart from possible overlapping, the division of responsibility does not appear to be by any means clear, and although two at least of the bodies concerned—the Council for Scientific and Industrial Research and the Nature Conservancy—are responsible to the Minister for Science, the position of the latter was never mentioned. Earl Jellicoe was clearly hoping for a good deal of voluntary agreement, but on the amenity issue he himself said very little—the record of his Ministry as a co-ordinating authority is not at all encouraging in this context.

These points were not discussed further in the debate on May 23, nor was the question of such alternative sources of supply as the use of sea-water mentioned. However, when the Water Resources Bill received its second reading in the House of Lords on November 22, this aspect and the question of research were repeatedly stressed. In introducing the Bill, Lord Hastings directed attention to the research function of the proposed Water Resources Board. Besides making known to the several research agents the questions to which answers would be needed, the Board would organize the necessary field work through the river authorities, and would also be empowered to carry out research if it seemed to be the most appropriate agency for a particular project. He also submitted that the Board had a key role in planning the development of water resources, and without promising the disappearance of such controversial issues as the water schemes discussed under the Manchester Corporation Bill, he maintained that once the Bill was operative the Board would be able to review alternative ways of meeting a particular need. Moreover, it would be able to reach an informed judgment on the practical merits of any particular proposal, in which due account would be taken of the amenity aspect.

In the earlier debate Lord Jessel had put the cost of distilled sea-water as 10*s*. per 1,000 gal. as against an average of 3*s*. for treated or potable water. In the meantime, a good deal of further attention has been directed to the use of sea-water as well as to the re-use of industrial effluent, and it was not surprising that this aspect should be particularly prominent in the debate on November 22. A symposium on the re-use of water in industry had already been arranged in April by the Industrial Water and Effluent Group of the Society of Chemical Industry, although the papers presented were not published in *Chemistry and Industry* until December. The symposium brought out the way the high cost of town water already induced industry to keep its consumption to a minimum. With the need for water of low hardness for some processes, obtainable only by de-ionizing, and, on the other hand, the increasing emphasis on the treatment of effluent to ensure that the water discharged from industrial processes was substantially free from toxic constituents, the electroplating industry, for example, had found that very considerable economies could be obtained by re-using water in plating processes. Probably the greater part of the water used by industry is for cooling purposes and this water can commonly be used again without difficulty.

Obviously, any national water policy should give the maximum encouragement to the re-use of water in such ways; but it is no less important that every encouragement should be given to the possibility of obtaining fresh water from the sea. It was, in fact, stated at the first European symposium on "Fresh Water from the Sea", held at Athens during May 31–June 4, that, with the rapid growth of industry and populations, every country will before long face a water shortage and will require to supplement its supplies by producing fresh-water from sea-water. At present, evaporation is the only commercially feasible method of de-salination and is likely to remain so for the next decade, though ultra-filtration or reverse osmosis is regarded as offering the most promising of the more-distant prospects. Dr. R. S. Silver claimed, however, that distillation was now only a fifth of the cost of what it was in the early 1950's and that distillation was a completely proved and practical process at costs competitive in many areas with those of normal water catchment methods.

Production of drinking-water from sea-water in Britain, using cheap sources of waste steam, for example, is estimated as costing at present rather more than 3*s*. per 1,000 gal., but it is predicted that within five or ten years this could be reduced to 2*s*. per 1,000 gal. Lord Balfour of Inchrye in the House of Lords debate said that in the United States four or five demonstration plants were already producing water at 6*s*.–7*s*. per 1,000 gal., a quarter of the cost in 1952, and that plant to supply 50 million gal. a day now under investigation was expected to give a cost of 2*s*. 6*d*. per 1,000 gal. Lord Balfour urged that the closest possible touch should be maintained on this subject with the United States.

Lord Balfour, who had also cited three examples of such distillation plants in actual use, had stressed the research responsibilities of the Water Resources Board in this connexion. Those responsibilities were equally emphasized by Lord Silkin, who was concerned with the possibility of some confusion of responsibility between the Water Resources Board and those to the Central Advisory Water Committee, and by Lord Fleck. The latter thought that it was important that industrial water should have an adequate place in the organization established, and that Britain should have regard also to the International Hydrological Programme of Unesco, the World Health Organization, the Food and Agriculture Organization and the World Meteorological Organization. Lord Burden also strongly urged the need for directing adequate attention to the distillation of sea-water, and the Earl of Malmesbury again pointed to the risk of duplication of research if the Water Resources Board was not adequately represented on the Hydrological Research Committee.

In replying on the debate, Earl Jellicoe welcomed the references to research and agreed as to the need of directing attention to the possibilities of distilling salt water. The need for the complementary use of surface- and ground-water was also recognized, and the stimulation and co-ordination of research in that field would come within the orbit of the Water Resources Board as well as of the Committee on Hydrological Research, on which the Board would be represented. On the question of amenity, which had been raised again very firmly by Lord Silkin, Lord Fleck (who referred particularly to the Nature Conservancy), Lord Henley, and Lord Chorley, Earl Jellicoe was scarcely more forthcoming than in the earlier debate. Without denying that the defence of amenity might perhaps be strengthened in the Bill, he reiterated that the Bill necessarily represented a balance of interests. Meanwhile, it seems very clear that the Bill demands very close scrutiny from the point of view of research as well as of amenity—including in this context the position of inland waterways generally, though they may also have a supply function. Particularly, it needs close scrutiny in order to ensure that the allocation of responsibility is sufficiently clear to enable effective action as well as efficient diffusion of information to take place. In its present form there is some room for doubt whether it is likely to ensure the co-operation which the Government itself desiderates to command adequate resources and avoid duplication or overlapping of effort, quite apart from providing adequate defence or hearing for what may be minority, but no less vital, national interests.

WATER-RESOURCE PLANNING

Design of Water-Resource Systems

New Techniques for Relating Economic Objectives, Engineering Analysis, and Governmental Planning. By Arthur Maass, Maynard M. Hufschmidt, Robert Dorfman, Harold A. Thomas, Jr., Stephen A. Marglin and Gordon Maskew Fair. Pp. xviii + 620. (London: Macmillan and Co., Ltd., 1962.) 90*s*. net.

THE appearance of an American manual on the design of water-resource systems during the passage of the English Water Resources Bill through Parliament is indeed fortunate, for the Bill proposes the setting up of research groups for which such a manual would be invaluable.

Any organized community needs water for a great variety of uses. Domestic purposes and the disposal of waste products immediately spring to mind, but water is also needed for industrial and agricultural purposes, for transportation and power production, and for recreational facilities. These uses often overlap, sometimes supplementing, but more often conflicting with each other in any tightly packed community. So it is necessary to turn to multi-purpose projects in the use of water. These multi-purpose schemes will cost different amounts according to their composition, while subsequent financial returns will also differ according to the basic design. This brings us to an operational field where economists, engineers and politicians all overlap.

The engineer can indicate what can be done and how much it will cost. The economist can perhaps more readily indicate what is worth doing and how essential it is to the community, while it is more often than not the politician who eventually decides because of the large sums of public money involved. Arriving at the right answer to provide a reliable guide for the politician involves the close co-operation of the engineer and the economist, and this manual on the *Design of Water-Resource Systems* is an attempt to effect this kind of co-operation.

Under the direction of Prof. Arthur Maas, all the interests involved were brought together at Harvard University in a water-research programme and this book of 620 pages, 93 tables and 98 diagrams is the final report of the group inquiry. The methodology of system design, as defined in the book, involves four related steps: identifying the objectives of design, translating these objectives into design criteria, using the criteria to devise plans for the development of specific water-resource systems, and evaluating the consequences of the plans that have been developed.

The book presents the first attempt to work out methods of choosing the right and most economic design method of multi-unit, multi-purpose, water-resource development in any given river basin. New methods of river-system design and a comparison with those now in use are featured. The two chief new techniques considered are the construction of mathematical models that produce optimal decisions and the simulation of systems on high-speed digital computers. The treatment is necessarily detailed and in places somewhat complex; but for those who need only the main principles, there are four generalized chapters which, taken together, give a comprehensive account of the investigation and its results.

W. G. V. Balchin

POPULARIZATION OF SCIENCE

Atoms and the Universe

An Account of Modern Views on the Structure of Matter and the Universe. By Prof. G. O. Jones, Prof. J. Rotblat and G. J. Whitrow. Second, revised edition. Pp. 275 + 37 plates. (London: Eyre and Spottiswoode, 1962.) 30*s*. net.

IT is nowadays generally thought desirable that the intelligent layman should have a knowledge of the work of the scientist. Just what form this popularization should take is less clear. One approach is to emphasize the wonder and excitement of modern scientific achievement so that it can be read as romance. Another approach is to try to explain the scientific method and the way scientists think. Still another approach is strictly practical—how to build your own radio telescope, for example. The best examples of popularization combine some aspects of all these approaches, and if, with all this, real scientific accuracy can be retained, the result is exciting for the layman and respected by the scientist.

The present volume is an excellent example of the best of such books. It is the second edition of a review of nuclear and atomic physics which has already established an enviable reputation. The authors lead the reader from the discovery of the electron and the nature of the elements to the age and size of the universe. They emphasize the unity of the scientific view of Nature and show that the division into physics, chemistry and the biological sciences is largely one of convention and convenience. To discuss the whole of modern physics in 250 pages is clearly impossible; but the authors have made a careful selection of those phenomena to be included. Invariably these are clearly and simply described. After all, most of the concepts of modern physics can be extremely simply described in words. It is only when a quantitative mathematical formulation is required that complexity is revealed.

This second edition is satisfyingly up to date, including the most recently discovered hyperon and the latest in high-energy accelerators. The last third of the book is an excellent sketch of our present knowledge of the solar system, galaxy and universe. This is the oldest of the branches of physics with a 5,000-year history at least. Yet it still abounds with open questions, and now that man is becoming able to make faltering forays into space the whole subject is one of the highest excitement for both scientist and layman.

The authors have wisely chosen to abandon almost completely the historical approach which is so often used in popular scientific books. Instead, they describe the physical world, starting with its smallest constituents, continuing with the binding of atoms into solid matter and finishing with the universe. This emphasizes the unity and beauty of the physicists' picture of our environment which is the concept which gives most thrill and satisfaction to the scientist himself. He is normally less moved by the romanticized history of his subject. However, there is one interesting feature of the scientific scene which is obscured by the non-historical approach, though the authors occasionally refer to it. These are the conditions historical, philosophical, social, psychological, technical and economic which make a scientific break-through possible. The flash of insight may occur, but it is the result of a very large number of pre-conditions. The scientist must be looking with sufficient flexibility of mind at the right class of phenomena with the right class of instrument. The last requirement, which depends on engineering and industrial developments often far removed from physics in their motivation, may be the limiting factor in many cases. Developments in vacuum engineering, electrical engineering, and precision mechanical engineering underlie much of what is described in this book. History suggests that as soon as a new equipment becomes possible to build, the physicist will appear to use it to further our knowledge and sometimes to give a really fresh insight.

The authors do not overlook what might be called the philosophical aspects of physics, and in the introduction and an appendix, and in a chapter on the transition from classical to modern physics, touch on many of the interesting aspects of how scientists operate and think.

There are few errors or mistakes in such a book. However, the statement on p. 50 that the apparatus for identifying the antiproton was 'ingenuous' is surely an understatement!

It is hoped that this excellent little book reaches an even wider public, and enhances people's interest and understanding of the physical world in which, for better or worse, they find themselves.

E. B. PAUL

TERTIARY FLORA OF SPITSBERGEN

Studies in the Tertiary Flora of Spitsbergen

With Notes on Tertiary Floras of Ellesmere Island, Greenland, and Iceland—a Palynological Investigation. By Svein Manum. (Skrifter Nr. 125.) Pp. 128 + 21 plates. (Oslo: Norsk Polarinstitutt, 1962. Distributed by Oslo University Press.) 26 Kr.

THIS book describes the pollen and spore flora of Palæocene to Eocene beds in West Spitsbergen. Seventy pollen and spore types are described (15 Sporites, 54 Pollenites and 1 uncertain), and many of these are illustrated by line drawings and photographs. Most of the types described could not be referred to species previously described, and a number of new species are made. The palynology is largely qualitative, but a few quantitative analyses were made. In addition to the Spitsbergen material samples from the Lower Tertiary of Ellesmere Island, Greenland and Iceland are described and compared with the Spitsbergen flora. Though the floras from these regions are not identical, they appear to be composed of the same families and genera. The commonest pollen grains in most of the samples are coniferous, and of the inaperturate type.

The Arcto-Tertiary flora has received much attention since Heer's pioneer studies in the late-nineteenth century. The previous records are largely of macroscopic plant remains, and Dr. Manum makes an interesting comparison of these with the microfossil record. There are curious divergences. Among the macrofossils, *Metasequoia occidentalis* is very common, but few *Metasequoia*-type pollen grains are found. Conversely, *Sciadopitys* pollen occurs in all the samples, but its macroscopic remains have not been found. In general, macroscopic remains of Pinaceae are rare, but pollen of Pinaceae-type is fairly abundant. In the Angiosperms *Cercidiphyllum arcticum* is the most abundant macrofossil, but pollen grains of this genus have not been found. On the other hand, *Betula* and *Alnus* have been found—both macrofossil and microfossil.

The new plant records from Spitsbergen make little difference to Heer's original interpretation of the climate as temperate. Dr. Manum shows, however, that the original conception of an Arcto-Tertiary flora of deciduous type is too simple an interpretation of the vegetation, and that, as might be expected, there must have been a diversity of temperate vegetation types, both deciduous and evergreen, the latter containing an important element of evergreen conifers.

R. G. WEST

CHEMISTRY FOR ENZYMOLOGISTS

Molecular Biochemistry

By Edward M. Kosower. (McGraw-Hill Series in Advanced Chemistry.) Pp. xii + 304. (New York: McGraw-Hill Publishing Company, Ltd., 1962.) 97*s*.

ONE may ask: Is biochemistry to-day other than molecular? However, this book is far from being yet another addition to the accelerating flow of works on general biochemistry. The title stems from the concept of molecular biology, and the author defines his subject as the study of the detailed chemical mechanisms of the chemical transformations in biology. In fact, he presents an analysis of selected enzymological reactions within a sophisticated framework of modern physico-organic chemistry.

The three parts of the book are entitled: "Biochemical Patterns", "Chemical Patterns" and "Physical Patterns". The first part, of 63 pages, surveys some of the main integrated enzymological pathways: glycolysis, tricarboxylic acid cycle, terminal oxidation and phosphorylation, etc. Within the space allowed the author can do little more than enumerate the reactions and indicate possible mechanisms. For the chemist-turning-biochemist there are pitfalls to be avoided: clear distinction between hypothetical and experimentally established reactions is not always made clear. Particularly unfortunate is the chapter on photosynthesis. After a résumé of carbon dioxide fixation reactions and discussion of semi-conductors, the author presents a speculative mechanism for oxygen evolution (p. 34). It is not clear why this was put forward, rather than a scheme based on the experimental studies of Hill, Duysens, Kok, Witt and others.

The second and largest section of the book is devoted to mechanisms of the reactions summarized earlier. Armed with an extensive knowledge of chemical theory, the author systematizes a wide range of scattered material which the enzymologist could not hope to encompass unaided. The subjects treated include enolization, hydration and dehydration, hydrolysis, organic phosphates, pyridinium compounds and oxido-reductions. The treatment of the nicotinamide coenzymes in the light of pyridinium chemistry is outstanding, as might be expected from the author's deep interest in the subject. A fitting climax is provided by the final section, which is devoted to the fundamental problem of enzymology: the nature and function of active sites. Compressed within nineteen pages are not only useful evaluations of published work but also the author's original views on approaches to the problem.

The text is unusually free from errors and is carefully indexed. References are numerous and up to the minute. One can criticize the excessive use of inverted commas, which can give the text a disjointed appearance. This book will be useful to all who delve into the mysteries of enzyme action.

E. F. Hartree

ACTIVITY IN ANALYSIS

Analytical Chemistry

Vol. 2. Edited by Carl E. Crouthamel. (Progress in Nuclear Energy, Series 9.) Pp. vii + 400. (London and New York: Pergamon Press, 1961.) 100s. net; 15 dollars.

THE first volume of this series consisted of a collection of papers from among those presented at the second International Conference on the Peaceful Uses of Atomic Energy held in 1958. The papers in this second volume have not previously appeared though references to the 1958 Conference proceedings occur frequently in several of the sections. The eight topics dealt with here, each by an authority in his field, are those considered likely to have a marked influence on the future of analytical methods associated with nuclear energy.

The first chapter on "Recent Advances in Counting Techniques" opens with an excellent account of gas ionization proportional counters and their use in non-dispersive X-ray analysis. The theory and construction of these counters are dealt with in detail. This is followed by sections on p–n junction and surface barrier counters, and on scintillation spectrometry.

The second chapter, on the "Applications of Electron Spin Resonance Spectroscopy", gives a full account of the theory of electron spin and of the many types of interaction affecting the energy-levels of the 'spinning' electron. This is followed by a description of many applications, including investigations on transition elements, donor concentrations in semi-conductors, organic free radicals and radiation-induced free radicals. Some notes on microwave and radiofrequency electron spin resonance equipment conclude this section, but the experimental approach is not covered as completely as the theoretical.

The next chapter, on "Recent Advances in the Separation and Analysis of the Transuranium Elements", covers the analytical chemistry of ten elements and closes with a footnote on the discovery of element 103. This is followed by one on "Applications of Liquid–Liquid Extraction in Inorganic Separations". The general concepts of the process are lucidly described and there is a good account of positive and negative synergistic effects. Many examples of fundamental research on separations of potential use in analysis are given.

A review on "Flame Photometry and Atomic Absorption Spectroscopy" describes the principles of the methods and in the case of flame photometry emphasis is placed on high-temperature oxycyanogen flame photometry and on Dean's technique of using as carrier an organic solvent which has previously extracted and concentrated the elements of interest. The limits of detection of a large number of metals, under different experimental conditions, are given in tabular form.

A chapter on "Recent Advances in Ultra-violet, Visible and Infra-red Spectrometry" covers the period of the past decade. The determination of a number of metals including technetium, of interest as a corrosion inhibitor, is described followed by practical details of the pressed pellet technique in infra-red analysis and of absorption spectrometry in molten salts.

The penultimate chapter considers two aspects of the interaction of beta particles with matter. The first deals with backscattering and describes the effect of atomic number of the backscatterer, and the energy degradation and angular distribution of the backscattered beta particles. The second details the excitation of characteristic X-rays by beta particles and the design and uses of X-ray sources based on this phenomenon.

The volume ends with a general account of ion exchange chromatography, including the use of hydrous oxide and heteropolyacid salt inorganic exchangers and cellulosic exchangers. Organic applications are included.

A good feature of this volume is the comprehensive bibliography at the end of each chapter. The editor's aim of including the basic principles of new techniques in addition to covering recent advances is amply fulfilled in this book. The subject index is scanty, and it might have been better to omit the lengthy author index, which includes all the names in the references, and devote more space to the former.

D. I. Coomber

ADVANCES IN INORGANIC CHEMISTRY

Advances in Inorganic Chemistry and Radiochemistry

Vol. 4. Edited by Prof. H. J. Emeléus and Dr. A. G. Sharpe. Pp. viii + 344. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962. 88s.

THE previous volumes in this series have each strongly emphasized the chemistry of a particular group of elements, but in the present volume it is the subject of organometallic compounds which recurs throughout the book. They are the subject of the very complete and up-to-date review on "Olefin, Acetylene, and π-Allylic Complexes of Transition Elements", by R. G. Guy and B. L. Shaw, and figure extensively in the review on the stereochemistry of nickel, palladium and platinum by J. R. Miller, and in "The Use of Nuclear Magnetic Resonance in Inorganic Chemistry", by E. L. Muetterties and W. D. Phillips. There is a certain amount of overlap, particularly between the first two of these chapters, but this is not serious as Miller emphasizes the actual stereochemical structures adopted by the compounds of these elements while Guy and Shaw pay major attention to the compounds themselves. The article on nuclear magnetic resonance, a technique which has played a fundamental part in the elucidation of the structures of organometallic compounds, only deals with the spectra of these compounds as particular examples but also deals very fully with most of those aspects of nuclear magnetic resonance spectroscopy which would be of interest to a physicist or physical chemist interested in the spectra of inorganic compounds.

K. W. Bagnall writes on "The Chemistry of Polonium". Extensive reviews were written on this little-known element as recently as 1957, but in spite of this its reactions are being so actively investigated that a new review is amply justified. On the other hand, I was rather disappointed in "Condensed Phosphates and Arsenates", by E. Thilo, as there is no great extension to the discussion given by Van Wazer in his book of 1958 while the treatment is rather classical with only brief reference to some of the modern physical methods which have been used in the study of these compounds. Finally, J. D. MacKenzie reviews "Oxide Melts". The examination of melts is rapidly becoming of major importance as they provide opportunities for carrying out reactions in a non-aqueous medium at an elevated temperature. Oxide melts are little understood at the moment but this review summarizes the known properties of these systems. There are no articles on radiochemistry in the present volume.

D. W. A. Sharp

PHYSICAL AND CHEMICAL TABLES FOR X-RAY CRYSTALLOGRAPHY

International Tables for X-ray Crystallography

General Editor: Dame Kathleen Lonsdale. Vol. 3: Physical and Chemical Tables. Edited by Caroline H. Macgillavry and Gerard D. Rieck. Pp. xvi+362. (Birmingham: The Kynoch Press, 1962. Published for the International Union of Crystallography.) 115s.

THE fifty years which have elapsed since the discovery of X-ray diffraction by crystals have witnessed the development of X-ray crystallographic techniques as a structure-determining tool of unprecedented power and catholicity of application. By it the complexities of mineral structures have been rationalized, the exotic configurations of natural products elucidated and the elaborate architecture of the giant globular proteins mapped out. Fortunately, it was recognized early that this diversity would make discipline in the presentation of results, and uniformity of nomenclature and convention particularly desirable. The first attempt to provide such an authoritative basis was by the *Internationale Tabellen zur Bestimmung von Kristallstrukturen* of 1935. In 1946 the International Union of Crystallography decided on a complete revision and extension of these tables under the general editorship of Dame Kathleen Lonsdale. Two volumes, one on space groups, one with mathematical tables, have already appeared; Volume 3, dealing with physical and chemical tables, represents the completion of the 1946 project.

Section 1 describes the general physical properties (morphological, mechanical, optical, magnetic, electrical and thermodynamic) which should be determined in a preliminary investigation of any crystal; it provides data on immersion media for measurement of refractive-indices and detailed methods for determining crystal density. The preparation, mounting and setting of crystal specimens is also discussed. Section 2 is concerned with data needed for analysing basic interaction of X-rays and crystals: tables of wave-lengths and absorption edges are followed by lists of filters, monochromators and standard crystals the diffraction spacings of which can be used for calibration. A set of best values of the universal physical constants is provided together with a valuable resolution of the confusing situation about the use and standardization of Å, A and X units. Section 3 is concerned with the next phase of an X-ray crystallographic investigation: the measurement of photographically and counter recorded intensities and their correction for the effects of absorption of the radiation by the specimen. Atomic scattering factors for all the elements are collected together and a critical appraisal of the methods used to derive them is provided. Other sections provide important interatomic distances determined in inorganic, organic and metallic systems; deal with small-angle scattering, texture and line-broadening analysis in polycrystalline material; and catalogue the precautions to be taken against radiation injury.

Unlike the two preceding volumes these tables are concerned with experimental techniques and data from physical measurement. Discussion of the former is necessarily prone to the vagaries of subjective appraisal and the latter will have to be amended as better results become available. This volume does not, therefore, have the air of authoritative finality possessed by its more mathematical predecessors, but its comprehensiveness and the sound judgment in appropriately weighting the various topics is a triumph for its Dutch editors, Prof. Caroline Macgillavry and Dr. Gerard Rieck, and their assistants.

No X-ray crystallographical laboratory worthy of the name will fail to add this magnificently printed and luxuriously bound volume to the two they already should possess.

STRUTHER ARNOTT

NITROGEN RELATIONS OF PLANTS

Nitrogen Metabolism in Plants

By H. S. McKee. Pp. 728. (Oxford and London: Clarendon Press: Oxford University Press, 1962.) £5 5s.

PRIOR to the publication of this large and important new book, Dr. McKee, now of the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, had already a reputation as the writer of valuable review articles on nitrogen metabolism in plants which have appeared at intervals over the past twenty-five years and have been indispensable to workers in that field. This book represents a synthesis of these earlier reviews, now brought up to date and extended by a widening of the field covered and by the incorporation of historical matter.

As might be anticipated, the main sequence of treatment is to proceed from the uptake of nitrogen compounds from the soil and their transformation within the plant to the occurrence, biosynthesis and breakdown of amino-acids and amides, leading to the composition and synthesis of proteins and of alkaloids. Valuable chapters on the storage and transport of nitrogenous substances in the plant and on the nitrogen-cycle are included, as well as a substantial treatment of the biological fixation of nitrogen, extending to sixty-three pages.

Dr. McKee writes interestingly, with a historical approach, one effect of the citation of so many old writings being that the bibliography and author index together account for some two hundred and fifty pages. Some of the material that he has gleaned from these older sources is of considerable interest, as, for example, is a report (of which I myself anyway was unaware) of the occurrence of root nodules on a species of *Rhamnus*. In other cases the reader is left in doubt whether an old paper is worth hunting for: thus he is not told whether any attempt to maintain aseptic conditions was made in nineteenth-century work alleged to show uptake of amino-acids by higher plants. In a few instances there is a lack of balance between the old and the new. Thus quotations from a highly conjectural paper dated 1829 are prominent in a short account of carnivorous plants, while there is no reference to the invaluable monograph by Lloyd published in 1942. It could also be considered an omission that, though the preparation of food protein from leaves is mentioned, no references are cited in this connexion. As examples of a tendency to repetition may be noted similar statements concerning the form of nitrogen in snow on pp. 437 and 440, the constitution of insulin on pp. 301 and 351, the origin of combined nitrogen in sedimentary rocks on p. 434 (the same information is given twice on that page), and fixation in *Cycas* nodules on pp. 45 and 71.

For the most part Dr. McKee is very well informed, but on p. 78 it is wrongly stated that Pommer classed as a fungus the organism which he recently isolated from alder nodules and held to be the true endophyte. Though he felt unable to decide its precise systematic position, Pommer thought that the isolate was probably an actinomycete or related organism. The description on p. 114 of the content of an article by Cheniae and Evans on nitrate reductase is inaccurate; moreover, if this enzyme does form part of the fixation mechanism it seems more likely to me that oxidized forms of nitrogen will precede rather than follow ammonia formation. Very few misprints have been noted. The text would have been brightened by a few illustrations.

Despite these minor strictures, with which not all readers may agree, there is no doubt that this volume will make a very valuable addition to the libraries of the many institutions and individuals interested in the nitrogen relations of plants. It would indeed be a tribute to the industry of one man that he should even attempt to review so large a sector of plant physiology and chemistry, let alone bring the venture to a highly satisfactory conclusion, as Dr. McKee has done. He has every reason to take pride in the publication of this book—a signal event in the history of Australian and Commonwealth botany.

G. Bond

MICROFAUNAS OF EUROPE

Leitfossilien der Mikropaläontologie

Ein Abriss herausgegeben von einem Arbeitskreis deutscher Mikropaläontologen. Pp. viii + 432; Tafeln und Tabellen (in separate volume) mit 61 Tafeln und 22 Tabellen. (Berlin-Nikolassee: Gebrüder Borntraeger, 1962.) D.M. 182.80.

These volumes—text and plates—are the work of fifteen German micropalæontologists closely connected with the oil industry. The treatment is severely practical; micropalæontology may be a branch of biological science, but its aim is to enable exact stratigraphical correlations to be made. The fossils described and figured are those which are most useful from this point of view. Even twenty tiny siderite nodules, characteristic of the zone of siderite (base of Wealden 3), make an unexpected appearance on Plate 30. On the other hand, no consideration is given to fossil pollen, and fossil spores are only cursorily discussed. This omission can be easily defended since the two sides of micropalæontology appeal to quite distinct groups of people. In the present work an admirable uniformity of treatment has been attained; the authors appear to have the same views on the species problem and on stratigraphical subdivision. Presumably this is the result of many conferences and considerable editorial care.

The first part of the text consists of a discussion of taxonomic problems and palæontological and stratigraphic nomenclature worthy to rank with the famous chapters in Arkell's *Jurassic System*. Later sections are stratigraphically arranged, commencing with a discussion of some Palæozoic microfaunas which includes short accounts of the ostracods of the Upper Devonian, of zoning in the productive Coal-measures of the Ruhr, of foraminifera from the Middle Devonian, Upper Devonian, Lower Carboniferous and Zechstein. A fine well-illustrated section deals with zonal division based on conodonts in the Devonian and Lower Carboniferous. The discussion of the succeeding Mesozoic begins with a short account of micropalæontological divisions in the German Keuper and continues with chapters on the Lias, Dogger and Malm. In these, after short but valuable discussions on stratigraphy and faunal assemblages, descriptions of zonal fossils, chiefly ostracods, occupy many pages. The two succeeding chapters on the Lower Cretaceous are devoted to the German Wealden and the marine rocks of north-west Europe and Tethys. Both are of considerable interest to British readers, since they contain detailed discussions of stratigraphy and facies and the succession of ostracods and foraminifera which are directly relevant to British stratigraphy. Equally important in this regard is the chapter describing the Upper Cretaceous rocks of north Central Europe in which the emphasis is on biostratigraphy and foraminiferal succession, 67 species of foraminifera being described and figured.

The text ends with two chapters on the Tertiary of Germany, one a valuable general account of the stratigraphy, facies and microfauna (chiefly foraminifera) of north-west Germany, the other a very detailed study of the Rhine Graben, with a description of characteristic foraminifera and ostracods.

The volumes are well produced, with wide margins and excellent plates. Many species are illustrated three times, once as an isolated object in half-tone, a second time as a component of a typical assemblage of microfossils, and a third time as a line drawing in the margin of a stratigraphic table. The good practical sense of the authors can even be seen in the colour of the binding, which can be easily picked out on the bookshelf, and the bold lettering of the title on the spine. The price may seem high, but the sheer bulk of information presented is quite remarkable. Anyone interested in the zonal stratigraphy and microfaunas of Europe will find this work indispensable.

A. Wood

MACROMOLECULES: BIOSYNTHESIS, STRUCTURE AND FUNCTIONS

The Structure and Biosynthesis of Macromolecules

Edited by D. J. Bell and J. K. Grant. (Biochemical Society Symposium No. 21, held at Senate House, University of London, 27 and 28 March, 1961.) Pp. v + 132 + 4 plates. (Cambridge: At the University Press, 1962.) Cloth 30s.; Paper 20s. net.

This volume has to be judged both as a collection of essays and reviews published for the dissemination of information and as a record of the proceedings during part of the fiftieth anniversary meeting of the Biochemical Society.

As a contribution to the scientific literature, the volume presents a mixture of interesting history and exciting news. When the lectures were delivered, in the spring of 1961, two of them were particularly timely: M. F. Perutz gave a lucid account of the work of J. C. Kendrew and himself on the success in the determination of amino-acid sequences in proteins by X-ray crystallographic techniques. This was a fine progress report of a subject which will produce a more definitive statement soon. J. Monod's lecture was noteworthy for its scientific content, the description of the controlling steps in enzyme formation, and for the fine presentation. Both these lectures made very personal impressions of the authors' recent interests and achievements. Prof. Monod's account of the prediction of the existence of messenger RNA from his work and its subsequent experimental demonstration by British and American workers has a very special intellectual appeal.

P. Doty gave a stimulating account of the consequences of the established structures of nucleic acids in terms of physicochemical behaviour in solution, and J. N. Davidson described polynucleotide biosynthesis with more examples from mammalian systems than one usually gets in such accounts.

E. L. Smith's account of protein structure in relation to enzymatic activity, with special reference to papain, was an interesting if somewhat circumscribed survey of progress in that field. His judgment and contributions in the field of protein chemistry of enzymes is undisputed. His interpretations of kinetics and mechanisms are, however, not quite so unambiguous, and the reader would be advised to consult them side by side with other views.

W. Z. Hassid and E. L. Hirst discussed, respectively, the biosynthesis and structure of polysaccharides. Both these articles will serve for some time as authentic accounts of important chapters in biochemistry.

Prof. F. G. Young, who introduced the symposium, presents us with a very stimulating account of various problems and ideas in modern biochemistry. The meeting, as a whole, was a good reflexion of those aspects of biochemistry to which British biochemists have made their greatest contributions in recent years. The resulting volume should make exciting reading for those who were unable to enjoy the lectures and useful reference for all of us.

H. Gutfreund

Marine Molluscs of Victoria

By J. Hope Macpherson and C. J. Gabriel. (Handbook No. 2.) Pp. xv+475. (Victoria, Australia: Melbourne University Press; London and New York: Cambridge University Press, 1962.) 63s.

THERE is no doubt that students of the marine biology of south-eastern Australian shores will find this book of considerable help in their investigations. For amateurs taking their first steps into conchology, it will also be of great value. There are comprehensive, and yet not over-powering, introductions to each of the five classes of the phylum Mollusca and sensible notes on methods of collecting. The descriptions are basically sound, and provide adequate comparisons between different genera and species; these compensate for the lack of keys which would have made the book much more useful to everyone. There is a very good glossary.

This work, however, is not intended as an introductory text-book, and it is beyond the elementary stage that some features could have been improved. It is regrettable that measurements have been presented only in inches and too frequently as, 'size 1¼"'. This is not sufficient. Size ranges must be available for collectors and students. Although executed with a high degree of technical skill some of the black-and-white figures could have been more accurate—in *Xenogalea thomsoni*, for example, the columella callus plate is ridged, not smooth as illustrated in Fig. 177. This shell is also much more handsome than this figure shows—indeed, it is doubtful if there is a place to-day for a shell-book which does not at least have some of the illustrations (certainly the cowries and murex shells) in colour. The value of a good coloured photograph of a shell to a working systematist cannot be over-estimated.

Greater detail of distribution outside Victorian and Australian waters would have been a useful addition and a map showing the principal localities in Victoria itself a great help. There can be no virtue in making up common names for all shells, some of which are far from common, as has been done in this book. This does not present a good example to students and amateurs, who must become familiar with the binominal system of nomenclature from their earliest days if their collecting is intended to be at first purposeful and then scientific. NORMAN TEBBLE

Destructive and Useful Insects

Their Habits and Control. By C. L. Metcalf and W. P. Flint. Fourth edition. Revised by Prof. R. L. Metcalf. (McGraw-Hill Publications in the Agricultural Sciences.) Pp. xii+1087. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1962.) 135s. 6d.

METCALF and Flint has been a standard reference book since its original appearance in the 'twenties. Dr. R. L. Metcalf, professor of entomology at Riverside, California, has carefully revised the whole book and entirely re-written the chapters on "Insect Control" and "The Applications of Insecticides", as well as greatly increasing the size of the sections on classification and on the morphology, physiology and biochemistry of insects. Most of the illustrations of the earlier editions are retained but all have been printed from new blocks which, with the improved paper, give the new edition a refreshingly clean and tidy appearance. Only a very few of the figures (for example, the fly in Fig. 2.16) are less pleasing than the old. All have been re-lettered and labelled in full. A small error has been introduced into Fig. 4.15, where two different structures are called the hypopharynx.

The thinking in the book remains rather old-fashioned—you name your pest and spray it according to the recipe. Perhaps in future editions more attention could be given to the long-term ecological effects of continued insecticide treatment. Where insecticides have caused pest outbreaks it is not enough to term them 'side-effects' and explain them away as the result of 'improper' application of insecticides. At present one cannot tell what long-term effects treatment may have on other species, beneficial or otherwise. Obviously there is still much to learn in this rapidly advancing field in which the full testing of one new insecticide would take years of careful experiment.

This book should have at least as good a run of useful life as the earlier editions it so worthily replaces.

G. C. VARLEY

Animal Ecology

By Prof. S. Charles Kendeigh. Pp. x+468. (London: Prentice-Hall International, 1961.) 70s.

THIS volume is intended for undergraduates with at least one year's training in zoology and appears to form the basis of a lecture course in animal ecology given by the author at the University of Illinois. It would be easier to assess its full worth if one could also attend the practical course at which one hopes the major defect of the book would be remedied. Here is a large work with approximately 1,500 references and an astronomical number of facts. It could have been of more value to the student had there been fewer facts and more guidance on how to assess their worth. As in all branches of biology, the value of any ecological work relies on a full understanding of the methods and techniques utilized and the rigorous interpretation of the results obtained. It is a pity that this is not communicated to the reader.

Nevertheless, this comprehensive and reasonably well-illustrated account may be read with interest. Following introductory chapters devoted to the scope and history of the subject and the general nature of environmental responses, there are accounts of different habitats (streams, lakes, ponds, forests, etc.), ecological processes, and community dynamics (dispersal, migration, food and energy relationships, speciation). The final part deals with the geographical distribution of communities (temperate deciduous forest, coniferous forest, tundra, grassland, desert, tropical and marine communities).

R. H. HEDLEY

South African Lepidoptera

By L. Vári. Vol. 1: Lithocolletidae. (Transvaal Museum Memoir, No. 12.) Pp. xix+238+112 plates. (Pretoria: The Transvaal Museum, 1961.) n.p.

THIS is the first volume of a proposed series of taxonomic revisions of the world genera and southern African species of the lepidopterous superfamily Tineoidea. The family Lithocolletidae is characterized, and keys are provided to genera and species. Twenty-four new genera and more than a hundred new species are described. Where knowledge of a species permits, an account is given of the type of leaf-mine formed by the larva. Mention is also made of the distribution of each species, but not of some of the genera. Critical examination of the type species and the previously included southern African species of each genus has resulted in the lumping or splitting of many genera, the transference of many species from one genus to another, and the discovery of many synonyms, necessitating many changes in nomenclature. The descriptions of new species are unfortunately not accompanied by a differential diagnosis, and there is no clear statement that type material of species other than new has been examined. It is disputable whether there is much advantage to be gained from quoting the sometimes long original descriptions of previously described species. The selection of a male from a Meyrick syntype series of one male and one female and its designation as holotype, instead of lectotype, is incorrect.

The numerous drawings of wing-venation and genitalia and the excellent coloured reproduction of paintings and colour-photographs merit special mention.

From the outset, this work will be indispensable to those engaged in taxonomic work on the group and to collectors of African microlepidoptera. A. WATSON

MARTIAN BIOLOGY: THE EXPERIMENTALIST'S APPROACH

By S. M. SIEGEL, L. A. HALPERN, C. GIUMARRO, G. RENWICK and G. DAVIS
Union Carbide Research Institute, Tarrytown, New York

RECENTLY, Salisbury, in a provocative article[1], has constructed a speculative picture of possible Martian life-forms, and of possible biogeochemical cycles in which such forms might participate.

We are in general agreement with the concepts which Salisbury has presented; as experimentalists, however, we question his reservations about the use of simulated environments. Speculation or even sound theorization notwithstanding, the sole model for life now available to us is life on Earth. Because statistical factors influence evolution, one would expect even identical planets to exhibit appreciable divergences in life-forms. Nevertheless, Earth and Mars might contain local environments in which convergent forms could arise. Thus, Salisbury suggests that the higher terrestrial plant, rather than the lower forms traditionally postulated in astronomical circles, is a good model for explaining some of the phenomena which have been held to indicate the presence of life on Mars. On the other hand, he distinguishes higher plants as plants which "universally require oxygen". This distinction suggests a divergence between the terrestrial model and its Martian counterpart which we believe may not exist.

Higher Plants at Low Oxygen Pressures

Mature air-grown plants, including *Coleus*, marigold and *Alyssum*, can survive many days under near-anaerobic conditions in spite of their aerobic history[2]. Lettuce, tomato, cucumber, bean, marigold, *Zinnia*, rye, corn, *Ageratum*, *Dianthus*, *Celosia*, rice, turnip, broccoli and other common plants germinate well and produce sizable seedlings in an atmosphere containing 5 per cent oxygen or less (ref. 3, Table 1). Several of these plants—notably cucumber, rye, corn, rice and *Celosia*—produce some seeds which are facultative anaerobes.

Table 1. GERMINATION PERCENTAGE AFTER 3-6 DAYS

Kind of seed	Atmosphere Argon (or nitrogen)	2% oxygen + 98% argon	5% oxygen + 95% argon	Air
Lettuce	0	78	78	98
Marigold	0	33	—	57
Zinnia	0	22	—	57
Celosia	22	81	—	89
Alyssum	0	21	—	80
Portulaca	0	50	—	55
Carrot	0	50	—	33
Onion	0	65	—	69
Cucumber	17	88	96	100
Bean	0	—	60	53
Coleus	0	—	54	91
Tomato	0	—	33	91
Dianthus	7	—	50	82
Ageratum	—	—	33	84
Cabbage	0	9	71	95
Turnip	0	16	21	90
Beet	0	—	40	50
Rye	40	50	95	95
Barley	—	—	28	80
Corn	29	—	—	80
Rice	17	24	24	23

If our results with more than 30 higher plant species are at all representative, they indicate that, among terrestrial plants, many (perhaps most) require little oxygen; some (perhaps many) might qualify as microphilic aerobes; and a sizable minority, perhaps 10 per cent, can function anaerobically.

Low Oxygen–Low Temperature Interaction

Many terrestrial plants are hardy enough to survive freezing and to grow during the more moderate phases of the extreme diurnal temperature cycles reported for Mars. The south tropical latitudes of Mars are notably mild[4,5]. Furthermore, we have found that cultivation of seeds in atmospheres containing little oxygen enhances the resistance of the seedlings to freezing and lowers the minimum temperature required for germination. Three-week-old cucumber seedlings grown from seed in air or in an atmosphere of 2 per cent oxygen and 98 per cent argon were cooled to −10° C, kept at that temperature for 1 h, and then allowed to thaw. As the air-grown seedlings thawed, they collapsed, presumably because of mechanical damage by ice crystals (Fig. 1, left). In contrast, the sturdy seedlings grown in an atmosphere of 2 per cent oxygen were not damaged (Fig. 1, right). At temperatures below 10° C, cucumber seeds will germinate in air to an extent no greater than 10 per cent in 10 days. If, however, the seeds are incubated in an argon atmosphere (containing only 0·005 per cent oxygen) 50–90 per cent germinate in 20 days at 6°–7° C, and subsequent shoot extension at a rate of 10 mm a day has been noted. Even under aerobic conditions, germination of cucumber seeds exposed daily to temperatures of −7° C for 8 h and 23° C for 16 h was about 30 per cent in 2–3 days and 50 per cent in 4 days. Cucumber is not noted for its resistance to cold.

Hardier species can germinate when exposed daily to temperatures of −7° C for 16 h and 23° C for 8 h. For the common 'rock garden' species, *Dianthus* and *Ageratum*, germination is 10 per cent in 3–4 days in these temperature conditions. Germination for these species was doubled or tripled when the daily freezing period was shortened to 8 h.

We have already reported[6] the germination of winter rye and the development of green winter rye seedlings under a synthetic atmosphere which simulates in most respects the atmosphere believed to exist on Mars[5], using a temperature-cycle of +20° C (day) and −10° C (night). In one respect our synthetic atmosphere differs considerably from the real Martian atmosphere in that we added 1–2 per cent by weight of water in the soil, which results in a substantial partial pressure of water in the enclosed atmosphere. The best available information indicates a much lower (perhaps 2 orders of magnitude) average water content in the Martian atmosphere than is present under our conditions. However, it must be borne in mind that astronomical observations of the Martian atmosphere as a whole would not, in all probability, reveal the presence of localized bodies of water, or of microclimates at the surface of Mars which are much higher in water content than for the total Martian atmosphere.

Xerophytes

Xerophytes are also markedly conditioned by their gaseous environment. Only a few specimens, of *Euphorbia clandestina* and *Gymnocalycium friedrickii*, were tested, but the results were none the less striking.

In this experiment, specimens were grown in 16·l. jars either in air or in a synthetic atmosphere of volume composition as follows: oxygen, 0·09 per cent; carbon dioxide, 0·24 per cent; argon, 1·39 per cent; and nitrogen, 98·28 per cent.

This gas mixture was used at a total pressure of 0·1 atmosphere (after De Vaucouleurs[5], who provides a good general treatise on aerography). The plants were placed

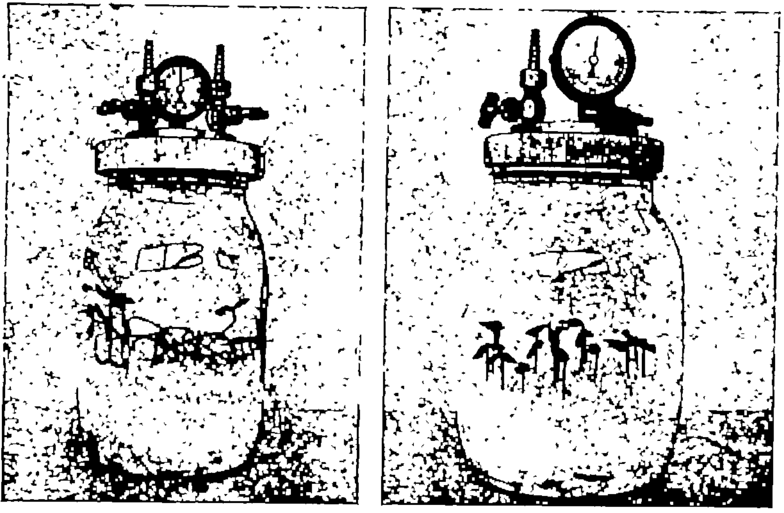

Fig. 1. Left: air-grown 3-week-old cucumber seedlings damaged by freezing (−10° C); right, freeze-resistant cucumber seedlings grown for 3 weeks in an atmosphere of 2 per cent oxygen plus 98 per cent argon

Both groups had been kept at −10° C for 1 h and allowed to thaw for 1 h before being photographed

in a 1:1 mixture of potting soil and 'Perl-lome' ('Perl-lome', a product of Certified Industrial Products, Inc., Hillside, New Jersey, is a derivative of perlite. De Vaucouleurs suggests that a felsitic rhyolite covers the Martian surface, and perlite is such a volcanic mineral). The water content of the soil and 'Perl-lome' mixture was about 1–2 per cent by weight.

After about a month at 25° C, the jars were transferred to an unheated greenhouse. Temperatures were recorded and observations on plant conditions were made during January, February and March 1962. The mean daily maxima for these months were +5° C, −2° C and +8° C, respectively. The mean nightly minima were −9° C, −12° C and 0° C.

By the end of February the four *Euphorbia* specimens grown in air were dead, but the four grown in the synthetic atmosphere were normal in appearance—green and erect by contrast with the yellow, shrivelled group grown in air. At the end of March, only one of the plants in the synthetic atmosphere appeared normal. The remaining three were no longer erect, although they retained green coloration and were not desiccated. Six *Gymnocalycium* plants were grown in each atmosphere. Three of those grown in air survived through February, and all those grown in the synthetic atmosphere still appeared normal at the end of February. During March, the remaining plants grown in air died, while only one of those grown in the synthetic atmosphere died.

After 3 months of cold, the plants were examined and analysed. The *Euphorbia* plants grown in air had lost height, diameter and fresh weight (Table 2). Plants grown in the synthetic atmosphere showed a gain in these measurements, and all had retained some green coloration, even though only one out of four was scored as 'normal'. All the *Gymnocalycium* plants lost weight, regardless of the atmosphere in which they were grown.

Table 2. CHANGES IN WEIGHT AND SIZE OF XEROPHYTES UNDER EXPERIMENTAL ATMOSPHERIC CONDITIONS

Height (cm)			Diameter (cm)			Fresh weight (g)			Dry
Initial	Final	Change	Initial	Final	Change	Initial	Final	Change	wt. (g)
*Euphorbia**, grown in air									
11·4	10·1	−1·3	1·0	0·5	−0·5	12·0	5·4	−6·6	4·7
*Euphorbia**, grown in synthetic atmosphere									
11·6	11·9	+0·3	1·0	1·1	+0·1	12·0	12·5	+0·5	5·5
Gymnocalycium†, grown in air									
						13·9	5·2	−8·7	2·2
Gymnocalycium†, grown in synthetic atmosphere									
						14·5	6·3	−8·2	1·7

* Four specimens in each atmosphere.
† Six specimens in each atmosphere.

Comparative chemical and biochemical tests made on *Euphorbia* plants grown in the two atmospheres revealed an interesting pattern of similarities and differences. Spectrophotometric determination of chlorophylls[7] in acetone extracts of the leaves (which form an apical whorl) showed that none was present in the group of dead plants that had been grown in air. Chlorophylls *a* and *b* were present in the usual 3 : 1 ratio in extracts from plants grown in the synthetic atmosphere. Longitudinal sections (3–5 mm) from the aerial portions of the plants were tested by immersion in several common reagents. Tissues from the two groups were similarly treated. Marked differences were observed in peroxidase activity and in the results of non-carbohydrate aldehyde determinations. (A mixture of 10^{-3} M pyrogallol and 10^{-3} M hydrogen peroxide in phosphate buffer (*p*H 4·5) was used as the peroxidase developer.) Even sharper were the distinctions in the results of tests for reducing sugar, starch, phosphorylase activity (tissues were immersed in 1 per cent glucose-1-phosphate at *p*H 6·6 for 1 h at 25° C, then stained with KI_3 (after S. Siegel, *Bot. Gaz.*, 114, 139; 1952) and indole; all results were negative for the air-grown group and positive for the test group. Determinations of element composition for the two groups reflected no striking differences. (The determinations were made by Micro-Tech Laboratories, Skokie, Ill.)

Germination Without Liquid Water

Xerophytism is a relative condition, and it seems reasonable to suppose that plants other than typical desert forms may be able to adapt to dry conditions. The seed, generally a rich source of hydrophilic colloids, is well known for its imbibitional powers. Accordingly, some experiments were carried out in which dry seeds were supported in closed jars, in air at a pressure of 75–125 mm of mercury, above a source of liquid water. Germination was slow as compared with usual rates, but the results (Table 3) show that a number of species can germinate and produce seedlings without difficulty under such conditions. When winter rye was placed in the synthetic atmosphere at a total pressure of 75 mm of mercury, 150 mm above a source of liquid water, germination was reasonably prompt and considerable root growth was noted after several days (Table 4).

Table 3. GERMINATION OF SEEDS IN WATER VAPOUR IN AIR AT 25° C

Kind of seed	Germination after 13 days (per cent)	Root-length after 13 days (mm)
Phlox	52	7·3
Cucumber	37	7·7
Marigold	18	1·0
Portulaca	63	4·9
Alyssum	26	8·3
Turnip	88	16 5
Carrot	40	18·3
Celosia	100	18·2
Rye	100	20·5

Table 4. GERMINATION OF RYE IN EXPERIMENTAL ATMOSPHERE IN WATER VAPOUR AT 25° C

Incubation time (days)	No. germinated	Germinated (per cent)	Longest roots (mm)
1	6	4·5	1
4	45	33·8	5
5	100	75·8	15

Other Factors

In our laboratory we are also investigating the effects of substratum and of ultra-violet radiation on plant growth under conditions of low temperature and low oxygen supply. We have observed that remarkably high levels of ultra-violet radiation are sometimes needed to suppress the growth of higher plants (energy of 10^{6} ergs/

cm^{3} is only marginally effective as an inhibitor of growth in bean embryos).

Animal Forms

Most of our investigative efforts have been concerned with the flowering plant. Other plants also show promising behaviour at low oxygen-levels. The fungus *Alternaria* and gametophytes of the fern *Pteridium* have been grown in an atmosphere of 5 per cent oxygen and 95 per cent argon.

Among animal forms, the ciliate *Colpoda* grows in hay-infusion cultures prepared and maintained under the synthetic atmosphere. HeLa cells, which can grow for 4 days under strictly anaerobic conditions more rapidly than in air, are being examined for adaptability (including clonal selection) to anaerobic culture of indefinite duration at low temperature in media of high osmotic concentration. *Planaria* have thus far shown rigidly aerobic behaviour, but they have been maintained for as long as 23 days in 5 per cent oxygen and 95 per cent argon and 16 days in 2 per cent oxygen and 98 per cent argon, as compared with 93 days in air.

The most striking results have been obtained with the common brine shrimp, *Artemia salina*. In sea-water (salinity 3 per cent) or diluted sea-water (salinity 1 per cent) in both liquid and agar media, active, viable *Nauplius* larvæ have been hatched in approximately equal percentages in air, in air reduced to 0·03 atmosphere, and in the synthetic gas mixture of 0·1 atmospheres. Typical values for hatching, based on findings with several hundred eggs per trial range from 35 to 45 per cent in 2 days.

It seems likely that marine organisms could adapt to the requirements of existence in a variety of synthetic environments—existence in a medium which is 'dry' in the physiological sense and has a depressed freezing point[8]. If, as in the case of *Artemia*, such organisms were also facultative anaerobes or near anaerobes, their prospects for survival would be good indeed.

Members of other invertebrate phyla also possess unusual ranges of tolerance to low pressure, low oxygen, and low temperature. The small black ant, *Monomorium minutum*, retains general activity and positive phototaxy after more than 15 h in 1 per cent oxygen and 99 per cent argon. The nematode *Cephalobus* remains active indefinitely under anaerobic conditions or at total pressure of 0·03 atmosphere and shows remarkable tolerances to freezing and to salinity well in excess of that of sea-water.

Conclusions

Our results show that complex terrestrial organisms can survive and grow under conditions which constitute an extreme departure in one or more respects from the normal terrestrial environment. Of particular importance was the finding that tolerance of plants to low temperatures could be conditioned in a favourable manner by synthetic atmospheres low in oxygen. It would be reasonable to expect other combinations of stress factors to give rise to unusual and exciting biological responses.

While it is impossible to project these findings on to present-day attempts to anticipate the nature of life elsewhere in the solar system, it is reasonable to conclude that the 'plasticity' of organisms as we recognize them is far greater than the teachings of modern biology indicate. Thus, whatever environments may be encountered during future explorations of the solar system there is every prospect that those which are not too extreme relative to Earth, for example, those expected on Mars may well support forms of life that we could recognize readily.

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FUNCTIONS OF THE TWO FORMS OF LACTIC DEHYDROGENASE IN THE BREAST MUSCLE OF BIRDS

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TWO basically different molecular forms of lactic dehydrogenase occur in the tissues of most animals[1-3]. They differ in amino-acid composition, immunochemical structure, electrophoretic mobility, relative rates of reaction with analogues of the coenzyme nicotinamide–adenine dinucleotide (NAD) and several other physicochemical and enzymatic properties. One of the enzymes, the more negatively charged at *p*H 7, predominates in the heart musculature of most vertebrates, and is called the *H* enzyme. The other, called the *M* enzyme, predominates in many skeletal muscles.

The *H* enzyme consists of four identical sub-units of molecular weight about 30,000 as does the *M* enzyme. But the *M* sub-units differ from the *H* sub-units in many properties, including amino-acid sequence, as tryptic digests give different fingerprint patterns. When these two different sub-units are produced in the same cell, they combine with each other in groups of four in essentially random fashion to give the two 'pure' enzyme types—H_4 and M_4—and three 'hybrid' enzymes having the composition H_3M_1, H_2M_2, and H_1M_3. This accounts for the five electrophoretically distinguishable forms of lactic dehydrogenase detected in most animals examined.

This article is concerned with the metabolic functions of these two basically different types of lactic dehydrogenase. It was noted previously that the conversion of pyruvate to lactate by the *H* enzyme is strongly inhibited by fairly low concentrations of pyruvate. By contrast, the *M* enzyme functions with increasing efficiency at these same pyruvate concentrations and is inhibited only by very high pyruvate concentrations (Fig. 1)[3,4]. It was therefore suggested that the *H* enzyme could not permit the rapid production of lactate and would favour complete oxidation of glucose via the Krebs cycle; the *M* enzyme, on the other hand, should permit the rapid conversion of pyruvate to lactate and the accumulation of an oxygen debt under anaerobic conditions. In support of this suggestion the *H* enzyme predominates in the heart, a highly aerobically oriented and well-oxygenated tissue; and the *M* enzyme predominates in skeletal muscles many of which function only on sudden occasions and then relatively anaerobically with the consequent production of lactic acid and oxygen debt.

Table 1. LACTIC DEHYDROGENASES OF BREAST MUSCLE

	Common name	Scientific name	Substrate inhibition $\frac{\text{NADH}_L}{\text{NADH}_H}$	Electrophoretic forms				
				M_4	M_3H	M_2H_2	MH_3	H_4
1	Wilson's petrel	*Oceanites oceanicus*	3·8	−	−	−	+	+ +
2	Dovekie	*Plautus alle*	3·5					
3	Ruby-throated humming-bird	*Archilochus colubris*	2·8	−	+	+ +	+ +	+ +
4	Chimney swift	*Chaetura pelagica*	2·2	+ +	+ +	+ +	+ +	+ +
5	Black-bellied plover	*Squatarola squatarola*	2·1					
6	Golden-crowned kinglet	*Regulus satrapa*	2·1					
7	Black-capped chickadee	*Parus atricapilla*	1·9					
8	Woodcock	*Philohela minor*	1·9	+ +	+ +	+ +	+ +	+ +
9	Herring gull	*Larus argentatus*	1·8	+ +	+ +	+ +	+ +	+ +
10	Brunnich's murre	*Uria lomvia*	1·7					
11	Warbling vireo	*Vireo gilvus*	1·7	+ +	+ +	+ +	+ +	+
12	Common pigeon (wild)	*Columba livia*	1·6	+ +	+ +	+	+	−
13	Black duck	*Anas platyrhynchos*	1·6	+ +	+ +	+ +	+	−
14	White-crowned sparrow	*Zonotrichia leucophrys*	1·6					
15	Black and white warbler	*Mniotilta varia*	1·6	+ +	+ +	+ +	+	−
16	Pintail duck	*Anas acuta*	1·6	+ +	+ +	+ +	+	−
17	Bronzed grackle	*Quiscalus quiscula*	1·5					
18	Starling	*Sturnus vulgaris*	1·5	+ +	+ +	+ +	+	+
19	House sparrow	*Passer domesticus*	1·5					
20	Sparrow hawk	*Falco sparverius*	1·5	+ +	+ +	+ +	+	−
21	Yellow-shafted flicker	*Colaptes auratus*	1·5	+ +	+ +	+ +	+	−
22	Mourning dove	*Zenaidura macroura*	1·4					
23	Rough-legged hawk	*Buteo lagopus*	1·4					
24	Mallard duck (wild)	*Anas platyrhynchos*	1·4	+ +	+ +	+ +	+	−
25	Great blue heron	*Ardea herodias*	1·3	+ +	+ +	+	+	+
26	Blue jay	*Cyanocitta cristata*	1·3					
27	Horned grebe	*Podiceps auritus*	1·3	+ +	+ +	+	−	−
28	Black-billed cuckoo	*Coccyzus erythrophthalmus*	1·3	+ +	+ +	+	−	−
29	Belted kingfisher	*Megaceryle alcyon*	1·2	+ +	+ +	+	−	−
30	Towhee	*Pipilo erythrophthalmus*	1·2	+ +	+ +	+	−	−
31	Barred owl	*Strix varia*	1·2					
32	Virginia rail	*Rallus limicola*	1·1	+ +	+ +	+	−	−
33	Laysan duck	*Anas platyrhynchos*	1·08					
34	Domestic duck	*Anas platyrhynchos*	1·08	+ +	+	−	−	−
35	Domestic fowl	*Gallus gallus*	0·95	+ +	−	−	−	−
36	Chinese partridge	*Bambusicola thoracica*	0·9					
37	Guinea fowl	*Numida meleagris*	0·8	+ +	−	−	−	−
38	Ruffed grouse	*Bonasa umbellus*	0·8	+ +	−	−	−	−
39	Ring-necked pheasant	*Phasianus colchicus*	0·7	+ +	−	−	−	−
40	Reeve's pheasant	*Syrmaticus reevesi*	0·7					
	Domestic fowl, pure M_4		0·95	+ +	−	−	−	−
	Domestic fowl, pure H_4		3·4	−	−	−	−	+ +

Sources of organisms Nos. 1–5, 7–11, 13, 16–23, 25–29, 31, 32, 37–39 from Dr. W. Drury and Mr. J. Baird, Massachusetts Audubon Society; Nos. 33, 36, 40 from Dr. C. Gandal and Mr. W. Conway, Bronx Zoo (New York); No. 14 from Drs. J. King and D. Farner, Washington State University; No. 6 from Dr. C Sibley, Cornell University, No. 24 from Messrs. W. Palas, M. Lundy and J. Appel, U S Bureau of Sport Fisheries and Wildlife North-east Region; and Nos. 12, 15, 30, 34, 35, 37 were collected by us or obtained from commercial sources in the Boston area. We examined several specimens from each of the following species: Nos. 1, 14, 24, 34, 35 and 37; and one specimen from each of the other species. No individual variation outside the limits of analytical error was detected in the substrate inhibition or electrophoretic results.

Tissue homogenates were made in 0·25 M sucrose or, in a few cases, in 0·1 M *tris* at *p*H 7 and centrifuged at 100,000*g* in a Spinco ultracentrifuge. The supernatants were assayed for lactic dehydrogenase activity as described in Fig. 1 and subjected to starch gel electrophoresis as described by Cahn *et al.* (ref. 3). The different electrophoretic forms usually separated at *p*H 7·0 but in the case of the petrel and charadriiform birds, *p*H 8·6 was required. The intensity with which the various electrophoretic forms were stained by nitro blue tetrazolium is indicated by. + +, intense staining, +, weak staining; or −, little or no staining.

Substrate inhibition. Further support for this suggestion concerning the different metabolic roles of *H* and *M* lactic dehydrogenase has now come from examination of the breast (pectoral) muscles of more than thirty species of adult birds. The results are summarized in Table 1. The degree of substrate inhibition is indicated by the ratio $\frac{\text{NADH}_L}{\text{NADH}_H}$, which is the ratio of the lactic dehydrogenase activity at $3·3 \times 10^{-4}$ M pyruvate to the activity at 10^{-2} M pyruvate.

Pyruvate does not inhibit the breast muscle enzyme of the pheasant, grouse, guinea fowl, partridge or domestic fowl. Usually these birds fly only occasionally and then in short bursts. After they have been flushed a few times in rapid succession their breast muscles are greatly fatigued[5], owing in all probability to the accumulation of a severe oxygen debt. The majority of birds, which fly more often or in longer bursts, show even more substrate inhibition. The strongest substrate inhibition, similar to that shown by the heart, is found in the breast muscle of the storm petrel, the humming-bird and the swift, which spend much of their waking lives in active flight, and the dovekie, which flies swiftly with a rapid wing beat for long distances and swims under water by means of its wings. The rough-legged hawk spends much time in the air, but usually glides in such a leisurely way that little breast muscle activity would seem to be involved. It shows only a moderate degree of substrate inhibition. There is therefore a good qualitative correlation between the degree to which the breast muscle of birds can maintain continuous activity and the degree to which the lactic dehydrogenase is inhibited by high pyruvate.

The correlation is also apparent, on a smaller scale, within taxonomic sub-divisions of the birds. Among the passerine birds, for example, the towhee (a ground

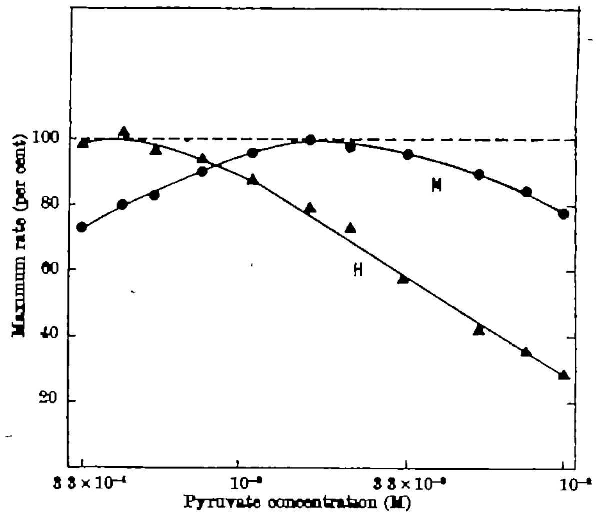

Fig. 1. Effect of pyruvate concentration on the activity of the pure *H* and *M* enzymes of the domestic fowl. The enzyme preparations were described by Cahn *et al.* (ref. 3). Lactic dehydrogenase activity was measured by following the oxidation of NADH spectrophotometrically at 25° C in potassium phosphate buffer (0·1 M, *p*H 7·5)

finch) gives a low value consistent with its reluctance to fly often or in more than short bursts, whereas the jay, sparrow, starling and grackle, which give higher values, fly more frequently and in more sustained bursts. Birds like the warbler, vireo, chickadee and kinglet which hunt insects among the branches and in the undergrowth do not usually fly in such sustained bursts as the starling or grackle, yet they give a high value. This can be associated with the fact that they flick their wings incessantly while hunting; in addition, the kinglet has a habit of fluttering its wings even while perching[6].

The trend is also demonstrable within a single species, for example, *Anas platyrhynchos*, the duck. The mallard (*A. p. platyrhynchos*) and the black duck (*A. p. rubripes*) both exhibit a moderate degree of substrate inhibition and often fly in a sustained manner. The domestic duck (*A. platyrhynchos*) and the Laysan duck (*A. p. laysanensis*)[7] have lost the capacity for sustained active flight and their breast muscle lactic dehydrogenase exhibits virtually no substrate inhibition.

The correlation between the degree of substrate inhibition and flight habits is good, but may not be perfect. The dovekie and the murre seem to have rather similar flight habits; but their degrees of substrate inhibition differ markedly. Their flight habits may differ more than has been generally assumed[8]. What is probably more important, their underwater habits differ. Murres dive more deeply and for longer periods than dovekies[9–11]. Murres must, therefore, have the capacity for developing a pronounced oxygen debt. This would account, at least in part, for the lower degree of substrate inhibition shown by the breast muscle lactic dehydrogenases of murres.

The woodcock has flight habits more like those of the galliform birds than those of its relatives, the charadriiform birds (for example, murre, gull, plover and dovekie), which are all capable of sustained active flight. The substrate inhibition exhibited by the woodcock breast seems more in keeping with its ancestry than its present flight habits.

Another problem is raised by birds like the rail, which show little substrate inhibition although they can migrate long distances. Except in the migratory season they fly very little and in short bursts. The capacity for sustained active flight may be less important than the practice of sustained active flight in determining the degree of substrate inhibition. Possibly the degree of substrate inhibition changes at the time of migration. However, our results with a strongly migratory race of the white-crowned sparrow (*Zonotrichia leucophrys gambeli*) do not support this possibility. Drs. J. R. King and D. S. Farner kindly supplied us with the breast muscles of two groups of captive sparrows.

(1) Three birds in the non-migratory state. These birds had been kept on short photoperiods (8 h/day) from the previous winter, when they were captured, until June 29, 1962, when they were killed.

(2) Three birds in a state of migratory unrest (*Zugunruhe*). These birds were kept on the short photoperiods until June 4, 1962. They were then exposed to long photoperiods (20 h/day) until July 12, 1962, when they were killed. King and Farner have previously shown that a strong urge to migrate develops in such 20-h birds but that the 8-h birds are devoid of the urge[12,13]. The results are presented in Table 2. The two groups had the same degree of substrate inhibition, although the concentration of lactic dehydrogenase in the migratory group was double that of the non-migratory group. Still, this experiment does not rule out the possibility that actually migrating birds, in contrast to caged birds with a strong urge to migrate, would have an enhanced degree of substrate inhibition.

To summarize the results presented so far, the correlation between substrate inhibition and flight habits is remarkably good, although there are some possible discrepancies which need to be examined further.

Table 2. LACTIC DEHYDROGENASE FROM BREAST MUSCLE OF WHITE-CROWNED SPARROWS

Migratory state	Substrate inhibition (NADH*L*/NADH*H*)	Enzyme concentration* (units/g wet wt.)
Non-migratory	1·55 1·62 1·66	163 105 178
Mean	1·61	149
Migratory unrest	1·54 1·63 1·59	361 349 476
Mean	1·59	395

* A unit is that lactic dehydrogenase activity which causes unit decrease in absorbance at 340 mμ/min, when measured in a final volume of $3{\cdot}0$ ml. and a pyruvate concentration of $3{\cdot}3 \times 10^{-4}$ M, as described in Fig. 1.

Relative Proportions of H and M. Electrophoresis of breast muscle extracts in starch-gel reveals that the different degree of substrate inhibition in the breast muscle of different birds is largely due to different proportions of the *M* and *H* sub-units (Table 1). The galliform birds and the domestic duck have the pure *M* enzyme (M_4) and at most a trace of the M_3H hybrid. A small proportion of *H* sub-units occur in the rail and the towhee as shown by the presence of considerable amounts of the M_4 and M_3H_1 forms together with a little of the M_2H_2 form. The species showing intermediate degrees of substrate inhibition contain nearly as many *H* sub-units as *M* sub-units because the M_1H_3 and even the H_4 forms are detectable. Finally, birds which show strong substrate inhibition have mostly *H* sub-units.

Taxonomic differences in the properties of H and M. The results in Table 1 illustrate that the differences in substrate inhibition among the various species is due mainly to differences in the relative proportion of *H* and *M* sub-units and not due to great differences in the catalytic properties of the sub-units themselves. However, it should be pointed out that there are small differences in the properties of the *M* and *H* sub-units in the various taxonomic groups of birds. The results in Table 1 indicate small differences between the *M* enzyme of the various galliform birds. This has been confirmed by a comparison of reaction rates with a number of coenzyme analogues as well as by immunological methods as illustrated by the complement-fixation results in Table 3. Complement-fixation methods[14] also reveal differences between the *H* enzymes of different galliform species (Table 3). Details of these investigations will be presented in a future publication.

Table 3. COMPLEMENT-FIXATION TEST, USING ANTIBODIES DIRECTED AGAINST THE *M* AND *H* ENZYMES OF THE DOMESTIC FOWL

Species	Relative percentage of complement fixed	
	M enzyme versus anti-fowl *M*	*H* enzyme versus anti-fowl *H*
Domestic fowl	100	100
Guinea fowl	98	99
Ring-necked pheasant	59	12
Domestic turkey	54	48
Ruffed grouse	40	48

The pure *M* and *H* enzymes of the domestic fowl and the two antisera were those described earlier (ref. 3). Crude extracts (100,000*g* supernatants) of breast muscle and heart were the source of *M* and *H* from the other species. The antisera were tested by the method of Wasserman and Levine (ref. 14) at a 1/5,000 dilution against eight different concentrations of the corresponding enzyme. Peak fixation occurred at the same enzyme concentration for all species. The results are expressed as percentages of complement fixed at peak fixation relative to the domestic fowl value.

Discussion. Insects have provided results analogous to those presented here on birds. The flight muscle of the locust and other insects is capable of sustained, highly aerobic activity and contains a high level of α-glycerophosphate dehydrogenase but only a trace of lactic dehydrogenase. In contrast, the jumping muscle of the locust, which functions only in occasional brief bursts, has more lactic dehydrogenase than glycerophosphate dehydrogenase[15-18]. We have found that the lactic dehydrogenase of the jumping muscle of the locust (*Schistocerca gregaria*) and the cave weta (*Pachyrhyma fascifer*) resembles the *M* enzyme of birds in having a low degree of substrate inhibition.

A possible histological basis for our breast muscle results is provided by the work of George *et al.*[19], who have demonstrated two types of muscle fibre in breast muscle. One type, called the white fibre, is broad, pale in colour, rich in glycogen and poor in mitochondria; the other, called the red fibre, is narrow, red and rich in fat and mitochondria. Only white fibres are found in the chicken, but the pigeon, which is quite a strong flier, has both fibre types. Since the pigeon was shown in the present work to contain both *H* and *M* sub-units in its breast muscle, one is tempted to guess that the *H* sub-units are produced by the red fibres and the *M* sub-units by the white fibres. However, in the pigeon and all other birds examined, the hybrid forms of lactic dehydrogenase occur in the proportions one would expect if both *H* and *M* sub-units were produced in the same cell. For this reason, it seems unlikely that the two fibre types produce different lactic dehydrogenase sub-units.

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LOCOMOTION IN WHALES

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THE best authenticated maximum speed of swimming in whales is 15 knots, although bursts of 20–25 knots have been claimed by some observers. Using the equations relating to rigid and streamlined bodies, Gray[1] was able to show that the power available in the locomotory muscles of dolphins was insufficient to overcome the drag of the body at these high speeds. From this he concluded that the flow of water around the whale's body must be almost completely laminar, and suggested that this might be due to the acceleration, by the action of the caudal flukes, of the water particles in the boundary layer. It is proposed to show that the anatomical facts favour Gray's hypothesis.

Parry[2] reviewed Gray's Paradox, as it was called, and gave a quantitative treatment of the dynamics of the tail, based on the equations of v. Holst and Kuchemann[3] relating to the flight of birds. He, too, concluded that the power available was only consistent with the assumption of laminar flow. Kramer[4], in attempting to explain the apparent absence of turbulence, invoked the structure of the skin and subjacent oil sinuses as being responsible.

The task of measuring the water flow around the body of a swimming dolphin is perhaps insurmountable; but there is a great deal of indirect evidence by which it is possible to deduce the average orientation of the slip-stream and the relative velocities of its components. In the first instance, most of the observations of whales and dolphins swimming at high speed have been made while the animals were at, or near to, the surface, and it was considered by me that in such circumstances there must often have been a strong pressure gradient postero-dorsally along the flanks, and consequently an average displacement of water in this direction. Furthermore, since, for reasons which will be explained later, it is considered that there is only one true power stroke in the tail, this being the upstroke, it was presumed that there must be a predominant upwash of water in the direction of the tail. It is noteworthy that in all the fast-swimming odontocetes, the entire dorsal surface of the body, posterior to the dorsal fin, is strongly 'faired'; while on the ventral surface this 'fairing' does not extend beyond the level of the anterior extremity of the caudal peduncle.

Fig. 1 is a photograph by F. C. Fraser showing a common dolphin, *Delphinus delphis*, just breaking surface in still water. The photograph was taken in the North Sea in the morning sunlight and is in itself unique; but it also provides a great deal of information about the average displacement of water around the animal's body. The bow-wave is spread out in two extremely thin sheets of water on either side of the body, like the wings of a butterfly. The thinness and clarity may be judged by the lack of distortion in the reflexion of the body, dorsal fin and those of the sheets themselves, in the undisturbed water in the vicinity. One would imagine that if the animal were just submerged these two sheets of water would be closed over the back, like the wings of a butterfly at rest. Posteriorly, there is a powerful upwash of turbulent water over the dorsal edge of the caudal peduncle, and two trailing vortices in the wake. This would correspond in the submerged condition to a powerful acceleration of water over the dorsal aspect of the flukes.

If it be accepted that the foregoing assumptions are true, it follows that the shearing stresses at the surface of the skin, due to the flow of water, should be at an average maximum in a postero-dorsal direction. It will be seen now that the detailed mechanical structure of the skin is consistent with this hypothesis. According to Johnston and Whillis[5] the main function of the papillary form of the corium is that of preventing the epithelium from

Fig. 1. A common dolphin just breaking surface. (*Photo: F. C. Fraser*)

being stripped off the surface of the skin by shearing stresses, and it is interesting in this respect, that the dermal ridges on the palm of the human hand are orientated in such a way, that when a shaft is grasped by the hand, the ridges lie approximately parallel with the long axis of the shaft.

The dermal ridges are extremely well developed in whales and have been described by a number of authors—Japha[6], Schumacher in Bolk[7], Parry[8], van Utrecht[9] and von Naaktgeboren[10], and it is generally stated that the ridges lie parallel with the long axis of the body. However, the stratum corneum is perfectly smooth and undifferentiated, so that it is quite impossible to determine their total orientation by superficial examination, and the conclusions of most of the above authors have been arrived at largely from the examination of sections of isolated pieces of skin.

In order to investigate this subject more fully, the stratum corneum was removed from the entire body of a preserved, juvenile common dolphin, *Delphinus delphis*, by the laborious, but effective, process of rubbing it down with a very fine grade, waterproof, abrasive cloth. The skin was then examined over the whole surface of the body through a binocular microscope. It was found that the dermal ridges lay parallel with the long axis of the body only in certain clearly defined areas, and that the latter represented an extremely small porportion of the total surface area. The whole system follows a pattern now to be described (Fig. 2).

Over the forehead, and along the entire length of the upper two-thirds of the flanks, the ridges passed obliquely upwards and backwards, making an angle of approximately 30° with the long axis of the body. The ridges on the caudal peduncle were also thus orientated, except near the insertion of the flukes, where they swept posteriorly towards the caudal notch. From the insertion of the rostrum, a long, narrow band of ridges passed backwards, dividing round the eyes, and, undulating along the ventral limit of the flanks, terminated at the anterior ventral extremity of the tail. Another band of ridges, commencing at the rostrum and posterior part of the mandible, passed obliquely downwards over and under the flippers. On the chin, throat and belly, the ridges were poorly developed but there was some orientation of those behind the flippers towards the central axis of the abdomen.

On the flippers, dorsal fin and flukes, the ridges lay almost horizontally, but tended to become orientated towards the normal of the anterior curvature of these appendages, that is, along the lines of minimum width. Anterior to the dorsal fin, the ridges on the upper surface of the head and chest formed a narrow, horizontal band which divided round the base of the fin and then swept upwards in conformity with those on the flanks. Along the faired edge behind the dorsal fin the ridges of the two sides converged in a multiple chevron pattern without the least trace of horizontal ridges.

It is the small band of ridges reaching down to the flippers which probably provides the clue to the mode of locomotion. These lines are strongly reminiscent of the theoretical 'downwash' effect shown by Harris[11] on wind-tunnel models of the dogfish. It will be recalled that the pectoral fins of the side-gilled selachians, unlike those of the bony fishes, provide for lift of the thorax by a hydroplaning effect against the downward and forward thrust of the heterocercal tail. It was then considered that in the living dogfish there might also be an 'upwash' effect, due to the action of the tail, which would not be demonstrable in the wind-tunnel model. Although the dermal denticles are arranged like the scales of bony fishes, in oblique, parallel rows, the spines of these denticles are orientated on the ventral half of the body in the direction of the theoretical downwash, while those on the dorsal half are orientated obliquely upward and backward, in the direction of an apparent upwash (Fig. 3). It will be seen that there is some resemblance in the orientation of these spines to that of the dermal ridges of the dolphin.

It is apparent from the arrangement of the pectoral muscles of the dolphin that the main function of the flippers is also that of maintaining a hydroplaning effect. The muscles of extension are poorly developed and the trapezius is vestigeal, the greater part of the work of extension evidently being performed by the pressure of water on the ventral surface of the thorax and flippers. On the other hand, the muscles of flexion, abduction, adduction and circumduction are all well developed. These are the muscles which act antagonistically to the pressure of the water.

Turning now to the action of the tail, it has been stated, Parry[8], that the tail and flukes oscillate inde-

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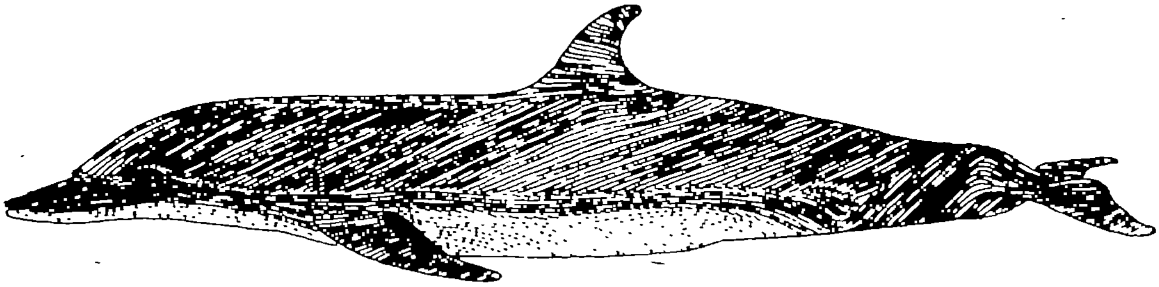

Fig. 2. Diagram of common dolphin, *Delphinus delphis*, showing orientation of the dermal ridges

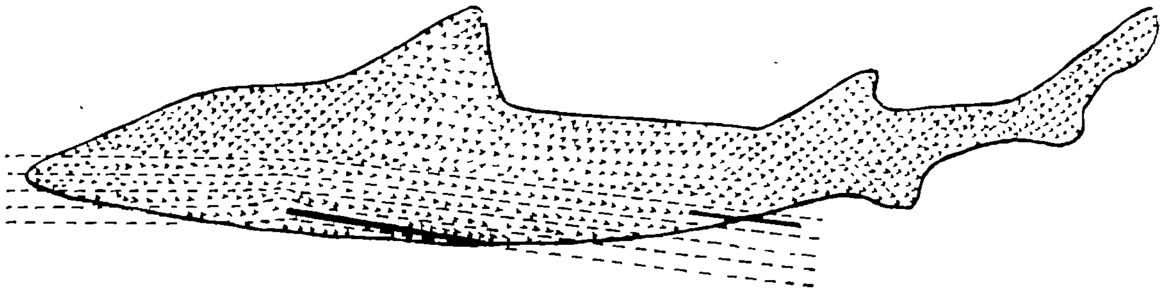

Fig. 3. Diagram of a dogfish (adapted from Harris) showing the theoretical downwash and the orientation of the dermal denticles

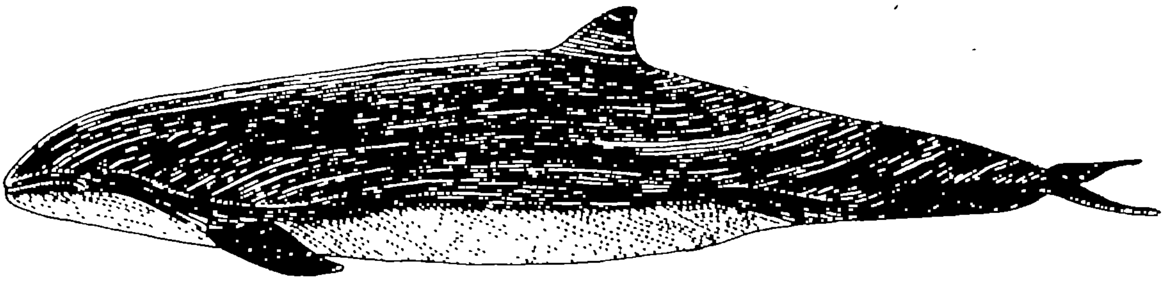

Fig. 4. Diagram of a common porpoise, *Phocaena phocoena*, showing orientation of the dermal ridges

pendently and sinusoidally in a direction at right angles to their course, this movement being compared with similar oscillations in the wings of birds. Closer inspection shows that such movement is true in both these animals only when the time factor is ignored. In cetaceans, the epaxial locomotor musculature, when the spinalis and longissimus dorsi are included, is approximately double that of the hypaxial muscles mass, while in birds, the pectoralis major, which depresses the wing, is many times the weight of the pectoralis minor, which raises the wing. The power and velocity of the upstroke of the tail in the dolphin are apparently greater than those of the downstroke, and provide the greater part of the forward thrust. The forward momentum, thus imparted, together with the buoyancy, assists materially in returning the tail to the horizontal position on the downstroke. Conversely, in the bird, the downstroke provides the main forward thrust and raises the body, while the wind velocity and gravitation on the body provide most of the lift of the wings during the upstroke.

It is clearly important, in assessing the power available, that some agreement should be reached about the identification of muscles which constitute the locomotor organs in whales. Slijper[12] has accurately demonstrated the fusion and interdependence of the whole epaxial muscle mass from occiput to tail, yet he denies any asymmetry in the weight of the epaxial and hypaxial locomotor muscle masses. Kermack[13], in his tabulated lists relating to fin whales, obviously recognizes such an asymmetry. An asymmetry of function, in the case of the bottle-nosed dolphin, is demonstrated in the serial film shown in Parry's[2] paper. It will be seen that 10 frames are required to return the tail from maximum elevation to the horizontal position. From maximum depression to the horizontal position only 5 frames are required. The extra thrust of the upstroke is also seen in the independent movement of the flukes. On the downstroke there is no clearly defined angle of attack between frames 1 and 9, that is, not until the tail is clear of the upwash. On the upstroke, however, the angle of attack changes rapidly between frames 16 and 18.

That Slijper[12] made this observation from Parry's film is apparent from his modified reproduction of the serial which he uses to demonstrate the interdependent movement of the flukes; thus frames 1 and 2 in Slijper's paper represent frames 2 and 9 from Parry's film, while frames 5 and 6 in Slijper represent frames 16 and 18 from Parry's film.

The effect of a more powerful upthrust would be essentially similar to that of the heterocercal tail of the dogfish, that of tending to push the body downwards and forwards. The poor development of the dermal ridges on the throat and belly seems to indicate that the latter are pressure areas. The antero-posterior circulation round the hydrofoil of the flukes induced by a powerful upthrust would produce an acceleration of water particles over their dorsal aspect, and consequently obliquely over the flanks. This excess of velocity over the observed momentum of the animal is that to which Gray referred, and is analogous with that part of the velocity of the slip-stream which produces the 'lift' on an aircraft wing. It is expected that during rapid swimming the amplitude of the tail beat would be much smaller than that shown in Parry's film, the frequency of oscillation being correspondingly greater. It is possible that in these conditions there is never any appreciable angle of attack on the downstroke.

If the orientation of the dermal ridges does indeed indicate the average flow pattern round the dolphin's body, it would clearly be erroneous to use the total length of the animal in the calculation of Reynold's number. The calculation of total resistance would appear to be a rather complex matter involving a great number of different values of *Re.* and a correspondingly large number of 'drag coefficients'. No attempt has been made here to give a quantitative assessment of the drag on the dolphin's body, not only because of the departure from simple boundary layer phenomena expected by the accelerations, described here, but also because very little agreement can be found in the literature about the exact value of *Re.* at which the transition from laminar to turbulent flow occurs. For example, Gray[1] gives the figure as *Re.* 5×10^5, Kramer[4] $2{\cdot}8 \times 10^6$, while Sutton[14] states that the slightest disturbance will cause the flow to become turbulent above *Re.* 2×10^5. It may be stated, however, that if Kramer's value is used, then the oblique flow over the body of a 6-ft. dolphin at 15–20 knots would be 90 per cent laminar, irrespective of any moulding of the skin due to dermal ridges, or subdermal oil sinuses. As previously stated, the dermal ridges are not apparent on the external skin of a healthy dolphin, and the oil ducts, which lie deep to the panniculus carnosus, are only conspicuous in the thoracic region, where they are associated with the pectoral musculature. The panniculus carnosus is very well developed in cetaceans and takes the form of broad, but discrete sheets, covering over the muscles of the shoulder, chest, neck and abdomen. From the general appearance and disposition of its components it would seem that its main function is that of smoothing out the transverse contours of the subjacent muscles.

A paper by van Heel[15] recently received, concerning experimental work with the common porpoise, *Phocaena phocoena*, stated that these animals "never showed their flukes and seldom jumped . . . in smooth water a conspicuous surface wave could be observed if the animals were moving quickly or near the surface". This observation seemed to indicate that *Phocaena* is a rather slower swimmer than *Delphinus*, but that there is an average upward displacement of water in this species also. Van Utrecht's statement that the dermal ridges lay at a slight angle to the long axis of the body was found to be true over the greater part of the flanks and the dorsal surface, but the head, belly and caudal peduncle showed the familiar 'upwash' and 'downwash' orientation (Fig. 4).

Van Utrecht's observation that the typical pattern becomes visible at the surface is most clearly seen by careful examination of the fine 'brush marks' in the marginal zones between broad patches of pigment. If the foregoing hypothesis were correct then it is true to say that the streaming lines of colour which are to be seen on many species of odontocete provide, at least, an approximate guide to the flow of water over the body, and it is interesting in this respect that there is scarcely a species which does not show the 'downwash' effect behind the eyes.

Note. A reference discovered after the present article had been submitted for publication shows that Sokolov[16] had made a similar observation. Without giving details he states: "Oblong epidermal septa in the thoracic skin of quick-swimming whales as well as that of the flukes of all Cetacea are always arranged in a definite direction, namely, along the lines of flow of water over the whale's body when swimming".

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OBITUARIES

Prof. D. M. Steinberg

PROF. D. M. STEINBERG, who died in November at the early age of fifty-three, was one of the leading figures in the Russian entomological world and well known abroad as an active participant at international entomological and zoological congresses. He was born at Leningrad in a highly cultured family, his father being professor of the theory of music in the Musical Academy and the maternal grandfather the composer N. A. Rimsky-Korsakov. After graduation from the University of Leningrad in 1930, D. M. Steinberg worked for a short time at the Oceanographic Institute at Vladivostok, but soon joined the All-Union Institute for Plant Protection in Leningrad and also lectured at the Medical Institute. In 1945, he moved to the Zoological Institute of the Academy of Sciences, where he spent the rest of his life.

D. M. Steinberg's scientific interests have been very wide, but his works are characterized by their thoroughness, critical insight and often an original approach to the subject. Most of his eighty papers deal with the comparative morphology of insects and their physiological ecology; more recently he turned his attention to taxonomy and produced a monograph on wasps of the family Scoliidae in the *Fauna of the USSR*. Among his outstanding works may be mentioned his doctor's thesis on the morphogenetic potentialities of the larval hypoderm of insects (1942); comprehensive field and laboratory investigations on the web-worm (*Loxostege sticticalis*), containing a wealth of data on its ecology, behaviour (particularly, flight in relation to the dynamics of the air) and physiology, with special reference to the conditions of sexual maturation (1935); a valuable review of Russian work on insect morphology for the period 1917–57 (1957), etc. His latest interest was in the theoretical aspects of biological control of insect pests, in which connexion he spent three months in 1961 in Canada, studying research methods. He also visited Iran (1955) and Ethiopia and the Sudan Republic (1959) for entomological investigations. He organized and led research expeditions of the Zoological Institute to Kazakhstan (1949–51) for a survey of the insect fauna in the steppe areas where the development of forest shelter-belts was planned; and Turkmenia (1952–53) for investigations of the insect of the deserts scheduled for irrigation; the object in both cases was to discover the natural components of the fauna likely to become important pests under the conditions of new land use. D. M. Steinberg's influence on the development of entomology in his country was very great, and his premature death leaves a wide gap in the senior ranks of Soviet entomologists.

B. P. UVAROV

Dr. Richard E. Trees

DR. RICHARD E. TREES, a theoretical physicist at the National Bureau of Standards, U.S. Department of Commerce, died on October 27. Dr. Trees had made distinguished contributions to the quantum theory of complex atomic spectra.

Born in Philadelphia in 1920, Dr. Trees received his B.S. degree in naval architecture and marine engineering at the Webb Institute of Naval Architecture in 1943. He received his M.S. and Ph.D. degrees in physics from the University of Pennsylvania in 1949 and 1951 respectively.

He joined the National Bureau of Standards in 1951 as a physicist. In 1955, Dr. Trees was the recipient of a Rockefeller Public Service Award, which enabled him to spend a year in research at Princeton University. He received a Meritorious Service Award from the U.S. Department of Commerce in 1958.

Prof. T. H. Berlin

PROF. THEODORE H. BERLIN, of the Rockefeller Institute, a leading American theoretical physicist, died on November 16.

Dr. Berlin went to the Rockefeller Institute, New York, in February 1961 to develop a school of physics and mathematics with Prof. George E. Uhlenbeck, a physicist, and Prof. Mark Kac, a mathematician. He was formerly professor of physics in the Johns Hopkins University.

Born in New York City on May 8, 1917, Dr. Berlin received the B.S. degree in chemical engineering from the Cooper Union Institute of Technology, and the M.S. and Ph.D. degrees from the University of Michigan.

His Ph.D. thesis was on the quantization and electric interaction in diatomic molecules. During the Second World War, while a graduate student at the University of Michigan, he worked on the proximity fuse.

He joined the faculty of the Johns Hopkins University in 1946, and was appointed assistant professor in 1947. He was then named associate professor of physics in Northwestern University and, in 1955, returned to Johns Hopkins University as a full professor.

Dr. Berlin in 1952 and 1953 was a member of the Institute for Advanced Study, Princeton University, as a Guggenheim Fellow. At the University of Michigan he studied under Prof. Uhlenbeck, and at the time of his death was engaged in writing a volume on statistical physics with Prof. Uhlenbeck.

A Fellow of the American Physical Society, Dr. Berlin has served as associate editor of the *Journal of Chemical Physics* and the *Physical Review*, publications of the Society. Last year he served for a time as the editor of *Physical Review Letters*.

Mr. C. W. Wood

CYRIL WARCUP WOOD, senior science master at St. Bees School (Cumberland) from 1945 until 1959 and since 1960 executive editor of the new Commonwealth and International Library of text-books, died in an Oxford nursing home on December 20, at the age of sixty-two. He had recently been appointed a director of the Pergamon Press.

Cyril Wood, who was born in Yorkshire, held a first-class honours degree of the University of Manchester and was also a master of science of the same University. He was chief examiner in physics-with-chemistry for the Northern Universities Joint Matriculation Board and assistant examiner in the General Certificate of Education for the Examining Boards of Oxford, Cambridge and Wales. Before joining the staff of St. Bees, Mr. Wood taught chemistry in Leeds Grammar School for twelve years.

During his lifetime Mr. Wood wrote fifteen general science and chemistry text-books.

NEWS and VIEWS

The British Broadcasting Corporation:

Sir Harold Bishop, C.B.E.

SIR HAROLD BISHOP, director of engineering since 1952, will retire on May 10, the fortieth anniversary of the date on which he joined the B.B.C. He is the first man to complete forty years' service with the B.B.C. Sir Harold received his technical education at the City and Guilds College, being awarded the associateship of the College in 1920. He was later awarded the fellowship of the City and Guilds Institute and the fellowship of the Imperial College of Science and Technology. After two years with H.M. Office of Works and a year with Marconi's Wireless Telegraph Co., Ltd., he joined in 1923 the British Broadcasting Company, which in 1927 became the British Broadcasting Corporation. He was senior superintendent engineer from 1923 until 1929, assistant chief engineer from 1929 until 1943, and chief engineer from 1943 until 1952, when he was appointed to his present post. He was awarded the C.B.E. in 1938 and created Knight Bachelor in 1955. Sir Harold was chairman of the Radio Section of the Institution of Electrical Engineers in 1941–42, a member of the Council from 1944 until 1947, vice-president from 1948 until 1953, and president in 1953–54, and was awarded the Institution Premium in 1935. He was president of the Association of Supervising Electrical Engineers from 1956 until 1958 and president of the Television Society in 1961 and 1962.

Mr. F. C. McLean, C.B.E.

SIR HAROLD will be succeeded as director of engineering by Mr. F. C. McLean, who is at present deputy director of engineering. After taking his degree at the University of Birmingham, Mr. McLean joined Standard Telephones and Cables, Ltd., in 1925 as an engineer on radio projects and spent six years in the Paris laboratories of the Company. In 1937 he joined the Station Design and Installation Department of the B.B.C. and became assistant to the chief engineer in 1947. He was promoted to assistant chief engineer in 1951 and deputy chief engineer in 1952, and has been deputy director of engineering since 1960.

Mr. M. J. L. Pulling, C.B.E.

THE new deputy director of engineering will be Mr. M. J. L. Pulling, who is at present assistant director of engineering. Mr. Pulling was educated at Marlborough College and at King's College, Cambridge. After a period in industry he joined the B.B.C. Engineering Information Department in 1934. He became assistant to the assistant chief engineer in 1937 and superintendent engineer (recording) in 1941. From 1949 until 1956 he was senior superintendent engineer (television) and was appointed controller (television service engineering) in 1956. He took up his present post in January 1962.

Mr. D. B. Weigall

MR. PULLING will be succeeded as assistant director of engineering by Mr. D. B. Weigall. On leaving the University of Oxford in 1933, Mr. Weigall joined the B.B.C. as a student apprentice. He was a member of the Research Department from 1935 until 1939 and was seconded as chief engineer to the Malaya Broadcasting Corporation from 1940 until 1942 and afterwards to the Ministry of Information. In 1946 he joined the Department of the

Superintendent Engineer (Sound) and became a senior engineer in the Planning and Installation Department in 1948. From 1961 until 1962 he was in the External Broadcasting Engineering Department and became chief engineer (external broadcasting) in May 1962.

Dr. K. R. Sturley

THE new chief engineer, external broadcasting, will be Dr. K. R. Sturley. On leaving the University of Birmingham, Dr. Sturley joined Marconi's Wireless Telegraph Co., Ltd., in 1936. He remained with them until 1945 when he joined the B.B.C. and was appointed to his present post as head of the Engineering Training Department.

Marconi Director of Engineering :

Dr. E. Eastwood, C.B.E.

DR. E. EASTWOOD has been appointed director of engineering and research of Marconi's Wireless Telegraph Co., Ltd. Born in Rochdale, Lancashire, Dr. Eastwood was educated at Oldham High School and later at the University of Manchester and Christ's College, Cambridge. He joined Marconi's in 1948, from the Nelson Research Laboratories of the English Electric Company, and was appointed deputy chief of research. In 1954 he became chief of research, and in 1962 he was appointed to the new post of director of research. He was awarded the Wakefield Gold Medal of the Royal Aeronautical Society in 1961, in recognition of his contributions towards safety in the air, and he was awarded a C.B.E. in the 1962 New Year Honours List. In 1961 he was elected to the Board of Marconi Instruments, Ltd., and was appointed director of research for the entire English Electric Group in August 1962. He is at present giving the 1962–63 series of Faraday Lectures for the Institution of Electrical Engineers. In this newly created post, Dr. Eastwood will be responsible for all engineering and research work within the Marconi Company.

Mr. R. J. Kemp, formerly deputy engineer-in-chief of the Company, is appointed deputy director of engineering and research.

1962 James Clayton Prize

THE Institution of Mechanical Engineers has awarded the 1962 James Clayton Prize to: Dr. H. E. Merritt, for his contributions to mechanical engineering science by theoretical and practical investigations leading to his solution of many problems involving tooth gearing, in part communicated to the Institution in the form of papers and lectures; Dr. D. G. Christopherson, for his broad contribution to mechanical engineering science by way of his researches into lubrication, elasticity and plasticity, and through his services in the development of engineering education. This Prize is drawn from the Clayton Trust Fund, and represents one-quarter of the annual income of the Fund (nearly £6,500 each year). It is awarded annually to a member or members of the Institution who in the opinion of the Council contribute most in the year to modern Engineering Science by way of research, invention, experimental work or a treatise or paper on a modern engineering subject, or originality in engineering design or by service to engineering.

The Association of Universities of the British Commonwealth

THE report for the year ended July 31, 1962, of the Executive Council of the Association of Universities of the British Commonwealth records a membership of 118 universities, 10 university colleges and 4 other approved institutions of higher education (Pp. 36. London: Association of Universities of the British Commonwealth, 1962). While the final total of 967 vacancies for 1961–62 was not significantly higher than in the previous year (943), the number of applications received for the posts announced fell from 1,634 to 1,398, and the number of announced cases in which the appointment went to a candidate secured by the Association's advertisements rose from 127 to 151; during the year 4,741 specific inquiries about advertised posts were handled. The Association continued to provide the secretariat for the Commonwealth Scholarship Commission in the United Kingdom and to assist in the selection and placing of Scholars and Visiting Fellows, and has also assisted with the competition for the Frank Knox Memorial scholarships, with the Marshall scholarships, the Commonwealth University Interchange, the Drapers' Company scholarships and the admission of candidates to universities in France by virtue of examinations passed in Britain. A note is appended on the work of the Committee of Vice-Chancellors and Principals.

University Support in Canada

THE report on University Support 1961–62 of the National Research Council and the Medical Research Council of Canada records direct expenditure by the former of 8·85 million dollars, of which 82·8 per cent was for some 1,000 research grants to members of university staffs and 17·2 per cent for 675 postgraduate scholarships and fellowships (Pp. vii+138. N.R.C. No. 6862. Ottawa: National Research Council and Medical Research Council, 1962. 50 cents). Of the total expenditure, 8 million dollars was provided by the Council, 700,000 dollars by the Atomic Energy Control Board, 24,460 dollars by the Fisheries Research Board and 120,937 dollars by the North Atlantic Treaty Organization Science Committee. Of expenditure totalling 965,892 dollars for indirect support, 43·6 per cent was to scientific organizations and functions, 36·5 per cent for the publication of six scientific periodicals and the remainder for administration of the programme. The total of 9·81 million dollars compares with 8·17 million dollars in 1960–61 and 3·32 million five years ago. The Medical Research Council's programme of support totalled 3·36 million dollars in 1961–62 compared with 3·31 million dollars in 1960–61 and 894,357 million dollars five years ago. Of the total, 82·7 per cent was for 383 research grants to Canadian institutions and 15 per cent for 60 fellowships, 24 associateships and 24 summer scholarships. Lists of fellowships and scholarships and of research grants are included in each report.

Management Studies in Technical Colleges in Britain

VIGOROUS further development of postgraduate courses of high quality is recommended in the first report of the United Kingdom Advisory Council on Education for Management, 1962, *Management Studies in Technical Colleges* (Pp. viii+19. London: H.M.S.O., 1962. 2s. net). The report points out that the technical colleges provide a wide range of courses in management studies, including the new diploma in management studies, a national award introduced in 1961. It is of great importance that the diploma course should be of a high standard at all centres where it is offered, based on teaching of high quality, with rapid build-up of full-time courses, and concentrated at a relatively limited number of centres, where exploration and development of the field of knowledge are possible. Some full-time staff may be obtainable from industry, and the special needs of management departments should be recognized in settling staff complaints and regard paid to opportunities for research and consultancy. A sustained effort is needed to ensure a continuous flow of information about the purposes of management education and the available facilities. Besides the courses with 2,360 students at forty-three technical colleges (including four colleges of advanced technology) in October 1962, twelve universities are offering courses, in eight of which postgraduate full-time courses of a year's duration are available. The importance of close co-operation between the colleges and the firms providing students is stressed, and the report includes recommendations for the training and development of teachers, such as overseas visits, interchange of staff, and the use of part-time staff.

The Sixteenth Arthur Stanley Eddington Memorial Lecture

IN the sixteenth Arthur Stanley Eddington Memorial Lecture, "On Having a Mind", delivered on November 9, 1962, Prof. W. Kneale concludes that we must retain the Platonic notion of mental events which are distinct from anything in the physical world and manifest a special kind of connectedness (Pp. 56. Cambridge: At the University Press, 1962. 4*s.* 6*d.* net; 95 cents). The occurrence of such events is part of what we ordinarily intend to assert when we speak of the existence of minds and a presupposition of all the more interesting things we want to say about them. He believes that an analysis of perception which does justice to the complications of our ordinary talk about it must be a casual theory of some kind, and that the distinction of primary and secondary qualities, as drawn by Locke at his best, is a very important discovery of philosophy. He does not attempt to consider how it came about that some organisms have minds, nor yet what advantage, if any, an organism can gain from having a mind, but in this part of our search for truth he thinks we are still like children playing with pebbles on the shore of a great ocean. He suggests that the survival of the Platonic theory of mind during the past three centuries in spite of fairly obvious difficulties is not due to the dominance of a religious interest among philosophers but to its leaving the way open for the development of natural science.

U.K. Population Trends in the Late 'Fifties

IN the third part of his statistical review for 1960, the Registrar General remarks on the change in the pattern of population growth which took place in the late 'fifties (*The Registrar General's Statistical Review of England and Wales for the year 1960.* Part III: Commentary. Pp. xiv + 281. London: H.M.S.O., 1962. 21*s.* net). Between 1959 and 1960 the home population increased by 369,000 persons, compared with 277,000 and 202,000 in the previous two years. Natural increase accounted for 255,000, the remainder being due to net migration. As there was a not inappreciable outward movement, total immigration was considerably heavier. Live births at 785,000 were higher than in any year since 1947, and it seems reasonably certain that ultimate family size has been rising. The marriages of 1936–41 achieved about two children apiece, those of 1946 are likely to achieve 2·2, the most recent cohort for which the Registrar General makes an estimate are likely to have between 2·3 and 2·4. A rise of 20 per cent in total family size over a period of 15 years is obviously an important shift and needs explanation, though this is not the task of the Registrar General, who confines himself to the role of chronicler. It is, however, to be noted that the 1962 total of births is likely to exceed that of 1960 by a considerable margin. As regards deaths, the principal comparison made is between 1950 and 1960, two years not very different climatically. Infectious diseases, and particularly tuberculosis, have fallen very considerably, from 47·0 per 100,000 in 1950 to 12·3 in 1960. As regards cancer, an increase of 53 per cent in the standardized mortality ratio is noted for cancer of the lung among males and a 35 per cent increase for females. Expectation of life at birth was 68 years for men and 74 for women, at age 45 the figures were 27 and 32 years, and at age 65, 12 and 15 years, respectively. Marriage rates continued high, the gross nuptiality table for 1960 implying no fewer than 96·4 per cent of females marrying by the age of 50.

Antiquities from Benin, Nigeria

BENIN sculptures are well known and can be seen in most of the major ethnographical museums. Frequently they were made by the *cire perdue* method and the artists showed great skill in their productions. Bronze is commonly the material used, but brass also occurs. As there is no tin available in Nigeria, this latter alloy must have been imported, perhaps from the southern Sudan. These sculptures have been made in Benin for a very long time; indeed, since the sixteenth century. Did the early Portuguese traders have any influence on a native art existing in the country at the time? But it was only really after the punitive expedition of 1897, when the country was opened up, that these splendid sculptures became known in England. Nowadays they are much sought after and command a large price.

Captain A. W. F. Fuller, who was born in 1882, and came of a family long settled in Sussex, started the hobby of collecting at an early age, and his collections from Oceania and Africa became famous. Towards the end of his life he became interested in the Chicago Natural History Museum, and there all his collections are to be found. The Museum already had a good number of specimens from Benin, and these together with the Fuller examples are now on show. A well-illustrated catalogue has been produced, and for those who cannot visit Chicago itself this will be of great interest (*The Art of Benin: a Catalogue of an Exhibition of the A. W. F. Fuller and Chicago Natural History Museum Collections of Antiquities from Benin, Nigeria.* By Philip J. C. Dark. Pp. iii + 74 + 48 plates. Chicago: Chicago Natural History Museum, 1962. 2 dollars).

Russian Geology in Translation

FOR some years the American Geological Institute has been conducting an extensive programme of English translation from Russian, Chinese, and other 'difficult' languages, much of which has been published in its monthly periodical *International Geology Review* (55 dollars per year; 15 dollars to educational institutes and personnel: American Geological Institute Translations Office, 2101 Constitution Avenue N.W., Washington 25, D.C.). A special supplement to the October 1962 issue of this journal, in the form of an 89-page *Catalogue of Translations of Russian Papers in Geology, Solid-Earth Geophysics, and Related Sciences through 1961*, is likely to meet a present need. It records fifteen Russian periodicals in the earth sciences of which cover-to-cover translations are made by American agencies; and it lists a further 1,652 papers from other serials (dating from 1953 to 1961) of which translations are available, with the sources whence copies may be obtained. One noteworthy omission from the list of cover-to-cover translations is the *Bulletin of the Moscow Society for Natural Research* (*Geological Section*), now being produced in English, on behalf of the National Science Foundation, by the Israel Program for Scientific Translations (61, Geological Section, 31, 1st, 2nd, 3rd Editions. Pp. iv + 444. (Jerusalem: Israel Program for Scientific Translations. Available from the Office of Technical Services, U.S. Department of Commerce, Washington, 25, D.C., 1961.) 4.50 dollars.) Beginning in 1963, *International Geology Review* will be expanded to carry selected papers from the *Palaeontological Journal* (*U.S.S.R.*), from the *Izvestiya* (Geological Series) of the U.S.S.R. Academy of Sciences, and from *Soviet Geology*, and it will thus make the results of much Russian geological work more readily accessible to the Western reader.

Bibliography on Soft X-ray Spectroscopy

AN *Annotated Bibliography on Soft X-ray Spectroscopy* has been compiled in connexion with a research programme on the electronic structure of metals and alloys in progress at the U.S. National Bureau of Standards (*Monograph* No. 52: *Annotated Bibliography on Soft X-ray Spectroscopy.* Pp. iv + 108. Washington, D.C.: Government Printing Office, 1962. 1 dollar). The bibliography contains about 550 references and is considered to be a complete coverage of the soft X-ray literature for the period 1950–60. Some references to earlier work are included but the general review references, listed separately, cover fairly adequately previous work. The first group of references in the monograph is of general survey and review papers and books. The main list of references follows, grouped by year and arranged alphabetically by author within each year. The emphasis is on the application of soft X-ray spectroscopy to the study of valence band electronic states in metals and alloys. The spectral region 25–800 Å involving ruled glass grating spectrometers is therefore of principal interest. However, considerable data have been gathered, primarily by the Russians, on valence electronic states by means of high-energy transitions where crystal spectrometers are satisfactory. These references and any X-ray work leading to the distribution of valence electronic states are included regardless of the transitions used. In addition to soft X-ray data, references on all pertinent aspects of the apparatus and experimental problems are included in the bibliography. A number of indexes are given at the beginning of the monograph for ready reference to papers containing the specific data to which the bibliography applies. In the literature the spectra are presented in a variety of energy units, and in compiling the bibliography the units given in the original papers have been retained. A conversion chart of units is appended to the bibliography. It is intended to continue the bibliography and to issue supplements periodically.

Protecting Turtles in Sarawak

A Government Ordinance now protects the greater turtles of Sarawak from disturbance, under penalty of a 1,000 dollar fine. This is the penalty for any unauthorized vessel approaching within half a mile of three islands off the coast of Sarawak, Talang Talang Besar, Talang Talang Kechil and Satang Besar, on which the turtles nest. Carefully conserved by the curator of the Sarawak Museum, Dr. Tom Harrisson, the turtles have provided more than twenty million eggs for local consumption in the past fifteen years (*World Wildlife News*, No. 7; October 1962). Many other eggs have been removed to hatcheries, where the young turtles have been reared safely and released again at sea when they are big enough to fend for themselves.

Incidence and Distribution of Asthma in Australia

An analysis was made of asthma cases in public hospitals in Queensland in the year 1957. Asthma was responsible for 2,673 cases, or 1·62 per cent of the total. It predominated in male children and in adults more than 55 years of age. It predominated in females in the intervening years. A striking fall in the male incidence in the second decennium suggested a favourable prognosis for most asthmatic boys. The apparent incidence of asthma varied considerably from place to place. The variation could not be explained simply in terms of latitude, altitude, temperature, rainfall or distance from the coast. Some of the variation between hospitals was probably due to different criteria for admission. The seasonal distribution changed significantly at about the Tropic of Capricorn. To the south, the main incidence was in autumn; to the north, no seasonal patterns had a wide prevalence. The investigators, Drs. E. H. Derrick and R. H. Thatcher, showed that an aetiological study of asthma must take into account variation in the complex of causes including sex, age, season and locality (Seventeenth Annual Report of the Council of the Queensland Institute of Medical Research for the year ended 30th June, 1962. Pp. 12. Brisbane: Queensland Institute of Medical Research, 1962).

The Society for Visiting Scientists

The report of the secretaries on behalf of the Council of the Society for Visiting Scientists made to the fifteenth general meeting on December 11 refers to the success of the three discussion meetings dealing, respectively, with colour television, the turkey X disease, and matching men and machines. Activities were somewhat restricted by the change of premises from Old Burlington Street to 19 Albemarle Street, and reference is again made to the difficulty of finding new and adequate sources of income.

The Scottish Field Studies Association : Programme of Courses

The Scottish Field Studies Association has arranged the following courses during 1963: *Garth:* geography (April 13–20); spring in the Highlands (May 18–25); geology of Glen Lyon (June 8–15); insect natural history (June 29–July 6); grasses, rushes and sedges (July 6–13); mountain and moorland ecology (August 3–10); mountain flowers (August 10–17); plant ecology (August 17–24); lichens (September 7–14); *Isles of Great Cumbrae:* seashore biology (May 11–18); marine biology (June 1–8, June 8–15, August 24–31); *Black Isle:* geography (June 1–8); *Pitlochry:* archæology (June 1–8); *Isle of Arran:* birds of Arran (April 13–20); geology of Arran (June 1–8, September 7–14); wild flowers (July 6–13); *Strathpeffer:* Highland birds (June 8–15); geographical field studies (July 6–13); *North Ballachulish:* geology of Glencoe (July 6–13); *Alnmouth:* birds of Northumberland (June 1–8); a geographical survey of the Hebridean highlands and islands (April 11–17). Further information can be obtained from the Scottish Field Studies Association, 179 West Regent Street, Glasgow, C.2.

Announcements

Dr. Bernard Kirsch, research physicist with the Phillips Petroleum Company's Atomic Energy Division at Idaho Falls, has been appointed senior scientist at the Nuclear Science and Engineering Corporation, Pittsburgh, Pa.

Changes of name of subsidiary companies of the Derritron Group have been announced as follows: Beme Research and Development Ltd., to Derritron Research and Development Ltd.; Chapman Ultrasonics Ltd., to Derritron Ultrasonics Ltd. (managing director, Mr. C. T. Chapman); Doran Instrument Co. Ltd., to Derritron Instruments Ltd.; Electronic Vibrators Ltd., to Derritron Electronic Vibrators Ltd.; L. S. B. Components Ltd., to Derritron Transformers Ltd.

A programme of twelve courses on the use of isotopes has been arranged by the Atomic Energy Research Establishment Isotope School during the period February 11–December 13. The courses will range between five and twenty-three days in length, with tuition fees between £20 and £60. Further information can be obtained from the Wantage Research Laboratory (A.E.R.E.), Wantage, Berkshire.

A symposium on "Communications in Industrial Relations", organized by the Central London Productivity Association in conjunction with the Department of Management and Social Science, Northampton College, will be held at the Northampton College of Advanced Technology, London, on January 30. Further information can be obtained from Miss C. M. Burnell, Room 101, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Erratum. In the communication by C. Chlouverakis entitled "Adipose Tissue Lipoprotein Lipase Activity in Rats of Two Different Ages", which appeared on p. 1103 of the December 15 issue of *Nature*, the tenth paragraph should read "The average enzyme activity so defined was found to be 19·36 ± 8·25 (mean ± *S.D.*) units in young, and 3·81 ± 2·92 (mean ± *S.D.* as above) units in old rats ($t = 5·3$, $P < 0·0001$)".

FLUORIDE, TEETH AND THE ANALYST

A MEETING on "Fluoride, Teeth and the Analyst", held by the Society for Analytical Chemistry at the Wellcome Building, Euston Road, London, N.W.1, on November 7, under the chairmanship of Dr. A. J. Amos, provided an opportunity for discussion between chemists of widely different interests and specializations.

Dr. H. J. Cluley (General Electric Co., Ltd., Hirst Research Centre, Wembley, Middlesex) spoke on "The Determination of Fluoride in Inorganic Materials by Pyrohydrolysis". Fluorine, he said, interfered with the determination of a number of elements and was itself frequently difficult to determine. One particular difficulty arose from the lack of selectivity of the methods available for the determination of fluoride, so that for fluoride in inorganic materials a preliminary separation of the fluoride from most other elements was almost invariably necessary. The most common method of separation was that of Willard and Winter in which fluoride was distilled as fluosilicic acid; however, this was subject to serious interference from aluminium, boron and gelatinous silica and hence was of little practical value to the silicate analyst.

Against this background, an alternative means of separation was required and 'pyrohydrolysis' offered a method of rapidly separating fluorine from a variety of inorganic materials. This technique involved heating the sample, usually mixed with a suitable 'accelerator', in a current of steam or moist oxygen; the fluorine was evolved as hydrogen fluoride which could be condensed and determined by titration.

The method originated in the atomic energy field where it was observed that the fluorides of uranium and of aluminium, bismuth, magnesium, vanadium, zinc, zirconium and rare earths were rapidly hydrolysed on heating in a current of steam. Other fluorides such as those of calcium, strontium and barium were more reluctant to part with their fluorine but the addition of an accelerator, for example, U_3O_8 or alumina, facilitated their hydrolysis. More recently, tungstic oxide and vanadium pentoxide had been used as accelerators.

Originally, a platinum reactor tube had been used for the pyrohydrolysis. It had now been shown practicable to use a silica reactor tube in conjunction with a glass water-cooled condenser, since the concentration of hydrofluoric acid vapour was always low and the time of contact with the apparatus was short. Typically, pyrohydrolysis was continued for 15–30 min at approximately 1,000° C, with a fast flow of steam to give 3–5 ml. of condensate per min.

Where conventional methods require lengthy separations, for example, the determination of fluorine in silicate materials, the rapidity afforded by the pyrohydrolysis technique is of particular advantage. Dr. Cluley described how the technique had permitted the rapid determination of 5 per cent of fluorine in opal glass, giving results of high precision. He had also used pyrohydrolysis to determine 0·02–0·10 per cent fluorine in very small dentine samples which were supplied by Dr. G. N. Jenkins (who was to speak later) and which originated from 'low' and 'high' fluorine areas.

Dr. J. Longwell (deputy government chemist, Laboratory of the Government Chemist, Clement's Inn Passage, Strand, London, W.C.2) dealt with the fluoridation of water supplies from the analyst's point of view. Not enough was known about the vast amount of work that had been devoted to this subject during recent years in the United States and in Great Britain; it had been the subject of detailed study without parallel in preventive medicine. He recalled how the condition of children's teeth had improved during the Second World War when sugar and sweets were rationed and how their condition had since worsened, and showed colour slides to illustrate the gravity of the problem.

Surveys of children's teeth in the United States had shown how the numbers of decayed, missing and filled teeth were much lower or nil in the areas where naturally occurring fluoride was present and the practice of fluoridation of water supplies at a level of 1 p.p.m. had since been adopted. The improvement in dental health that had been observed after fluoridation for some fifteen years in the United States was described and illustrated.

In 1952, a group from Great Britain went to North America to study the fluoridation of water and, on returning, recommended that fluoride be added to the water supplies of several selected communities before the general adoption of fluoridation. Studies were first carried out on the availability of fluoride in food and drink and on the amounts that would be ingested with and without fluoridation of the water. Fluoridation was started in Kilmarnock, Watford and Anglesey in 1955–56 and a report had just been published by the Ministry of Health *et al.* giving the results obtained after five years of fluoridation (see *Nature*, 195, 760; 1962).

The methods used to add fluoride in a steady and controlled concentration to the water supplies in Britain were outlined and emphasis was laid on the care taken to ensure control—which was two-fold. First, the amount of fluoride actually added was recorded as was the volume of water pumped and from these figures the concentration of fluoride ion in the water was calculated. Chemical determinations at the water plant, using a visual colorimetric zirconium–alizarin lake method were confirmed at the Laboratory of the Government Chemist, using distillation from perchloric acid to remove any interfering ion, followed by colorimetric determination. More than 20,000 samples examined in the five years gave an average of 0·94 p.p.m. fluorine, with a standard deviation of about 0·1 p.p.m. fluorine. The improvement in the deciduous teeth of children in the three study areas, as a result of fluoridation, was illustrated; the reduction of about 60 per cent in the incidence of caries in 3- and 4-year-old children was similar to that observed in the United States, and even more interesting was the increase in the percentage of children with completely sound teeth, from 32 to 60 per cent at 3 years and from 22 to 42 per cent at 4 years.

In time, teeth of older age groups would be improved and teeth of older children and adults in natural fluoride areas were examples of what could and should be achieved by fluoridation. Dr. Longwell ended his talk by showing coloured photographs of teeth of two children, typical of many, living in a natural fluoride area.

Dr. G. N. Jenkins (Reader in Oral Physiology, Medical School, King's College, University of Durham, Newcastle upon Tyne) concerned himself with the role of analysis in investigating the mode of action of fluoride in tooth decay. Fluorine was present in teeth as a result of an ion exchange reaction with the hydroxyapatite of the calcified tissues, and hence in areas with a water of high fluorine content the fluorapatite of the teeth was also high. Topical application of high concentrations of fluoride led to other reactions—double decomposition leading to the deposition or adsorption of calcium and magnesium fluoride, and hence the mechanisms involved by such methods, for example, in toothpastes, were certainly very different and incompletely understood.

Studies of the distribution of fluorine in the tooth showed a very definite pattern, the concentration being much higher in the outer enamel layers than deeper in the tissues of the tooth. Nevertheless, the concentrations even in these deeper enamel layers were higher in teeth from high than from low fluoride areas. The lesser solubility of fluorapatite compared with hydroxyapatite suggested a possible mode of action in reducing the incidence of caries, and detailed investigations of solubility were described in which the outer surfaces of teeth from high and low fluoride areas were compared. The large variation tooth-to-tooth made valid comparison very difficult and the slightly lower solubility found for teeth from a 1 p.p.m. fluorine area, as compared with a zero fluorine area, was not statistically significant. At 2 p.p.m., however, a statistically significant lowering by 21 per cent in the solubility was recorded. The choice of analytical method was of importance in the solubility determinations; in some conditions different results were obtained, according to whether phosphorus or calcium was measured, and workers in the United States had investigated the problem by gravimetric determinations of solubility on powders ground from successive layers of the teeth. By this method, too, the teeth with highest fluoride content were also the most soluble. Experiments with radioactive fluoride revealed that the tooth took up fluoride more readily in the nieghbourhood of a caries blemish, and it had been found that the differences between the fluoride of enamel from areas of high and low intake of fluoride were greater when carious enamel was studied.

Other properties of the fluoride ion were discussed that might be related to its action in reducing dental decay. Since it favoured the precipitation of calcium phosphate from unstable and metastable solutions, such as saliva, it might intermittently reverse the removal of calcium salts in, and thus slow the development of, a dental cavity. The role of fluoride as an enzyme poison might also be related to its decay-inhibiting properties. Attempts were therefore being made to study the fluoride content of the 'dental plaque' (the muco-bacterial film on the tooth surface)—an analytical problem of some magnitude when it is realized that only about 10 mg of plaque can be obtained from one person. It had been shown to be significantly higher in subjects from 'high fluoride' areas. Partial release of the bound fluoride in plaque might occur if the *p*H became sufficiently acid, as it probably did after eating carbohydrate. It had been found with saliva that the addition of fluoride at a concentration similar to that of plaque was effective not merely in preventing the production of acid from carbohydrate by salivary bacteria but could actually bring about an increase in *p*H.

That fluoridation of public water supplies was a subject of great topical interest was evident from the lively discussion that followed each paper. The analyst, it was clear, had an important part to play by developing and applying sensitive methods that were essential not only to ensure its proper regulation and control but also to provide the basic tools for the study of the fundamental mechanism by which fluoride exerted its beneficial effect.

S. A. Price

GROWTH AND NUTRITION OF A WHOLE PLANT

ANYONE interested in the growth of the whole plant should find a recently published memoir interesting, for they will find it a refreshing revision course dealing with some theories of contemporary botany in a more stimulating way than is usually found in text-books*. Prepared by Prof. F. C. Steward and three colleagues it contains, in Parts 2–5, information about the anatomy, growth and development of mint grown with a wide range of nutrient-levels (some of which led to deficiency symptoms) and different light and temperature conditions. Parts 4–6 have details of many chemical analyses, involving chromatographical techniques, carried out on roots, stems and leaves of plants grown under a wide range of environmental conditions. The final section is a synthesis of the earlier parts.

Although the morphology of the plant does make it readily acceptable to sampling procedures, the decline in popularity of mint as an experimental plant (discussed in Part 1) must largely be due to the fact that large numbers of plants are usually needed for experiments associated with analysis of growth. Raising plants from seeds is more satisfactory from many points of view than from cuttings. There are no troubles in keeping stock plants alive, no labour-demanding jobs such as preparing cuttings, and less variation in initial size of plant. The rapid growth rate of mint is obviously valuable; but the fact that it produces much lateral growth and a large number of small leaves is a disadvantage when compared with plants producing a few large leaves from a main axis. The unbranched plant, particularly one the form of which is similar under a wide range of environmental conditions, will, unlike mint, have similar efficiencies of light utilization in, for example, long- and short-day conditions. Would the results of the many chemical analyses that are presented in Parts 4–6 of the memoir have been similar if a plant with a single main axis had been used?

These first seven parts contain a great deal of information about effects of length of day, night temperature and mineral nutrition on some cell constituents; some information in future parts on effects of amount of incident radiation, spectral composition of light and perhaps other environmental variables would obviously give a more complete picture. Finally, most botanists would agree that the chemical analyses which were done on mature tissues should be extended to the growing regions, not only to apical meristems, but also to all organs throughout periods of cell division, expansion and differentiation if a better understanding of organization of growth is to be obtained.

P. Newton

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* Growth, Nutrition and Metabolism of *Mentha piperita* L. Parts 1–7. Memoir 379, Cornell University Agricultural Experimental Station, Ithaca, New York. August 1962, 144 pages.

THE CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE

IT is not uncommon to find that scholarly academic work is undertaken in underdeveloped countries while practical problems of vital interest to the economy of the State are neglected. Sometimes technological laboratories equipped with foreign capital do not function satisfactorily because of lack of suitable personnel. Some of these problems are no doubt due to the type of training given to students sent to Europe and elsewhere, but others to lack of proper incentives presented by the Governments concerned. None of these criticisms can, however, be applied to the Central Food Technological Research Institute at Mysore, which, under its director, Dr. V. Subrahmanyan, is rapidly building a reputation in South-East Asia. The report of its work during 1960–61 covers many aspects of the food industry and includes biochemistry, nutrition and dietetics*.

Emphasis is properly placed on the solution of problems which have a direct application in Asia, but some of the results are of interest to the European technologist. The frying of banana chips, the preparation of buttermilk soup powders, the canning of drinking-water, the utilization of passion fruit waste or the colorization of vanaspati with tumeric would be unusual subjects for study by the Western scientist. But the preparation of foods for infants and children from largely plant sources is well known to be of vital importance not only in India and Asia but also in many other parts of the world. In this respect, the experience of the Indian workers with isolated proteins from groundnut and soya warrants careful attention, especially with regard to the method of presentation to the consumer. Greatest success has been achieved with a high-protein blend of materials designated 'multi-purpose food' which is made on a large scale and has been used in school-meal programmes as a supplement "to overcome the deficiency of the lunch". The manufacture of this product has now been handed over to private industry.

Similarly, the sections of the report dealing with meat and fish technology, packaging, storage and pesticide research have applications in any tropical country. Of particular value is the work on fish, as this source of protein has been somewhat neglected in the past; work is being done to extend the use of traditional methods as well as the development of new products. Sun-drying, salting and curing are likely to be extensively used for many years, and an understanding of these processes is essential to encourage and standardize the production of fish and other foods of high acceptability and nutritive value.

It is a pity that the report of the Institute for 1960–61 was not published more promptly, as for more recent information it is necessary to consult Indian journals some of which have only a limited circulation. Clearly, however, the workers at Mysore are aware of the importance of increasing food supplies to meet the needs of the growing population and are making a real contribution to the problem from the technological side. One hopes that the limitation of human fertility is receiving the same attention elsewhere.

D. S. MILLER

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* Central Food Technological Research Institute, Mysore. Annual Report, 1960–61. Pp. v+112. (Mysore: Central Food Technological Research Institute, 1962.)

POTASSIUM–ARGON AGES FROM WESTERN OAHU, HAWAII

By IAN McDOUGALL

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THE large shield volcanoes which comprise the Hawaiian Islands have been the subject of intensive study for many years. Little is known concerning the absolute age of the lavas or the time involved in building these shield volcanoes. Previous workers have based their estimates of age on superposition and geomorphological arguments. A study of the age of the Hawaiian Islands by using the potassium–argon method, mainly on whole-rock samples, is in progress. In this preliminary communication results are given of ages from Western Oahu.

The Waianae Volcano comprises the western half of Oahu, and the volcanic sequence has been divided into the Lower, Middle and Upper Waianae Series[1,2]. The volcano has been profoundly eroded into wide, flat-floored valleys up to 3,000 ft. deep, separated by steep, rugged ridges.

Specimens were collected from the Mauna Kuwale hornblende–biotite–trachyte[1,2], and basaltic rocks of the Middle and Upper Waianae Series. Biotite was separated from the trachyte and used for dating this rock. The age of each of the basaltic rocks was determined on whole-rock samples; about 10 g of sample was used for each argon extraction. The extractions were made in a high vacuum line by fusing the sample, adding a tracer of argon-38, followed by purification of the argon over hot copper oxide and calcium. The isotopic composition of the argon was determined on a Reynolds-type mass spectrometer by the static method[3]. Potassium was measured on a Perkin Elmer flame photometer using lithium as an internal standard; sodium was added as a buffer to both the sample solutions and the standards. Results are given in Table 1, and details of the samples are listed in the appendix. Replicate potassium analyses agree to better than 1 per cent. Duplicate argon extractions agree to better than 2·5 per cent in those cases where the air argon correction is less than 60 per cent; where this correction is greater the precision decreases owing to marked error magnification. The absolute accuracy is probably ± 3 per cent, increasing to ± 6 per cent in those samples with a high air correction, excluding uncertainties in the decay constants. The marked disagreement between replicate argon extractions for *GA*560 is partly because of the high air correction, and partly because of sampling difficulties, as this basalt is amygdaloidal. The fact that the rocks show no evidence of alteration, and that they have never been deeply buried, renders it unlikely that appreciable loss of argon has occurred.

Biotite from the Mauna Kuwale trachyte gives an age of 8·4 ± 0·2 million years, corresponding to the Lower to Middle Pliocene, based on the time scale of Kulp[4]. The basalts of the Middle and Upper Waianae Series yield Upper Pliocene ages ranging from 3·5 ± 0·3 to 2·75 ± 0·1 m.y.

Stearns and Vaksvik[1] included the Mauna Kuwale trachyte in the Lower Waianae Series, regarded as the oldest exposed rocks of Oahu. The trachyte is overlain conformably to the south-west by basalts mapped as Lower and/or Middle Waianae Series[2]. Because silicic lavas are generally associated with the declining stages of activity in the Hawaiian volcanoes and the Lower Waianae Series is composed predominantly of tholeiitic basalts, it was suggested[1] that, alternatively, the trachyte may be associated with an older volcano buried by the later Waianae lavas. The potassium–argon age of the trachyte may lend support for this hypothesis, as the Middle and Upper Waianae Series volcanics are much younger; the results clearly show that there was a prolonged hiatus between the extrusion of the trachyte and the outpouring of the lavas of the Middle and Upper Waianae Series.

The Middle Waianae Series lavas are only slightly older than those of the Upper Waianae Series. Hence, extrusion of the lavas of these two series apparently occurred rapidly, and the change in composition from dominantly tholeiitic to dominantly alkali–olivine basalts, on which the division between the two series is made, occurred over a relatively short period of time.

These results provide some hitherto non-existent control as to the absolute age of the volcanics of Western Oahu, and show that the estimates of age based on geomorphological arguments are qualitatively correct. Thus, Stearns and Vaksvik[1] suggested that the deep valleys of Oahu probably were eroded during early Pleistocene time, by correlating the emerged coral reefs with shifts of sea-level induced by glaciation. They concluded that the building of Koolau Volcano (Eastern Oahu) ceased in the late

Table 1. POTASSIUM–ARGON AGE MEASUREMENTS ON ROCKS FROM WESTERN OAHU, HAWAII

Specimen No	Sample	Potassium analyses (wt. %)	Average potassium (wt. %)	$^{40}Ar^{*}/^{40}K$	Air correction (%)	Calculated age (m.y.)	Stratigraphic position
GA 553 (1)	Whole-rock	1·217, 1·219	1·218	0·000162	58	2·77	Upper Waianae Series
(2)	Whole-rock	1·217, 1·219	1·218	0·000161	56	2·75	
GA 556 (1)	Whole-rock	1·446, 1·448	1·447	0·000161	51	2·75	Upper Waianae Series
(2)	Whole-rock	1·446, 1·448	1·447	0·000160	56	2·73	
GA 554 (1)	Whole-rock	0·638, 0·638	0·638	0·000183	76	3·13	Middle Waianae Series
(2)	Whole-rock	0·638, 0·638	0·638	0·000172	70	2·93	
GA 560 (1)	Whole-rock	0·479, 0·483	0·481	0·000192	89	3·27	Middle Waianae Series
(2)	Whole-rock	0·479, 0·483	0·481	0·000214	85	3·65	
GA 397 (1)	Biotite	6·13, 6·19	6·16	0·000484	41	8·26	Lower Waianae Series?
(2)	Biotite	6·13, 6·19	6·16	0·000495	47	8·46	

$^{40}Ar^{*}$—radiogenic component $\lambda_{\beta}=4{\cdot}80\times10^{-10}$ yr.$^{-1}$ $\lambda_{e}=0{\cdot}584\times10^{-10}$ yr.$^{-1}$

Tertiary or early Pleistocene, and that as the Waianae lavas are overlapped by the Koolau Volcanics, then eruption from the Waianae centre probably extended farther back into the Tertiary. The potassium–argon measurements indicate that the profound erosion of the Waianae Volcano occurred during the past 2·7 million years, and probably mainly during the Pleistocene.

Normal and reversed polarities recently have been reported from the Hawaiian lavas[5]; by using the potassium–argon method it should be possible to date these reversals.

The potassium analyses were made by J. A. Cooper and the separation of biotite from the Mauna Kuwale trachyte was carried out by R. Rudowski. I thank D. A. Davis and O. Johnson of the U.S. Geological Survey for their co-operation during my stay in Oahu.

APPENDIX

GA 553: Alkali-olivine basalt of the Upper Waianae Series, 2·3 miles south-east of Kaena Point. 21° 33′ N., 158° 15′ W., altitude about 1,120 ft. Massive, slightly vesicular, intergranular basalt with average grain size about 0·1 mm, and composed of plagioclase, iddingsitized olivine, titanaugite, iron ore and about 15 per cent intersertal, poorly crystallized feldspar, probably a potassium-rich oligoclase.

GA 556: Basaltic andesite (hawaiite) of the Upper Waianae Series, 1·4 miles west-north-west of Puu Palailai. 21° 21·4′, 158° 6·6′, altitude about 550 ft. Massive, light grey rock consisting of olivine, augite and iron ore embedded in a felted mass of laths of andesine. Some interstitial oligoclase and also sporadic biotite.

GA 554: Tholeiitic basalt of the Middle Waianae Series, western cliff of Keaau Ridge. 21° 29·1′, 158° 13·6′, altitude about 400 ft. Dark grey, massive rock with plagioclase and olivine phenocrysts up to 10 mm set in a fine-grained (0·1 mm) intergranular groundmass of plagioclase, augite, iron ore, minor olivine and intersertal oligoclase.

GA 560: Tholeiitic basalt of Middle Waianae Series, Kolekole Pass Road. 21° 28·1′, 158° 7·3′, altitude about 950 ft. Massive, dense, slightly amygdaloidal rock composed of sporadic plagioclase and serpentinized olivine phenocrysts set in a groundmass of clinopyroxene, plagioclase, iron ore and minor intersertal green glass.

GA 397: Hornblende–biotite–trachyte from flow 400 ft. thick at Mauna Kuwale. 21° 27·8′, 158° 9·6′, altitude about 300 ft. Dark grey, massive rock composed of phenocrysts of andesine, hornblende and biotite set in a feldspathic microcryptocrystalline groundmass which has a fluidal texture. Phenocrysts average 0·2 mm in length.

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[1] Stearns, H. T., and Vaksvik, K. N., *Hawaii Div. Hydrog., Bull.* 1 (1935).

[2] Macdonald, G. A., *Hawaii Div. Hydrog., Bull.* 5 (1940).

[3] Reynolds, J. H., *Rev. Sci. Instr.*, 27, 929 (1956).

[4] Kulp, J. L., *Science*, 33, 105 (1961).

[5] Tarling, D. H., *Nature*, 196, 882 (1962).

CLASTIC QUARTZ, PROVENANCE AND SEDIMENTATION

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QUARTZ grains in sandstones commonly attract attention by the provenance inferences which can be derived from the nature of their inclusions[1,2]. Krynine[3], Bokman[4], Keller and Littlefield[5] and Doty and Hubert[6] are recent workers who have deduced significant information about the nature of source rocks from a study of the differing varieties of quartz grains, their shape and inclusions.

These investigations, however, do not exhaust the inferences which can be made from quartz grains. This has become apparent during an examination of Carboniferous sandstones in Derbyshire[7] and Scotland[8,9]. All these sandstones were laid down on shallow shelves of varying degrees of stability and are characterized by a dominance of orthoquartzitic textures and shallow-water structures such as ripple marks and cross-bedding[7,8,9]. The preliminary results proved so interesting that additional shelf sandstones of Cretaceous age were examined together with a variety of older Palæozoic sandstones of more mixed tectonic setting. Within them all it is possible to detect a three-fold grouping of the clastic quartz grains. The information deduced from this grouping gives a new insight into provenance make-up and abrasion during the time of deposition of the sandstones.

The basis of this new work is thin-section microscope analysis involving a determination of whether the clastic quartzes are unstrained, strained or compound in nature. The diagnosis requires the use of crossed polars and various magnifications depending on the grain size. Three-fold grouping is adopted for ease of graphical representation; further subdivision would lead to graphical complications.

The definitions of unstrained, strained and compound grains naturally need to be rigid to ensure reproducibility of results by the same or different operators. Unstrained grains are those which do not show undulatory extinction no matter how feeble. They are single crystals. Strained grains are single crystals also in which all degrees of undulatory extinction occur from weak to intense. There must not be any precisely defined internal boundaries between the zones of variable extinction; gradation is the rule. Compound grains show multiple effects composed of the qualities of both strained and unstrained types, but the fundamental distinction rests in the fact that they consist of an aggregate of quartz crystals. Each of the component crystals is separated from the others by a sharp boundary. For all the grain types marginal authigenic outgrowths are ignored.

This method of grain analysis, it will be noted, does not necessarily give a direct indication of the petrographic nature of the specific rock types at source. Strained quartz, for example, could have been derived from acid igneous and metamorphic rocks or older sandstones;

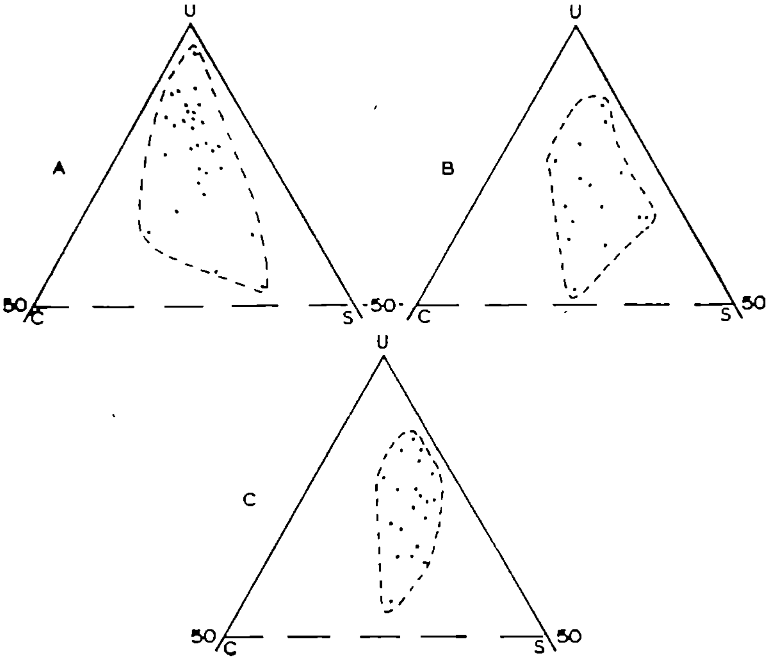

Fig. 1. Comparative UCS (Unstrained, Compound, Strained) diagrams for: *A*, Oil-Shale Group of the Lothians; *B*, Calciferous Sandstone Series of East Fife; *C*, Upper Carboniferous of north Derbyshire. 50 per cent base line for unstrained quartz indicated

similarly with unstrained quartz. The compound grains could have a metamorphic source (metaquartzite, schistose quartzite), sedimentary source (chert, orthoquartzite, re-cycled) or be vein quartz.

The selected sandstones were analysed using a mechanical stage and for each a hundred grains were classified by means of non-duplicatory traverses.

Fig. 1*A* shows the distribution pattern of 29 samples taken at random though fairly evenly spaced intervals through the Carboniferous Oil-Shale Group of the Edinburgh (or Lothians) area. The group has an approximate maximum thickness of 5,000 ft. The pattern is interesting because the field of point distribution appears to be restricted. The proportion of unstrained clastic quartz is clearly dominant over the compound and strained varieties, and a possible conclusion is that more unstrained quartz was being supplied by the source area to the basin of deposition. It seems reasonable to suggest, furthermore, that during the whole of Oil-Shale Group times the clastic quartz sources in the land area to the north-east[8] remained relatively constant both in proportion and nature. If provenance erosion had progressively uncovered older rocks with very markedly different proportions of the three quartz types then this, in all probability, would have been reflected into the resultant shelf (fluvial) sandstones.

The Calciferous Sandstone Series of East Fife are facies equivalents of the Oil-Shale Group and outcrop 20 miles or so to the north-east of Edinburgh. The continuity of sedimentation of the two areas during Carboniferous times can be demonstrated by features such as cross-bedding[8] within the sandstones. Fig. 1*B* suggests that the continuity is also expressed by the quartz grain distribution. The sandstones analysed are taken through the full thickness of the succession, about 4,000 ft.

The Upper Millstone Grit and Lower Coal Measures are at a higher stratigraphical level than the above Scottish rocks and Fig. 1*C* illustrates the field for 20 samples from northern Derbyshire. They are taken through a thickness of 3,000 ft. of strata and, once more, the limited point scatter suggests small variations in supply from the provenance. At the same time there is a noticeable broad similarity of distribution with that of the older Scottish rocks. This is probably significant because it is known from other considerations[2,8,9] that the land source of all these sandstones lay to the north and north-east of the Midland Valley of Scotland.

To extend the knowledge and assess the prospective value of this new technique random samples of Cretaceous shelf sandstones from Surrey were examined. Eleven of the specimens were from Hythe Beds and the rest from the overlying Sandgate Beds. In Fig. 2 the two sets of data are plotted separately, and it appears that there might have been slight differences in the provenance of the clastic quartz for each group of beds. The Sandgate Beds sandstones seem generally to have more strained quartz and less unstrained than the Hythe Beds. Possibly the change could be linked up with an alteration in the palæogeography of the London Platform massif immediately to the north[10]. The other feature of the distribution is the pattern which resembles those of the Carboniferous sandstones. The post-Carboniferous palæogeography is such that derivation from a land mass to the north of the British Isles is unlikely. Therefore the similarity may be fortuitous or express the re-cycling of Carboniferous sandstones originally deposited on the site of the St. Georges Land–London Platform. It is probable, however, that the Hythe and Sandgate grains have passed through at least one post-Carboniferous cycle of sedimentation.

Fig. 3 illustrates a random series of Lower Palæozoic (and one Pre-Cambrian) sandstones of shelf and geosynclinal facies. Considerable contrast in quartz grain distribution is evident between this and those of Figs. 1

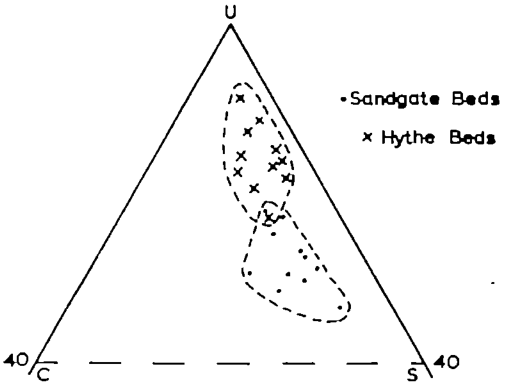

Fig. 2. UCS (Unstrained, Compound, Strained) diagram for Cretaceous sandstones of Surrey. 40 per cent base line for unstrained quartz indicated

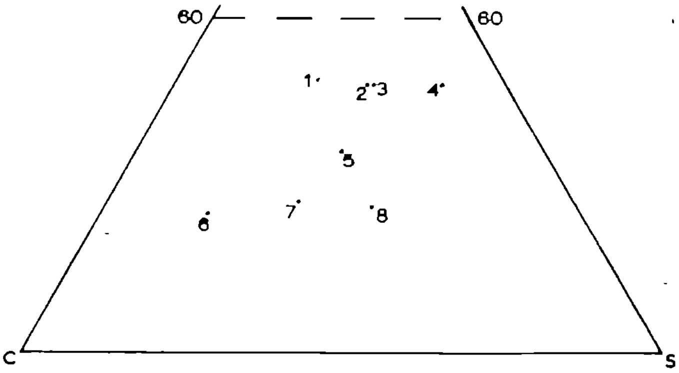

Fig. 3. UCS (Unstrained, Compound, Strained) diagram for single sandstone specimens. 1, Barmouth Grit (Camb.); 2, Penrith Sandstone (Perm.); 3, Wrekin Quartzite (Camb.); 4, Peeblesshire Greywacke (Sil.), 5, Stiperstones Quartzite (Ord.); 6, Greywacke, Harlech (Camb.); 7, Wentnor Grit (Pre-Camb.); 8, Leni Grit (Dalradian)

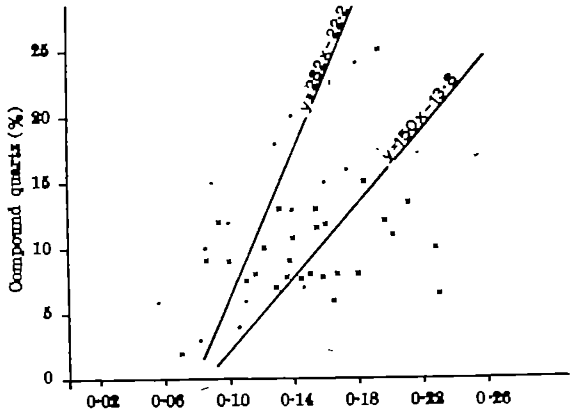

Fig. 4. Variation of compound quartz percentage and median diameter. Regression lines indicated for Calciferous Sandstone Series (•), ($y = 282x - 22{\cdot}2$) and Oil-Shale Groups; (×), ($y = 150x - 13{\cdot}8$)

and 2. It is clear that for most of the individual sandstones the provenance of the quartz grains was entirely different from that of either the Carboniferous or Cretaceous sandstones. What it does not show because of lack of systematic data through any specific sandstone group is the variation, if any, in provenance during the deposition of that group. Further work is needed.

Sandstones, however, are products not only of heredity as broadly indicated here but also of environment. On shallow water shelves the constant agitation of the waters must affect the clastic grains either in terms of sorting or abrasion. To confirm the presence of these processes the Oil-Shale Group and facies equivalent Calciferous Sandstone Series sandstones were examined in greater detail. Cumulative curve data provided the median diameters of the sandstones and these were plotted against the percentage of compound quartz grains. Fig. 4 shows the distribution of points and corresponding regression lines. In both instances it is clear that the percentage of compound grains decreases as the median grain size decreases. This relationship could be explained by fluctuations in the provenance supply of debris, sorting in the depositional areas or physical disintegration of the grains into their smaller component parts. Other evidence[9] suggests that the provenance for both areas was similar and the detritus derived from it varied negligibly. There also seems to be no reason why compound grains should be differentially separated from other quartzes of approximately equal size and specific gravity. Hence it is probable that the regression lines reflect the abrasion and comminution of the compound grains. A lower compound quartz proportion means a greater degree of abrasion and vice versa.

A further point concerning inferred abrasive effects can be deduced from the regression lines by considering any given median diameter. If, for example, in Fig. 4, a median diameter of 0·14 mm is selected then the corresponding quartz percentages are 17·5 per cent for the Calciferous Sandstone Series and 7·5 per cent for the Oil-Shale Group. As provenance and sedimentation of sandstones were similar for both areas it is possible that the differences in percentages reflect the greater distance of transport, and thus abrasion, involved for the Oil-Shale Group debris. The Calciferous Sandstone Series clastic quartz derived from the common source area to the north-east[9] travelled at least 20 miles less than that of the Oil-Shale Group. Sorting does not appear to have played any part in the proportional distribution of the quartz grain varieties.

These conclusions relating the percentage of compound quartz to abrasion history in no way detract from the broad conclusions regarding similarity in provenance which can be drawn from the less refined detail of the ternary diagrams. These diagrams relate the three types of quartz. With the Scottish Carboniferous sandstones it is only because the ternary provenance patterns are similar that possible abrasion inferences can be made about one of those varieties of quartz.

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SENSITIVITY IN LEDA CLAY

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THE sensitivity (ratio of the natural strength to the remoulded strength) of Norwegian marine sediments reported in the literature[1] shows an inverse relationship to the existing salt concentration of the pore water. These findings are explained by the 'leaching theory' of sensitivity[2] which is based on the premise that sedimentation took place in sea-water or brackish water. The decrease in the salt concentration of pore water by subsequent leaching and/or diffusion causes the salt-flocculated structure to become 'metastable'. This reduces the remoulded strength much more than the natural strength.

The marine sediments of the St. Lawrence Lowlands (commonly referred to as Leda clay) are similar to the Norwegian sediments in clay mineral composition[3,4]. In both soils unaltered particles of similar minerals make up a considerable proportion of all size fractions. There are also similarities in many of the geotechnical properties the soil[5,6] and in the phenomenon of frequent and sudden earth flow slides in such deposits. The most characteristic property of both soils is the large loss of strength when remoulded. To date, however, it has not been possible to establish for Leda clay how much of the sensitivity can be attributed to the leaching of salts or to what extent other factors are involved.

Certain previous observations may be recounted since they support this aspect of dissimilarity between the Norwegian and Leda clays regarding sensitivity[6]. In the Ottawa area the pore water salt concentration of Leda clay is generally a fraction of a gram per litre and rarely exceeds 1–2 g/l. (In contrast, a wide range of pore water salt concentrations has been reported for Norwegian marine clays.) At the same time the sensitivity may vary from a low value of 10 to values of 200 and higher depending on how it is measured[7]. Usually, high sensitivities are accompanied by low plasticity indices and low liquid limits, and low sensitivities with higher plasticity indices and liquid limits, quite independent of salt concentration. There is also a tendency for the most sensitive portions of the profile to be more silty in nature although the differences are sometimes small or negligible.

In other studies being carried out on Leda clay from a deep excavation, radical changes were observed in

Table 1. SUMMARY OF COMPARISON TESTS FOR TWO SAMPLES OF LEDA CLAY

Sample No.	94–30	94–13
Undisturbed strength (falling cone)	1,930 lb./ft.^{2}	2,080 lb./ft.^{2}
Remoulded strength (falling cone)	1·3 lb./ft.^{2}	68 lb./ft.^{2}
Sensitivity	1,500	30
Geodetic elev.	124 ft.	140 ft.
Depth below surface	47 ft.	22 ft.
Mechanical analysis		
< 2μ (clay size)	71 per cent	83 per cent
2–20μ (silt size)	29 per cent	18 per cent
> 20μ	0	0
Liquid limit	39	69
Plastic limit	24	31
Plasticity index	15	38
Salt concentration (pressure extraction of pore water)	1·1 g/l.	0·6 g/l.
Dry density (field)	68·4 lb./ft.^{3}	61·7 lb./ft.^{3}
Surface area (glycol method)	58·7 m^2/g	72·0 m^2/g
Cation exchange capacity	16·0 m.equiv./100 g	15·9 m.equiv./100 g
*p*H	8·1	8·1
Natural moisture content	55·6 per cent	65·2 per cent
Water content at equivalent strengths	55·6 per cent	136 per cent
Cation content (leached 5 g soil with 200 c.c. of ammonium acetate)		
Ca	0·35 g/100 g soil	0·40 g/100 g soil
Mg	0·09 g/100 g soil	0·12 g/100 g soil
K	0·09 g/100 g soil	0·07 g/100 g soil
Na	0·17 g/100 g soil	0·04 g/100 g soil
Ratio, surface area/per cent clay	1·21	1·15

sensitivity in the absence of any large changes in the pore water salt concentration. With samples from strata of high and low sensitivity exploratory determinations were carried out in an attempt to establish other reasons for this apparently anomalous situation as compared with Norwegian clays.

The results given in Table 1 show that there are only small differences between the two samples with respect to percentage clay- and silt-size particles, surface areas, cation exchange capacity, low salt concentration, natural water content and *p*H. The large differences are in the behaviour of the materials as measured by the Atterberg limits and, of course, in sensitivity. The water content of sample 94–13 had to be increased to 136 per cent before it reached the low remoulded strength that sample 94–30 had at its natural moisture content of 55·6 per cent.

When electrolyte was added, each sample showed the same trend in strength behaviour although there were large differences in the magnitude of the response. Fig. 1 shows the drop in strength resulting from additions of sodium metaphosphate (this compound has a 1·1 mole ratio of Na_2O/P_2O_5) after remoulding the samples mechanically. These strengths were measured with a lightweight drop cone 1 min after mixing was stopped. It is the large change in strength of sample 94–13 that is of significance when compared with sample 94–30. By adding 1 g of sodium metaphosphate per 100 g of soil the strength dropped from 70 lb./ft.^{2} to about 1 lb./ft.^{2}, while the strength of the more sensitive soil changed by only a fraction of this. At higher concentrations flocculation occurred as indicated by the increase in remoulded strength. It is not unreasonable to assume that the stability of the structure of these two samples was quite different as evidenced by the response to the dispersant. In the highly sensitive soil, mechanical mixing was sufficient to cause a high degree of dispersion which could only be achieved in the clay of lower sensitivity by increasing the electrokinetic potential[8] and hence the amount of repulsion between adjacent particles.

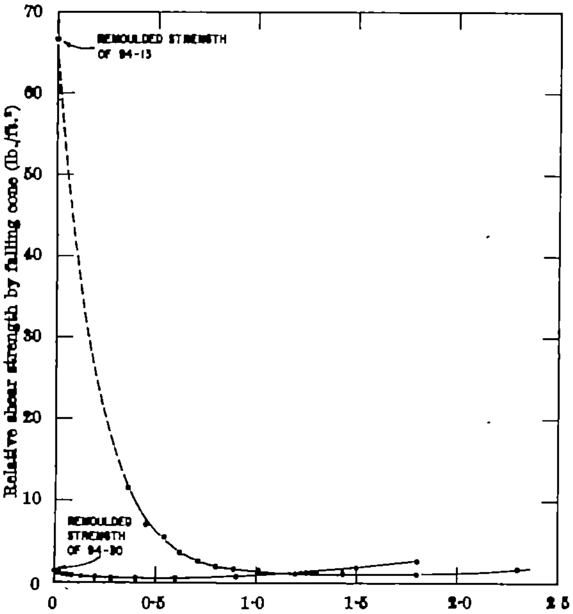

Fig. 1. Comparison of strength reduction of remoulded Leda clay of different sensitivity by adding sodium metaphosphate at the field moisture content

Addition of sodium metaphosphate also reduced the liquid limit and plasticity index. Sample 94–13, originally with a liquid limit of 68·9 per cent, was reduced to 67 per cent at 0·022 g of sodium metaphosphate per litre of pore water to 63 per cent at 0·09 g/l. and to 53·7 per cent at 0·26 g/l.

Fig. 2 shows changes, measured in shear strength, of sample 94–30 by increasing the electrolyte concentration with various salts at natural moisture content. With calcium chloride, sodium carbonate and sodium chloride the strength increased but with sodium metaphosphate the strength decreased. This behaviour can be explained in terms of the effect that changing the interparticle force has on structural arrangement[9] and this is consistent with the behaviour of Norwegian marine clays[1].

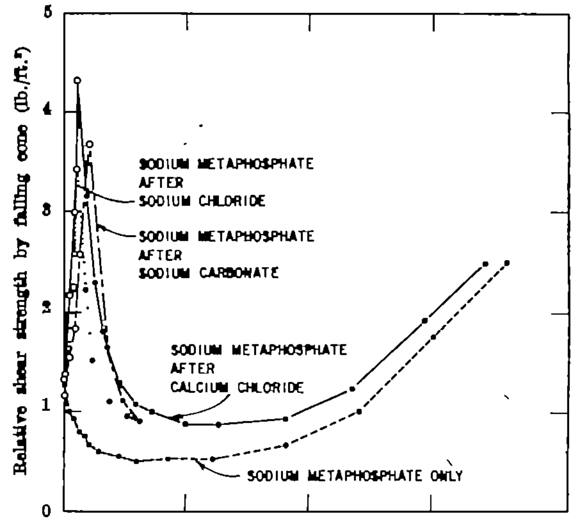

Fig. 2. Remoulded strength of 94–30 as a function of electrolyte content at the field moisture content

The higher sodium content of the leachate from 94–30 (Table 1) may be of significance in assisting more complete dispersion while remoulding and in this case may explain the difference in sensitivity between these two samples. There are apparently factors, therefore, other than reduction of the pore water salt concentration of sediments that have led to the present variation in sensitivity in Leda clay.

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ELECTRIC CHARGES AND THIXOTROPY OF CLAYS

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HYDROGEN clays prepared by washing in acid are unstable[1]; on washing in distilled water Al^{3+} is released as the lattice edges crumble[2] and this is followed by a slower release of Al^{3+} involving diffusion of exchangeable hydrogen ions to the edges[3]. In the time required to dialyse a hydrogen clay electrolyte free, the clay has become an aluminium clay, and the permanent charge is balanced by Al^{3+} ions in a Stern layer.

In a standard method (conductometric) for measuring cation exchange capacity by titration with alkali, the aluminium ion is converted to the hydroxide ($Al(OH)_3$), and the negative charge is balanced by alkali metal cations, which form a diffuse layer. Throughout a titration, almost up to the equivalent point, the viscosity is high, but within the last 20 per cent of added alkali the viscosity decreases by a factor of 10^3 (ref. 4), a discontinuity which suggests there might be some unevenness in the charge distribution, greatest in the later stages of neutralization.

Recent work on the hydrolysis of aluminium ion[5] suggests either the formation of a series of complexes $Al[(OH)_5Al_2]_n^{n+3}$, with a limiting OH/Al ratio of 2·5, or of single complexes such as $[Al_6(OH)_{15}]^{3+}$ or $[Al_8(OH)_{20}]^{4+}$, the value of the charge not being known. Conductivity measurements with aluminium salts (Cashen, G. H., in preparation) show that there is a discontinuity at five-sixths of the neutralization, and a probable interpretation of the charge balance for a clay, at the same stage of neutralization, is that five-sixths (83 per cent) is then balanced by alkali metal cations in a diffuse layer, and the remaining one-sixth by complex aluminium ions in a Stern layer. Because there must be some mobility to account for Al^{3+} ions coming together to produce, say $[Al_6(OH)_{15}]^{3+}$, it seems reasonable to suggest that the distribution of the complex ions is in a state of minimum energy, that is, as far apart as possible under their mutual repulsions; and the hypothesis now advanced is that this is so; as a result, the clay particles are positively charged near the periphery by an excess of complex ions in the Stern layer.

The hypothesis, advanced to explain the particular results of work on kaolin, aluminium ion and alkali hydroxides, could be general, and a polar nature might arise for any non-spherical particle, whenever diffuse and Stern layers co-exist.

A mathematical treatment of the interaction between clay particles would be formidable if, in addition to the Gouy repulsive and London attractive forces, forces of a circular dipole nature were taken into account. It is obvious, however, that there could be preferred mutual orientations, for example, to produce the "house of cards" structure in clays, and the decrease in viscosity towards complete neutralization needs no further comment.

Qualitatively, and even semi-quantitatively, the hypothesis explains some puzzling phenomena in colloid chemistry, and provides an alternative explanation for others. For brevity, the following reasoning deals only with clays and clay problems.

Thixotropy. Freundlich[6] demonstrated the effects of added (and adsorbed) divalent and trivalent anions on the coagulation and thixotropy of positively charged ferric oxide sols (prepared from ferric chloride), and was surprised when the same valency rules did not apply to cations and negatively charged 'hydrogen' bentonite. No thixotropic sols were produced by $AlCl_3$ or $Th(NO_3)_4$, the hydroxides and chlorides of divalent metals had little effect, and it needed alkali hydroxides (the most efficient) or chlorides to form strongly thixotropic pastes. He discussed this behaviour in terms of bound water, but his results are much more intelligible if it is recognized that the 'hydrogen' clay was an aluminium clay. Buzágh and Szántó[7] found that the optimum thixotropic behaviour of a 'hydrogen' bentonite occurs between 80 and 90 per cent neutralization—in good agreement with the 83 per cent the hypothesis would predict.

Thixotropic behaviour of clays finds practical application in the preparation of drilling muds. The 'red lime muds' are made highly alkaline by adding both lime and caustic soda, and quebracho extract is used as a liquefier[8]. This again is a two cation system but the conditions are such that large negative edge charge should develop, a state which, according to the usual conceptions, should lead to enhanced deflocculation. If the high alkalinity is essential in these muds, the most probable conclusions are: (1) the negative edge charges increase the kind of polarization suggested in this communication; (2) it must be the calcium ions that are preferentially adsorbed at the edges, a deduction that follows naturally from the argument already advanced. Van Olphen experimented with the trihydric phenols, as typical of components of the quebracho extract, and found that only those with an adjacent pair of hydroxyl groups, and thus able to complex calcium ions, were efficient liquefiers. He discussed the adsorption of anions containing calcium in this state at the crystal edges, but it is much more probable that the action of the quebracho extract is to change the Na^+–Ca^{++} ratio by removing calcium ions from the particle surfaces.

Another example of the effect of a Na^+–Ca^{++} system is the almost perfect petrifaction of soft-bodied sea animals in the Solnhofen slate[9]. The slate contains more than enough calcium carbonate to ensure that the initial clay must have been calcium-saturated and sea-water provided the sodium ion; it is reasonable to suppose that a rapid gelling of the clay around the animal cut off access of oxygen, and if this was not the immediate cause of death it could certainly be the preventive of decomposition. It is, of course, possible that the phenomenon is of a co-operative nature. Peselnick[9] compared the elastic constants of Solnhofen slate and of single crystals of calcite and concluded that the slate behaved as an aggregate of randomly oriented single crystals of calcite. Boswell[10] states that calgon (sodium hexametaphosphate) makes chalk suspensions (whitening) more mobile, a deflocculating effect indicating that sodium ions have replaced calcium ions complexed by the calgon, and that the chalk is negatively charged.

A similar argument might well explain why clay is necessary to produce the thixotropic behaviour of the marine quicksands examined by Freundlich and Juliusburger[11], but here there is no internal evidence of the presence of calcium ion, or of any other polyvalent ion that could act in the same way.

Oriented Flocculation. After treating kaolins with normal sodium chloride at *p*H 3, Schofield and Samson[4] noticed a change in the nature of the flocculation when the kaolins were washed with distilled water. They explained this by a change from face-to-face (salt) flocculation to a more bulky edge-to-face state when removal of the salt allowed interaction between positively charged edges and the negatively charged planar faces. It has

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been shown that, after washing with distilled water, the edges are uncharged; positive edge charges are lost as aluminium ion, released from the lattice at the crystal edges, appears in the exchangeable form (Cashen[2] and unpublished data); consequently, it is concluded that the edge-to-face flocculation is that for an Na^+–Al^{3+} system.

Effects of Shear. (i) Assuming that the state of least energy for two particles is when they are symmetrically placed at right angles to one another, Gouy forces will not exert a moment tending to disturb the orientation. For small rates of shear, attractive forces between the circular dipoles will predominate and will oppose change in orientation. At some critical rate of shear (and orientation) the Gouy forces will take over and exert a moment assisting the shear, because the repulsive pressures will be greatest where the particles are closest together; the viscosity of the suspension then exhibits the drop characteristic of thixotropic systems as the rate of shear is gradually increased.

(ii) Low[12] prepared a sodium clay by a two-stage process described by Low and Anderson[13]. A clay suspension was passed through exchange resins in the hydrogen and hydroxyl forms and analysis gave the cation status as 60 per cent H^+ and 40 per cent Al^{3+}. The sodium clay, obtained by adding the requisite amount of sodium hydroxide, thus contained $Al(OH)_3$ as the only impurity. While standing undisturbed for six days the water tension of the suspension gradually increased, that is, the vapour pressure gradually decreased. (Experimental conditions are not given fully, and the system may have been adsorbing carbon dioxide from the atmosphere, with formation of the complex aluminium ion.) On the sixth day the suspension was stirred and the water tension decreased almost to zero. Low recognized that the mutual particle orientations were changing with time, but considered that the osmotic activity of the cations should not be affected by stirring. He ascribed the slow gelation to a gradual extension of a water structure, of lower free energy, to rather large distances from the particles. Quite apart from the effect of ionic charges on the structure of water, possible changes in ionic concentration cannot be ignored in a complete interpretation. If, initially, two particles are situated in the same plane, the diffuse layers extend, theoretically, in parallel to infinity (apart from edge effects); on rotation of both particles through 90° to the face-to-face position, the cations tend to pack between the particles (which gives rise to the repulsion) and the 'outer' liquid is depleted of ions. A rotation of one particle only would lead to some intermediate distribution; stirring, which tends to produce the face-to-face orientation, consequently leads to lower ionic concentrations in the 'outer' solution, and the vapour pressure rises. In so far as the free energy of the water is concerned, work done on thixotropic systems by application of shear is analogous to that on salt solutions by application of pressure.

(iii) Similar reasoning would suggest that the freezing-point for the "house of cards" structure in a clay should be lower than the freezing-point of a mechanically sustained ungelled state. It is lower, but the anomalous freezing-point depression is usually explained by the rigidity of the gel structure which so limits the size of the ice crystals that the surface area–size ratio is large when compared with that for macroscopic ice crystals (Weiss and Frank[14]). In a pressure–temperature diagram the sublimation curve would be above that for macroscopic ice, and for equilibrium at some given vapour pressure, the microscopic crystals must be colder than the macroscopic to prevent sublimation between the two types of ice crystal.

The concept that gel rigidity should prevent growth of ice crystals is completely counter to agricultural experience. When soil clods are frozen slowly, the growth of ice crystals compresses the soil which is being dried and produces a favourable, stable crumb structure; when the freezing is fast, the clods are shattered to such an extent that the tilth produced is much too fine[15].

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REACTION OF LIME WITH FERRIC OXIDE

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It has recently been suggested that the adsorption of lime by fired kaolin and kaolinitic clay, in presence of water, may be considered to be a semiconductor surface reaction[1]. It was also found that the adsorption of lime decreased with the increase in the electrical conductivity of these materials[1,2]. In this article the reaction between lime and αFe_2O_3 (obtained from fired laterite) in the presence of water is considered. The problem arose during a study of the puzzolanic activity of laterite.

At low electrical conductivity of Fe_2O_3 it reacted very little with lime. At high conductivity, reactivity increased but remained constant over a wide range of conductivity, that is, saturation in reaction was obtained. On firing laterite with admixture of small quantities of Cr_2O_3, TiO_2, ZnO, NiO and CoO the nature of the reaction between Fe_2O_3 thus obtained and lime remained generally unaltered. On firing with Cu_2O, at low values of conductivity, the reactivity increased with the decrease in conductivity and at high conductivity, saturation was obtained.

The parallel change in reactivity with conductivity, when considered with the fact that Fe_2O_3 is a '*p*' type semiconductor in an oxidizing atmosphere, indicates that the reaction between lime and Fe_2O_3 in presence of water is a '*p*' type reaction on a '*p*' type semiconductor. The opposite behaviour in Fe_2O_3 obtained when laterite is fired with Cu_2O indicates that Fe_2O_3 may have changed into an '*n*' type semiconductor and the reaction is a '*p*' type reaction on a '*n*' type semiconductor.

A quantity of laterite was powdered, sieved through B.S. 200 mesh and fraction passing through was thoroughly mixed and the samples from it were analysed chemically and by X-ray and differential thermal anaysis.

About 200 g of the sample was drawn from this lot for each experiment and was fired in ordinary atmosphere,

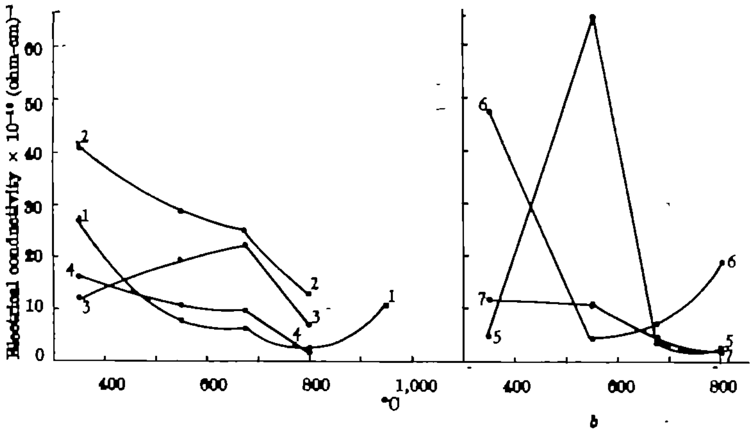

Fig. 1. Variation of room temperature electric conductivity of ferric oxide with firing temperature. *a*, Curve 1, Fe_2O_3 from fired laterite; curves 2, 3, 4 from laterite fired with admixture of Cr_2O_3, TiO_2 and ZnO. *b*, Curves 5, 6, 7, Fe_2O_3 from laterite fired with NiO, Cu_2O and CoO

as such or with admixture of 1 per cent by weight of Cr_2O_3, TiO_2, ZnO, NiO, Cu_2O and CoO. Firing temperatures for natural laterite were 350°, 550°, 675°, 800° and 950° C and those with oxides were 350°, 550°, 675° and 800° C. The firing period was 4 h in each case. The mass obtained on firing was examined visually and under a microscope. No sintering could be detected which meant that the specific surface area remained unchanged. The fired mass was washed clean of colloidal suspensions, dried at 110° C for 24 h and sieved through B.S. 300 mesh. A large portion of the sieved mass had the linear size below the 'screening length' (10^{-4} cm).

The amount of lime removed by the material from a saturated lime solution, at constant temperature 35 ± 0·5° C and in an atmosphere free of carbon dioxide, at intervals of 1 min was measured by the method described earlier[3]. Adsorption was rapid initially and the process continued at a slow rate for a long time and equilibrium conditions was not obtained even after 48 h but a near equilibrium condition was obtained after 5 min. Lime removed by 1 g of the material during the first 5 min was taken as a measure of its reactivity.

For electrical conductivity measurements the samples were made into tablets using a 20-mm cylindrical mould and plunger. 3 g of samples was placed in the mould and compressed in a hand-operated hydraulic-press at a total pressure of 1,000 lb. (Electrical conductivity was found to vary slightly with pressure.) The tablets, taken out of the mould, were coated on the flat ends with a dilute suspension of aquadag in alcohol. These were then dried at 110° C for 24 h and cooled to room temperature over fused calcium chloride and soda lime.

Resistance of the tablets was measured at ordinary temperatures and in ordinary atmosphere with a General Radio megohm bridge by placing the tablets between two flat platinum electrodes under a slight pressure. In some of the specimens resistance was found to increase slowly during measurements; and in such cases resistance at the establishment of contact was taken. There was also a slight change in conductivity with temperature but this was neglected in the present experiments.

Chemical analysis of the laterite sample is (per cent): loss on ignition 14·09, silica and insolubles 30·87, iron (Fe_2O_3) 35·04, aluminium (Al_2O_3) 18·12, calcium (CaO) 0·56, magnesium (MgO) 0·91 and alkalis nil.

X-ray diffraction pattern of the powdered laterite samples shows the presence of goethite, kaolinite and quartz. The differential thermal analysis curve shows endothermic peaks at 125°, 330° and 565° C and an exothermic peak at 940° C. These are identified as due to goethite, kaolinite and gibbsite.

Specific electrical conductivity of the samples of the natural laterite powder passing B.S. 300 mesh, washed, dried and tableted, was found to be in the order of 9×10^{-8} $(\text{ohm-cm})^{-1}$ at ordinary temperature. Its lime reactivity at 35 ± 0·5° C. was 8·18 mg/g.

X-ray powder patterns of the fired samples used for lime and resistance measurements show mainly the pattern of αFe_2O_3. Variation of (room temperature) electrical conductivity of Fe_2O_3 with firing temperature is shown in Fig. 1*a*. Up to 800° C conductivity generally decreases with the firing temperature with a slight hump at 675° C. A rise in conductivity is observed in the samples fired at 950° C.

Curves 2, 4, 5 and 7 (Fig. 1*a* and *b*) show variation in the electrical conductivity with firing temperature of the laterite samples with admixture of Cr_2O_3, ZnO, NiO and CoO. When fired with Cr_2O_3, the room temperature conductivity of Fe_2O_3 is higher (at the corresponding temperature) than the product obtained by firing laterite alone. Decrease of conductivity is, however, maintained. With ZnO, conductivity at 350° C is lower, at 550° and 675° higher and at 800° C of the same order as that of the ordinary laterite fired at the corresponding temperatures. With NiO, when fired at 350°, 675° and 800° C, the conductivities are much lower and show a gradual decrease but when fired at 550° C conductivity has a high value ($6 \cdot 5 \times 10^{-8}$ $(\text{ohm-cm})^{-1}$). With CoO (curve 7) the conductivities are low but these also decrease with firing temperature. Curve 3 (Fig. 1*a*) shows the effect of TiO_2. Conductivity increases up to 675° C and falls to a low value at 800° C. Curve 6 (Fig. 1*b*) shows effect of Cu_2O. There is a large increase in conductivity when fired at 350° C; at 550° C it decreases but again increases at 675° C and 800° C. There are humps at 675° C in the conductivity-firing temperature curves for natural laterite as well as for laterite fired with '*n*' type oxides (Fig. 1*a*). When fired with '*p*' type oxides the hump has shifted to a lower temperature (550° C).

Curve 1 (Fig. 2*a*) shows the variation of lime reactivity with (room temp.) electrical conductivity of Fe_2O_3 obtained from laterite fired without admixture. At low values of conductivity, reactivity increases with conductivity but at high values the reactivity tends to a saturation value. There is a reversal in the specimen fired at 950° C. Here conductivity in increased but reactivity decreases.

Curves 2, 3 and 4 (Fig. 2*a*) show the variation of reactivity with conductivity of the samples obtained after firing with Cr_2O_3, TiO_2 and ZnO. These are similar to curve 1 except for a slight decrease in reactivity at high conductivity which might indicate desorption. The nature of the curves from samples fired with NiO and CoO

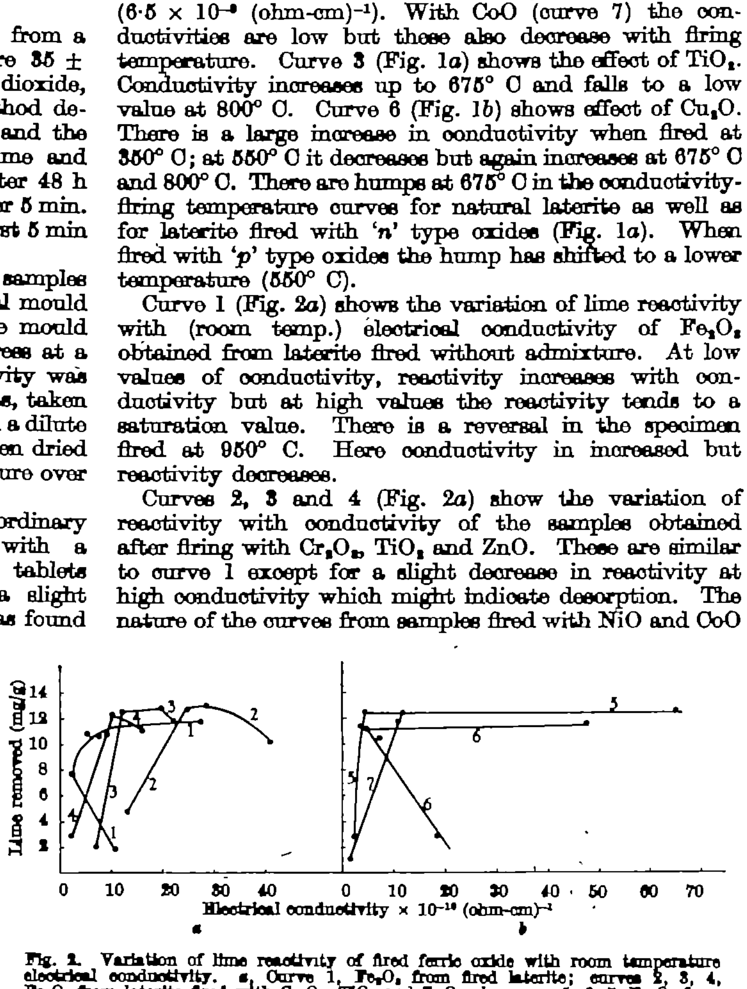

Fig. 2. Variation of lime reactivity of fired ferric oxide with room temperature electrical conductivity. *a*, Curve 1, Fe_2O_3 from fired laterite; curves 2, 3, 4, Fe_2O_3 from laterite fired with Cr_2O_3, TiO_2 and ZnO. *b*, curves 5, 6, 7, Fe_2O_3 from laterite fired with NiO, Cu_2O, CoO

(Fig. 2*b*, curves 5 and 7) is similar to curve 1. Curve 6 is for samples heated with Cu_2O. At high conductivity saturation is obtained but at low values the reactivity decreases with conductivity.

Specific electrical conductivity of pure Fe_2O_3 is of the order of 10^{-12} (ohm-cm)$^{-1}$. Higher conductivity obtained in these experiments may be the result of impurities present.

Fe_2O_3 can be either a '*n*' type or a '*p*' type semiconductor depending on the valence state of the cation. Oxidation changes pure Fe_2O_3 into a '*p*' type semiconductor. In these experiments Fe_2O_3 has been produced by heating laterite in an oxidizing atmosphere. A possibility is that the Fe_2O_3 used is a '*p*' type oxide. Further, on heating with Cr_2O_3, TiO_2, ZnO and Cu_2O (lower valency cations) an increase in conductivity is observed at corresponding firing temperatures in almost all cases. With higher valency cations there is a decrease in conductivity. These facts confirm that Fe_2O_3 used here is a '*p*' type semiconductor.

The decrease in conductivity of Fe_2O_3 with temperature may be due to annihilation of 'holes' by combination with electrons provided by Al_2O_3 (from the decomposition of gibbsite and entering into a solid solution in Fe_2O_3). The fall in conductivity (as well as of reactivity) at 800° C may be due to combination of 'holes' with electrons from enhanced quantity of Al_2O_3 entering into a solid solution with Fe_2O_3. This is possible because of the breakdown of kaolin structure into Al_2O_3 and SiO_2. (There is also a large change in the electrical resistivity of kaolin between temperatures 700°–800° C) (ref. 3). Increase in conductivity when fired at 950° C may be due to more Al_2O_3 entering into solid solution.

Introduction of donor impurities into a semiconductor crystal raises the Fermi level of the crystal towards its conduction band. Under equilibrium conditions, for a '*p*' type reaction, a shift of the Fermi level towards the conduction band adversely affects the number of particles involved in a chemisorption process. Thus the observed fall in reactivity with decrease in electrical conductivity for Fe_2O_3 obtained from fired laterite (Fe_2O_3 was being fired in presence of Al_2O_3) may be explained, if it is assumed that the reaction is a '*p*' type reaction on a '*p*' type semiconductor. Effect of admixture of '*n*' type oxides as Cr_2O_3, TiO_2 and ZnO can be explained in a similar way.

In the sample heated at 950° C the reaction is probably '*p*' type on a '*n*' type semiconductor.

On heating with Cu_2O in the range 550°–800° C reactivity varies with conductivity in opposite direction. This may happen if the reaction is assumed to be a '*p*' type reaction on a '*n*' type semiconductor. Fe_2O_3 may be changed into a '*n*' type semiconductor through Fe^{3+} changing to Fe^{2+} through reduction by Cu.

NiO and CoO have scarcely affected the reactivity of Fe_2O_3 in the way they are expected to do.

The humps at 675° C in the conductivity–firing temperature curves (Fig. 1*a*) may be due to change of Fe_2O_3 from its antiferromagnetic state to a paramagnetic one when fired at that temperature. (~ 675° C is the Néel temperature for Fe_2O_3.) If this is so, then the chemical reactivity of Fe_2O_3 becomes connected with its magnetic property through its semiconducting property and one expects that such a connexion should exist. The explanation of some of the observed results would then need some modification.

The reaction between Fe_2O_3 (obtained from fired laterite) and lime in the presence of water is a semiconductor surface reaction. The reaction type is a '*p*' type reaction on a '*p*' type semiconductor. Fe_2O_3 may be changed into a '*n*' type semiconductor by heating with Cu_2O above 550° C or with active Al_2O_3 above 800° C.

These conclusions need verification with pure Fe_2O_3 and experiments with this in view are in hand.

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FIXATION OF RADIOACTIVITY IN SOLID MEDIA: STABILITY OF GLASSES AND GLAZES

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AT the Working Meeting on Fixation of Radioactivity in Stable, Solid Media[1] a standard leaching test was proposed as a basis for the evaluation of the stability of glasses and glazes. The objective was to set up a test which would be easy to carry out and which could be performed on a wide variety of substances in any laboratory. The test was primarily devised to produce a numerical basis on which the relative merits of various materials could be argued, and not necessarily to have any close relation to actual leaching conditions.

The proposed test was very simple, the apparatus being merely a slightly modified Soxhlet extractor. Pure distilled water boiling in the flask of the extractor supplied a continuous stream of condensate from a glass finger which passed over the sample in a thimble arranged with an intermittent siphon. In the initial applications of this test the extraction thimbles were made of 'Pyrex'. These glass thimbles had the advantage of making easily visible the specimen undergoing test. 'Vycor' flasks were first used, but 'Pyrex' flasks proved to be satisfactory and far less expensive.

Leaching conditions. The 1-litre 'Pyrex' boiling flasks containing 200 ml. of water were heated with 'Glass-Col' mantles operated from 'Variacs'. The optimum voltage settings were found to be 70 V to the lower half of the mantle and 60 V to the upper half. This gave a steady reflux rate and maintained a temperature of 92°–98° C in the water of the extractor thimble (according as the thimble was only partly or was completely filled). Temperatures were measured with a copper-constantan thermocouple placed beside the sample in the thimble. The siphon emptied every 5 min under the foregoing conditions.

The principal advantage of this form of the test lies in the reproducibility of the conditions. It is easily possible to support a relatively bulky specimen in the thimble so that its entire surface comes into contact with the hot water. Further, the material tested is not subjected to any abrasive action—as might be the case if it were placed directly in a flask of boiling water. Any abrasion of the sample would certainly give fictitiously high results as to material leached, with abraded particles exposing too much area.

Preliminary test of leaching apparatus. Since the dithizone colorimetric method for determining lead is very sensitive and accurate, the first leaches were made on crushed samples of lead glass. Crushed fragments of 30 per cent lead monoxide lead glass passing a 6-mesh screen and retained on a 30-mesh screen were used. Two weighed samples were taken by 'quartering', every care being taken to keep the particle-size distribution nearly the same in each. The extractions were done over successive 12-h periods with 400 ml. of water in the boiler. The distilled water showed the equivalent of 0·01 mg lead/l., and thus contributed only 1 per cent of the total lead found after a 12-h leach. A blank run, with no lead glass in the extractor, showed a concentration of only 0·02 mg lead/l. after a 5-day leaching period. Thus all blanks are quite negligible.

The rates dropped over the first two or three 12-h periods then reached nearly constant values for a total of seventeen leaching periods. Omitting the abnormally high initial period, we found average leach rates for the two samples of 0·50 ± 0·06 mg and 0·52 ± 0·04 mg lead/12 h/g glass.

Since the amount of lead removed was quite accurately a linear function of time, rather than linear with the square root of time, diffusion of lead to the surface of the glass was certainly not the limiting factor. In all probability the lead was released in proportion to the amount of glass actually decomposed by the hot water.

Similar experiments made with a cobalt glass, in which the ion was present in only very small quantities, gave similar results. A leach rate of about 1γ per day of cobalt was found.

Radioactive glazes. Mr. M. I. Goldman, of the Department of Civil and Sanitary Engineering of the Massachusetts Institute of Technology, supplied us with samples of a glass prepared with small added amounts of cæsium-137 tracer. They contained about 10 per cent alumina, 60 per cent silica, 10 per cent boric oxide and 20 per cent calcium oxide. The leaching period of thirty days was used, with 200 ml. of water in the boiler. After each leaching period the entire contents of the boiler was removed and the flask washed with nitric acid to remove any adsorbed material. Solution and washings were made up to a convenient volume and an aliquot taken for radioactive analysis. In all the experiments silica collected in the boiler as a tenacious encrustation of siliceous matter on the walls of the flasks. This was removed by rinsing with dilute hydrofluoric acid and added to the total sample.

The most consistent results were obtained with a small amount of cæsium salt added to the water in the boiler which probably prevented adsorptive losses.

A γ-count of the whole pellet was taken in the well of a scintillation crystal. Aliquots of the leachate were also analysed. The samples were followed for a period of six months.

If one assumes the pellet to be a sphere (it was actually more nearly hemispherical), we may calculate the equivalent depth to which the glaze was leached. The result of this estimate is:

$$\Delta r = \frac{r}{3}\frac{\Delta V}{V} = \frac{1}{3}r\frac{\text{counts in leachate}}{\text{total count of pellet}} = 0{\cdot}00005 \text{ mm per day}$$

in which r is calculated from the known area of the pellet. This result is very rough and is possibly optimistic by as much as a factor of five.

For accelerated tests it is evident that samples of large surface area will be required. In the equipment we were limited for mechanical reasons to particles at least sufficiently large to be retained on a 30-mesh screen. If it were desirable to test smaller particles the apparatus could be modified, but with smaller particles the uncertainty of exposure of the surface increases.

The release of activity is certainly proportional to the area of the exposed surface and consequently in comparisons between different materials a measure of the surface area will be required. For granular material of irregular shape this is a difficult problem. For non-porous fractured glasses between standard sieve sizes 6 and 30, a procedure based on the work of Gross and Zimmerley[2] is suggested as perhaps the best method of getting an estimate of surface area. The method depends on correlations between measured rates of dissolution of silica specimens of known areas with the results of sieve analyses. The procedure is to determine the sieve distribution in weight per cent of a 100-g sample in the 6–30 range. The approximate area can then be calculated directly from Table 1 for crushed silicas and glasses.

Table 1. COMPUTATION AREAS OF CRUSHED SILICEOUS MATERIALS

Mesh No.	Area (cm)^2 per g of material retained on this mesh size
7	55·6
8	59·6
10	63·8
12	69·4
14	76·1
16	86·4
18	95·3
20	103
25	112
30	127

The amount of radioactive isotope required in a test specimen varies greatly with the nature of the isotope and the type of measuring equipment available to the operator. For highly stable glazes it is essential to incorporate enough radioactive material so that meaningful leaching tests can be made in a reasonable length of time. This means that decisive experiments will often require a high-level laboratory and equipment for remote handling.

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SOME BOILING AND TRIPLE POINTS BELOW 0° C

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As part of the temperature standardization programme of this laboratory we have built an apparatus for realizing the normal boiling point of oxygen, which is a primary fixed point on the International Practical Temperature Scale (IPTS), with a standard deviation accuracy of about 10^{-4} deg. C. In addition, the normal boiling or sublimation points and triple points of other, sufficiently pure, substances in the range 0° C to −196° C could be determined with comparable accuracy.

The apparatus is generally similar to that described by Stimson[1], and consists of a solid copper block, containing four thermometer wells and a cavity in which about 1,000–2,000 cm^3 (at N.T.P.) of the test gas is condensed. The block is contained in an evacuated housing which is surrounded by liquid nitrogen, or, where appropriate, dry ice and acetone. A radiation shield and two thermal guards on the thermometer wells help to maintain an isothermal environment, and for normal boiling or sublimation point determinations, the block temperature is maintained constant with an indicated precision of better than 10^{-4} deg. C, using a vapour pressure sensing servo loop described elsewhere by me[2]. The pressure transducer is backed by a reference pressure of one atmosphere (the normal or standard atmosphere = 1,013,250 dynes/cm^2), given by a precision manostat described by Berry[3]. Either stick type or capsule type platinum resistance thermometers could be used in the apparatus and details of calibration and measurement equipment used with the thermometers may be found in ref. 3.

Purest available materials were used. In the cases of oxygen, nitrogen, argon, krypton, xenon and carbon dioxide purities ranged from 99·99 to 99·999 per cent. The carbon monoxide available was of somewhat lower purity and this resulted in a lowered accuracy for its boiling point determination. Methane of about 99·99 per cent purity was prepared by Dr. J. H. Colwell, of the National Research Council Pure Chemistry Division, and it gave some evidence of forming a constant boiling mixture with about 100 p.p.m. nitrogen.

At the boiling points of the various substances, the vapour was removed from over the liquid in steps of about 100 cm^3, and any resulting changes in the boiling point (which could be detected to better than 10^{-4} deg. C) were observed. By this means the effect of residual impurities could be estimated in most cases to about 10^{-4} deg. C. Triple points were determined from the melting curve, by extrapolating the melting plateau back to the time of intersection with the linear warm-up curve. Triple points determined in this way were reproducible to a few $\times 10^{-4}$ deg. C.

Below 0° C the IPTS is defined by means of platinum resistance thermometers, using for interpolation the Callendar–Van Dusen equation:

$$R_t = R_0 \, [1 + At + Bt^2 + Ct^3(t - 100)]$$

R_t and R_0 are the resistances at temperature $t°$ C and at 0° C respectively, while the coefficients A, B and C are determined by calibrations at the steam, sulphur (or zinc) and oxygen points. It is required, as a rough control of the purity of the platinum, that the coefficient $\alpha (= A - 100B)$ must be greater than 0·003920.

It is likely that different thermometers satisfying the specifications of the IPTS would indicate slightly different temperatures in regions removed from the calibration points. Furthermore the IPTS does not extend below the oxygen point. In order that our measurements should not be limited in accuracy by these considerations we have defined a scale IPTS*, which is the IPTS as realized by a particular group of six thermometers of high purity ($\alpha >$ 0·003925), and is taken to extend by means of the Callendar–Van Dusen equation to temperatures below the oxygen point.

Below the oxygen point the thermometers were also calibrated in terms of the scale N.B.S. 1955, which is maintained by the U.S. National Bureau of Standards. This scale can be related to scales maintained at the Pennsylvania State University, at the National Physical Laboratory, Teddington, and at the Physical-Technical and Radio-Technical Measurements Institute, U.S.S.R., by means of data reported by Barber[4]. The scale N.B.S. 1955 is, at all temperatures, less by 0·010 deg K than the earlier provisional scale of Hoge and Brickwedde[5].

The difference between the scales IPTS* and N.B.S. 1955 is given for the range −183° C to −222° C in Table 1. Interpolation accuracy is better than 10^{-3} deg. C to −210° C reducing to about 5×10^{-3} deg. C near −222° C. Table 1 may be used with any platinum resistance thermometer, which has been calibrated on the IPTS, to extend its useful range to temperatures below the oxygen point. There is evidence that, for thermometers having $\alpha >$ 0·003924, Table 1 is accurate to within 2×10^{-4} deg. C down to about −200° C.

Table 1. DIFFERENCES BETWEEN TEMPERATURE SCALES IPTS* and N.B.S. 1955

IPTS*	IPTS*–N.B.S. 1955	IPTS*	IPTS*–N.B.S. 1955
−183° C	0 $\times 10^{-3}$ deg. C	−204° C	304 $\times 10^{-3}$ deg. C
−186	1·4	−206	390
−188	5·7	−208	495
−190	15·2	−210	622
−192	30·6	−212	780
−194	52·9	−214	973
−196	83·4	−216	1,209
−198	122·7	−218	1,496
−200	171·8	−220	1,845
−202	232	−222	2,273

Table 2. NORMAL BOILING POINTS (SUBLIMATION POINT FOR CARBON DIOXIDE) AND TRIPLE POINTS IN THE RANGE 0° C TO −200° C (IPTS*), WITH BRACKETED VALUES ON THE SCALE N.B.S. 1955

Gas	Normal boiling point	Triple point
S	+444·6000†	—
H_2O	+100·0000†	+0·0100‡ (273·1600)
Hg	—	−38·8644§
CO_2	−78·5097	−56·6000
Xe	−108·1065	−111·7694
Kr	−153·3416	−157·3799
CH_4	−161·4925	−182·4735
O_2	−182·9700† (90·1800)	—
A	−185·8553 (87·2934)	−189·3342 (83·8044)
CO	−191·420 (81·706)	—
N_2	−195·7167 (77·3547)	—

† Primary fixed points on the IPTS. ‡ Fundamental defining point. § IPTS value of mercury triple point from Dengler (ref. 6).

In Table 2 the measured values of the fixed points are recorded on the scale IPTS* with bracketed values (where appropriate) on the scale N.B.S. 1955. In addition, the primary fixed points used to define the IPTS are listed and also a recent value of the mercury triple point, given by Dengler[6]. The mercury triple point (or the normal freezing point, which is $5 \cdot 2 \times 10^{-3}$ deg. C lower) has frequently been used as a calibration fixed point in this region.

In general, there have been rather few precision measurements of the foregoing boiling and triple points by other workers. The argon triple point has been determined by Michels, Wassenaar, Sluyters and De Graaff[7]. However, they used their own scale in which the coefficients in the Callendar–Van Dusen equation were determined from calibrations at the oxygen boiling point, mercury freezing point and carbon dioxide triple point, estimated thermodynamic values of the latter two points being used. It can be shown that the argon triple point value determined here agrees to within about 2×10^{-3} deg C of Michels's value when allowance is made for the difference between the two scales.

The argon triple point has been determined by Flubacher, Leadbetter and Morrison[8], and the krypton triple point by Beaumont, Chihara and Morrison[9]. In each case the quoted value is 6×10^{-3} deg. C higher than our value. However, it now appears (private communication) that there is a positive systematic error of between 5 and 7×10^{-3} deg. C in Morrison's measurements and the agreement can thus be considered to be quite good.

I thank Mr. N. J. Scardina for help with the experimental measurements, Mr. R. J. Berry for help in the use of the manostat, and Dr. H. Preston-Thomas for useful discussions.

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TRANSFER OF ENERGY FROM BACTERIOCHLOROPHYLL TO A REACTION CENTRE DURING BACTERIAL PHOTOSYNTHESIS

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REVERSIBLE changes in absorption occur in purple bacteria when illuminated[1,2]. Part of these changes take place in the near-infra-red region, where the bulk, if not all, of the absorption is due to bacteriochlorophyll. In *Rhodospirillum rubrum* the absorption difference spectrum shows a decrease in absorption of about 2 per cent, with a maximum at about 880 mμ, and an increase around 795 mμ. Since bacteriochlorophyll has a major absorption maximum at 880 mμ and a minor one at 800 mμ, the changes in absorption were attributed to a small fraction of the bacteriochlorophyll[2], which we call *P* 890 or *P*. *P* 890 may be the photochemically active molecule, that is, the 'reaction centre'[1]. The light-induced reversible 'bleaching' at 880 mμ was more rapid and occurred at lower intensities of exciting light in the absence of hydrogen donors under oxidizing conditions[2]. A similar bleaching could be brought about in water extracts on addition of a mixture of potassium ferri- and ferro-cyanide with an oxidation-reduction potential of about 0·5 V (refs. 3 and 4). It has been suggested[2-4] that the light-induced absorption change around 880 mμ reflects the oxidative bleaching of *P* 890. The energy of light absorbed by the bacteriochlorophyll type *B* 890 may be transferred to *P* 890 (ref. 1), which becomes oxidized on receiving energy. Oxidized *P*, (P_{ox}), might oxidize in its turn the hydrogen donor, possibly by way of one or more cytochromes[2,5-8].

If the transfer from *B* 890 to *P* 890 occurs from the fluorescing excited state, the efficiency of the transfer to *P* is lowered on bleaching of *P*, and the fluorescence yield of *B* 890 will increase[1]. In this article it will be shown that the increase in fluorescence is correlated with the bleaching of *P*, and that this correlation can be explained quantitatively by the hypothesis of energy transfer already mentioned. It will also be shown that the bleaching occurs with high quantum efficiency.

Rhodospirillum rubrum, strain 1, was grown in a modified Hutner medium, similar to that used by Cohen-Bazire *et al.*[9]. In most of our experiments 3-day-old cultures were used. After centrifugation, the bacteria were resuspended in fresh medium or water, at a higher concentration than in the growth medium. The bacterial suspension was finally transferred to an absorption cuvette, at a thickness of 1 mm.

All measurements were carried out in a split-beam difference spectrophotometer[10]. Absorption was measured continuously by means of two modulated mutually compensating measuring beams. The intensity of these beams was so low as not to cause measurable changes in absorption. These changes were brought about by an unmodulated actinic monochromatic beam of 589 mμ, which did not cause a deflexion of the detecting apparatus, since this apparatus only responds to modulated light. For measurements of fluorescence the measuring beams were cut off, and the actinic beam modulated; thus the modulated actinic beam also functioned as exciting beam. Since the fluorescence yield changed slowly compared with the modulating time, which was 1/50 sec, the average intensity of the modulated beam was taken as the actinic intensity, which is given in 10^{-9} einstein cm^{-2} sec^{-1}.

Changes in absorption in the infra-red occurred at rather high intensities of actinic light, if the bacteria were suspended in growth medium[2]. We found that the kinetics of the changes on illumination depended on the length of the dark period and on the intensity of the preceding illumination. Figs. 1 and 2 show recordings of the light-induced spectral changes of absorption and fluorescence on illumination. The measurements of change in absorption and fluorescence, represented in each figure, were carried out shortly after each other at the same intensities and with the same light and dark periods. For bacteria suspended in malate, the fluorescence intensity at the beginning of the illumination is *f*. The fluorescence increases, starting from this initial level *f*. At least two phases can be distinguished in both absorption (*a*, *b*) and fluorescence (a_f, b_f); the absorption decreases *a* and *b* are correlated with fluorescence increases a_f and b_f. For bacteria washed and suspended in aerobic distilled water, the changes occur at much lower intensities of actinic light and at a higher initial rate than for bacteria in malate.

In Figs. 3 and 4 the steady-state absorption difference, light minus dark, and the steady-state level of fluorescence are plotted as a function of the actinic intensity, *I*. For bacteria suspended in growth medium (Fig. 3) the change

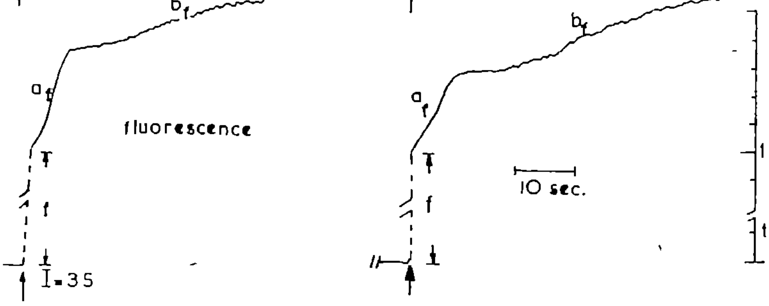

Fig. 1. *Rhodospirillum rubrum*, suspended in malate medium. The top tracings are time recordings of the light-induced absorption change at 880 mμ. The bottom tracings are recordings of the total fluorescence, occurring in the wave-length region above 850 mμ. The switching on and off of the actinic and the fluorescence exciting beams is indicated by upward and downward pointing arrows. The average intensity of these beams (*I*) was 3·5 × 10^{-9} einstein/cm^2 sec, the wave-length 590 mμ. The actinic and fluorescence exciting light was given after 10 sec dark time (left-hand tracing) and after 15 sec dark time (right-hand tracing) for both absorption change and fluorescence

in absorption starts at intensities above $I = 0{\cdot}5$. The increase in fluorescence yield (upwards curvature of fluorescence versus intensity curve) also starts above this intensity. In aerobic water both the decrease in absorption and the increase in fluorescence yield start below this intensity (Fig. 4). In Fig. 3, f is plotted as a function of I. Since f is proportional to I, the fluorescence yield at the start of the illumination is independent of the intensity. This means that under these conditions the change in fluorescence yield (and this is also true for the increase in absorption) is slow compared with the response of the apparatus. For bacteria in aerobic water the change in fluorescence yield is more rapid. The upward bending of the fluorescence-intensity curve starts below $I = 0{\cdot}2$, as we found by plotting a few points, measured at low intensity, on a ten times larger scale than that of Fig. 4. The slope of this intensity curve was estimated at $I = 0$ and is given in Fig. 4 by the dashed straight line. Similarly the absorption change at 880 mμ starts from $I = 0$.

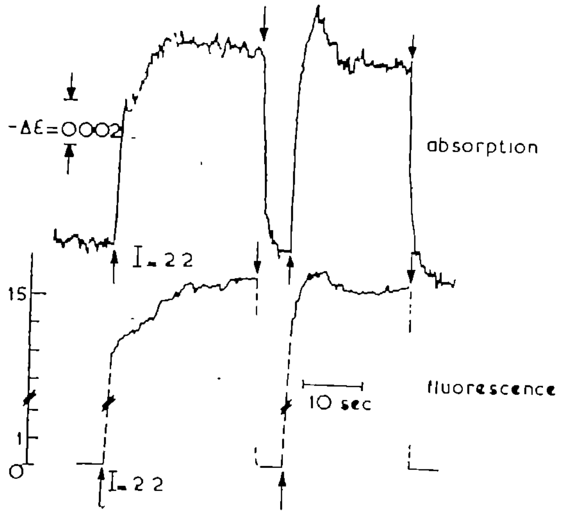

Fig. 2. *Rhodospirillum rubrum*, washed and suspended in distilled water. Time recordings of light-induced absorption change at 880 mμ and of total fluorescence. The first illumination, at the left-hand side of the figure, was given after about 5 min darkness, the second after 5 sec. The intensity of the light was $2{\cdot}2 \times 10^{-9}$ einstein/cm² sec

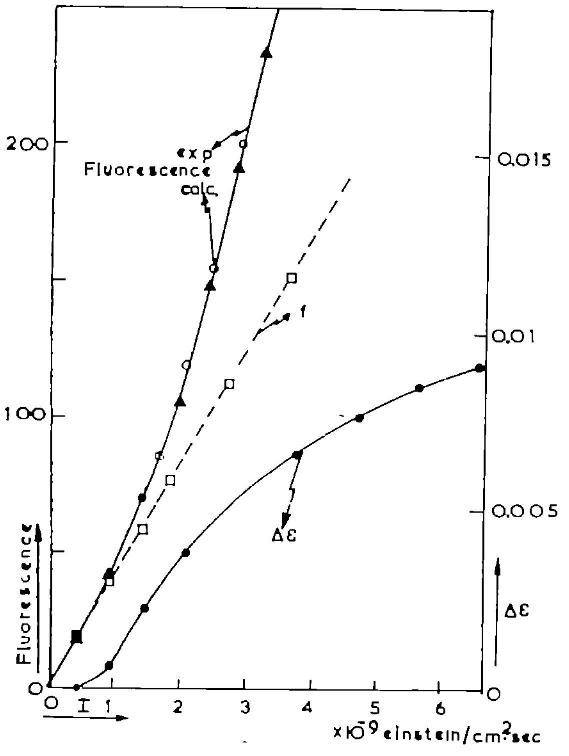

Fig. 3. *Rhodospirillum rubrum*, suspended in malate medium. The change in absorption at 880 mμ and the level of fluorescence in the steady-state are plotted as a function of the actinic intensity, I, of a wave-length of about 590 mμ. The initial fluorescence intensity f is also plotted as a function I. The calculated fluorescence intensities (see text) are indicated by open circles. The amount of fluorescence (left scale) is expressed in arbitrary units. The absorption difference (right scale) is given in optical density units

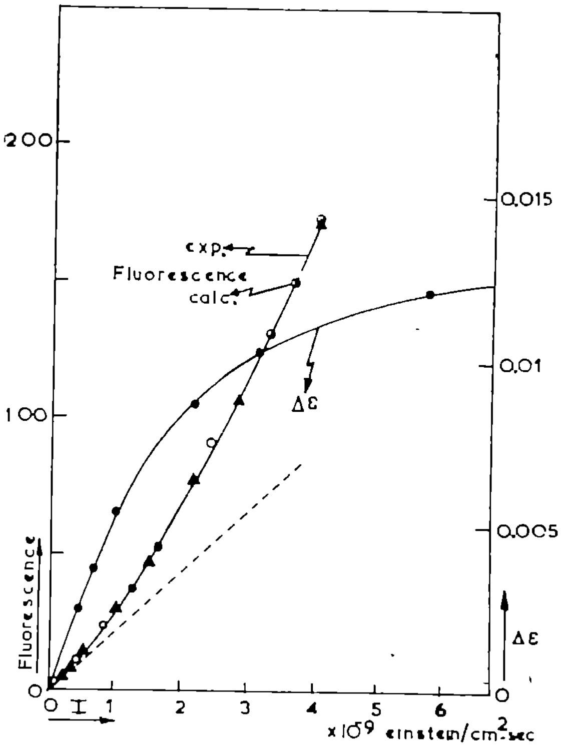

Fig. 4. *Rhodospirillum rubrum*, suspended in distilled water. The meaning of the symbols is the same as in Fig. 3. The slope of the fluorescence versus intensity curve at $I = 0$ is given by the dashed straight line

At – 170° C the changes in absorption at 880 and 795 mμ still occurred on illumination. The reactions were reversible: in the dark the original steady-state was again reached. For dried bacterial extracts this reversibility has been observed by Arnold and Clayton at 1° K (ref. 11).

We found that at – 170° C both the absorption spectrum and the absorption difference spectrum show a shift of the maximum at 880 mμ of about 20 mμ to longer wave-lengths. This shift provides an additional argument for the assumption that the change in absorption around 880 mμ is due to the bleaching of a pigment similar to B 890. It has been reported that in *Rhodospirillum rubrum* the fluorescence band of B 890, which has a maximum at 900 mμ, shifts about 20 mμ to longer wave-lengths on cooling to liquid nitrogen temperatures[12].

The quantum requirement for P 890 bleaching was measured for an aerobic aqueous bacterial suspension, to which in most experiments 10^{-3} M phenylmercury acetate was added in order to minimize the rate of P 890 restoration in the dark. The quantum requirement was calculated from the measured initial rate of the absorption change at 880 mμ, the intensity of the actinic light (590 mμ) and the absorbance of the suspension. It was assumed that the specific absorption coefficient of P was equal to that of bacteriochlorophyll at the infra-red maximum ($\epsilon = 9{\cdot}6 \times 10^{4}$ l. mol^{-1} cm^{-1} (ref. 13)), and that the absorption coefficient of P_{ox} was zero. The quantum requirements calculated from the initial rates measured at two intensities ($I = 0{\cdot}2$ and $0{\cdot}4$) for samples taken from the same culture were 3·3 and 3·1 (bacteria suspended in water to which phenylmercury acetate had been added), 3·1 and 3·6 (bacteria washed and suspended in water) and 3·2 and 3·2 (bacteria washed and suspended in water to which phenylmercury acetate had been added). These numbers are lower if the absorption coefficient of P_{ox} is not zero.

It seems that the observed correlation between increase in B 890 fluorescence and P 890 bleaching can be quantitatively described by the following hypothesis. Excitation energy is transferred from B 890 to P 890. On excitation of P 890 this component is bleached, which prevents further transfer of energy from excited B 890, causing an increase in the fluorescence of B 890; P 890 is assumed not to fluoresce at the wave-length of measurement. We postulate that the excited bacteriochlorophyll, $[B^*]$, looses its energy in 3 different ways: (1) fluorescence; (2) internal conversion; (3) transfer of energy to P 890. The change in B^* concentration, $d[B^*]/dt$ is then given by:

$$\frac{d[B^*]}{dt} = c_1 I - (k_1[B^*] + k_2[B^*] + k_3[B^*]\,[P]) \qquad (1)$$

where I is the intensity of exciting light and c_1 and the k's are rate constants for absorption, fluorescence, internal conversion and energy transfer; $[P]$ is the concentration of unbleached P 890. It is possible that other terms, containing variable parameters, should be added to the right side of equation (1). However, for the interpretation of the present experiments, the terms given appear to be sufficient. During quasi steady-state, that is if $[B^*]$ does not change appreciably in 10^{-8} sec ($k_1 \approx 10^{+8}$), $d[B^*]/dt$ is small compared with the other terms and we may put $d[B^*]/dt = 0$. This condition certainly is fulfilled at the relatively low intensities used. Solving for I we obtain:

$$I = (k + k'_3\,[P])\,[B^*] \qquad (2)$$

where $k = (k_1 + k_2)/c_1$ and $k'_3 = k_3/c_1$.

The intensity of fluorescence is given by:

$$I_{fl} = k_1[B^*] \qquad (3)$$

The fluorescence yield is:

$$\varphi_f = \frac{I_{fl}}{I} = \frac{k_1}{k + k'_3[P]} \qquad (4)$$

At zero intensity, all P 890 is in the non-bleached form: $[P] = P_0$. At high intensities, at which all P 890 is bleached: $[P] = 0$. The change in absorption is equal to:

$$\Delta\epsilon = c_2(P_0 - [P]) \qquad (5)$$

in which c_2 is a constant, equal to the difference in absorption coefficient of the unbleached and bleached forms of P 890. Eliminating P from equations (4) and (5) we get:

$$\frac{1}{\varphi_f} = \frac{k + k_3P_0}{k_1} - \frac{k'_3}{k_1c_2}\,\Delta\epsilon \qquad (6)$$

According to equation (6) $1/\varphi_f$ and $\Delta\epsilon$ are linearly related. We tested this by plotting $1/\varphi_f$ as function of $\Delta\epsilon$ in Fig. 5. Fig. 5 shows that $1/\varphi_f$ is in first approximation linearly dependent on $\Delta\epsilon$, which supports our hypothesis. The values $1/\varphi_f$ and $\Delta\epsilon$ for the same intensities were obtained from the experimental curves given in Fig. 3 and 4. The values of $1/\varphi_f$ at $\Delta\epsilon = 0$ is found by taking the slope of the fluorescence-versus-intensity curve at $I = 0$. In Figs. 3 and 4 calculated intensities of fluorescence are also given. These were calculated from the $\Delta\epsilon$ curves by substituting the experimental value of $\Delta\epsilon$ in equation (6). The two constants k'_3/k_1c_2 and $(k + k_3P_0)/k_1$ in the right side of equation (6) were obtained from each of the lines of Fig. 5. The first constant is equal to the slope of a line, the second is equal to $1/\varphi_f$ at $\Delta\epsilon = 0$. The experimental and calculated curves of fluorescence appear to be similar.

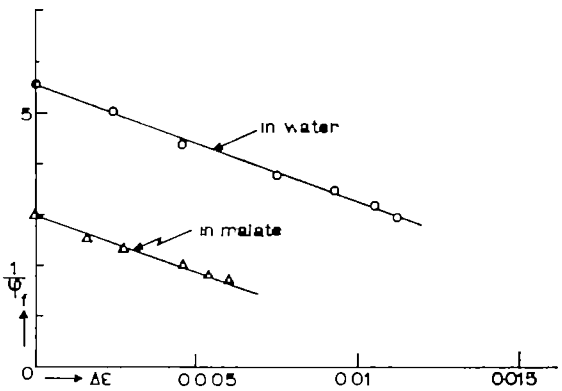

Fig. 5. The inverse of the fluorescence yield, $1/\varphi_f$, plotted as a function of the absorption change, $\Delta\epsilon$, for bacteria, suspended in growth medium (open triangles), and in water (open circles). The two curves are not comparable, since the fluorescence intensities were not measured absolutely

The quantitative correlation between fluorescence increase and P 890 bleaching, the low quantum requirement for this bleaching, and the fact that the bleaching occurs at very low temperature, strongly argue in favour of our hypothesis that the energy transfer from bacteriochlorophyll to P 890 occurs through inductive resonance.

The high efficiency for bleaching of P 890 indicates that P 890 may be an intermediate in bacterial photosynthesis. P 890 is suggested to be a pigment similar to B 890; it is, however, non- or weakly fluorescent and photochemically active; it may act as the photosynthetic reaction centre. It has been shown[1] that the transfer of energy with good efficiency to such a reaction centre is theoretically possible taking into account the concentrations and the fluorescence yield of bacteriochlorophyll *in vivo*.

Summarizing, we may say that the light-induced decrease in absorption at 880 mμ in the photosynthetic bacterium *Rhodospirillum rubrum* is quantitatively correlated with an increase in fluorescence yield of the bacteriochlorophyll type B 890. This correlation can be quantitatively explained by energy transfer from bacteriochlorophyll to a pigment P 890, present in a small amount, which pigment is bleached on excitation. The quantum requirement for P 890 bleaching was found to be about 3·2 or lower. The results indicate that the bleaching of P 890 is a primary photochemical process, and that P 890 acts as a reaction centre in bacterial photosynthesis.

After this manuscript was completed, Dr. R. K. Clayton kindly send us preprints of papers submitted for publication (*J. Photochem. Photobiol.*), giving evidence and conclusions in agreement with this and earlier papers from our laboratory. The main new evidence was that in an aqueous extract of an aged culture of a green mutant of the purple bacterium *Rhodopseudomonas spheroides* the optical density at the long wave-length maximum was reduced to about one-half on illumination; the difference spectrum was essentially similar to that of 'normal' purple bacteria[8]. This indicates that the specific absorption of these special bacteriochlorophyll molecules is reduced to one-half or less, and that thus in normal bacteria probably an appreciable change in absorption occurs in the few P 890 molecules, instead of a small change in all B 890 molecules.

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DOES THE NEURONE SOMA ACTUALLY GENERATE ACTION POTENTIALS?

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IT has been shown by Oomura *et al.*[1] that the neurone of a marine pulmonate mollusc, ***Onchidium verruculatum***, could generate the action potential in various sodium-free sea-waters, and that not sodium but calcium ions played an important part in the electrical activities. They also reported that the excitability in the soma was relatively low compared with that in the axon or axon–soma junction. With direct stimulation of the soma, it was assumed that the spike potential was initially generated in a remote triggering region, say, the axon or axon hillock, and electrotonically spread to the soma, then the action potential was generated in the soma membrane. It was still not certain, however, whether the electrical activity recorded in the soma was only a reflexion of the spike potential in a remote active region, and the soma membrane remained unexcited[2]. This uncertainty was tested as follows. (1) The excitability of the soma membrane was directly demonstrated in neurones which were ligated at the axon–soma junctional region; (2) indirectly evidenced by the transmembrane impedance measurements. The impedance measurements by means of the intracellular microelectrodes were first made on the neurones. With these techniques, we were able to demonstrate that the soma membrane was really excitable during activity.

Materials used were dissected œsophageal ganglion cells of a marine pulmonate mollusc, ***Onchidium verruculatum***. In the ligature experiments the neurones were tied with single silk filaments at the axon–soma junctional region. For the impedance measurements three microelectrodes were inserted into a single neurone. The first microelectrode (*Rr*) was for recording transmembrane potential, the second one (*Rc*) served as an arm in an a.c. impedance bridge, and the third one was for polarizing the membrane and connected to an electronic stimulator through a high resistor (Fig. 1). The resistances of the microelectrodes utilized in the impedance experiments ranged from 10 to 20 MΩ. For the voltage clamp experiments a feedback amplifier was inserted between the first and the third electrodes[3].

Ligature experiments. Ligated soma showed some signs of injury. The resting potentials of about 40 mV were obtained which were somewhat smaller than those of unligated neurones. Spontaneous firings, observed frequently on penetration of the electrodes in the latter, were usually absent in the former. The ligated neurones never responded synaptically or antidromically. This indicates that the nervous function in the region of the axon hillock was satisfactorily blocked by the ligature. By direct transmembrane stimulations the action potentials could be generated in the soma, although their thresholds were higher than those of unligated ones. Shapes of the action potentials were quite indistinguishable from those in the unligated neurones, especially when the threshold of the latter was slightly increased by an application of a hyperpolarization (Fig. 2*A* and *C*).

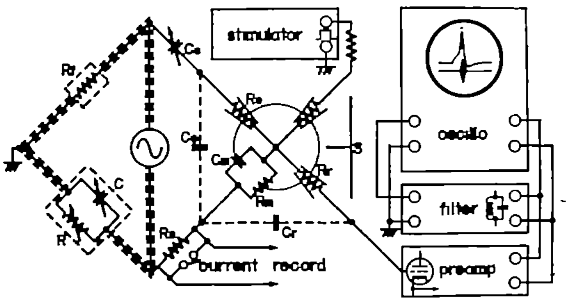

Fig. 1. Schematic presentation of the impedance circuit. *R* and *C*, variable resistor and capacity; *Rc* and *Cc*, resistance and capacity of the microelectrode in the bridge arm; *Rm* and *Cm*, membrane resistance and capacity; *Rr* and *Cr*, resistance and capacity of the recording microelectrode, respectively. *Cc*, variable air condenser (max. cap. 50 pF); *Rf*, fixed resistor (100 kΩ); *Rs*, current monitoring resistor, shunted in impedance measurements; *S*, shielding plate

Despite the similarity of the action potentials between two preparations, a marked difference was clearly demonstrated in the voltage clamp experiments as shown in Fig. 2*B* and *D*. In the unligated neurones under the voltage clamp condition, the inward membrane current appeared in the all or none manner. Moreover, the time course of the current showed close resemblances with that of the action potential. In the ligated neurones, on the other hand, the time course of the membrane current was much different. Similar to the results obtained by Hagiwara and Saito[4], the inward current was followed by a slowly developing outward current. Low intensity of the inward current and slow time course of the outward current might be attributable to an injury in the membrane. These results will be discussed elsewhere.

Impedance experiments. Frequencies of alternating currents applied to the bridge were 0·2–2 kc/s (adequate frequencies 0·5–1·2 kc/s). Impedance changes during

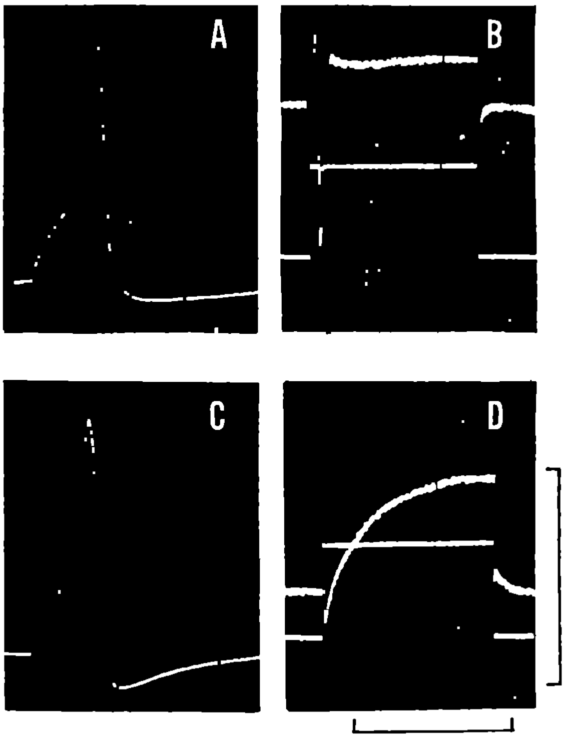

Fig. 2. Intracellular recordings from unligated (*A* and *B*) and ligated soma (*C* and *D*). *A* and *C*, action potentials initiated by short current pulses; *B* and *D*, relationships between the membrane currents (upper beams) and potential (lower beams) under voltage clamp condition. The resting potential of the unligated neurone was artificially increased. Note the difference in time courses of the membrane currents when the membrane was depolarized. Current gains are not identical. Upward and downward deflexions represent outward and inward currents, respectively. Resting potential, 40 mV; voltage scale, 100 mV; time scale, 100 msec for *A* and *C*, 400 msec for *B* and *D*

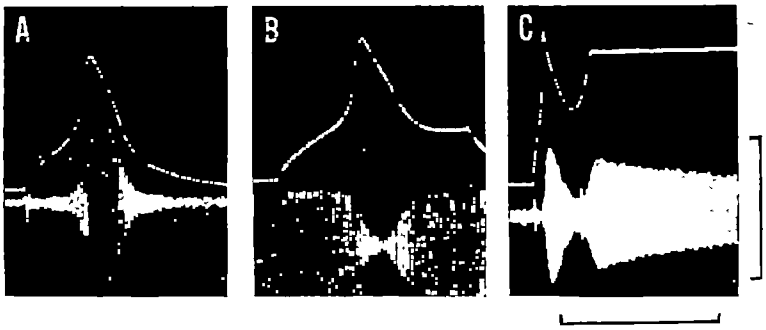

Fig. 3. Impedance changes during the action potentials. *A* and *B*, the bridge is balanced at the resting state and near the peak of the action potential, respectively; *C*, markedly prolonged action potential. Voltage scale, 100 mV, time scale, 100 msec for *A* and *B*, 400 msec for *C*

neuronal activities could not be detected by frequencies higher than 2 kc/s. Relatively large value of the membrane time constant (Cm 0·02 μF × Rm 5 MΩ) and presence of stray capacities (Co and Cr) decreased the accuracy of the measurements by the a.c. bridge in higher frequencies. After the bridge had been exactly balanced for the membrane impedance at the resting state, a suprathreshold outward current pulse was passed through the membrane. Considerable lack of balance of the bridge was recorded simultaneously with the action potential (Fig. 3*A*). Precise balancing of the bridge during the activity was also obtained at various stages of the action potential adjusting appropriate values of C and R (Fig. 3*B*). Time course of the impedance changes were more clearly demonstrated when the action potentials were prolonged by an administration of barium ions into the sea-water[1] (Fig. 3*C*).

These results suggested that the membrane impedance diminished considerably during the action potential. Although a quantitative analysis of the data was complicated by the presence of stray capacities of the electrodes, calculations indicated that the membrane resistance was reduced to about 100 kΩ and the capacity remained nearly unchanged. Now let us assume as follows: (1) the ratio of the area of the soma to that of the axon hillock is 20; (2) at the peak of the activity the membrane resistance might decrease to 1/100 of the initial value. If the active area is restricted within the axon hillock, the total resistance during activity might fall to about 1/5 of its resting value (about 1 MΩ). However, more marked reduction in the membrane resistance was obtained. It appears, therefore, unlikely that the membrane of the soma remains inexcitable and that the action potential recorded in the soma is the potential spread electrotonically from the axon or the axon hillock. We conclude that the soma of the giant neurones of *Onchidium* is excitable and generates the action potential.

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UREASE AND CITRASE OF PATHOGENIC AND SAPROPHYTIC FUNGI

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FUNGI, like bacteria, are very ubiquitous in their distribution throughout the world. Many saprophytic types play very essential parts in the biological cycles involving organic matter such as the decomposition of hemicellulose, lignins, etc., but are also involved in the synthesis of complex organic compounds. Their most important function is the breaking down of complex molecules and the synthesis of useful ones, both for the animal and plant kingdoms. There are numerous species of fungi in Nature, mostly saprophytic in their biological behaviour, and only about 50 species are capable of causing diseases in man and animals, some producing fatal infections such as *Coccidioides immitis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Candida albicans*, etc., while the vast majority give rise to chronic systemic or skin diseases. Fungus infections are very serious among birds and insects.

The outlook of systemic mycotic infections was very gloomy in the past, but with the introduction of the sulphonamides, tetracycline and penicillin in actinomycosis, nystatin in candidiasis[1], 'Amphotericin *B*' (ref. 2) in serious systemic mycoses and griseofulvin in dermatophytoses and deep mycoses[3,4], the overall prognosis is at present very favourable.

Moulds, yeasts and fungi are extensively used in fermentation in industry, in the dairy industry, the manufacture of citric acid, ascorbic acid, antibiotics and even steroids. They also produce ammonia from urea. Somehow, the enzymatic activities of pathogenic fungi have been utilized to a very limited extent in their biochemical studies. Lactic acid, ethanol, acetic acid and formic acid are formed from glucose by *Actinomyces bovis*[5] and acid phosphatase is produced from some strains[6]. *Cryptococcus neoformans* does not ferment sugars, assimilate potassium nitrate nor reduce nitrates into nitrites. The carbon of glucose, maltose, sucrose and galactose is utilized but not that of lactose, and urea may be split[7]. *Nocardia asteroides* did not liquefy gelatin nor coagulate milk, but nine out of eighteen strains of *Nocardia brasiliensis* coagulated milk, nine liquefied gelatin and three reduced nitrates to nitrites[8]. *Streptomyces* and *Nocardia* produced penicillinase[9]. On the basis of the fermentation of glucose, maltose, sucrose and lactose, *Candida* is classified into seven species. *C. albicans* produces acid and gas in glucose and maltose, acid in sucrose and has no effect on lactose. *C. tropicalis* produces acid and gas in all, but fails to ferment lactose. *C. pseudotropicalis* produces acid and gas in all except maltose. *C. krusei* and *C. parakrusei* produce acid and gas only in glucose. *C. stellatoidea* produces acid and gas only in glucose and maltose and *C. guilliermondi* causes no change in sugars[10]. *C. immitis* liquefies gelatin, coagulates milk, utilizes carbon from sugars, alcohols, amino-acids, organic acids, and amides; and nitrogen from peptone, amides, amino-acids, nitrate ions and ammonia ions, but these biochemical reactions do not help in its identification[11]. In *Sporotrichum schenkii* the sugar reactions are of no value. Nitrogen of amino-acids, urea and ammonium nitrate were utilized, and no amino-acid was an essential growth factor[12–14]. Nothing is known about the fermentative propensities of *Hormodendrum compactum*, but *Phialophora verrucosa* produces acid in dextrose and dextrin, but has no effect on milk and does not produce indol[15].

The aforementioned references indicated that very little work has been done concerning the enzymatic activities of pathogenic fungi, and that very little use of this enzymatic activity has been applied towards the identification of fungi. Since enzymatic studies have been very fruitful in the identification of pathogenic bacteria and there is the possibility of using the enzyme profiles of bacteria in the chemotherapy of infections, a proper analysis of the enzyme profiles of fungi may be promising.

On the basis of the urease and citrase profile of pathogenic bacteria, they have been classified into seven profiles. Profile 1 produces urease and citrase and includes *Proteus vulgaris*, *P. rettgeri*, *P. mirabilis* and *P.* OX_2. Profile 2 produces only urease and includes *P. morganii* and 69 per cent of *Staphylococcus*. Profile 3 produces urease aerobically and also citrase and includes the *Klebsiella aerobacter* group and 77 per cent of *P. aeruginosa*. Profile 4 may or may not produce urease and/or citrase and includes *P. OX K* and *P. OX* 19. Profile 5 may or may not produce citrase, and has *P. inconstans*. Profile 6 produces only citrase and has *Pseudomonas* (26 per cent), *Alcaligenes fecalis*, *Salmonella*, *Vibrio cholerae*, and *Escherichia intermedia*. Profile 7 fails to produce urease and citrase and has *Escherichia coli*, *Salmonella paratyphi*, *S. gallinarum*, *S. pullorum*, V. el Tor, *Staphylococcus* (31 per cent), *Enterococcus*, *Streptococcus pyogenes* (Lancefield *A*), *Shigella*, *Pneumococcus*, *S. viridans*, *Neisseria*, *Brucella*, *Pasteurella*, *Lactobacillus*, and *M. tuberculosis*[16].

The protozoa have no urease: *Trypanosoma cruzi*, *Leishmania donovani*, *L. tropica*, *L. brasiliensis*, *Trichomonas vaginalis* and *T. foetus*[17]. On the basis of Krebs's cycle substrates, pathogenic bacteria are grouped into eleven profiles. Profile 1 has no effect on these substrates and includes *Streptococcus pyogenes* (Lancefield *A*) *Pneumococcus* and *Enterococcus*. Profile 2 utilizes only the carbon of oxaloacetic acid and includes *Staphylococcus*. Profile 3 is *Shigella*, utilizing the carbon of acetate, and malate. Profile 5 is *Escherichia*, utilizing the carbon of acetate, *cis*-aconitate, oxalosuccinate, fumarate and malate. Profile 6 is *Alcaligenes*, which utilizes the carbon of acetate, citrate, ketoglutarate, fumarate and malate. Profile 7 is *Salmonella*, which utilizes the carbon of acetate, lactate, citrate, *cis*-aconitate, ketoglutarate, fumarate and malate. Profile 8 is *Pseudomonas*, which utilizes the carbon of lactate, acetate, citrate, oxalosuccinate, fumarate, malate and oxaloacetate. Profile 9 is *P. morganii*, which utilizes the carbon of all except citrate, succinate, oxaloacetate and pyruvate. Profile 10 is *Klebsiella aerobacter*, which utilizes the carbon of all substrates except isocitrate, succinate and pyruvate. Profile 11 is *P. vulgaris*, *P. rettgeri* and *P. mirabilis*, which fail to utilize the carbon of succinate and pyruvate[18].

Pathogenic bacteria which are active urease and citrase producers are either drug-resistant or they mutate to become drug-resistant when exposed to these therapeutic agents[19]. It was this interest concerning the urease and citrase propensities of pathogenic fungi which motivated these investigations.

Urease is a macroglobulin with a molecular weight of 473,000, has 37 sulphydryl groups, with an optimum *p*H of 7·2–7·5, is dialysable, adsorbed on the Seitz filter, but filterable through the 'Millipore *HA*', does not permeate through the bacterial cell wall, and catalyses the hydrolysis of urea into ammonia and carbon dioxide through the mechanisms of sulphydryl groups. It is inactivated by silver, mercury, copper, cadmium, lead, fluorides, halogens, borates, quinones, formaldehyde and hydrogen peroxide, is rapidly digested and inactivated by pepsin and papain hydrogen sulphide at *p*H 4·3. Jackbean contains 16 times more urease than soybean. Inactivation by mercury can be reversed by cysteine. Urease activity is expressed as the amount of urease which will form 1 mg of ammonia nitrogen from ureaphosphate at *p*H 7 at 20° C in 5 min. Crystalline urease has about 133,000 U/g. Pasteur observed urease activity in *Torula ureae*.

Thunberg in 1910 [20,21], observed an enzyme which oxidized citric acid. It occurred in animal tissues, moulds, cucumber seeds, bacteria, etc. Recent investigations indicate that such an enzyme did not exist. Citric acid is first converted into *cis*-aconitic acid by aconitase and then changed into isocitric acid, then dehydrogenation occurs and the enzyme is isocitric dehydrogenase. This enzyme is found in heart muscle, muscle, liver, kidney, adrenals and other animal tissues and plant tissues[22,23]. There may be two isocitric dehydrogenases in yeasts, one requiring DPN and the other TPN as coenzymes: isocitric dehydrogenase requires manganous or magnesium ions. Pyrophosphate inhibits the enzyme because these ions are removed. Iodoacetic acid also inactivates by reacting with apodehydrogenase[24,25]. The enzyme can be extracted with water and is concentrated by fractionation with ammonium sulphate and ethanol at low temperatures[26].

Most of the pathogenic and saprophytic fungi were given to us by Dr. M. Silva, mycologist in the Department of Dermatology at the Medical School, Columbia University. Our stock cultures were also included in this investigation. A total of 190 cultures constituting 46 genera of pathogenic and saprophytic fungi are reported in this work.

Culture media	Mycological urea	
	Mycological agar (Difco)	35 g
	Distilled water	900 ml.
	Boil to melt and sterilize in the autoclave.	
	Urea c.p. Merck	20
	Phenol red	0·012 g
	Distilled water	100·00 ml.
	Stir to dissolve urea, and sterilize by filtering through the Seitz filter	

After cooling the mycological agar to 45° C, the sterile urea solution was added to the medium with constant stirring. If the temperature of the agar is high urea will be broken down and the medium cannot be used. Mycological agar urea is then tubed and solidified in a slanting position. The *p*H is 7 and the colour of the medium is straw or pale orange. The tubes are then ready for inoculation.

Simmon's citrate (Difco) is prepared by boiling 24·2 g of the medium in 1,000 ml. of distilled water. It is then tubed, sterilized by autoclaving, and slanted to solidify. The colour of the medium is green. If the carbon of citrate is utilized, growth is due to the presence of the enzyme, and the colour of the medium becomes blue. One pellet of Herbox Bouillon (obtained from the Pure Food Co., Inc., Mamaroneck, New York) cubes is added to 250 ml. of distilled water. It is then heated to dissolve the cube. 5 g of Nobel agar are added and the mixture boiled to dissolve. The *p*H is about 4, but it is adjusted to *p*H 7 by adding 10 per cent sodium hydroxide. It is then tubed, autoclaved and slanted to solidify.

190 cultures, comprising 46 genera, split urea. They were active urease producers in live cultures, when incubated at room temperature for 10–15 days. The following cultures failed to show urease activity in 48-h growth: two of seven *Beauveria*, one of three *Oospora*, three of thirty-nine *Candida*, one of four *Trichosporon*, two of four *Monosporium*, one of two *Neurospora* and one each of *Dipodascus*, *Emmonsia* and *Sclerotium*.

The utilization of citrate apparently indicates the presence of an enzyme which is probably citrase or isocitric dehydrogenase. The following genera failed to utilize the carbon of citrate or lack the enzyme: *Microsporon*, *Coccidioides*, *Oidium*, *Saccharomyces*, *Eremascus*, *Dipodascus*, *Hormiscium*, *Hansenula*, *Thamnidium*, *Emmonsia*, *Rhizopus*, *Sclerotium*, *Sporoblastomyces*, *Neurospora* and *Schizosaccharomyces*.

The following genera actively utilize the carbon of citrate and apparently have the enzyme which promotes growth: *Streptomyces*, *Paracoccidioides*, *Blastomyces*, *Beauveria*, *Pityrosporium*, *Aspergillus*, *Syncephalastrum*, *Mucor*, *Penicillium*, *Coniophora*, *Trichosporon*, *Phoma*, *Gymnoascus*, *Cephalosporium*, *Verticillium*, *Fusarium*, *Isaria*, *Choenophora* and *Pythium*.

The intermediate group constitutes those genera which may or may not utilize carbon in citrate, that is, three of eight *Nocardia* failed to utilize carbon in citrate, fourteen of fifteen *Cryptococcus*, four of ten *Trichophyton*, nine of thirty-nine *Candida*, nine of eleven *Geotrichum*, one of five *Phialophora*, one of three *Fonsecaea*, one of three *Sporotrichum*, two of three *Histoplasma*, one of three *Oospora*, one of two *Cunninghamella* and one of four *Monosporium*.

190 fungi were cultured on 'Herbox' agar medium of pH 7·00. The growth on this medium was compared with that of mycological agar (Difco). The following genera of fungi grow just as well on this medium as on mycological agar, in other words, the growth is very abundant or luxurious: *Trichophyton, Candida, Microsporon, Phialophora, Fonsecaea, Sporotrichum, Paracoccidioides, Blastomyces, Coccidioides, Beauveria, Cryptococcus, Saccharomyces, Pityrosporium, Thamnidium, Aspergillus, Cunninghamella, Mucor, Coniophora, Phoma, Cephalosporium, Gymnoascus, Verticillium, Fusarium, Neurospora, Isacria, Pythium* and *Choenophora*. 27 of 46 genera studied grew very abundantly on Herbox agar medium.

'Herbox Bouillon' cubes are ready made and are inexpensive. If this medium proves to be an adequate medium for the growth of fungi, it will save time and be very economical.

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URICOLYTIC ENZYMES IN SOIL

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ALTHOUGH much work has been done on enzymes in soil, I have been unable to trace any report of the occurrence of uricolytic enzymes. The work reported here has been carried out in an attempt to ascertain whether detectable quantities of uricolytic enzymes are present in soil. News of Durand's[3] paper reached me only when this work had been finished.

Samples of garden soil (Waikanae silt loam) were taken at a depth of 0·5–3 in. immediately adjacent to a fowl run, which, with uric acid so readily available, could reasonably be expected to have correspondingly high uricolytic activity. The fresh soil was sieved through a 2-mm sieve, and stored at – 15° C. The pH of all samples of this soil was 6·6, and the moisture content varied from 17 to 19 per cent. Unless otherwise stated, soil extracts were prepared in the following manner.

Ten-g portions of soil were placed in 250-ml. stoppered conical flasks, and 15 ml. of buffer solution and 10 drops of toluene were added. The flasks were left without shaking for about 16 h at 24° C, and then filtered through a Whatman No. 32 paper. Both increase in temperature and in duration of extraction were found to increase the yield of enzymatic activity extracted from the soil, but for the purposes of this work the procedure already outlined was found suitable.

Two-ml. aliquots of the clear soil filtrate were incubated in 250-ml. conical flasks without shaking for approximately 24–48 h with sodium urate solution (~ 1 mg uric acid/ml.) and toluene. Control systems containing boiled soil extract, urate and toluene were incubated at the same time. Figures in Tables 1 and 2 show the difference between the amount of uric acid remaining in the control and that remaining after incubation with the soil extract which had now been boiled. Figures in Table 3 were calculated from results so obtained. Plating of the extracts on nutrient agar and microscopic examination showed that 0·5 ml. of toluene in the incubated systems suppressed bacterial growth. The uric acid concentrations were estimated according to Brown[1].

During initial experiments, it was found there was a very appreciable non-enzymatic breakdown of uric acid. Cupric copper has been shown to catalyse the auto-oxidation of uric acid in phosphate buffer, other bivalent metals being ineffective[2,3], and care was taken afterwards to exclude extraneous copper by using acid-washed glassware and glass-bidistilled water. Reasonably reproducible results could thus be obtained. Copper extracted from the soil could, however, have influenced results if boiling of the soil extract had changed its activity.

Preliminary experiments using 0·1 M buffers of pH 7·2 showed that efficiency of extraction varied in the ascending order: borate < *tris* < carbonate < phosphate. Accordingly, samples of soil were extracted at 37° C with 0·1 M phosphate buffers over the pH range 6–8·4 for 16 h, and with 0·1 M *tris*-acetate buffers over the pH range 7·5–9·5 for 64 h. A typical set of results appears in Table 1.

Table 1. EFFECT OF 0·1 M *tris* AND PHOSPHATE BUFFERS ON EXTRACTION OF URICOLYTIC ACTIVITY IN SOIL

Buffer used in extraction	pH	Fall in uric acid content (mg)
0·1 M phosphate	6·0	0·09
	6·6	0·65
	6·8	0·80
	7·0	1·25
	7·2	0·20
	7·6	0·15
	8·0	0·32
	8·4	0·88
0·1 M *tris*	7·5	0·19
	8·0	0·13
	8·5	0·33
	9·0	0·09
	9·5	0·05

2 ml. of soil filtrate, 2 ml. sodium urate solution, 2 ml. 0·5 M phosphate buffer of pH 7·0, and 0·5 ml. toluene were incubated without shaking for 48 h at 30° C.

Two pH optima for extraction were obtained at about pH 7·0 and 8·5, suggesting the possibility of the presence of two uricolytic systems.

Soil was extracted with phosphate buffers of pH 6·8 and molarities 2, 1, 0·5, 0·1, 0·05 and 0·01 M for 16 h at 37° C, and 2-ml. aliquots of each filtrate incubated with 2 ml. of urate solution and 0·5 ml. of toluene for 50 h at 30° C. There was little difference in the efficiency of extraction of uricolytic activity with buffers of concentrations between 2 M and 0·1 M, but at still lower molarities the amount of activity extracted fell markedly. This may, however, have been also a direct influence of salt concentration on the enzymatic reaction, not only a difference in extraction efficiency.

Two-ml. aliquots of soil extracts obtained in 0·1 M phosphate buffers of *p*H 7·0 and 8·4 were incubated with urate solution, toluene, and 2-ml. aliquots of 0·5 M phosphate buffers of different *p*H. The *p*H optima of both enzymes appeared to lie about *p*H 7·0. The temperature optima of the enzymes extracted in phosphate buffers of *p*H 7·0 and 8·4 appeared to lie at 30° C.

An examination of the thermal stability of the enzyme extracts was made by preheating the extracts for 15 min at each temperature (Table 2).

Table 2. EFFECT OF HEAT PRETREATMENT ON THE ACTIVITY OF ENZYMES EXTRACTED IN PHOSPHATE BUFFER OF *p*H 7·0 AND 8·4

Temperature of preheating (°C)	Fall in uric acid content (mg) Enzyme extracted at *p*H 7·0	Enzyme extracted at *p*H 8·4
20	0·487	0·345
30	0·553	0·497
37	0·751	0·894
40	0·894	0·959
45	1·530	1·300
50	0·748	0·748
60	0·163	0·244
70	0·00	0·00

Experimental details as for Table 1.

The activity of both the enzymes extracted at *p*H 7·0 and 8·4 rose to a maximum when they had been preheated at 45° C, and fell thereafter. This effect may be a result of activation of the enzymes, or of inactivation of some inhibitor.

A study of the stability of the enzymes extractable with phosphate buffer at *p*H 7·0 and 8·4 showed that when the soil was stored at − 15° C the amount of activity remained reasonably stable over the period of one month; extracts in 0·1 M phosphate buffer stored under the same conditions, however, lost some 50 per cent of their activity.

The effects of some metal chelators, sulphydryl compounds and sulphydryl reagents on the enzymes extracted with phosphate buffers of *p*H 7·0 and 8·4 were investigated (Table 3).

Table 3. EFFECT OF SOME INHIBITORS ON THE ENZYMES EXTRACTED IN PHOSPHATE BUFFER AT *p*H 7·0 AND 8·4

Inhibitor	Concentration in system (M)	Per cent inhibition Enzyme extracted at *p*H 7·0	Enzyme extracted at *p*H 8·4
Sodium cyanide	$2 \cdot 1 \times 10^{-3}$	100	14
Sodium azide	$3 \cdot 4 \times 10^{-4}$	100	86
Sodium diethyldithiocarbamate	$1 \cdot 3 \times 10^{-4}$	98	15
Sodium ethylenediamine tetraacetate	$7 \cdot 6 \times 10^{-4}$	52	20
Cysteine	$1 \cdot 8 \times 10^{-4}$	21	20
2,3-Dimercaptopropanol	$1 \cdot 8 \times 10^{-4}$	53	65
Sodium monoiodoacetate	$3 \cdot 6 \times 10^{-4}$	13	86
p-Hydroxymercuribenzoate	$1 \cdot 4 \times 10^{-4}$	27	93
Mercuric chloride	$1 \cdot 6 \times 10^{-4}$	80	72

2 ml. of soil filtrate, 2 ml. of sodium urate solution, 2 ml. of 0·5 M phosphate buffer of *p*H 7·0 and 0·5 ml. of toluene were incubated without shaking for 48 h at 30° C with 0·1 ml. of inhibitor solution. Controls of the same composition but with boiled soil extracts were incubated at the same time.

The two enzymes differed markedly in their behaviour with the metal chelators sodium diethyldithiocarbamate, sodium ethylenediamine tetraacetate, and sodium cyanide, and the sulphydryl group reagents sodium monoiodoacetate and *p*-hydroxymercuribenzoate.

A brief comparison has been made with the uricolytic activity of soil under permanent pasture. A sample of Taita soil, *p*H 5·2, at a depth of 0·5–3 in. from under grass–clover pasture, and the garden soil were extracted with 0·1 M phosphate buffers of *p*H 7·0 and 8·4 for 18 h at 37° C. Two-ml. aliquots of each filtrate were incubated with 2 ml. of urate solution, 2 ml. of 0·5 M phosphate buffer *p*H 7·0, and 0·5 ml. of toluene for 48 h. Under these conditions, and allowing for the different moisture contents, the pasture soil had approximately one-sixth the uricolytic activity of the garden soil.

It might possibly be concluded that the uricolytic activity in soil is due to organisms producing uricase, and that such organisms are likely to be more plentiful in a soil frequently or continuously supplied with urate.

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SOURCES OF ERROR IN THE WHEAT CYLINDER BIOASSAY FOR AUXIN

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WALKER, Hendershott and Snedecor[1] carried out a statistical investigation of the various sources of random variation in the bioassay of growth-substances separated from tissue extracts by paper partition chromatography. Their bioassay method utilized wheat coleoptile sections, placed in a small, corked assay tube with a chromatogram segment and 1 ml. of sucrose-buffer solution—a method similar to that of Hancock and Barlow[2] as used in this department[3,4].

They reached the following important conclusions:

(1) Variation between coleoptile sections within tubes provided a gross underestimate of experimental error; true replicate samples of the plant material should therefore be used.

(2) The failure of known or unknown growth substances to run consistently with the same R_F in a particular solvent made the greatest contribution to experimental error.

(3) Measurements of coleoptile growth should be used as data, without being transformed to percentages of the 'control' growth.

(4) Reducing the number of coleoptile sections per assay tube from the usual 10 or 5 to 2 or 1 and increasing the number of samples, greatly reduced the variance of the mean and gave the greatest information per hour of work. These last conclusions were based partly on calculation and partly on comparisons between different experiments.

In previous work here (Clark and Heath[4]), estimates of experimental error have been based on triplicate chromatograms, rather than on replicate samples of a rather limited supply of experimental plant material, using the mean of 5 coleoptile sections (from one tube) for each chromatogram segment. We have recently followed Walker *et al.* in examining the effects of using different numbers of sections per assay tube but with known concentrations of the growth regulator, indolyl-3-acetic acid (IAA—sodium salt) instead of segments from chromatograms of tissue extracts. Thus we were able to compare within single experiments the mean growth obtained with different numbers of sections. These experiments at once revealed large and unexpected effects of number of sections, both on the amount of 'control' growth without added auxin and the responses to a range of concentrations of IAA.

Statistical analysis of some of these data showed a highly significant ($P < 0{\cdot}001$) interaction between number (1, 2 or 5 sections per tube) and IAA concentration (0, 0·01, 0·1, 1, 10 or 100 p.p.m.). This took the form that while the single sections gave their greatest growth at 10 p.p.m., with little less at 100 p.p.m., and the pairs grew most at 1 p.p.m., the peak for 5 sections occurred at 0·1 p.p.m. after which the curve fell away to show practically no growth promotion at all at 100 p.p.m. This was later shown to be a consequence of the sections being enclosed in the assay tube, for the responses to IAA concentrations were found to be very similar with different numbers of sections when these were placed in uncorked assay tubes (each with a band of paraffin wax at the mouth to prevent spilling). Further research, to be described in a paper to be submitted to the *Journal of Experimental Botany*, has indicated that accumulation of respiratory carbon dioxide is the major factor causing the interaction found between number of sections and IAA concentration in corked assay tubes.

Heath and Clark had already shown, in their researches on chelating agents, that variations in technique and in sources of wheat seed could alter the pattern of response of coleoptile sections to growth regulators. We have therefore examined the response of a second strain of the variety 'Eclipse', obtained from Wye College, and of a different wheat variety, namely, 'Thatcher', to 0 and 100 p.p.m. IAA when 5 sections were grown in both corked and uncorked tubes. The results of these experiments (which were conducted on separate occasions) are summarized in Table 1.

Table 1. SECTION-LENGTH (CM) AFTER GROWTH FOR 24 H AT 25° C IN 0·5 ML. OF THE SOLUTIONS INDICATED

Initial section length, 1·0 cm. Means of two occasions; four replicate tubes per occasion

Variety	Eclipse (Reading)		Eclipse (Wye)		Thatcher	
Dates	1.9.60 and 11.9.60		24.4.61 and 26.5.61		12.9.60 and 13.9.60	
IAA p.p.m.	0	100	0	100	0	100
Corked	1 89	1·93	1·91	1·87	2·03	1·91
Uncorked	2·02	2·34	2·07	2·52	2 05	2·46

It was clear, therefore, that this effect was a result of variations in technique alone as it could be reproduced with other varieties of wheat. It was also of interest to know whether the effect on final section length of corking the tubes was paralleled by an effect on uptake of IAA by the sections. Measurements were made of the quantity of IAA remaining in 100 p.p.m. solutions after they had supported the growth of different numbers of sections for 24 h. Two methods of colorimetric estimation were used on different occasions; in one the IAA remaining in the solution was estimated 30 min after the addition of a Salkowski reagent[6] and in the other the solutions were partially resolved by paper partition chromatography before the addition of the reagent[6]. The results obtained by these two methods were consistent and in close agreement (Table 2).

Table 2. PERCENTAGE OF IAA REMAINING IN THE ASSAY TUBES; INITIALLY 0·5 ML. OF 100 P.P.M., EQUIVALENT TO 50 µG IAA

Means of two occasions

	Corked tubes		Uncorked tubes	
Sections/tube	5	1	5	1
Resolved solution	44	27	0	5
Unresolved solution	43	29	0	5

The reduced growth of 5 sections in corked tubes was accompanied by a reduced disappearance of IAA at 24 h, but apart from this there was little correlation between IAA disappearance and final section length. In the uncorked treatments (irrespective of whether 1 or 5 sections were present) almost no residual IAA could be detected, which indicated that we were in fact testing the effect of a known quantity of IAA rather than the effect of a maintained external concentration. (If this were incorporated into the section and if it then remained as free IAA, the internal concentration at 24 h, for single sections in uncorked tubes in the 100 p.p.m. IAA treatment, would be greater than 1 g IAA/kg fresh weight of coleoptile tissue.) As the method was developed for the assay of growth substances in small quantities of tissue extracts it is undesirable to increase the volume of solution used.

The main object of the investigation was to determine which was the most sensitive and useful method for assaying growth substances, and therefore concentration-response curves were obtained for each number of sections in both corked and uncorked assay tubes. For each number of sections (1, 2 or 5) a separate factorially designed experiment was carried out in 5 blocks of occasion; on each occasion 6 concentrations of IAA (ranging from 0 and 0·01 p.p.m. to 100 p.p.m.) were tested in both corked and uncorked assay tubes with four replicate tubes. The results for the two extreme cases (1 and 5 sections) are

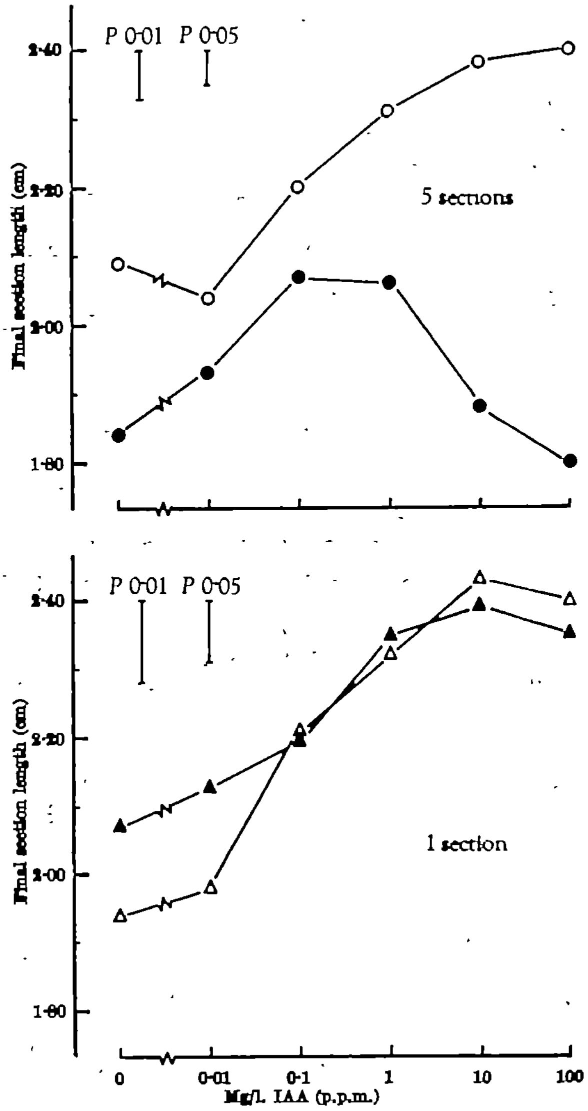

Fig. 1. The responses of 1 (▲ △) or 5 (● ○) sections to a range of IAA concentrations, in corked (▲ ●) and uncorked (△ ○) assay tubes after 24 h at 25° C. Initial section-length was 1·0 cm. Each point represents the mean of five occasions (between September 20, 1960, and November 16, 1960) with four replicate tubes on each. Least significant differences between two means are shown for P 0·05 and P 0·01

presented in Fig. 1. The results for 2 sections were intermediate in character but corresponded more closely to the curves for 1 section than to those for 5 sections. It is apparent that the range of growth-promoting concentrations is increased by the use of either uncorked tubes or fewer sections in corked ones. Walker *et al.*, on the basis of statistical considerations, recommended the use of fewer sections in each assay tube and on empirical grounds we also have reached this conclusion. The choice therefore is between 1 or 2 sections in either corked or uncorked assay tubes.

We have found that the accumulation of carbon dioxide can affect the growth response to IAA and it is evident from Fig. 1 that it can also affect the 'control' growth but in a more complex manner, for example, the respiratory carbon dioxide from a single section can give rise to a significantly greater section-length at 24 h, when it is allowed to accumulate in a corked tube. This effect reduced the magnitude of the IAA-induced growth in excess of the 'control'.

The main disadvantages of uncorked tubes would appear to be that the sections are exposed both to fluctuations in the composition of the atmosphere of the room in which the assay is performed, and to a slowly diminishing solution volume due to evaporation. This last should tend to maintain the external IAA concentration, which is not constant but is decreasing due to disappearance of IAA, presumably into the sections. Statistical analyses of the data showed that the error variance was no greater for uncorked tubes and when single sections were used the error variance was significantly less than in corked ones ($P < 0.05$).

From the significance-levels shown in Fig. 1 it can be seen that there is some loss of accuracy with single sections, but this can be improved, as Walker *et al.* pointed out, by increasing the number of replicate tubes. They recommended that either four replicate tubes with 2 sections in each or five replicate tubes with 1 section in each should be used at the discretion of the individual. We would endorse this view but would add that in our particular method the most sensitive technique would utilize single, 10-mm long sections in each replicate uncorked tube.

In certain circumstances it may be necessary or more convenient to use techniques in which the free diffusion of gases is restricted, for example, in either corked assay tubes or covered Petri dishes. In such techniques the quantity of carbon dioxide accumulated could be reduced by utilizing either shorter sections or shorter assay periods in conjunction with more accurate measuring techniques; by conducting the assay at a temperature lower than 25° C; by opening the vessels at intervals or by increasing the volume of enclosed air. Varying any of these factors could conceivably lead to considerable differences in response of coleoptile sections to growth regulators and such variations in technique may account for some of the observed discrepancies between different authors' results.

Of relevance in this connexion are the data of Barlow and Hancock[7], who observed effects on growth, with corked or uncorked tubes and 5 sections, essentially similar to those reported here. They concluded, however, that the relative effects of IAA concentration on final length or growth rate were similar in both corked and uncorked tubes. The data published in their Fig. 4 do not seem to support this statement, although the magnitudes of the differences were smaller at their lower assay temperature of 20° C.

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EFFECT OF ANTI-PLATELET ANTIBODIES ON THE *IN VITRO* UPTAKE OF 5-HYDROXYTRYPTAMINE BY BLOOD PLATELETS

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IT has long been known that blood platelets are antigenic and that anti-platelet antibodies produce shortening of the platelet life-span *in vivo* and agglutination and lysis of the platelets *in vitro*[1–3]. Further manifestations of platelet injury by antibody have more recently been shown by inhibition of clot retraction and by reduction of thromboplastin generation[4]. These have been important additions to the study of the various actions of antibodies on the platelets and have led to a better understanding of thrombocytopenic disorders in human beings. They have provided, however, but little knowledge of the profound biochemical changes that occur in the platelets when these are attacked by antibody. Investigations on this subject have begun in our laboratory. It is known that *in vivo* as well as *in vitro* blood platelets take up 5-hydroxytryptamine (5-HT, 'Serotonin') rapidly by an active transport mechanism partially linked to their content of adenosine triphosphate (ATP) (refs. 5–8). It was thought that antibodies acting on the platelets might inhibit this phenomenon. This article describes the results of experiments designed to test this hypothesis.

In these studies, an *in vitro* system was used in which various antibody-containing sera were added to samples of normal human platelet-rich plasma and incubated at 37° C in the presence of 5-HT labelled with carbon-14. Platelet radioactivity was determined at various intervals and taken as the measure of the platelet uptake of 5-HT.

The effect of anti-human-platelet serum prepared in the rabbit was first tested. The platelet agglutinin titre of this serum was 1/200 when measured by the technique of Dausset *et al.*[9]. The serum was added in the amount of 0·2 ml. to each aliquot of 1·8 ml. of citrated human platelet-rich plasma (platelet count 350,000–400,000/mm^3). (All platelet counts were done by the technique of Brecher and Cronkite[10].) After the subsequent addition of 0·2 ml. of a saline solution of labelled 5-HT (5-hydroxytryptamine (β-aminoethyl-β-C^{14}) (creatinine sulphate complex), Nuclear Chicago) (50 µg of ^{14}C-5-HT/ml. saline), the samples were incubated for various intervals at 37° C. Platelet buttons were obtained by centrifugation, washed once with normal saline, and re-suspended in 2 ml. of distilled water. The platelets were then disrupted by repeated freezing and thawing and the debris deposited by centrifugation. Aliquots of 1·5 ml. of the supernatants were dried on steel planchets and their radioactivity measured in an end-window gas-flow counter. Two sets of controls were prepared by replacing the heterospecific antiserum either with equal amounts of (1) serum from

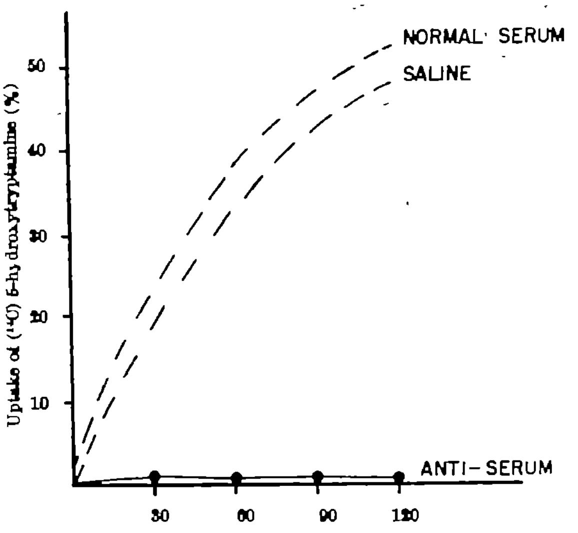

Fig. 1. The *in vitro* uptake of 5-hydroxytryptamine labelled with carbon-14 by normal human platelets was blocked by the action of a heterospecific antiserum

non-sensitized rabbits, or (2) saline. The platelet uptake of 5-HT in test samples and controls was derived from the platelet radioactive-counts and expressed in per cent of the total radioactivity added.

The platelet uptake of 5-HT in the controls increased progressively reaching the maximum average value of 47 per cent (41–52 per cent) between 90 and 120 min. In the controls containing 0·2 ml. of saline, platelets had similar values of 5-HT uptake as in the controls prepared with 0·2 ml. of normal rabbit serum. When, however, the heterospecific antiserum was used, the platelet uptake of 5-HT was consistently less than 1 per cent. One of such experiments is illustrated in Fig. 1.

The factor present in the heterospecific antiserum which was capable of blocking the *in vitro* uptake of 5-HT by normal human platelets was sensitive to dilution. When 0·2 ml. of progressively diluted samples of antiserum were used, the inhibiting effect was progressively reduced and no inhibition of 5-HT uptake was noticed when the total dilution of the antiserum in the system reached 1/880. Furthermore, these experiments showed that agglutination of the platelets at the end of the incubation period could be seen under the phase-contrast microscope only in the test samples with higher concentrations of antiserum (1/11–1/110). With dilutions of 1/220–1/440 there was no agglutination of the platelets, while there was still a marked reduction of the 5-HT uptake (11–29 per cent uptake).

The specificity of the reaction between the antiserum and the platelets was later demonstrated. Five absorptions of the antiserum with 'pure' preparations of normal human platelets almost completely removed the 5-HT inhibiting factor. (The saline platelet suspension used for the absorption of the antiserum contained 8·2 × 10⁶ platelets/mm³ while the contaminating white cells were less than 25 and the red cells less than 100/mm³. Each absorption of 1 ml. of antiserum was done with 0·25 ml. of platelet suspension.) This demonstration was necessary since it is known that antigen–antibody reactions not directed towards the platelets can cause release of 'Serotonin' from the platelets[11].

From these experiments it was concluded that since depression of 5-HT uptake by normal human platelets in contact with heterospecific antiserum occurred even in the absence of visible platelet agglutination, it was therefore not the consequence of platelet clumping but rather the expression of a specific metabolic damage produced by the antiserum directly on the platelets.

A second series of experiments was performed by the use of serum from four patients who had received multiple blood transfusions. It is known that multi-transfused patients often become iso-sensitized to blood platelets[12]. Iso-sensitization to platelets in the four patients was demonstrated by the remarkable shortening of the survival-time of isologous platelets as measured by the chromium-51-labelling technique[13], while the patients themselves had either a normal platelet count or had amegakaryocytic thrombocytopenia (Table 1). The experimental system used in the examination of the human sera differed somewhat from that adopted in the investigations with heterospecific antiserum. 1 ml. of patient's serum was added to each 1 ml. aliquot of normal platelet-rich plasma obtained by differential centrifugation of citrated venous blood from healthy donors. (In each 'Serotonin' experiment, platelets from the same donor were used as for the platelet survival study.) After addition of 0·2 ml. of the solution of 5-HT labelled with carbon-14, the samples were incubated at 37° C for various intervals. Two sets of controls were used, one in which the patient's serum was replaced by donor's serum, and the other in which the donor's platelet-rich plasma was replaced by the patient's own platelet-rich plasma.

Results of these experiments are summarized in Table 1. The serum from all four of these sensitized patients demonstrated a variable, but often marked effect in depressing the *in vitro* uptake of 5-HT by donor's platelets, while when in one experiment the patient's serum was used with the patient's own platelet-rich plasma, no depression in 5-HT uptake was observed. It was therefore evident that the 5-HT inhibiting factor present in the serum from multi-transfused patients behaved as an iso-antibody, being effective on the donor's platelets and ineffective on the patient's own platelets. Furthermore, it was demonstrated that the serum factor inhibiting the 5-HT uptake by donor's platelets could be removed almost completely by three absorptions with donor's platelets. (For the absorption experiments platelet concentrates in saline were used containing 5·9 × 10⁶ platelets/mm³ with a white cell count of less than 25/mm³ and a red cell count of less than 100/mm³. 1 ml. of serum was absorbed each time with 0·25 ml. of platelet concentrate.)

In all the experiments performed with the sera from the iso-immunized patients, no agglutination of the platelets could be seen under phase-contrast microscope, and no lysis of the platelets occurred as demonstrated by the unchanged platelet counts in the various samples before and after incubation. Furthermore, when the above four sera were tested for platelet iso-agglutinins by the method of Dausset *et al.*[9] a positive result was obtained with only two sera. This finding indicated a high degree of sensitivity of the 5-HT method in the detection of platelet iso-antibodies.

Further experiments were performed using sera from six patients with acute or chronic idiopathic thrombocytopenic purpura. The bone marrow in all six patients was abundant in megakaryocytes, while the platelet counts were greatly reduced (8,000–62,000/mm³). These cases are considered by many observers to be auto-immune.

Table 1. SEROTONIN UPTAKE TEST WITH SERA FROM MULTI-TRANSFUSED PATIENTS

Patient	Disease	Platelet count/mm³	No. of blood trans.	Uptake of 5-HT (per cent) Donor platelet-rich plasma Patient serum	Donor platelet-rich plasma Donor serum	Patient platelet-rich plasma Donor serum	Patient platelet-rich plasma Patient serum	Survival time of donor platelets in patient* (h)
L. C.	Aplastic anæmia	20,000	22	18·6	42·3	—	—	30
M. B.	Leukæmia	45,000	17	28·0	44·2	—	—	16
M. L.	Blood loss anæmia	340,000	29	9·8	40·2	39·0	37·6	8
S. Q.	Refractory anæmia	60,000	12	25·2	34·3	—	—	22

* Normal value 8–10 days.

Table 2. Serotonin Uptake Test with Sera from Idiopathic Thrombocytopenic Purpura Patients

Patient	Platelet count/mm^3	Uptake of 5-HT (per cent) Donor platelet-rich plasma: Patient serum	Uptake of 5-HT (per cent) Donor platelet-rich plasma: Donor serum
T. G.	8,000	35·6	32·9
J. M.	40,000	37·3	38·9
B. F.	10,000	42·4	44·0
A. M.	24,000	32·7	35·1
H. M.	38,000	33·5	36·1
S. M.	62,000	40·9	48·1

with auto-antibodies attacking the platelets in the circulation and resulting in shortening of the platelet life-span[14,15]. The serum from these patients, presumably containing an anti-platelet auto-antibody, exerted no effect on the 5-HT uptake of normal donor's platelets (Table 2). The negative result was repeatedly confirmed with platelets from various donors. It was concluded, therefore, that the anti-platelet factor presumed to be present in the serum of patients with idiopathic thrombocytopenic purpura injured the platelets through a different mechanism than did hetero- and iso-antibodies, or resulted in the development of thrombocytopenia by some other more indirect process.

It is believed that the inhibiting effect induced by specific hetero- and iso-antibodies on the *in vitro* uptake of 5-HT by blood platelets may be a useful indicator in further studies on the complex biochemical changes produced by antibodies on blood platelets.

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ETHYLENE PRODUCTION BY SUBCELLULAR PARTICLES FROM RAT LIVER, RAT INTESTINAL MUCOSA AND *PENICILLIUM DIGITATUM*

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It has been reported that all fruits, without exception, give off ethylene[1]. While the production of ethylene by fruits and certain micro-organisms has been the subject of extensive investigation over the past twenty-five years, the possible role of this compound in the metabolism of animals has been virtually ignored. Recently, Kakanov[2] provided evidence that both normal rats and pig ascarids produce ethylene. Although no attempt was made to remove ethylene from air supplied to the respiratory chambers or to maintain aseptic conditions, Kakanov's experiments were nevertheless interesting, particularly since rats with transplanted subcutaneous sarcoma, subcutaneous abscess or which had received a subcutaneous injection of aluminium oxide exhaled more ethylene than healthy rats.

With knowledge of the site of ethylene production in fruit[3,4] and a method for activation of the ethylene-producing system[4], a more direct approach to the question of ethylene production by animals and micro-organisms was made in our laboratory by attempting *in vitro* preparations capable of evolving the gas. In this article, mass spectrometric and gas chromatographic evidence of ethylene production by particulate fractions from rat liver, rat intestinal mucosa and *Penicillium digitatum* is given.

About 50–60 g fresh weight of liver or 2·5–3·5 g intestinal mucosa pooled from 4–6 rats were used in each experiment. The Wistar rats were killed by decapitation prior to the experiments. A 10-cm loop of the small intestine posterior from the pyloric sphincter was dissected, washed with demineralized water and blotted dry. The mucosa was squeezed from the gut with a spatula.

Penicillium digitatum (Sacc.) NRRL 1203 was grown in stationary culture under aseptic conditions. The growth medium[5] contained glucose (25·7 g), ammonium nitrate (4 g), potassium dihydrogen phosphate (13·61 g), magnesium sulphate (1·23 g), ferric nitrate (5 mg) and 1 ml. of Pratt's micro-element solution[6], dissolved in 1 litre of distilled water. The *p*H of the medium was 4·4. After two weeks, the growth (about 25 g wet weight) was gathered and either used immediately or frozen. The *p*H of the medium when gathered was 4·2.

To prepare subcellular particles from rat liver and intestinal mucosa, the tissues were homogenized in ice-cold 0·88 M sucrose (1 g wet weight per 10 ml. suspension) at low speed in the Servall 'Omnimixer'. The *p*H of the homogenate was maintained at 7·0–7·2 by the addition of 0·1 N potassium hydroxide. Particles from *Penicillium digitatum* were obtained from a homogenate of the fungus in 0·5 M sucrose–0·1 M potassium dihydrogen phosphate buffer of *p*H 7·0 (1 : 10 w/w). All homogenates were centrifuged at 4,000*g* for 7 min. The sediment was rejected and from the clear supernatant particles were separated by centrifugation at 12,725*g* for 15 min. The particles were carefully suspended in 25 ml. of reaction mixture (Table 1) and tested for ethylene production over 2- and 18-h periods at 25° C by gas chromatographic and mass spectrometric methods described earlier[4]. When particles were sonically treated, they were exposed for 4 min at 2° C to a Raytheon sonic oscillator (10 kc/s) tuned to 1·1 amp. Microscopic examination of the particulate fraction from *Penicillium digitatum* revealed the presence of intact spores and some incompletely fragmented mycelia in these preparations, which were found to be viable. Untreated particles from the animal tissues were stainable with janus green *B*.

Table 1. Ethylene Production by Subcellular Particles from Rat Liver, Rat Intestinal Mucosa and *Penicillium digitatum*
(μl. × 10^6/mg nitrogen/2 h)

Run No.	Treatment	Rat liver	Rat intestinal mucosa	*Penicillium digitatum*
1	Untreated	5·0	15·2	12·5
2	Untreated	8 0	16·2	—
3	Sonically treated	9·5	25·8	13·2
4	Sonically treated	13·0	13·8	31·6
5	Sonically treated	—	12·5	23·6

Female rats, 10 weeks old, were used.

Reaction mixture: 0·5 M sucrose, 0·125 M KH_2PO_4, 0·05 L-malic acid, 10^{-3} M $MgSO_4$, 10^{-3} M $MnSO_4$; 1·93 × 10^{-3} M adenosine triphosphate and 3 3 × 10^{-4} M diphosphopyridine nucleotide were added after sterilization of the mixture; sonication was performed prior to ATP addition; *p*H was adjusted to 7 with sodium hydroxide.

The results of our initial trial experiments with tissues from female Wistar rats, 10 weeks old, and *Penicillium digitatum* are given in Table 1. It is evident that particles separated at 12,725*g* from rat liver, rat intestinal mucosa and the fungus produced ethylene. Many steps in the preparation of these fractions could not be performed quantitatively and variation in nitrogen content of particulate fractions from different runs occurred. Thus, quantitative duplication of results from different runs was not to be expected. These initial experiments, along with mass spectrometric analyses which confirmed the identity of the gas as ethylene, served to show that subcellular fractions from sources other than the tissues of higher plants produce ethylene.

In our earlier work[4] with subcellular particles from tomatoes, we had shown that production of ethylene was related to the disintegration of the structure of the mitochondria. In the experiments reported here, to test the applicability of this finding to animal and fungal tissues, comparisons were made, within a single run, of ethylene production by particles which were untreated, sonically treated or aged *in vitro*. It should be noted first that some fragmentation of the particles probably occurred when the tissues were originally ground in the 'Omnimixer'. This is consistent[7] with the observed lack of lag-period in carbon dioxide production by the particles. Ethylene was also produced by a fraction separated at 40,210*g* for 15 min, which would contain fragments of both mitochondria and endoplasmic reticulum. It is evident from Table 2 that ageing of the untreated particles or sonic treatment of them increased ethylene production. Thus, as with tomatoes[4], ethylene production by subcellular particles from rat liver, rat intestinal mucosa and *Penicillium digitatum* appears to be stimulated by disintegration of the particles.

Table 2. EFFECTS OF *in vitro* AGEING AND SONIC TREATMENT ON ETHYLENE PRODUCTION BY SUBCELLULAR PARTICLES FROM RAT LIVER, RAT INTESTINAL MUCOSA AND *Penicillium digitatum*

		Ethylene (μl. × 10^{6}/mg nitrogen)						
		Rat Liver		Rat intestinal mucosa			*Penicillium digitatum*	
Treatment	Time (h) Run No.	1	2	3	4	5	6	7
Untreated	0–2	2·2	2·0	5·6	5·3	Trace	Trace	7·2
Untreated	2–18	2·7	4·3	12·4	11·8	7·6	10·5	8·0
Sonically treated	0–2	5·1	4·8	36 4	15·9	38 0	22·7	18·0
Sonically treated	2–18	2·2	2·2	52·4	23·5	15·0	31·0	24·0

Male rats, 16 weeks old, were used.
Reaction mixture as in Table 1.

The foregoing experiments, together with others[8] in which we have observed the presence of ethylene in exhaled gas from human subjects, lead us to suggest that the production of ethylene may be a general phenomenon of life processes and that its metabolic role may be a question of fundamental importance.

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A POSSIBLE MECHANISM OF ACTION OF CALCIUM AND SOME PSYCHOTOMIMETIC AGENTS ON MEMBRANES

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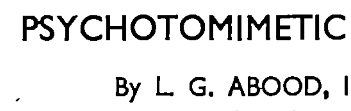
IN the course of investigating the mechanism of action of a group of anticholinergic psychotomimetic agents, it was observed that the agents seemed to influence those properties of excitable tissues that were particularly sensitive to a depletion of Ca^{2+}. The importance of Ca^{2+} in the maintenance of excitability in nerve and muscle has been recognized since 1883 [1], but it was only recently that there has been a revival of interest in it, following the observation of Hodgkin and Keynes[2] that an increased influx of Ca^{2+} accompanied excitation of the squid giant axon. This article discusses the types of Ca^{2+}-dependent systems in excitable tissues which are influenced by the agents, and the possible mechanisms involved.

The chemistry and pharmacology of the agents concerned within the present study are described elsewhere[3]. They encompass a wide variety of esters of heterocyclic imino and di-imino alcohols and substituted glycolic and other carboxylic acids. An example of this class, and that utilized in the present investigation, is *N*-methyl-2-pyrrolidyl methyl *cyclo*pentylphenyl glycolate (PMCG):



Resting potentials were recorded in single fibres of freshly dissected frog sartorius muscles with the glass capillary microelectrode (Ling–Gerard type) filled with 3 M potassium chloride and having a resistance of 20 megohms. A pair of muscles were first soaked in Ringer's solution for 30 min before the resting potentials of each muscle were determined. One muscle was then transferred to a solution of calcium-free Ringer's solution and the resting potentials were measured after spontaneous twitching ceased 40 min later.

The experimental muscle was simultaneously transferred to a calcium-free Ringer solution containing 10^{-4} M PMCG and the resting potential also recorded 40 min later. Comparable experiments were conducted in calcium-free Ringer's containing 4 mM EDTA. A set of experiments was performed where 10^{-4} M PMCG was present in the test solution prior to the immersion of the experimental muscle.

When frog muscles are transferred from a Ringer's to a calcium-free solution, there is a rapid drop in the intrafibrillar resting potential which results in spontaneous twitching, or fasciculation, of the muscle. Both the decrease in the resting potential and the fasciculations can be prevented if the calcium-free solution contained PMCG (Table 1). In the presence of the drug, the resting potential could be maintained at almost normal levels. After the resting potential has dropped 50 per cent in a calcium-free medium, it could be restored to almost normal by the addition of 10^{-4} M PMCG to the calcium-free medium. In this respect, the action of PMCG in restoring the resting potential is similar to that of Ca^{2+} and other bivalent alkali cations. It should be mentioned that similar effects could be demonstrated with a large number of homologues of PMCG.

The action of PMCG and Ca^{2+} appear to be similar in a number of respects. Both cations can form poorly dissociable salts with such acids as EDTA, phosphoric, and

Table 1

		Drug at zero time		Drug present 40 min later	
		Resting potential (−mV)	% Diff.	Resting potential (mV)	Reversal (%)
Ringer's	C	90 ± 3	—	91 ± 3	—
	PMCG	89 ± 3	−1	91 ± 4	0
Ca-free	C	65 ± 5	—	70 ± 6	—
	PMCG	80 ± 4	23 (89)	82 ± 5	12 (91)
Ca-free	C	44 ± 6	—	45 ± 5	—
EDTA	PMCG	74 ± 5	67 (82)	55 ± 5	22 (61)
NaCl	C	60 ± 3	—	61 ± 4	—
	PMCG	81 ± 4	29 (90)	74 ± 3	47 (82)

The effect of PMCG on the intracellular potential of frog sartorius muscle exposed to various solutions. The results are expressed as the mean ± standard deviation. The values in the parentheses refer to the per cent of the normal resting potential (−90 mV). The concentration of PMCG at zero time was 10^{-4} M and, when added 40 min later, 10^{-4} M.

citric, as evidenced by the fact that the acids interfere with the adsorption of the cations to a variety of substances including frog nerve, muscle and skin, as well as brain mitochondrial membrane and lipid monolayers. The adsorption of both Ca^{2+} and PMCG to frog sartorius muscle increases experimentally as the pH increases between 4 and 10. When freshly dissected sartorius muscles are exposed to isotonic saline, they immediately begin to fasciculate, while the lactate production increases five-fold. On the gradual addition of either Ca^{2+} or PMCG at equimolar concentrations to the saline solution, a similar pattern of decrease in the concentration-activity curve is obtained for both the frequency of twitching and lactate production.

On the basis of such studies with various biological preparations it seemed likely that one was dealing to a large extent with surface phenomena; so that it might be possible to demonstrate a similarity in the action of Ca^{2+} and PMCG on the physical properties of interfacial films. Preliminary studies have been conducted with lipid interfacial films, while more extensive studies involving complexes of phospholipids and crystalline proteins are in progress. The addition of 10^{-4}–10^{-6} M PMCG to a monolayer of either stearic acid or dipalmitylphosphatidyl choline will increase the rigidity of the monolayer in a manner similar to Ca^{2+}. At higher concentrations (10^{-3} M) the surface active properties of PMCG become manifest so that the effect is one of dispersion of the monolayer and reduction in the surface pressure resulting from the interaction of PMCG and the monolayer. It is of significance that the concentration range in which PMCG acts like Ca^{2+} on the monolayer is the range in which it restores the resting potential of sartorius muscle fibres. PMCG at concentrations in excess of 10^{-4} M will inhibit the adsorption of Ca^{2+} to monolayers of lecithin, and the inhibition appears to be of the competitive type.

The dispersity of anionic lipophilic colloidal suspensions is increased by the loss of divalent cations; and the preservation of a normal colloidal state in biological systems is dependent on a balance of monovalent and divalent cations. The ability of Ca^{2+} to impart rigidity to colloidal dispersions consisting of proteins and phospholipids is to a large extent attributable to its capacity to combine with acidic carboxylic and phosphate groups[4]. The addition of even trace amounts of polyvalent metals to monolayers of stearic acid and other substances containing carboxylic acid groups will impart rigidity by occupying two or more end groups[5]. Under certain conditions this effect can be antagonized by monovalent cations.

Harkins[6] has shown that crystalline proteins, such as catalase and cytochrome c, will form a monolayer of spherical or ellipsoidal molecules with barium stearate. In the absence of Ba^{2+} or a similar cation such a monolayer is incapable of forming. Interactions between lipids and proteins can occur through van der Waals forces between the non-polar hydrophobic residues of the fatty acids and similar functional groups in the hydrophobic face of the protein molecule. Combination can also occur as a result of hydrogen bonding, electrostatic attraction between oppositely charged groups of proteins and lipids, and adsorption of the hydrated polar molecules on to the highly hydrated protein molecules.

The lipophilic hydrocarbon residues of an air/water interfacial lipid film are arranged in closely packed parallel arrays, normal to the surface, while the hydrophilic polar tail is projected into the aqueous medium. Despite the length of the hydrocarbon chain and the restricted translational freedom, there exists considerable freedom of rotation about the long axes of the molecules[7]. In addition to diminishing the hydrophilia of the polar tails Ca^{2+} or Ba^{2+} can, by combination with adjacent acidic groups, restrict rotational freedom. There is a third factor which may be involved in explaining the increased cohesiveness Ca^{2+} imparts to the interfacial film, and that is the diminution in repulsion of adjacent molecules brought about by the neutralization of anionic groups. Finally, the van der Waals attraction between adjacent units in the monolayer is increased since the molecular weight of the repeating units is doubled.

It has been postulated that during membrane depolarization there is a loss of Ca^{2+}, along with various phosphates, and that the displacement of Ca^{2+} from the lipoprotein membrane results in the structural rearrangement responsible for altering membrane permeability to such ions as Na^+ and K^+ (ref. 8). If it is assumed that surface Ca^{2+} forms complexes with the phosphates groups of phospholipids or ATP-protein complexes, the replacement of the poorly hydrated Ca^{2+} by the well-hydrated Na^+ would increase the hydrophilic character and diminish the rigidity of the biomolecular lipophilic structure of the membrane. Simple displacement of Ca^{2+} from bound sites could result in a net inflow of water since the dissociated Ca^{2+} is more than twice as greatly hydrated as Na^+. Removal of Ca^{2+} with complexing agents such as EDTA and citrate leads to an analogous situation. The addition of numerous bivalent alkali earth metals as well as agents such as PMCG will restore a depolarized calcium-deficient membrane to its resting, electrically excitable state. Agents such as PMCG may act by virtue of their ability to complex with phosphate groups of both lipids and proteins with the net result that the cohesiveness and the lipophilic-hydrophilic balance of the membrane is partly restored. It must be pointed out, however, that it has not been possible as yet to duplicate the present experimental findings when frog spinal nerve roots or ganglion cells were used in place of sartorius muscle. Whether or not this difference is largely a matter of technique or is due to the more complex nature of nervous tissue is not known.

The interaction of PMCG with an excitable membrane, however, must obviously be quite different from that of Ca^{2+} since the polar–nonpolar aspects of the drug would permit a diversity of interactions with a complex lipoprotein membrane not possible with Ca^{2+}.

The objective of this investigation has been to explore the similarity and interrelationship of the two substances, and it remains to determine the unique physico-chemical properties of this class of agents underlying their mechanism of action. It is hoped that such investigations may not only help elucidate the mechanism of action of the drugs but also aid in the investigation of the properties of excitable membranes.

This work was supported jointly by grants from the U.S. Army Chemical Research and Development Laboratories, *U.S. Da Cml* 18–108 *G*41, and the Mental Fund, State of Illinois Project 1747.

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LETTERS TO THE EDITOR

PHYSICS

Particle Structure

It has been shown[1,2] that an empirical model of Nature exists in which many natural constants equal functions of π. The dimensional units are defined by the equations $C = 1$ and $\bar{\lambda} = \lambda_e = 2$ (λ_e is the Compton wave-length of the electron). Charge squared then equals $2a$ (a is the fine structure constant) and $M\lambda$ equals 4π (M is mass).

Particle structure can be described in terms of the properties of the particle and the constants characterizing the particle. In this suggested model the formation of the electron ($\mp\ \mp$) and the positron ($\pm\ \pm$) involves the combination of three units of rest mass π and field $2\exp - \pi^2/2$ to give a particle with rest mass 2π and a charge squared equal to $2\exp(-\pi^2/2 + 2\exp(-\pi^2/2 + 2\exp - \pi^2/2))$ or $2a$ (ref. 2). The energy of combination equals π. There are now two components each contributing a mass of π and a charge squared of a. Rest mass and energy are interconvertible but charge is constant and therefore conversion of energy to rest mass will only increase the rest mass of a component.

In the formation of the charged π meson the mass of the components equals $M\lambda/e^2 = 4\pi/2a$:

$$4\pi/2a + 4\pi/2a \rightarrow (8\pi/2a - 2\pi) + \text{energy}\ (2\pi)$$

Energy of combination equals 2π, indicating meson formation corresponds to a strong interaction similar to that involved in electron formation.

If components of opposite sign for charge and mass combine the result is a particle of zero mass and zero charge or a neutrino.

The charged π meson decomposes into a muon and a neutrino. The neutrino formed could have meson components or it could be a double neutrino since it has unique properties[3]. The masses involved are:

$$(8\pi/2a - 2\pi) \rightarrow (6\pi/2a + 2\pi) + 2(\pi/2a - 2\pi)\ \text{energy}$$

There appear to be two neutrino components or two neutrinos as indicated by the energy term.

Formation of the neutral π meson involves components of opposite charge:

$$(\mp) + (\pm) \rightarrow (\mp\ \pm) = \pi^0\ \text{meson}$$

$$4\pi/2a + 4\pi/2a \rightarrow [8\pi/2a - (1/2a - 2\pi)] + \ \text{energy}\ (1/2a - 2\pi)$$

Loss of energy on particle formation involves the charge factor since charge neutralization occurs.

Resonances may correspond to rest masses, for components, which are multiples of $4\pi/2a$. Combinations of these components may give particles of high mass and a very short life.

Elementary particles may combine to give higher mass particles. Helium is an example. The charged K meson may be a similar combination:

$$4(8\pi/2a - 2\pi) - (4\pi/2a - 1/2a)\ \text{energy} = K\pm$$

The neutral K meson has two distinct lifetimes involving two particles of slightly different masses. This suggests the dual formulation:

$$4(8\pi/2a) - [4(\pi/2a - 2\pi) - (1/2a \pm 2\pi)]\ \text{energy} = K^0$$

There are alternative possibilities, but this shows the various mesons can be accounted for with little deviation from accepted ideas. In all cases the calculated and observed rest masses differ by less than one electron mass. The model can be extended to hyperons[1]. The life of the particles may be related to the mass terms representing the particles, thus making possible extension of the model to these constants.

A simple model of Nature exists which is in good numerical agreement with many observations and which is readily expandable to include other observations.

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Neutron Capture in the Samarium Isotopes and the Formation of the Elements of the Solar System

Recent theories concerning the formation of the elements are based on neutron capture, and are subject to quantitative experimental tests through radiative capture measurements[1-3]. This communication reports 30 keV neutron capture measurements for the isotopes of samarium. 2–20-g samples of electromagnetically separated samarium isotopes as Sm_2O_3 were used with the *ORNL* 3-MV pulsed Van de Graaff, providing the neutrons via the lithium-7 (p,n) reaction. A Moxon-Rae counter detected the capture γ-rays as in an earlier tin isotope measurement[3].

The isotopes samarium-150 and -148 are shielded from r-process[1] production by stable neodymium-150 and -148. Thus the s-process only should have contributed to their creation in stars, and the products of their neutron capture cross-sections near 30 keV and their natural abundances should be nearly equal. From the measured cross-sections we find the ratio (Table 1, col. 5) to be 0·94 with a relative uncertainty estimated at 10 per cent. Combining additional data for the ratio only, we get 1·02 (± 6 per cent). The expected average ratio in the mass region 120–200 is perhaps a 1 per cent decrease per mass unit[4], or 0·98 for this case. The results thus strongly confirm the s-process nucleosynthesis prediction. The r-process abundances derived for the other isotopes are in reasonable agreement for the odd and even isotopes separately but show an unexpectedly large odd–even effect. The number (N_r) for samarium-152 was derived ignoring the minor s-process branch (β^- decay of 93 yr. samarium-151); however, a large but uncertain percentage of the branch returns to samarium-152. Thus the r-process abundance given in Table 1 for samarium-152 could be increased by a few per cent. resulting in even closer agreement with N_r of samarium-154.

The samarium-149 precursor of samarium-150 in the s-process additionally has one of the few extremely large thermal neutron cross-sections (40,800 barns at 0·0254 eV). That for samarium-147 is about 500 times smaller (90 barns). This ratio is even larger in the neighbourhood of the 0·1 eV resonance. The ratio remains greater than 400 for neutron temperatures up to 2,000° absolute. Thus the present results leave little possibility for significant neutron exposure in solid[2] or even molten bodies of solar system material either terrestrial or, as Murthy and Schmitt have recently shown[5], for representatives of the chondrites, carbonaceous chondrites and achondrites.

If we assume $N_s\sigma_c$ to be constant from atomic weight A = 148 to A = 150 the selective depletion (via thermal neutron capture) of samarium-149 should be apparent in the residual abundance assigned in Table 1, column 7, to the r-process. As the thermal cross-sections of samarium-148 and samarium-150 are comparable with that of samarium-147 or smaller[4], perhaps a

Table 1. NEUTRON CAPTURE IN THE ISOTOPES OF SAMARIUM

Samarium isotopes at 30 (±7) keV

A	N(Atom %)	Class	σ_c(mb)	$N_s\sigma_c$	N_s	N_r	A
144	2·87	p(m)	119 ± 55				
147	14·94	rs	1,173 ± 192	(2,850)	2·4 ± 0·4	12·5 ± 0·4	147
148	11·24	s–0	258 ± 48	2,900 ± 540			
149	13·85	rs	1,622 ± 279	(2,850)	1·8 ± 0·3	12·1 ± 0·3	149
150	7·36	s–0	370 ± 72	2,770 ± 535			
152	26·90	rs	411 ± 71	(2,850)	6·9 ± 1·0	20·0 ± 1·0	152
154	22·84	r–0	325 ± 61	0	0	22·84	154

The production process (column 3) is from ref. 1. The r-process abundances are derived by assuming $N_s\sigma_c$ constant for all the isotopes of samarium

more sensitive indicator is found in the $N\sigma_c(150)/N\sigma_c(148)$ ratio. First, the r-process is shielded, and secondly the abundance of samarium-150 is only about half that of samarium-149. The samarium-149 depletion is then:

$$\frac{\Delta N\,(149)}{N\,(149)} = \frac{7{\cdot}36}{13{\cdot}85}\,[(1{\cdot}02 \pm 0{\cdot}06) - 1]$$

which gives an upper limit of 4 per cent for selective depletion, applicable to solar system material as discussed here. It should be mentioned[7] that inclusion of minor secondary processes such as (p,γ) and (γ,n) reactions and neutron resonance effects can increase the uncertainties in this limit by perhaps a factor of two.

In summary the following conclusions may be drawn from the samarium results.

(1) The near equivalence of $N\sigma_c$ for samarium-148 and samarium-150 is in excellent agreement with the predictions of Burbidge *et al.*[1].

(2) At the same time these results provide some constraints for nucleosynthesis models[1,2].

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A New ²Π–X²Π Band System of Nitrogen Sulphide

THE spectrum of nitrogen sulphide is excited by means of a micro-wave oscillator of 2,450 Mc/s in sealed quartz discharge tubes containing a few mm of nitrogen and traces of sulphur. Besides the β- and γ-bands, a number of hitherto unassigned bands, degraded to the red, are recorded in the region 2650–2850 Å. These are photographed on a 6·6-m concave grating spectrograph in the second order at a dispersion of 0·57 Å/mm. Rotational structure of seven bands has been analysed and the results are reported in the present communication.

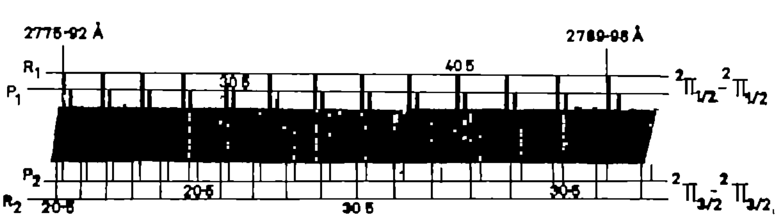

Fig. 1. (v' + 1) – 0 band of the new ²Π–X²Π system

Each band consists of two sub-bands with an average separation of 107 cm⁻¹. One such band at 2775 Å is shown in Fig. 1, where the rotational structure of the two sub-bands extends towards the longer wave-lengths as indicated by leading lines. The sub-band shown at the top in the spectrogram has two P and two R branches while the other sub-band with leading lines drawn below has only single series of P and R branches. Such a doubling in the case of P and R branches of only one sub-band is obtained in the $^2\Pi_{1/2}$–$^2\Pi_{1/2}$ component of a $^2\Pi$–$^2\Pi$ transition with an appreciable Λ-type doubling in the $^2\Pi_{1/2}$ states while in the $^2\Pi_{3/2}$–$^2\Pi_{3/2}$ component the Λ-doubling is negligible.

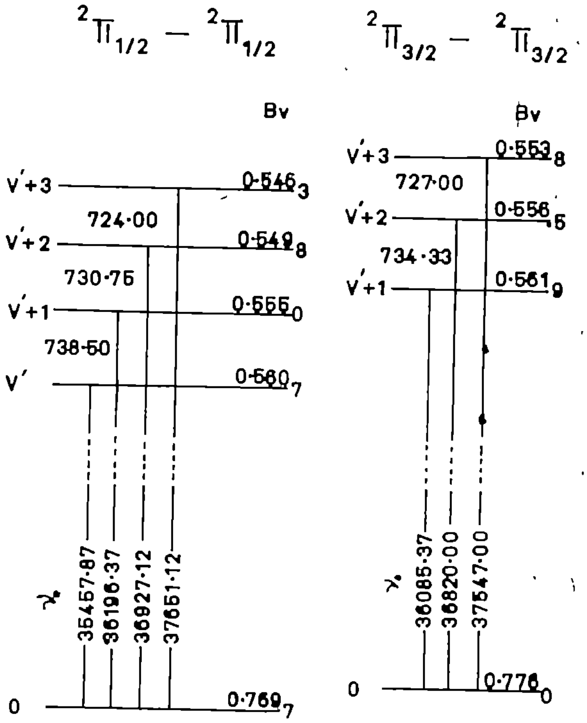

Fig. 2. Energy-level diagram of the vibrational levels of the ²Π–X²Π band system

Four bands of $^2\Pi_{1/2}$–$^2\Pi_{1/2}$ and three of $^2\Pi_{3/2}$–$^2\Pi_{3/2}$ transitions have been analysed and their respective vibrational levels are given in an energy-level diagram in Fig. 2. It is found that all the bands arise from different initial vibrational levels to the v'' = 0 level of the final $^2\Pi$ state. The band system belongs to a $^2\Pi$ (a)–$^2\Pi$ (a) transition with large spin-splitting in both the states. A comparison of the $\Delta_2F''_1$ (J + 1/2) and $\Delta_2F''_2$ (J + 1/2) values, that is, $R(J - 1) - P(J + 1)$, of the $^2\Pi_{1/2}$ and $^2\Pi_{3/2}$ sub-states of the final $^2\Pi$ level with those obtained for the v'' = 0 of final levels of the β- and γ-systems[1,2] shows good agreement, thereby indicating that all the three band systems have the $^2\Pi$ ground state as the common final level.

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Comminution: a Modified Logistic Growth Function

HOLMES's equation[1] rewritten in terms of specific surface area, S, can be derived from the differential equation:

$$\frac{dS}{dE} \propto S^{1-r} \tag{1}$$

where E is energy input per unit volume and r is Kick's law deviation exponent[1]. Equation (1) reduces to Kick's equation[2] when $r = 0$, Bond's third theory[3] when $r = \frac{1}{2}$ and Rittinger's equation[4] when $r = 1$. These equations suggest that material of unlimited fineness can be produced by milling.

A consideration of the expenditure of energy on the material under comminution, and the utilization of energy in causing fracture, indicates that: (*a*) the rate of change of the energy which can lead to fracture with energy input diminishes as energy input increases; (*b*) as the particles decrease in size, their resistance to fracture increases; (*c*) S reaches an upper asymptote, S_∞, as energy input increases. These lead to a differential equation which, reduced to its simplest form, becomes:

$$\frac{dS}{dE} = k\,\frac{S(1 - S/S_\infty)}{E} \tag{2}$$

where k is a parameter. Strictly E refers to net energy, defined as the difference between the gross energy and the energy required to maintain the mill in operation without relative movement. Equation (2) is a modified form [due to E in the right-hand side] of the Verhulst–Pearl–Reed logistic growth function[5,6]. The method involved here can be developed for differential grinding, which is analogous to competing populations.

Integrating equation (2) between non-zero limits gives:

$$E^k = A\,\frac{S}{1 - S/S_\infty} \tag{3}$$

where A depends on the initial values of S and E, which in turn depend on the material under comminution and its previous history. However, equation (3) may be used with confidence if the initial values of S and E are small; in most instances the experimental curves of S versus E will appear to extrapolate through the origin.

If values of S_x, S_{x+1} . . . corresponding to values of E_x, E_{x+1} . . . in geometrical progression:

$$\left(\frac{E_{x+1}}{E_x} = \text{constant} > 1\right)$$

are interpolated from the experimental data then:

$$\frac{1}{S_{x+1}} - \frac{1}{S_\infty} = \left(\frac{E_x}{E_{x+1}}\right)^k \left(\frac{1}{S_x} - \frac{1}{S_\infty}\right) \tag{4}$$

which has as its solution $S_x = S_{x+1} = S_\infty$. Thus, the value of $1/S_\infty$ is obtained from the point where the line of $1/S_{x+1}$ against $1/S_x$ intercepts the line at 45° to the axes through the origin. This method of finding an unknown asymptote has been used in other applications[7-9].

A plot of $\frac{S}{1 - S/S_\infty}$ versus E on a log log grid, or a plot of S/S_∞ versus E on a logistic log grid, will give a straight line if equation (3) is applicable. If S_∞ is so large that S/S_∞ can be neglected equation (3) reduces to a power form as with Holmes.

Fig. 1 shows some data[10] treated in this manner; excellent agreement is evident. In this example $k = 1$. The value of S_∞ could depend on the material under comminution, the conditions of breakage, mill dimensions and power input.

I thank Prof. H. J. King, Dr. A. Jowett and Mr. O. C. Dell for discussions.

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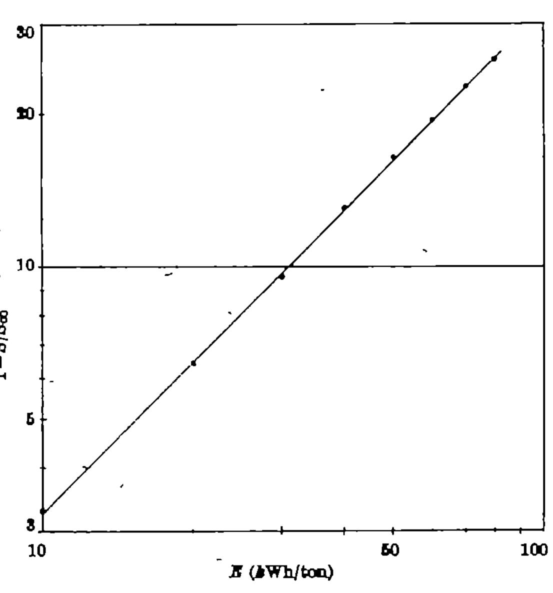

Fig. 1. Dry grinding of ferrosilicon with 30 mm balls (series XIV) (data from Svensson and Murkes, ref. 10). S_∞, 36,000; k, 1; A, 3·06 × 10^{-8}

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OCEANOGRAPHY

Occurrence of Abnormally High Fluoride Concentrations at Depth in the Oceans

DURING the course of an investigation in these laboratories of the constancy of ionic composition of sea-water and in particular of the distribution of fluoride ion in the oceans, unusually large variations in the fluoride/chlorinity ratio have been found.

A new method[1] for the determination of fluoride in sea-waters has been applied to the analysis of more than 300 samples collected from the surface and from depth. The surface samples showed no significant geographical variation in the fluoride/chlorinity ratio (mean value about 6·7 × 10^{-5}), except for a few samples of high chlorinity water from the Mediterranean, in which the ratio averaged about 6·4 × 10^{-5}, a phenomenon probably associated with precipitation of calcium. No significant change of the ratio with depth was found in the Pacific and Indian Oceans. In the deep waters from some other localities, however, much higher fluoride/chlorinity ratios were found, frequently amounting to a 30 per cent increase.

Particularly pronounced were the fluoride/chlorinity (parts per thousand) ratios of the deep-water samples from the North and South Atlantic west of the mid-

Table 1. FLUORIDE/CHLORINITY RATIOS OF SEA-WATER AT DIFFERENT DEPTHS

Sample No.	Position	Depth (m)	F/Cl parts per thousand ratio (× 10⁻⁵)
E 13	China Sea. 12° 23′ N., 110° 50′ E.	0	6·80
		1,000	8·29
		2,500	8·04
E 15	N. Atlantic, 42° 09′ N., 20° 27′ W.	0	6·93
		967	7·64
		3,413	7·87
B 20	N. Atlantic, 56° 42′ N., 54° 12′ W	0	6·68
		1,000	6·89
		3,109	8·87
B 21	N. Atlantic, 58° 36′ N., 52° 34′ W.	0	6·82
		1,829	6·85
		3,300	9·00
E 17	Mid-Atlantic, 10° 00′ N., 46° 58′ W.	0	6·78
		1,000	8·19
		4,500	8·01
E 18	Mid-Atlantic, 14° 18′ S., 28° 38′ W.	0	6·69
		1,000	8·19
		4,500	8·04
E 19	S. Atlantic, 53° 28′ S., 50° 20′ W.	1,070	8·00
		2,979	8·37
M 7	Mediterranean, 42° 17′ N., 04° 19′ E.	0	6·72
		1,936	7·31
M 2	Mediterranean, 41° 06′ N., 03° 35′ E.	0	6·41
		1,200	6·73
		2,250	7·02

Atlantic Ridge (Table 1). The high fluoride/chlorinity ratios found in deep waters may be caused either by submarine volcanic activity or by dissolution of fluoride minerals. The latter explanation seems likely for the samples from the North Atlantic since cryolite deposits are found on the east coast of Greenland. It is debatable, however, whether or not these Atlantic deep water masses would remain sufficiently unaffected by upwelling and other processes to retain their high fluoride content as far south as 53° S.

Many more samples will be needed before the significance of these results can be fully interpreted, but it is felt that the existence of these water masses having high fluoride/chlorinity might prove useful in the study of deep water circulation in the Atlantic.

We thank the many oceanographical institutions which have supplied water samples, and particularly Dr. R. Cox of the National Institute of Oceanography for arranging the collection programme.

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Size Analyses of Turbidity Current Sediment

SEDIMENT cores were obtained from the Iberia Abyssal Plain and the Biscay Abyssal Plain[1] on a cruise of R.R.S. *Discovery II* in 1958 organized by the Department of Geodesy and Geophysics of the University of Cambridge. These cores consist of beds of sediment deposited by turbidity currents, which are sometimes only a few cm thick but are more often 50–100 cm thick, and alternating with these beds is normal pelagic sediment, not thicker than about 40 cm. These cores have characteristics similar to those of abyssal plain sediments elsewhere described by Ericson and others[2].

Size analyses were made on two of the beds of sediment which had been deposited by turbidity currents to see how the grain size of the sediment varies with the depth in the bed. Such an investigation is necessary if quantitative studies are to be made on the settling of sediment from a turbidity current. Analyses made by other workers[2] have been only of the fraction of the sediment in which the particles are larger than 74μ. The analyses reported here are of sediment the size of which is in the range 2μ–64μ. The results of the analyses of one of the beds are given here. The position of the core from which the bed came and a summary of its lithology are presented in Table 1.

Table 1. DETAILS OF CORE 3742

Position	41° 22′ N., 14° 32′ W.	
Depth	2,919 fathoms	
Area	Iberia abyssal plain	
Lithology		
	0–26 cm	Brown pelagic sediment
	26–73 cm	Dark grey turbidity current deposit. Sharp contact
	73–100 cm	Light grey ooze, mottled colouring, disturbed. Passes with no sharp break into sediment beneath
	100–242 cm	Dark grey sediment grading down the core to a fine sand-turbidity current deposit

The analyses were made with a Coulter counter which was calibrated with pollen grains of known diameter[3]. The accuracy was estimated from repeated analyses of one sample of sediment. The Coulter counter measures the volume of sediment coarser than a given size with the assumption that all the grains are spherical and these values have been converted to weight per cent by assuming a specific gravity of 2·65. The instrument will disregard all sediment below a certain size; in the work described here the lower limit was for spherical grains having a diameter of 2μ so the results show size analyses for that part of the sediment which has a diameter greater than 2μ.

Two sets of analyses were made; in both sets the sediment was dispersed with sodium oxalate and in the second set carbonates were dissolved with hydrochloric acid. The results are shown in Figs. 1 and 2. Fig. 1 shows the percentage of sediment which is coarser than 10μ and the depth down the bed. The points (*a*) are for samples which were dispersed only, and the points (*b*) for samples which are carbonate-free. Fig. 2*a* shows histograms of the size analyses of the samples in which the carbonates remain and Fig. 2*b* of the samples which are carbonate-free. The percentage of calcium carbonate in three samples from this bed ranged from 23 to 24 per cent by weight.

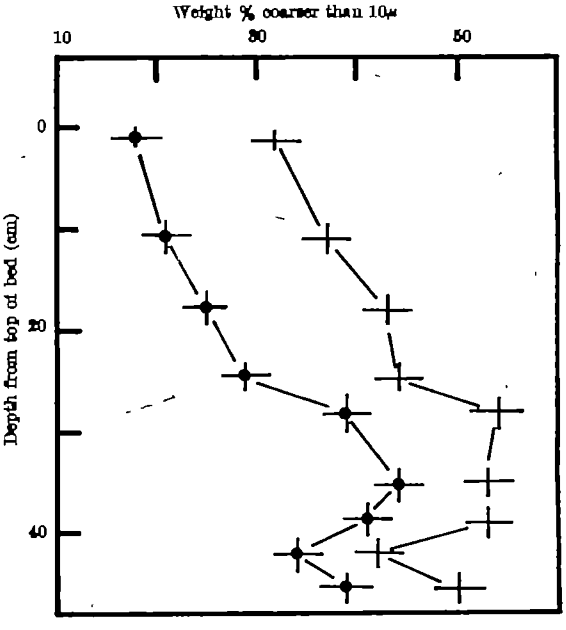

Fig. 1. Weight per cent coarser than 10μ and the depth from the top of the bed (cm). The right-hand curve (*a*) refers to samples which were dispersed only. The left-hand curve (*b*) refers to samples which are carbonate free. The vertical bars show the length of core from which the sample was taken and the horizontal bars represent two standard deviations

These results show that the sediment in the size range above 2μ diameter has a bimodal distribution in most of the samples with carbonates present and in some of the carbonate-free samples. The treatment with acid removes a part of the coarser size fractions. The sediment becomes coarser in grain size for a part of the way down the bed but the changes in size are less regular in the lowest 20 cm. This last feature was also observed in another turbidity

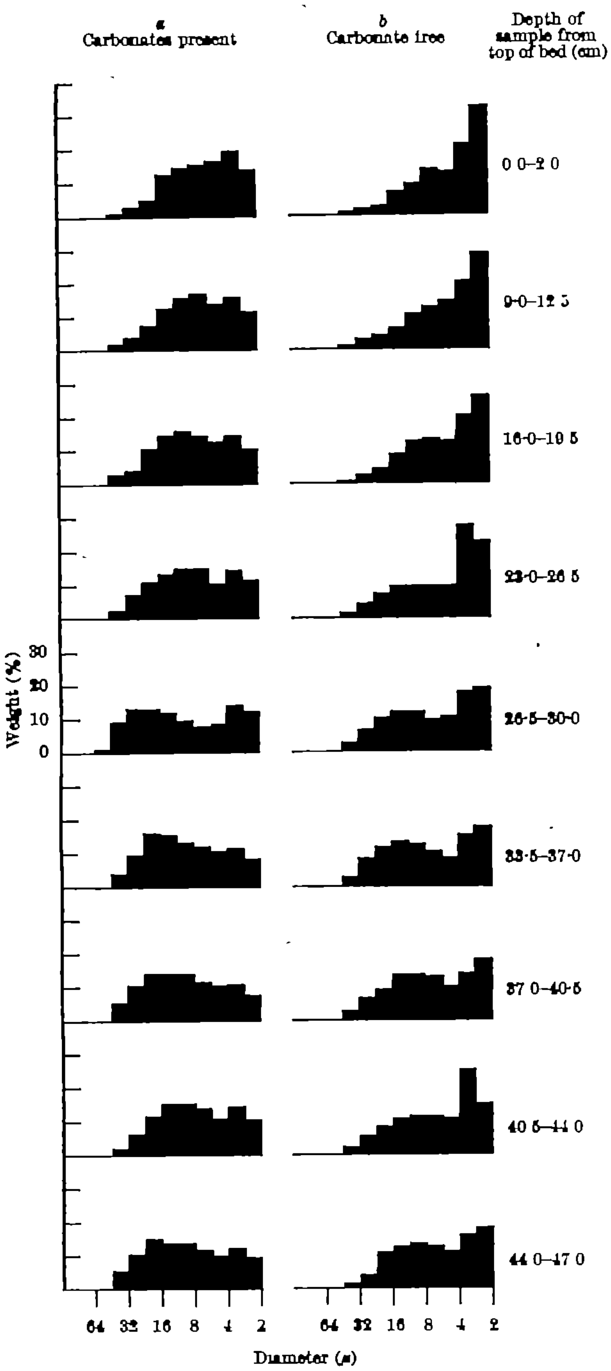

Fig. 2. Histograms of sediment size distribution. (a), Carbonates present; (b) carbonate-free. These histograms are for that part of the sediment coarser than 2μ (see text). They are arranged in order of depth from the top of the bed

current deposit from a second core nearby, analysed in the same way. Pipette analyses and water content determinations in several other beds deposited by turbidity currents also show it. It would be reasonable to expect such a feature if a turbidity current were turbulent at the time that settling of the constituent sediment started, for the change of the size distribution when successive increments were first deposited would not then be regular. If turbulence ceased then the change in size distribution with time would be regular and a graded deposit would result. Particles of 8μ diameter take approximately one month to fall freely through 100 m of water and the time to fall a certain distance increases as 1/diam.[2]; if 100 m is a reasonable figure to take for the thickness of a turbidity current before settling commences then it would seem there is time for any turbulence to cease.

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METALLURGY

Hydrocarbons in the Gases evolved from Steel in Vacuum Extraction at 600°C.

THE conventional method of determining hydrogen in steel is vacuum extraction at 600°–700° C. Small specimens are heated, and the gases evolved are pumped off and collected in a suitable measuring system. It is generally accepted that the bulk of the hydrogen present in the steel is evolved under these conditions and that hydrogen is the principal component of the gases. Initially, separation of hydrogen from other gases was considered unnecessary[1], but it has become common practice to pass the hydrogen through a palladium tube or to oxidize it with cupric oxide. The composition of the other gases, which may constitute 10–40 per cent of the total, is unknown. and there are considerable variations in analytical practice with regard to the elimination of moisture by freezing or reduction over ferro-manganese.

Hydrogen may react with carbon in the steel to form methane or other hydrocarbons; such reactions have been postulated within the steel to account for some embrittlement phenomena[2], and at the surface during vacuum extraction analysis to account for decarburization of hydrogen-charged specimens as well as the parallel increase of 'other gases' with hydrogen content in pickling or cathodic charging[3]. Small amounts of methane have been found in the gases evolved at room temperature, especially from welded specimens and at 400° C from pickled specimens[4], but on the rare occasions that the gases evolved at 600°–700° C have been analysed for methane, only about 3–6 per cent was present[5].

In this context it is of interest to record some evidence for the presence of hydrocarbons in the gases evolved in vacuum extraction at 600° C. The equipment used is based on apparatus developed by Bach, Dawson and Smith[6], in which the hydrogen is separated from the other gases by a heated palladium tube. It has two additional features which are of importance here: the gases can be circulated at will over heated ferro-manganese, and there is a freezing cell in the measuring system. Specimens of commercial mild steel wire (¾ in. long, ⅛ in. diam.) were pickled for 24 h in 50 per cent hydrochloric acid, washed, dried, and introduced into the apparatus through a mercury leg. The results for three parallel specimens are given in Table 1.

Evidently, the 'other gases' condense at – 79° C and are decomposed, reduced, or cracked by passage over ferro-manganese, yielding only hydrogen and non-volatile products. Since the volume of hydrogen formed is roughly four times that of the other gas it seems reasonable to postulate a hydrocarbon with 8 hydrogen atoms in the molecule.

Batches of similar specimens were analysed without circulating the gases over ferro-manganese, using the freezing cell in some cases and not in others; the results are summarized in Table 2.

When the freezing trap is not in use, the analysis yields appreciable quantities of 'other gases'; in addition there

Table 1. Vacuum Extraction at 600° C

	Specimen 1		Specimen 2		Specimen 3	
	Hydrogen (ml./100 g)	Other gases (ml./100 g)	Hydrogen (ml./100 g)	Other gases (ml./100 g)	Hydrogen (ml./100 g)	Other gases (ml./100 g)
Solid CO_2 in freezing cell	6·84	nil	6·84	nil	6·46	nil
Hydrogen removed; solid CO_2 removed to evaporate other gases	nil	0·90	nil	0·75	omitted	
Re-circulated over ferro-manganese	3·46	nil	3·65	nil	3·94	nil
Total hydrogen	10·30		10·49		10·40	
Ratio of other gases to volume of hydrogen produced	3·85		4·17			

Table 2. Vacuum Extraction at 600° C

Without solid CO_2		With solid CO_2	
Hydrogen (ml./100 g)	Other gases (ml./100 g)	Hydrogen (ml./100 g)	Other gases (ml/.100 g)
7·33	0·58	5·60	nil
6·94	2·20	5·60	nil
6·83	2·98	6·55	nil
8·10	2·78	6·47	nil
8·20	3·24	6·50	nil
7·34	2·70	5·63	nil
5·57	2·84	5·63	nil
Mean 7·187		6 058	

The difference is significant at 2 per cent level.

is an increase in the hydrogen yield, which suggests partial, and evidently somewhat erratic, cracking of the hydrocarbons in contact with the hot palladium tube.

It can be concluded that hydrocarbons are formed during vacuum extraction analysis for hydrogen in steel, and that precautions must be taken to ensure their complete decomposition, or the hydrogen figures will be too low.

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Reactivity of Deformed Metal Surfaces

For the understanding of both boundary lubrication and the effect of environment on mechanical properties, it is important to know what increase, if any, occurs in the reactivity of metal surfaces when they undergo deformation. If an increase in reactivity greater than that due to changes in surface area is observed, it is reasonable to attribute it to the generation of point defects and dislocations in the metal. The relative importance of changes in surface area, point defects and dislocations is still largely speculative and an investigation is being made here to elucidate this.

Small tensile specimens (0·25 in. wide × 0·02 in. thick) were formed from rolled sheets of high-purity copper, iron and aluminium. These were annealed in an argon atmosphere, then etched for a standard time, copper and iron in aqueous nitric acid and aluminium in aqueous hydrofluoric acid. After etching the specimens were washed in water and dried before use. During each experiment the test specimen was extended to a linear strain of 15 per cent in five strain-increments of 3 per cent while immersed in a solution of either stearic acid labelled with radioactive carbon-14 or elemental sulphur labelled with the radioisotope sulphur-35. *Cyclo*hexane was chosen as the solvent because of its low solubility for oxygen; stearic acid and sulphur are typical boundary lubricants. The experiments were generally carried out at a strain rate of 10^{-3}/sec, although some experiments with aluminium were carried out at a strain rate of 10^{-4}/sec. After each increment of extension the test specimen was allowed to interact with the solution for ten minutes and was then

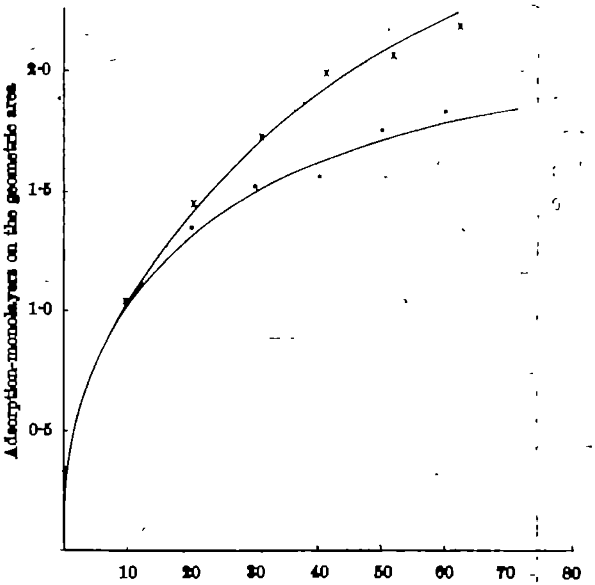

Fig. 1. Adsorption of stearic acid on copper versus time (including extension time). ●, Unextended; ×, extended at 6 per cent/min for 5 periods of 0·5 min

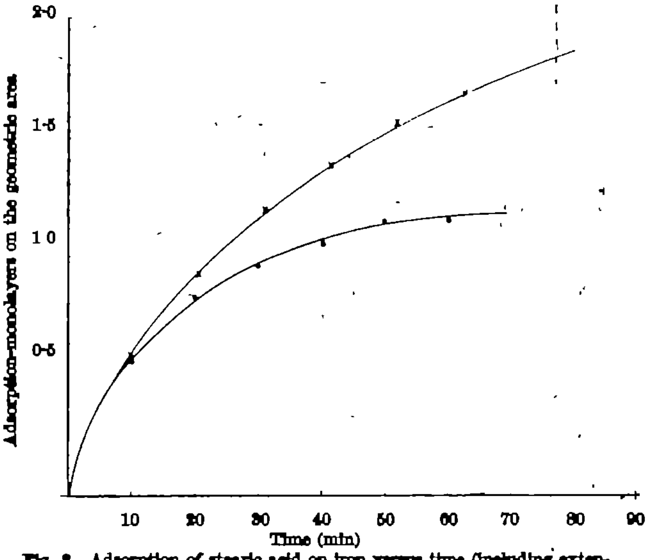

Fig. 2. Adsorption of stearic acid on iron versus time (including extension time). ●, Unextended; ×, extended at 6 per cent/min for 5 periods of 0·5 min

washed in benzene. The amount of reaction or adsorption which had taken place after each extension was determined by measuring the radioactivity on the surface of the specimen. This measurement was made in the centre of the specimen using an end-windowed Geiger-Müller counter of 0·2 in. diameter. Only the results obtained with stearic acid will be discussed here.

Fig. 1 shows the effect of deformation on the rate of adsorption of stearic acid on copper. The increased rate of adsorption on the deformed surface can be seen. Fig. 2 shows the effect of deformation on the rate of adsorption of stearic acid on iron. The increased rate of adsorption on the deformed surface is again evident. All three metals showed enhanced reactivity resulting from deformation and there was an approximately linear relation between the increase in adsorption on the deformed surfaces and the amount of deformation. In the experiments with aluminium the increase in reactivity was most marked if the deformation was carried out at the smaller rate of strain, due perhaps to the development of coarser slip in the metal under these conditions.

The increase in reactivity was not observed in specimens which were extended and then annealed before being allowed to interact with the solutions; the effect is thus not simply one of increased surface area resulting from deformation. Nor was increased reactivity observed in specimens which had been quenched from 600° C into cold water before allowing them to interact with the solutions. Such specimens would contain a large number of point defects; the effect is, therefore, not due to the creation of point defects in the deformed metals.

The effect is similar to that reported by Uhara *et al.*[1], who showed that the ability of copper wire to catalyse the decomposition of diazonium salts was enhanced by torsional deformation. They attributed this to a higher dislocation content of the deformed metal. It seems probable that the increased reactivity found in the present experiments is also due to a higher dislocation content.

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CRYSTALLOGRAPHY

Charge-Transfer Complex Crystal Growth at a Solution–Solid Interface

ORIENTED intergrowth (epitaxy) of organic compounds has been reported in many hundreds of cases[1]. Although some examples seem to involve van der Waals forces exerting an orienting effect[2], a more generalized description might be that on a solid surface anisotropic short-range forces can exist which will cause orientation of molecules on this surface.

In an attempt to deposit from solution oriented crystals of an electron donor, 1,4-diaminodurene, on an electron acceptor single crystal, *p*-chloranil, it was found that crystals of the 1 : 1 charge-transfer complex[3,4] were deposited instead. For example, when 6·8 × 10^{-3} M diaminodurene in petroleum ether is placed in contact with a chloranil crystal at 25°, small crystals of the complex are observed to grow first on the surface of the crystal and then on one another. Exhaustion of the amine is substantially complete in one day. Concentrations of amine 5–10 times higher lead to a deposit of amorphous complex; solutions 10 times as dilute gave some colour in solution but no deposit on the crystal.

Similar crystal growth occurs when compacted disks (0·5 g compacted at 3,000 atm. in a 13 mm die) of the amine and the quinone are placed a few mm apart in a poor solvent such as petroleum ether, the residual chloranil tablet becoming covered with a dense network of weakly held, randomly oriented needles of the complex in a few days. A beard-like stubble of crystals appeared on the edge of the chloranil tablet within minutes of submerging the pellets. Nucleation continued, first on the remaining chloranil surface and then by crystals growing on one another.

These observations all suggest that crystal growth is occurring in a small concentrated zone near the least soluble component in solution, but that there is some tendency for orientation to occur on the surface of that component.

In principle, this constitutes a novel technique of growing crystals in a controlled manner. In light of the difficulty of obtaining even small crystals of charge-transfer complexes which are regular enough for structural and electrical investigations[4], this technique of crystal growth is being further investigated. Thus far it appears that the early stages of growth seem to be quite regular but the crystal ends have a distinct tendency to grow two or more parallel spikes, that is, one crystallographic direction is favoured for growth.

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CHEMISTRY

Heterogeneous Recombination of Free Atoms

RECENT work by Reeves *et al.*[1] has shown that, under certain conditions, electronically excited molecules of O_2, N_2, NO, etc., can be formed by the heterogeneous recombination of free atoms on a metal surface. In these experiments light emission was observed from the gas just above the metal surface, showing that the molecules formed were desorbed in an excited electronic state. This evidence, and data on the kinetics of heterogeneous recombination[2,3], suggest that this desorption process is very rapid and that the molecules liberated may have excess kinetic energy. In this communication we report on an unsuccessful attempt to obtain evidence for such excess kinetic energy, by comparing the pressure exerted by a mixture of atoms and molecules on a surface where recombination takes place with the pressure on a surface where recombination is negligible.

The apparatus used consisted of metal foil 3 cm long, 1 cm wide and 0·1 mm thick mounted on a thin vertical glass or quartz fibre. Half of each face of the vane was coated with clear glyptal resin, which has a low coefficient for recombination of atoms, the coating being applied so that any differences in apparent pressure on the two types of surface would result in a couple on the vane. Deflexions of the vane were observed by means of a small galvanometer mirror mounted on the centre of the vane. The products of an electrodeless radiofrequency discharge through oxygen, nitrogen or hydrogen flowed vertically past the vane. Total pressures between 0·5 and 2 mm mercury were used, and atom concentrations of up to 20 per cent corresponding to atom flows of up to 8 μmole/sec were obtained.

The atom concentrations were determined by gas phase titration[4,5], and the fraction of atoms recombining on the vane, which was typically 80 per cent, was determined by comparing the intensities of the chemiluminescent emissions associated with the O + NO (ref. 6), N + N (ref. 7) or H + NO (ref. 8) reactions above and below the vane. The sensitivity of the suspension was determined by calculating the moment of inertia of the vane and measuring its period of oscillation when mounted on the fibre. It was possible to detect a difference of pressure of 0·05 dyne/cm^2.

Vanes of silver, copper, nickel and platinum were used; in no case could a deflexion attributable directly to atom recombination be detected, but in all cases there was a slow deflexion with a time constant of about 5 sec (the natural period of the vane-fibre system was about 0·5 sec). This deflexion increased with the atom concentration (but not linearly) and was independent of the total pressure for a given atom concentration. It was concluded that this deflexion was associated with unequal temperatures of the surfaces of the vane due to the heat released by recombination on the uncoated surface of the vane. Such effects have been discussed in connexion with the 'Crookes radiometer'[9,10]. The time constant of the deflexion corresponded to the time for the vane temperature to reach a steady state.

Since the smallest force which could be detected was equal to the force exerted by the atoms which hit the active surface and remained on the surface to recombine, it was concluded that molecules of oxygen, nitrogen and hydrogen formed by recombination on silver, copper, nickel and platinum surfaces do not leave the surface with an average of more than twice the normal thermal velocity.

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Heat of Formation of the Difluoromethylene Radical

Majer and Patrick[1] have recently reported a value for the heat of formation of CF_2 which differs appreciably from that reported by Margrave *et al.*[2] and regresses toward values from the older literature. This heat has been deduced from electron impact studies on $C_6H_5CF_3$ and $C_6H_4FCF_3$ and involves the appearance potentials of the $C_6H_5F^+$ and $C_6H_4F_2^+$ ions, respectively. Since those ions are not formed directly from the parent molecules, it is obviously dangerous to utilize appearance potentials for thermochemical calculations of any except the most qualitative nature, and a detailed consideration of the data seems to indicate that − 17 kcal/mole is only a positive limit for the heat of formation of CF_2.

A spontaneous unimolecular rearrangement of $C_6H_5CF_2^+$ to yield $C_6H_5F^+ + CF_2$ in large yield seems improbable but, if this happens, it must involve an excited state of the ion. A cyclic intermediate (7-membered ring) would require the breaking of two carbon–carbon bonds and no doubt a considerable activation energy. If the first step in the fragmentation reaction of $C_6H_5CF_3$ were:

$$C_6H_5CF_3 + e \rightarrow C_6H_5CF_2^+ + F + 2e \qquad (1)$$

and the second step were either a spontaneous unimolecular rearrangement:

$$C_6H_5CF_2^+ \rightarrow C_6H_5F^+ + CF \qquad (2)$$

or an ion-molecule process (highly improbable at the low pressures used):

$$C_6H_5CF_2^+ + F \rightarrow C_6H_5F^+ + CF_2 \qquad (3)$$

then the appearance potential of $C_6H_5F^+$ will be essentially that of $C_6H_5CF_2^+$ since the second and third reactions are undoubtedly exothermic. Majer and Patrick[1] did not consider the possibility of CF as a product. In either case, there seems little chance that the energy liberated in the second spontaneous step could be utilized to help make the first step to go in the normal ionization chamber.

One would estimate the energy needed for ionization of typical fluorocarbons to fall in the range 9–10 eV, and for breaking a C–F bond to fall in the 4–5 eV range[3-5]. The sum of these factors (13–15 eV) compares favourably with the 13·35 eV appearance potential for $C_6H_5F^+$ from $C_6H_5CF_3$, the 13·15 eV appearance potential for $C_6H_4F_2^+$ from *p*-$C_6H_4FCF_3$ and the 13·75 eV appearance potential for $C_6H_4F_2^+$ from *m*-$C_6H_4FCF_3$ as reported by Majer and Patrick[1], and thus tends to support the postulate that the apparent energy required for forming these ions is primarily determined by a reaction like (1) and that the exothermicities of reactions like (2) and (3) or those involving a cyclic intermediate are not being properly considered. Since the dissociation energy for a $C_6H_5{-}CX_3$ bond is approximately 10–30 kcal/mole less than for a $C_6H_5{-}X$ bond, one should correct the appearance potentials accordingly and this yields $\Delta H_f(CF_2) \approx -35 \pm 10$ kcal/mole, in good agreement with earlier work.

There seems to be little support for the 13·35 eV ionization potential of CF_2 reported by Reed and Snedden[6] since this would limit the C–C bond energy in C_2F_4 to a maximum of about 46 kcal/mole, which is much too low and incompatible with the properties of C_2F_4.

Another piece of evidence for a more stable CF_2 radical comes from the patent of Farlow[7], who prepared C_2F_4 from CF_4 and graphite in an induction-heated furnace. From these data, Stull[8] has calculated: $\Delta H_f^\circ = -46 \pm 5$ kcal/mole for CF_2.

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Orientation of Benzoic Acid adsorbed on Nickel

THE concept of aromatic systems interacting with metal surfaces has been used to explain data obtained by a variety of techniques. Suhrman and Schulz[1], using thin-film electrical resistance measurements, concluded that electrons are transferred to a nickel adsorbant surface by adsorbed benzene, triphenylmethane and naphthalene. The relatively low heat of adsorption of benzene on nickel powder was interpreted by Yu *et al.*[2] on the basis of the energy needed to destroy the aromatic ring. Blomgren *et al.*[3], in investigating adsorption at the mercury-aqueous acid solution interface using the electrocapillary method, explained the amounts adsorbed as resulting from perpendicular orientations for straight chain aliphatic compounds and parallel orientations for planar aromatic substances. By determining the amount of adsorption from aqueous solution of various aromatic substances on to solid electrodes by bulk-phase depletion measurements, Conway *et al.*[4] concluded that the molecular orientation was probably flat.

Additional evidence to support the hypothesis of aromatic system-metal interaction is given in the present work. The amount of benzoic acid adsorbed per unit of apparent area of nickel surface was determined and used to calculate the molecular area of the adsorbed molecule. Adsorption measurements were also made with stearic acid, a material the orientation of which is assumed to be perpendicular, in order to calibrate the surface. 5×10^{-5} molar solutions of the carbon-14 labelled acids in spectranalysed carbon tetrachloride were prepared. Nickel planchets were then contacted with these solutions by floating them in 20-ml. beakers containing 10 ml. of the solution for a number of periods up to four days. They were then removed, drained, and immediately counted. A correction was made for material that deposited due to evaporation during planchet drainage. The counts were then converted to weights and the molecular areas calculated using these values and the apparent surface area.

The molecular area of the adsorbed acids at equilibrium was found to be 22 ± 3 Å^2 for stearic acid and 44 ± 7 Å^2 for benzoic acid (both at the 95 per cent confidence-level, 20 samples each). The molecular area of benzoic acid, using models[4], is 56 Å^2 at a planar orientation and 20 Å^2 at a perpendicular orientation. Thus, the data indicate an interaction between the aromatic ring and the metal surface even in the presence of the competing carboxylic acid function. It is interesting to note that while the aromatic system is adsorbed when on the same molecule with a carboxylic acid function it does not in any way impair the adsorption of stearic acid on to chrome plate from benzene[5].

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Paper Chromatography of Organic Acids

THE identification of non-volatile organic acids on paper chromatograms is usually dependent on the use of a suitable pH indicator which is applied to the developed and dried paper by spraying[1]. This procedure is inconvenient and an even distribution of indicator on the paper is difficult to obtain.

Hartley and Lawson[2] incorporated the indicator in the developing solvent, and in this way obtained an automatically developed chromatogram by simply drying the paper. Of the acidic solvents used by Hartley and Lawson ethyl formate/formic acid/water (12 : 5 : 3) was found to be the best, but even this did not give sufficient separation of those acids commonly occurring in plant tissues (Table 1, col. *A*).

Table 1

Acid	R_F values (20° C) *A* (ref. 2) Ethyl formate/formic acid/water (12 : 5 : 3)	 *B* Butyl formate/formic acid/water (10 : 4 : 1)
Tartaric	0·67	0·21
Citric	0·71	0·24
Oxalic	0·76	0·40
Succinic	0·79	0·56
Lactic	0·84	0·64
Bromophenol blue	0·95	0·90

n-Butanol/acetic acid/water (30 : 5 : 10) has been used as a solvent for paper chromatography of organic acids[3]. In this solvent, however, oxalic acid streaks badly, and the possibility of esterification resulting from the presence of free butanol and acetic acid makes it unreliable.

A solvent in which esterification is not possible has been found to be more satisfactory. The best solvent was found to be *n*-butyl formate/formic acid/water (10 : 4 : 1). Commonly occurring plant acids were readily separated by this solvent (Table 1, col. *B*). It is fast flowing and bromophenol blue can be incorporated in it to give an automatically developed chromatogram.

Five typical plant acids—tartaric, citric, oxalic, succinic and lactic acids—were chosen as reference substances. 50 µg of each acid was spotted 1·5 in. from the edge of a sheet of Whatman No. 1 paper. The solvent was prepared by mixing 10 parts of *n*-butyl formate, 4 parts of formic acid (98 per cent) and 1 part of water by volume. To the solvent was added sodium formate[2] (0·05 per cent w/v) and bromophenol blue (0·02 per cent w/v). Development was by the ascending technique at room temperature. The paper was suspended in a tank, previously saturated by small paper strips dipping into solvent dishes, with the lower edge dipping into a trough of solvent. The development of the sheet took about 4 h, and it was not found necessary to equilibrate the paper in the tank before running.

After the solvent had run a sufficient distance the paper was removed and allowed to dry overnight at room temperature. The acids appeared as yellow spots against a blue background, and all produced well-defined spots with no streaking. Bromophenol blue had an R_F value high enough to show up all the acids examined.

This method has been found to be particularly suited to the identification of acids separated by silica-gel chromatography using gradient elution with *n*-butanol–chloroform. Acids which are eluted together from the column, as for example succinic and lactic acids, are readily separated by the technique. The solvent system is also applicable to many more naturally occurring acids.

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Separation of Zirconium and Hafnium by Reversed Phase Partition Chromatography

THE technique of reversed phase partition chromatography is being increasingly used for the separation of metals of similar chemical behaviour. Siekierski and Fidelis[1,2] describe the application of this technique to the separation of the rare-earth metals, using tributyl phosphate absorbed on kieselguhr as the stationary phase, and nitric acid in various concentrations as the eluant. Pierce and Peck[3,4] use di-(2-ethylhexyl) *o*-phosphoric acid on polyvinyl chloride/acetate copolymer ('Corvic'), also for the separation of rare earths. Hamlin *et al.*[5] use tributyl phosphate on Kel-*F* for the isolation of uranium from many metals, while Pierce and Peck[6] use dithizone/carbon tetrachloride/chloroform on cellulose acetate[7] for the isolation of indium. Biochemical separations have also been described[8]. A number of metal separations are described by Cerrai and Testa[9] using trioctyl phosphine oxide on cellulose powder.

It has been shown that a partial separation of zirconium and hafnium can be achieved by solvent extraction from nitric acid solution using tributyl phosphate[10] and that the addition of inorganic nitrates such as sodium nitrate may improve the separation[11,12]. It seemed probable, therefore, that the technique used by Siekierski and Fidelis would give a separation of zirconium and hafnium, using the same stationary phase, tributyl phosphate on kieselguhr, and an eluant containing nitric acid and an inorganic nitrate.

The chromatographic column was prepared according to the method described by Siekierski and Fidelis, except that the dimensions were somewhat larger, namely, 1 cm diameter and 13–15 cm length, using 4·8 g kieselguhr and 3·0 ml tributyl phosphate (column I). Column II was also prepared, with a larger amount of tributyl phosphate (4·8 g kieselguhr and 6·0 ml. tributyl phosphate).

As this investigation was initiated with the object of determining hafnium in reactor grade zirconium (that is, < 100 p.p.m. hafnium) the separation of a small amount of hafnium from a larger amount of zirconium was investigated. 1·0 g hafnium-free zirconium nitrate was dissolved in 24 ml. of the chosen eluant, and 1 ml. of a hafnium solution containing 10 μg hafnium and about 1 μc ^{181}Hf tracer (Radiochemical Centre, Amersham) was added. 5 ml. of this solution was added to the top of the column, and after this had been run through at a flow-rate of about 1 ml./min, elution was continued with the chosen eluant, collecting each successive 10 ml. fraction, which was first counted for ^{181}Hf and then treated with ammonia solution to precipitate the zirconium (and also the hafnium, but the quantity of this is negligible).

Table 1

Eluant	Column No.	Hafnium in eluant fractions	Zirconium in eluant fractions	Separation
5% HNO_3 + 50% $NaNO_3$	I	0–10 ml. 42% 10–20 ml. 35%	2% at 0–10 ml. 10% at 10–20 ml. bulk at 20–30 ml.	some
12.5% HNO_3 + 50% $NaNO_3$	I	0–10 ml. 22% 10–20 ml. 30% 20–30 ml. 18%	10% at 20–30 ml. bulk at 30–40 ml.	some
5% HNO_3 + 50% NH_4NO_3	I	0–10 ml. 44% 10–20 ml. 43% 20–30 ml. 6%	1% at 0–10 ml. 3% at 10–20 ml. bulk at 20–30 ml.	some
10% HNO_3 + 50% NH_4NO_3	I	0–10 ml. 32% 10–20 ml. 33% 20–30 ml. 15%	2% at 0–10 ml. 10% at 10–20 ml. bulk at 20–30 ml.	some
12·5% HNO_3 + 50% NH_4NO_3	I	0–10 ml. 26% 10–20 ml. 47% 20–30 ml. 11%	5% at 20–30 ml. bulk at 30–40 ml.	reasonable
20% HNO_3 + 50% NH_4NO_3	I	0–10 ml. nil 10–20 ml. 93% 20–30 ml. 7%	10% at 10–20 ml. bulk at 20–30 ml.	some
20% HNO_3 + 50% NH_4NO_3	II	0–10 ml. 8% 10–20 ml. 66%.	2% at 20–30 ml.	good
25% HNO_3 + 50% NH_4NO_3	I	0–10 ml. nil 10–20 ml. 75% 20–30 ml. 18%	2% at 10–20 ml. 3% at 20–30 ml. bulk at 30–40 ml.	reasonable
25% HNO_3 + 50% NH_4NO_3	II	0–10 ml. 24% 10–20 ml. 44% 20–30 ml. 20%	1% at 10–20 ml. 2% at 20–30 ml. 5% at 30–40 ml. bulk at 50–60 ml.	good

Table 1 shows the eluants investigated, and the amounts of hafnium and zirconium in the different fractions. It can be seen that quite promising separations of zirconium and hafnium can be achieved, particularly with column II. Quantitative recovery of hafnium does not, however, occur so that any analytical application is limited. It should, however, be possible to use an isotope dilution technique, ascertaining the recovery of hafnium by the addition of ^{181}Hf tracer, and then determining the hafnium spectrographically, after addition of a known small amount of specpure zirconium (for example, 5 mg) as standard and carrier. By this means the lower limit of detection of hafnium in zirconium by the spectrographic method should be reduced by an order of 10, that is, the precision at the normal impurity level (10–40 p.p.m. hafnium in reactor grade zirconium) should be considerably improved.

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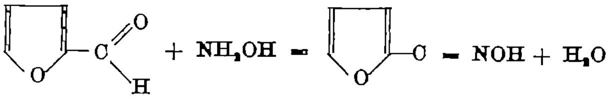
Polarographic Determination of Fural

A NUMBER of chemical and physico-chemical methods for the determination of fural have been devised. The most commonly used are gravimetric determination with phloroglucine[1], photometric determination with aniline[2] and with orcine[3], etc. Polarographic determination based on reduction of the aldehydic group present has also been described[4].

During an examination of potential utilization of some condensation reactions for determination of certain aldehydes significant in food technology[5,6] a new method of polarographic determination of fural was devised; this was based on conversion of fural to oxime, or as the case may be, to a semicarbazide. Fural reacts with hydroxylamine to form fural-oxime, which is very suitable for polarographic determination.



Fural-oxime is reduced on the mercury-drop electrode, and the waves are suitable for analytical evaluation. The condensation of fural with hydroxylamine occurs best in slightly acid medium/Britton-Robinson's buffer pH 1·98 in the medium of 1×10^{-2} N sulphuric acid. In the medium of this sort fural-oxime is reduced in one single well-developed and measurable wave. The half-wave potential in Britton-Robinson's buffer pH 1·98 is $-$ 0·73 V (standard calomel electrode).

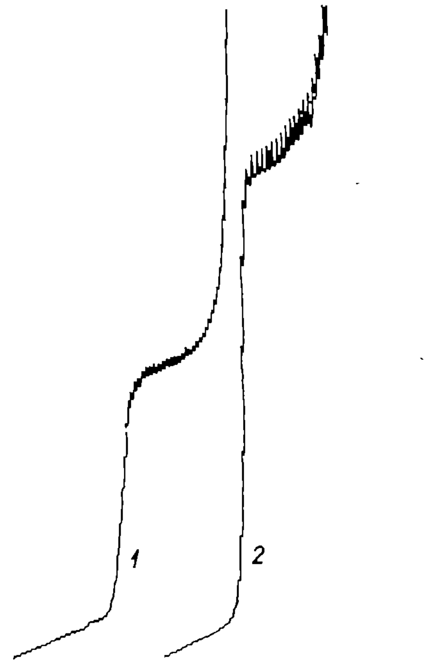


Fig. 1. Condensation of fural with hydroxylamine. (1) 1 ml., 1×10^{-3} N fural solution + 9 0 ml. phosphate buffer pH 7·20; (2) 1 ml. 1×10^{-3} N fural solution + 1 ml. 2 N hydroxylamine hydrochloride, 3 ml. 1×10^{-2} sulphuric acid + 5 ml. water. Mercurosulphate electrode, h, 60 cm, 4 V, sensitivity 1 : 20

The advantage of the method lies above all in that the oxime formed is reduced at a more positive potential in a four-electron wave, while in direct polarography the aldehydic group is reduced but in a two-electron wave. Hence, the method is doubly sensitive as compared with the direct polarographic method. This is of special advantage in analyses of samples with a low fural content, such as is the case with various food materials.

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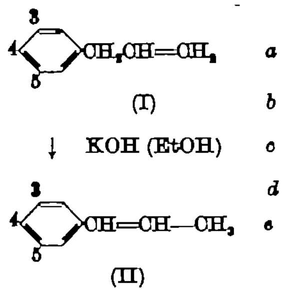
Composition of the Myristicin Fraction from Oil of Nutmeg

The aromatic ether fraction of oil of nutmeg has been previously shown[1] to consist of eugenol (I*a*), isoeugenol (II*a*), safrole (I*c*) and myristicin (I*d*). Vacuum distillation yields a fraction (b.p. 109°–112°/1 mm mercury; 60 g from 1 kg of 'W.I.' oil of nutmeg (George Lueders and Co.)) which consisted of a substance heretofore accepted both chemically[1,2] and pharmacologically[3] as the single compound, myristicin (I*d*).



	3	4	5
a	OCH$_3$	OH	H
b	OCH$_3$	OCH$_3$	H
c	O—CH$_2$—O		H
d	O—CH$_2$—O		OCH$_3$
e	OCH$_3$	OCH$_3$	OCH$_3$

The isomerization of this fraction with alcoholic potassium hydroxide yielded (*trans*) isomyristicin (II*d*), isolated by crystallization of the distilled reaction mixture. The mother liquors of this isolation, on analysis by vapour phase chromatography, provided the first indication of the complexity of the above 'myristicin' fraction. Of the four peaks observed (Fig. 1), No. 1 was easily identified as methyl isoeugenol (II*b*) by its infra-red spectrum and direct comparison to a commercial sample. Peak No. 3 was to a large extent *trans*-isomyristicin not removed by crystallization. The remaining two peaks were isolated using a Beckman Megachrome preparative V.P.C. instrument using a substrate of silicone 710, on firebrick at 220°. The presence of a methylenedioxy group (by nuclear magnetic resonance) and the absence of absorption in the 963–967 cm^{-1} region of the infra-red spectrum of peak 2 strongly suggested that the isomer might be *cis*-isomyristicin. That *trans*-propenyl aromatic ethers possess a characteristic absorption band in the above region (which is transparent for the *cis*-isomer) has been shown for isosafrole[4], anethol[5], isoeugenol, methylisoeugenol[6] and asarone[7]. Further, it has been shown recently[7,8] that the *cis*-isomer of the stereo-isomeric pairs invariably precedes the *trans*-isomer during vapour chromatography. This peak was verified as *cis*-isomyristicin by its conversion to *trans*-isomyristicin (II*d*) and its synthesis from myristicin, both with alcoholic potassium hydroxide.

The fourth peak was also isolated by preparative chromatography and showed the absence of a methylenedioxy group, but the presence of a strong band at 957 cm^{-1}. Isoelemicin (II*e*) was synthesized by the potassium hydroxide isomerization of elemicin[9] and was found to possess an identical infra-red spectrum.

Methylisoeugenol may be assumed to be in the propenyl form in the original sample of nutmeg distillate. There is an unresolved peak at this identical time in the original 'myristicin' fraction, and a search for the logical precursor, methyl eugenol, has been unsuccessful[1].

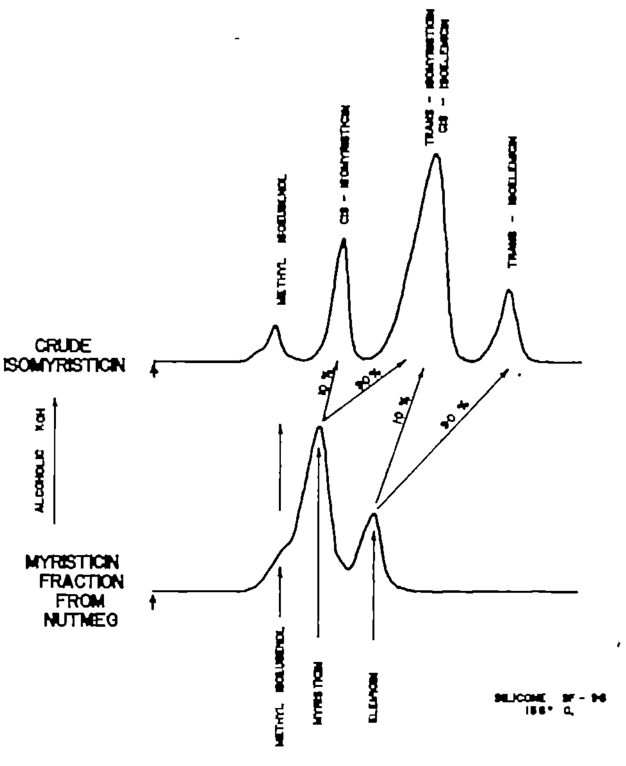


Fig. 1

The implied presence of elemicin (I*e*) in the original myristicin fraction was confirmed by the successful separation of it from myristicin by the low-temperature chromatography (158°, silicone '*SF*–96') as shown in the lower part of Fig. 1. Repeated fractional distillation was ineffective in increasing the myristicin content of this constant boiling fraction over 70 per cent. Consequently, in assigning chemical and biological properties to the substance as isolated from nutmeg allowance must be made for this congeneric contaminant.

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Synthesis of some 3-Deoxy-heptulosonic Acids (3-Deoxy-2-keto-heptonic Acids)

A suitable synthesis for 3-deoxy-aldulosonic acids has been published by Kuhn *et al.*[1]. After successfully repeating this work, resulting in the isolation of crystalline 3-deoxy D- and L-*erythro*-hexulosonic acid[2], we investigated the synthesis of some 3-deoxy-heptulosonic acids.

Starting from D-glucose, we followed the procedure as described for the hexulosonic acids (Fig. 1).

By this method the compounds II and III were obtained, both characterized by their spectra. However, we were

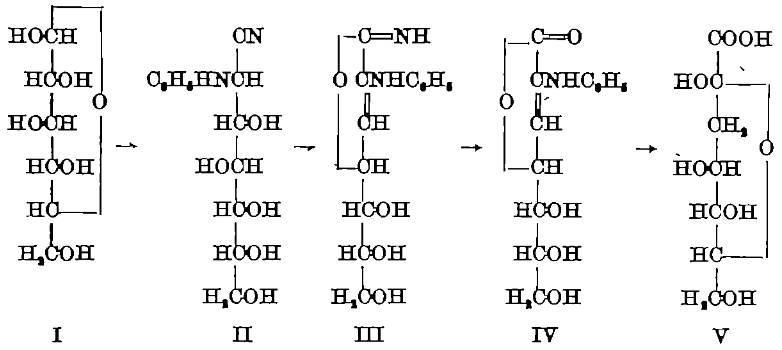
Fig. 1

unable to isolate the intermediate IV. We obtained a syrup in which the acid V was detectable by colour reactions. We did not succeed in preparing the acid in a pure form from this syrup.

After modifying the reaction conditions (treatment of compound III in water at 95°–100° C for 1 h) compound IV was obtained as a crystalline substance (melting point 158°–159° C). The structure was established by micro-analysis and by the infra-red and ultra-violet spectra. Stirring compound IV in water with 'Amberlite *I.R.* 120 H' at 70° C for 40 min afforded a crystalline compound with melting point 167° C (dec.), $[\alpha]_D^{23} = -5{\cdot}8°$ (H_2O; $C = 2{\cdot}8$).

This substance is the lactone of the keto acid (V), as was indicated by micro-analysis and by the absence of a strong acidic reaction. The keto group proved to be enolized, as was shown by potentiometric titration, which revealed the presence of a weak acid (equiv. weight 191·5; calc. 190·2). This agrees with the absence of the typical reactions of the 3-deoxy-2-keto acids, such as the coloration with *o*-phenylene diamine and the periodate–thiobarbituric acid reaction. Furthermore, the structure of the enol-lactone, as given in Fig. 2, was confirmed by ultra-violet and infra-red spectra, bromine consumption, colour reaction with ferric chloride, and consumption of 2 moles of periodate. The ultra-violet spectrum shows a maximum at 229·5 mμ ($\epsilon = 6{,}000$) in acidic solution, which shifts to 261·0 mμ ($\epsilon = 5{,}500$) at *p*H 8. In the infra-red spectrum (in methanol) absorption bands appeared at 1,761 cm⁻¹ (γ-lactone C=O, conjugated with C=C bond), 1,654 cm⁻¹ (C=C). No absorption band of a keto group was present.

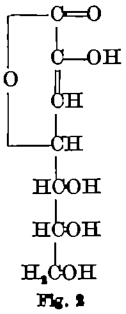
Fig. 2

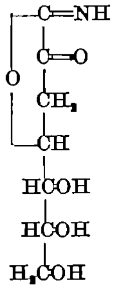
Fig. 3

For comparison we prepared 2,4-dihydroxy-crotonic acid-γ-lactone and its 4-propyl derivative by known methods[3]. These substances show the same characteristic bands in the infra-red (in methanol) and ultra-violet spectra and the same chemical properties. These spectral data are in accordance with reports on analogous compounds[4].

The enol-lactone was also obtained by treatment of the intermediate III with 'Amberlite *I.R.* 120H' in water at room temperature for 4 h. In this reaction a second crystalline substance could be isolated in addition. This material contains nitrogen and is no intermediate, because it could not be converted into the 3-deoxy-heptulosonic acid or its enol-lactone. It probably results from a side reaction. Micro-analysis and the infra-red spectrum showed the structure to be a 1-imino-2-keto-γ-lactone (Fig. 3). Absorption bands at 3,170 cm⁻¹ (N—H), 1,730 cm⁻¹ (C=O), 1,656 cm⁻¹ (C=N) in potassium bromide.

Starting from D-galactose we obtained only the corresponding 1-imino-2-keto lactone, as was established by micro-analysis and the infra-red spectrum.

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BIOCHEMISTRY

A Sensitive Qualitative Test for Sulphoxides on Paper Chromatograms

A number of sulphoxides have been found to occur naturally in plants[1] (for example, alliin, *s*-methyl-L-cysteine sulphoxide, glucoiberin). To aid in the detection of sulphoxides on paper chromatograms, a specific sensitive test has been devised. The test, which is based on the reaction of sulphoxides with hydriodic acid to form iodine[2], is presented here.

The test is carried out by lightly spraying a chromatogram with an aqueous solution of soluble starch, sodium iodide and hydrochloric acid. The reagent solution is prepared by mixing ten volumes of a solution of soluble starch (5 per cent) and sodium iodide (0·5 per cent) (stable one week in the dark) with one volume of concentrated hydrochloric acid immediately before use. The chromatogram is quickly placed in a desiccator containing dry sodium hydroxide. The paper is supported in an unfolded condition with minimal contact to avoid uneven colour development and artefacts. The desiccator is evacuated with a mechanical pump for 30–60 min in the dark. Sulphoxides are revealed as brown spots which develop slowly in about 15 min. The contrast can be improved by a second spraying with a 5 per cent solution of soluble starch to yield a purple colour.

This procedure is reproducible and sensitive. Methylcysteine sulphoxide can be clearly detected at a concentration of 0·01 μmole per 20 μl. (covering about 3 cm² of Whatman No. 1 paper). Other sulphoxides tested (methionine sulphoxide and glucoiberin) have a similar sensitivity, which is comparable with that of the ninhydrin reaction. Even at high concentration these sulphoxides react slowly, giving a brown colour. The brown spots are stable for several weeks in the desiccator, but disappear within 24 h after removal. The purple colour obtained from the second spraying (5 per cent soluble starch) fades in a few hours.

The protein amino-acids and the common sugars gave no reaction. Neither thioethers nor sulphones gave a positive test. Colour formation with di-*n*-propyl thiosulphinate was consistent with a sulphoxide structure. Dimethyl thiosulphonate, diethyl thiosulphonate and toluene sulphinic acid formed a purple colour immediately (that is, without drying) with about one-tenth the sensitivity of sulphoxides (0·1 μmole/20 μl.). 'Cysteine disulphoxide'[3], which probably has a thiosulphonate structure[4], also reacted without drying, but could be detected only at a concentration of 1 μmole per 20 μl. These results agreed with the known reduction[5] of thiosulphonates with hydriodic acid. A weak positive test was obtained with sinigrin, but the explanation for this reaction is unknown. Strong oxidizing agents such as peroxides, peracids, permanganate and periodate form an immediate purple colour with the sulphoxide reagent. Low concentrations of oxidizing agents react at once or not at all. The test is specific for sulphoxides, since they are the only compounds that give a positive test and do not react immediately.

After chromatography, 0·01 μmole of methylcysteine sulphoxide can be detected on a small chromatogram (18 × 18 cm) and 0·05 μmole on a large chromatogram (56 × 56 cm). The type of solvent used did not affect the sensitivity of the test. However, after chromatography with either phenol/water (8 : 3) or *n*-butanol/acetic acid/water (9 : 1 : 2·5), methionine and methylcysteine spots gave a positive test owing to oxidation during evaporation of the solvent after chromatography. The same effect has not been observed with other thioethers or after the use of basic solvents. To avoid oxidation of methionine and *S*-methylcysteine during chromatography[6], they can be converted to their sulphoxides prior to chromatography[7].

More stable thioethers may be chromatographed as such and then detected as sulphoxides after chromatography by treating the chromatogram in a closed chamber with hydrogen peroxide vapours at room temperature for 30 min, followed by thorough drying.

Subsequent to a sulphoxide test, a ninhydrin reaction can be obtained after decolorization of the spots by placing the chromatogram in an ammonia atmosphere for 1 h. This test has already proved useful in the study of the *in vivo* conversion of *S*-methylcysteine to its sulphoxide in crucifers[8], and in the detection of sulphoxides in plant extracts.

We thank J. F. Carson and F. F. Wong for gifts of dimethyl thiosulphonate, diethyl thiosulphonate and di-*n*-propyl thiosulphinate and A. Kjaer for gifts of glucoiberin and sinigrin. This work was partially supported by funds from the Rockefeller grant for research in plant physiology in the College of Agriculture at Cornell University.

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Influence of 5-Bromosalicylhydroxamic Acid on Serum Cholesterol-level

This communication presents the results of clinical experiments on influence of 5-bromosalicylhydroxamic acid (BSH) on serum cholesterol-level. BSH was formerly suggested by one of us as an antitubercular agent[1].

W. Johnson *et al.*[2] have given clinical evidence that BSH is an effective inhibitor of isoniazide inactivation. It is known that this inactivation is the result of acetylation of isoniazide[3]. On the other hand, it is known that acetic acid is a precursor of cholesterol in the rat[4] and the biosynthesis of cholesterol can start from acetate[5].

These two facts led us to examine the activity of BSH on cholesterol-level in blood serum. The substance has been shown to be of low toxicity[1,6,7], and this encouraged us to carry out our experiments directly in clinics.

Twenty patients with high cholesterol blood level (above 200 mg) were given orally 4 g of BSH daily. Within twelve days 9 patients (45 per cent) responded favourably to the treatment, and cholesterol-level was reduced by 8–31 per cent (average 20 per cent) (Table 1).

Table 1

No. of patient	Initial cholesterol-level (mg per cent)	Cholesterol-level after twelve days of treatment (mg per cent)	Reduction of cholesterol-level (per cent)	Cholesterol-level ten days after the treatment (mg per cent)
1	238	174	26·9	212
2	272	193	29·1	220
3	220	193	12·3	—
6	248	212	14·8	212
7	193	138	28·3	—
10	248	171	31·0	264
14	287	264	8·0	287
18	248	217	12·5	230
20	207	172	16 9	241
Average	240 ± 9·9	192 ± 11·9	20 0	238 ± 10·5

Ten days after the end of treatment, the cholesterol-level was again examined in most of the patients and was found to be clearly higher than immediately after treatment. A typical example is given in Fig. 1. This seems to indicate that lowering of cholesterol-level was due to the treatment with BSH.

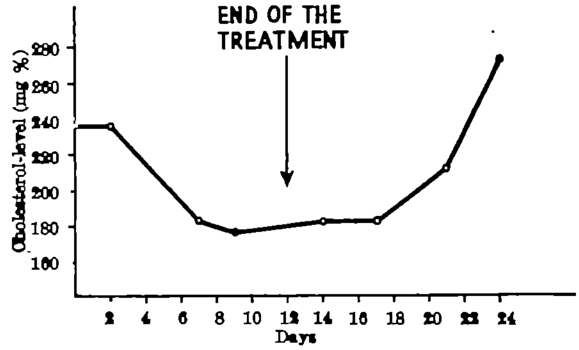

Fig. 1. Influence of BSH (4·0 g daily) on the cholesterol-level in serum

Further experiments will be carried out with the patients which did not respond to the treatment with BSH. In addition to these experiments a number of people with a normal serum cholesterol-level have been examined and their cholesterol, blood sugar and amino-nitrogen in plasma were determined.

The results are summarized in Table 2.

Table 2

No. of persons	Serum cholesterol (mg per cent)			Sugar in blood (mg per cent) (King's method)			Nitrogen of amino groups in plasma (mg per cent) (Frame *et al.* modified by Russel)		
	Hours after the application								
	0	2	4	0	2	4	0	2	4
12	154	167	170	93·5	74 1	78·1	5·1	5·4	5·0
S.E.	±13·3	±14·4	±13·3	±5·1	±2·6	±3·4	±0·17	±0·23	±0 12

It appears that BSH can also slightly raise the serum cholesterol-level in normal persons, and this indicates that

BSH definitely acts on the cholesterol-level, whether it is by reducing or increasing it. The latter seems to characterize cases with a normal cholesterol-level.

BSH certainly reduces the sugar content of blood and it appears that a little increase of amino-nitrogen is possible.

A sedative side-effect of BSH was also noticed in the course of experiments[8].

According to McIsaac and Williams[6] BSH is partly metabolized in the human body into 5-bromosalicylamide. We therefore also examined this substance from the point of view of its activity against hypercholesteraemia. Five patients were treated with 5-bromosalicylamide (2 g daily), but this did not seem to influence the cholesterol-level in blood serum.

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Anthocyanins of the Squill

In the course of an investigation of the cardiotonic glycosides and flavonoid content in the squill (*Scilla maritima* L. or *Urginea maritima* Baker), several anthocyanins were identified, we believe, for the first time.

There are two principal varieties of squill: the red, and the white, with intermediate forms[1]. In the many samples examined, we found the following differences in colour: (*a*) completely white in both the innermost and outer tissues; (*b*) cream colour, yellowish or yellow-green; (*c*) red-brown; (*d*) red-violet. When the transverse sections are treated with 2 N hydrochloric acid the changes occur only in groups *c* and *d*; in the others only a few cells appear to be coloured. The changes in colour most frequently occur in the cells which are under the skin.

According to del Amo[2] the squill is distributed in the Iberian Peninsula and Balearic Islands, in populations the individuals of which belong to the same cariological type. We used for our investigation some 250 bulbs collected by him during 1957–60. Cariological determination gives the following distribution for the bulbs collected: 5 populations towards the north of the Ebro River on the coast of Cataluña which are triploid; 25 populations towards the south of the Ebro on the Mediterranean coast, central part of the Peninsula and Portugal which are all hexaploid; 20 populations collected in the Balearic Islands which are tetraploid. Anthocyanin content has also been examined in other related species: *U. undullata* (Desf.) Steinh., *S. obtusifolia* Moris, *S. lilio-hyacinthus* L., and *S. verna* Huds.

Extractions were carried out by immersing the scales in the presence of absolute ethanol, the final alcoholic concentration being 85 per cent; the dry weight of the bulb was approximately 17 per cent. This was repeated until no more colouring matter could be extracted. No hydrochloric acid was added to the extraction liquids; otherwise the anthocyanins can be altered in part, this being indicated by the appearance of a brown streak near the origin in the chromatogram[3]. A glucoside of pelargonidin which exists in some bulbs was also changed by hydrochloric acid. This does not conform with Lamort's views[4], who maintains that pelargonidin and its glycosides are very stable.

The extracts were concentrated in vacuum and banded on Whatman No. 3 paper, directly or after extracting the major part of the cardiotonic glycosides and flavonoids with ethyl acetate and mixtures of butanol/chloroform[5]. The banding was repeated three times for the isolation of the anthocyanins: first with butanol/2 N hydrochloric acid (1 : 1), afterwards with butanol/acetic acid/water (4 : 1 : 5) and finally with acetic acid/water (15 : 85). The anthocyanins were completely eluted with 70 per cent ethanol. For the identification of the anthocyanins, sugars and acylated residues the general techniques were used[6,7].

The most abundant anthocyanin found in the squill varieties examined was cyanidin-3-monoglucoside, free or acylated with caffeic acid. R_F of the acylated pigment: 0·27, 0·38, 0·42 respectively on the solvents mentioned λ_{max} methanol 0·01 per cent conc. hydrochloric acid = 527 mμ (Lange spectrophotometer II). In some cases other unidentified bands of cyanidin glycosides were found, with lower R_F values when alcoholic solvents were used; some of these glycosides seem to be acylated with *p*-coumaric acid. Two populations of tetraploid plants, examined in August, were found to contain a pelargonidin glycoside, in quantities of about 30–40 times less than those of the cyanidin. It ran in the paper as pelargonidin-3-monoglucoside isolated from the carnation, but was very sensitive to hydrochloric acid. The main difficulty for the identification of some of these pigments is due to the presence of lyophilic substances, which separate well in the chromatograms from the cyanidin-3-monoglucoside, and badly from the other pigments. We are trying to achieve this separation using cellulose powder columns with anhydrous solvents.

Table 1. Anthocyanin Content of Squill Varieties expressed as Chrysanthemin (mg/g Dry Weight)

Polyploidy	Date of determination	No. of populations	Chrysanthemin	
			In dark	In light
Triploid	Dec.–Jan.	5	0·00–0·03	1·16–1·76
	May–June	5	0·03–0·18	1·06–1·13
Tetraploid	Dec.–Jan.	8	0·19–1·22	0·57–3·55
	May–June	14	0·06–0·73	0·10–1·60
Hexaploid	Dec.–Jan.	7	0	0
	May–June	12	0	0·00–4·10

The quantitative estimation of the total anthocyanin content of the bulbs was carried out by a colorimetric method (colorimeter Zeiss Elko II, filter *S* 53). The aqueous solutions were made 0·1 N in hydrochloric acid, and the results expressed in cyanidin-3-monoglucoside (chrysanthemin), the same chromatographically isolated pigment used as reference standard. Table 1 records the results of two seasons: in winter when the plants still had leaves, and in late spring when all the leaves were dry. For each population three bulbs of different sizes were examined, immediately after being removed from the earth and after exposing the scales for one month to indirect daylight. When they were exposed to direct sunlight, the anthocyanins were altered after a short time and the scale assumed a brown colour.

Among the bulbs not exposed to light, the greatest content of anthocyanins was present in the tetraploids, which generally are those of greater weight and stronger colour. The triploids contained less and the hexaploids were completely white and contained no appreciable anthocyanins, although small bands of anthocyanins can be seen on the chromatograms. During the seasons mentioned some 60 hexaploid bulbs were examined recently pulled from the earth, only two of which, in the month of May, contained anthocyanins (0·2 mg/g). These results are not given in Table 1 because we have been unable to confirm the cariological determinations.

When the bulbs were exposed to daylight, the anthocyanin content generally increased with the red-violet colour; the white and cream bulbs acquired stronger yellow colour and occasionally yellow-green. From the bulbs examined in these conditions, the lesser increase in anthocyanin content corresponded to some tetraploids; while the relatively greater increase was observed in the triploids. Approximately half the hexaploids remained without offering appreciable quantities of anthocyanins; however, some bulbs examined in May presented the highest content on exposure to light.

No generalization can be made yet on the relation between anthocyanin content and cariological data; we can say that the collected tetraploids were rich in pigments; the hexaploids we found in the central part of Spain were the whitest and the poorest in anthocyanins; the Mediterranean hexaploids when exposed to light generally increased greatly in content of anthocyanins. We have been looking for leucoanthocyanins, but thus far no reactive spots were found when the paper was sprayed with *p*-toluenesulphonic acid.

When the bulbs of the three types were not completely submerged in the ground, or when they suffered some shock, anthocyanins appeared in appreciable quantities. In those exposed to the cold during the winter their frozen parts were red, and rich in anthocyanins including the whitest hexaploids.

Anthocyanins did not appear on the leaves in appreciable quantities, except in the plants grown in darkness, coinciding with the decrease in chlorophyll content. These results are in agreement with those of Kandeler[8], who increased the anthocyanin content in the shoots of red cabbage by the elimination of the chlorophyll with antibiotics. In the other species examined, anthocyanins did not appear, except in the *U. obtusifolia*, which had in the bulbs and in the leaves quantities similar to the tetraploid squills. These plants did not show the Liebermann reaction for cardiotonic glycosides.

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Neutron Activation Analyses of Snake Venoms: Presence of Copper

LITTLE is known about the mechanism of the spreading of snake venoms through the body of the victim. Duran-Reynals[1] postulated a "spreading factor" in snake venoms, which was identified by Chain and Duthie[2] as hyaluronidase. While the spreading of elapide venoms may be correlated to some extent to their hyaluronidase content, very little[3] or no hyaluronidase was found in viperide venoms.

An attempt was made to follow the spreading of snake venoms in experimental animals by the use of radioactivity techniques. So far *in vitro* labelling of venoms has been reported only with iodine-131 (ref. 4).

Recently, radioactive venoms of a relatively high specific activity were obtained at the Rogoff Medical Research Institute at Beilinson Hospital[5] by *in vivo* feeding of cystine labelled with sulphur-35 to specimens of *Vipera palestinae*.

Neutron activation was used for the detection of trace elements in different venoms, on the assumption that the presence of specific trace elements could be used for tracing and following the path of the venom, or part of it, through tissues of experimental animals.

The only trace element reported in snake venoms is zinc. Concentration in viperide venoms are in the range of 1,000–3,000 p.p.m. (for example, that of *Bitis arietans*, 1,100 p.p.m., and in elapide venoms, 3,000–5,600 p.p.m.)[6]. Since zinc is widespread in animal tissues, the search was directed towards other elements.

Small samples of venom were weighed, sealed in polyethylene vials and irradiated in *IRR*-1 (the Israel Research Reactor at Soreq). The γ-ray spectra of the irradiated samples were determined using a 3 × 3 in. sodium iodide (Tl) scintillation counter and a multichannel analyser. Under these conditions, the only activities observed were due to sodium-24 and chlorine-38. When the venoms were first dialysed several times against water to remove non-protein-bound electrolytes, the activities of sodium and chlorine were considerably reduced. Other γ-ray peaks were observed and their decay was followed. The major peaks were assigned to 5·1 min copper-66 and 12·8 h copper-64, both produced by (*n*,γ) reactions with copper. A more precise assay of the protein-bound copper was carried out by fast radiochemical separation with carrier and β-counting of the precipitated CuCNS. The decay of the β-activity also consisted of two components with half-lives of ~ 5 min and ~ 13 h, due to copper-66 and copper-64 respectively. Fast coincidence measurements of the γ-rays resulting from the annihilation of the positron emitted by copper-64 provided an additional method of analysis and confirmed the results. Since γ-ray spectrometry is hampered by the interference due to the activation of sodium, chlorine and other impurities, most analyses were carried out using chemical separation and β-counting, which has a sensitivity of better than 10^{-9} g copper.

When the relative copper contents of samples were to be compared, the faster, though less-sensitive, method by coincidence counting was used (sensitivity ~ 10^{-7} g copper). This method can be made specific and is non-destructive.

A summary of various results is given in Table 1.

Table 1. COPPER CONTENT OF VARIOUS SNAKE VENOMS

Venom	Treatment	Copper concentration (±10%) (p.p.m.)
Vipera palestinae	dialysed	800
Naja naja	dialysed	1,600
Naja naja	non-dialysed	1,100
Echis carinatus	dialysed	3,900
Bitis arietans	dialysed	3,900

For comparison, the copper contents of normal mammalian tissues range from 10 to 30 p.p.m.[7].

A check on the zinc concentration was carried out using dialysed *Vipera palestinae* venom, which was found to contain 670 ± 10 per cent p.p.m. The zinc was determined by chemical separation of the irradiated sample and counting the β-activity of 52-min zinc-69 in the $ZnHg(CNS)_4$ precipitate.

Variation of the duration of dialysis against distilled water, using different venoms, did not reveal any change in the copper concentration of the protein fraction, which indicates that the copper is firmly bound to the protein.

An ion exchange chromatographic separation of *Vipera palestinae* venom on DEAE cellulose yielded two protein fractions containing the neurotoxin and the hæmorrhagin of the venom respectively[8]. Copper assay of the two fractions, by the coincidence method, showed that the copper was mainly in the neurotoxic fraction.

Activation analysis was also used for the determination of the copper content in various mouse tissues. Attempts to detect an increase in the copper concentration in different organs after the introduction of venom were unsuccessful, due to the dilution of the small quantity of venom (about 1 mg, with copper concentration of about 1,000 p.p.m.), in the larger sample of animal tissue (about 100 mg containing ~ 10 p.p.m. copper). Since the variation in the distribution of the copper within the analysed tissues was about ± 50 per cent, it was difficult to detect extra copper due to the venom, even under optimal experimental conditions, when the muscle containing freshly injected venom was frozen with liquid nitrogen immediately after the injection.

Experiments to detect other trace elements in snake venoms as well as to study the bond strength of the copper in the venom and the distribution of copper in the different venom fractions are in progress.

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Enzymatic Reduction of δ-Keto Acids to the Corresponding Optically Active Hydroxy Acids

OPTICALLY active lactones of δ-hydroxy fatty acids contribute considerably to the total character of natural butter flavour[1]. Investigations have shown that there are several micro-organisms capable of producing optically active γ- and δ-hydroxy acids from the corresponding keto-compounds. One of the most suitable micro-organisms for this conversion is *Saccharomyces cerevisiae* or normal bakers' yeast[2,3].

To examine the character of this conversion, the enzymology of the reduction of δ-ketodecanoic acid by *S. cerevisiae* was investigated, part of which is described here.

An acetone powder of *S. cerevisiae* was prepared by dehydrating normal bakers' yeast (Kon. Ned. Gist- and Spiritusfabriek, Delft) twice with 10 parts (v/w) of acetone at −25° C and filtering off the yeast. The last traces of acetone were removed from the yeast *in vacuo*. The powder, together with the keto acid, was incubated in phosphate buffer ($pH = 6·0$) in the presence of, respectively, NAD *, NADP, isocitric acid, fumaric acid, and combinations of the coenzymes and the acids mentioned (Table 1).

Table 1. REDUCING CAPACITY OF AN ACETONE POWDER OF *S. cerevisiae*

Co-factors/co-substrates added	Remaining keto acid (%)	Hydroxy acid found * (%)
Blank	92	3
NAD	95	—
NADP	74	16
NADP + Mg^{2+}	85	9
DL-Isocitric acid	95	?
Fumaric acid	96	—
NAD + DL-Isocitric acid + AMP	97	—
NADP + DL-Isocitric acid	66	22
NADP + DL-Isocitric acid + Mg^{2+}	83	8
NADP + fumaric acid	65	21

* As equivalent amount of keto-acid.

The reaction mixtures contained: 100 mg acetone powder, 0·25 mmole potassium phosphate buffer pH 6·0, 30 μmoles δ-ketodecanoic acid (potassium salt) and the coenzymes/co-substrates indicated here. Total volume: 5 ml. The amounts of co-factors/co-substrates added were: NAD, 1·5 μmoles; NADP, 1·5 μmoles; AMP, 10 μmoles; DL-isocitric acid (tri-sodium salt), 30 μmoles; fumaric acid, 40 μmoles; magnesium chloride, 1 μmole. The mixtures were incubated at 30° C for 3 h *in vacuo*.

After incubation the mixtures were acidified to $pH = 1$ and the keto- and hydroxy-acids extracted from the reaction mixtures with ether. The keto acid was determined spectrophotometrically as the 2,4-dinitrophenylhydrazone in alkaline solution at 530 mμ, and the hydroxy acid as the Fe-(III)–hydroxamic acid complex at 515 mμ.

Table 1 clearly shows the specific need for NADP and the stimulating influence of the DL-isocitric acid and fumaric acid on the reduction when NADP is also present.

A cell-free homogenate of *S. cerevisiae* was prepared by disintegrating bakers' yeast by means of a mechanical shaker of the same type as the destructor used by Nossal[4]; afterwards the intact cells and the cell debris were centrifuged off. The crude cell-free homogenate was fractionated by centrifugation and the various cell compounds (cell walls, mitochondria and 'soluble fraction') incubated with δ-ketodecanoic acid in phosphate buffer (pH 6·0) in the presence of DL-isocitric acid and NADP. These experiments showed that the δ-ketodecanoic acid reducing enzyme (enzyme system) is present mainly in the mitochondria. Experiments with dialysed mitochondria revealed which co-factors were specifically needed for the reduction of δ-ketodecanoic acid to δ-hydroxydecanoic acid. The following co-factors appeared to be absolutely necessary: NADPH, coenzyme-A, ATP and Mg^{2+} (or Mn^{2+}). Hence, the aforementioned reduction takes place via the coenzyme *A*-ester of the keto acid. Since NADPH cannot be replaced by NADH, the reduction appears to be NADPH-dependent. For the specific rotation of the δ-hydroxydecanoic acid lactone, obtained via the reduction of the corresponding acid with isolated yeast mitochondria, an $[\alpha]_D^{20} = + 55·5°$ (in methanol; lactone concentration: 29·3 g/l.) was found which is in agreement with that for the lactone obtained via the reduction with intact cells[2,3]. Hence, the reduction with isolated mitochondria is stereospecific, just as that with intact cells.

The ability to reduce δ-keto acids to the corresponding hydroxy acids, however, is not confined to micro-organisms only; extracts from pigeon liver and ox liver also possess this property so that it may be stated that the enzyme (enzyme system) in question occurs in preparations originating from quite different biological objects. Pigeon liver and ox liver were homogenized in 0·03 M phosphate–carbonate buffer (pH 9·0) at 0° C in a Bühler homogenizer for 30 sec at maximum speed. After filtration through muslin, the crude homogenates were first centrifuged for 15 min at 1,000*g*; afterwards the supernatants were centrifuged for 60 min at 100,000*g* (pigeon liver) and 20,000*g* (ox liver). Of both enzyme solutions obtained, 2-ml. portions were incubated *in vacuo* with 2 ml. 0·1 M phosphate buffer (pH 6·5), 30 μmoles δ-ketodecanoic acid (potassium salt), 1 μmole NADP, 15 μmoles DL-isocitric acid (tri-sodium salt), 0·4 μmole magnesium chloride, and 0·4 μmole manganese chloride, for 3 h at 38° C.

After incubation these pigeon- and ox-liver preparations contained, respectively, 67 and 77 per cent non-converted keto acid and 20 and 7 per cent hydroxy acid. The isolated hydroxy acid was identified by its lactone-flavour after heating the acid *in vacuo*, and by paper-chromatographic analysis of its hydroxamic acid in the systems benzene/isopropylalcohol/water/glacial acetic acid (5 : 2 : 5 : 1) and benzene/water/glacial acetic acid (5 : 5 : 1) both in accordance with Keeney's[5] method. In both cases the R_F-value of the hydroxamic acid spot was in agreement with that of the hydroxamic acid obtained from authentic δ-hydroxydecanoic acid lactone. Moreover, in both cases only one hydroxamic acid spot could be observed on the chromatograms. Although the foregoing is no absolute proof that the reaction product isolated is identical to δ-hydroxydecanoic acid, this is highly probable. Unfortunately, the amounts of lactone obtained were insufficient to determine its rotation.

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* NAD, NADP, NADH and NADPH represent, respectively, the oxidized nicotinamide adenine dinucleotide, its phosphate and both the reduced stages ATP is adenosine-5′-triphosphate; AMP is adenosine-5′-monophosphate.

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Deoxyribonucleic Acid Base Composition of Kappa and *Paramecium aurelia*, Stock 51

CERTAIN stocks of *Paramecium* contain cytoplasmic particles called kappa[1]. Kappa-bearing *Paramecia* (killers) are capable of killing other *Paramecia* (sensitives) and are resistant to the toxic agent which they produce[1]. The killer trait requires both the cytoplasmic particles and the dominant *K* gene for the maintenance of kappa, and is one of the most extensively studied examples of nucleo-cytoplasmic relations on a biological level[2-5].

In stock 51, kappa is of two morphological types, the so-called bright particle (which contains a refractile *R* body) and a non-bright particle which lacks this body[4]. The bright particle is associated with its ability to kill sensitive *Paramecia*[4]; the non-bright particle with the ability to convert sensitive paramecia of the proper genotype to killer animals[5]. In different stocks of killer animals, different kinds of killing are exhibited, characteristic of a particular stock; in stock 51, the most extensively investigated killer, the affected animals show an aboral hump before death[1].

The origin and phylogenetic relations of kappa to other organisms, to paramecia, to other cytoplasmic particles of paramecia, and to other DNA-containing particles of other organisms needs to be investigated using as many traits as possible. Since the equivalence of base composition of DNA would seem to be a minimum requirement for extensive base sequence homology and therefore genetic compatibility[6], a knowledge of the average base composition of the DNA of both host and particle might serve as a valuable trait. The present study reports the determination of the base composition of the DNA of kappa, of killer and sensitive *Paramecia*, and of *Aerobacter aerogenes*, used in the cultivation of the *Paramecia*.

The DNA base composition were determined using the caesium chloride density gradient centrifugation technique. The buoyant density of DNA is linearly related to its mole per cent guanine plus cytosine (G + C) content and requires only 1–2 μg DNA in the purified state or as the cell lysate[6]. The samples were each centrifuged together with *Pseudomonas aeruginosa* ^{15}N DNA (used as a reference of known density) in approximately 7 M caesium chloride at 44,770 r.p.m. until equilibrium was obtained in the Spinco Model *E* analytical ultracentrifuge (22 h). The banded DNA was photographed using ultra-violet absorption optics and the negatives traced with a Joyce-Loebl microdensitometer. The results are given in Table 1.

Lysates of killer *Paramecia* showed at least three well-defined bands. Each preparation of kappa (purified by the method of Smith[7]), of the strain of *A. aerogenes* used, and of the sensitive *Paramecia* used (isogenic with the killer, but lacking kappa) gave only a single band. Three bands were found in the lysate of the killer organisms at buoyant densities of 1·715, a minor or 'satellite' band at 1·696, and a major band at 1·689 g/c.c. corresponding respectively to the buoyant density of the single bands of DNA of *A. aerogenes*, kappa, and *Paramecia* found when each was run separately. Using a stock 51 *Paramecia* bearing a mutant kappa particle 51_{m43} (ref. 8) the DNA band of the host (1·689 g/c.c.) appeared to be the 'satellite' band with respect to kappa DNA (1·696 g/c.c.). This is not surprising since these particles are known to reach an average concentration of 3,000 per animal.

Table 1. COMPARISON OF MEAN GUANINE–CYTOSINE CONTENT OF DNA OF KAPPA, *Paramecium aurelia* AND *Aerobacter aerogenes**

	Buoyant density of DNA (g/c.c.)	Percentage G + C	DNA preparation‡
Killer paramecia†	1 716, 1·696, 1·689	56, 36, 29	lysate
A. aerogenes	1·715	56	purified
Kappa	1·696	36	lysate
Sensitive paramecia†	1·689	29	part purified

* The mole per cent guanine plus cytosine content was estimated by density gradient centrifugation of DNA in caesium chloride, using ^{15}N-labelled *Pseudomonas aeruginosa* DNA as the reference (density, 1·742 g/c.c.).

† Stock 51, killer *Paramecia* were grown on 0·15 per cent infusion of baked lettuce plus 0·1 per cent calcium carbonate inoculated 1 day before use with *A. aerogenes* (ref. 12). The *p*H of the medium was adjusted to a value of 6·5–7·5 prior to use; the sensitive animals were cultured axenically (ref. 13).

‡ Lysates were prepared by suspending 1 × 10^{5} *Paramecia*, or 2 × 10^{6} kappa, in 0·1 M ethylenediamine tetraacetate (EDTA) plus 0 15 M sodium chloride, *p*H 8·0, and adding sodium lauryl sulphate to a final concentration of 3 per cent; DNA was purified by Marmur's method (ref. 14).

On the basis of the mole per cent G + C content predicted from the buoyant density of the DNA, the results indicate that the DNA of the *Paramecia* is sufficiently different from kappa that extensive base sequence homology seems unlikely. It should be pointed out, however, that the presence of unusual bases in the DNA could affect the relation of the buoyant density of the DNA and the base composition[6,9]. The possibility of an odd base has not been excluded.

The base composition of the DNA of the *Paramecia* studied was in agreement with the range of mole per cent G + C contents for related ciliated protozoa (22–35 per cent)[10]. The DNA base composition of kappa, which strains gram negative, is close to certain *PPLO* organisms[9] and the cytoplasmic particle (polar bodies) of the flagellate *Strigomonas oncopelti*[11]. Whether these similar base compositions are fortuitous or indicative of a close taxonomic relation is not known at the present time.

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Effect of Cortisone on Collagen Formation in the Chick Embryo

CORTISONE given to adult animals decreases the amount of alkali-soluble[1] and neutral salt-soluble[2,3] collagen in the skin. In the urinary excretion of hydroxyproline, however, no effect has been found[4,5]. In the chick embryo, a marked increase in the content of free hydroxyproline occurs in the tissues 6–10 days after a single injection of

1 mg of cortisone acetate[6,7]. However, when cortisone is given to the pregnant rat, the contents of free and bound hydroxyproline in the tissues of the new-born young are decreased[8]. This discrepancy has been attributed to the fact that 1 mg of cortisone greatly reduces the weight of the treated chick embryos and hence the absolute amounts of free and bound hydroxyproline in the cortisone-treated embryos are lower than in the controls, although their content per unit of dry tissue is higher[7]. The present work indicates that cortisone also decreases the content of free hydroxyproline per unit of dry tissue, but when cortisone is given as a single injection, a marked increase later occurs in the content of free hydroxyproline.

In the present work the mode of action of cortisone on collagen formation in chick embryos is studied with respect to the following four fractions: (1) free hydroxyproline; (2) peptide-bound hydroxyproline soluble in 80 per cent ethanol; (3) hydroxyproline soluble in 1 M sodium chloride, insoluble in ethanol, designated as hydroxyproline of soluble collagen; (4) total hydroxyproline. The values for normal embryos and the methods of analysis have been presented in an earlier report[9]. In addition, the proline present in fraction (1) was determined by the method of Troll and Lindsley[10].

After a single injection of 100 µg or 300 µg of cortisone acetate on to the chorioallantoic membrane on the 10th day of development, a decrease in the content of free hydroxyproline and proline per unit of dry matter occurred during the following days (Fig. 1). This first stage is followed by a second stage with an increase in the content of free hydroxyproline. The free proline, however, does not exceed the level of the controls. To exclude the possibility that the increase in the free hydroxyproline might be due to a delay in reaching the maximum observed in the controls on the 14th day of development, a series was made, in which 300 µg of cortisone was injected on the 8th day of development. In this series the values were 55·5 µg per 100 mg of dry weight in the control embryos and 97·3 µg in those treated on the 14th day of development. When an increasing dose of cortisone correlated to the daily weight of the embryo was given daily from the 8th to the 15th day, the contents of free hydroxyproline and proline on the 16th day were less than that of the controls even with a daily dose of 3 µg/g embryo.

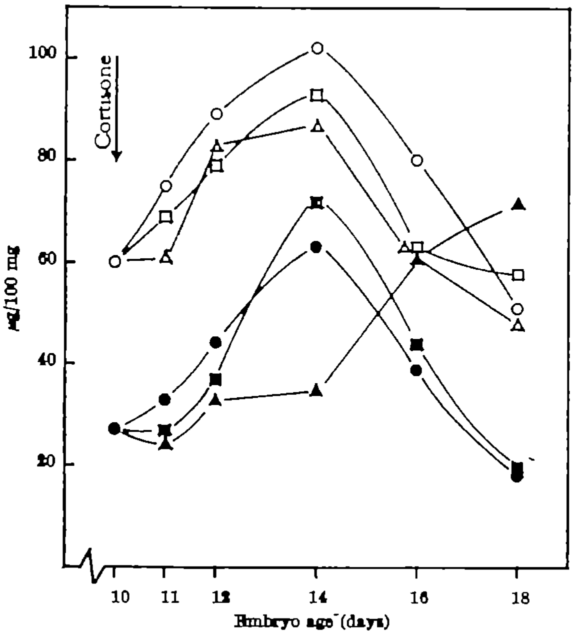

Fig. 1. Free hydroxyproline and proline in µg/100 mg dry substance of the embryo. Cortisone injected on the tenth day of development. Solid symbols, hydroxyproline; open symbols, proline;, ● ○, controls; ■ □, 100 µg cortisone acetate; ▲ △, 300 µg cortisone acetate

No marked change in the content of peptide-bound hydroxyproline was found after the administration of cortisone. Since this fraction was determined as the difference between the values before and after hydrolysis of the ethanol-soluble fraction, the methodical error was rather large. Therefore possible small changes in this fraction could not be detected.

The administration of cortisone as a single injection had no significant effect on the hydroxyproline of soluble collagen during the four following days when this was expressed per unit of dry matter. However, when this value was expressed as a percentage of the total hydroxyproline, a decrease was observed. When analysed six to eight days after the cortisone injection, the content of soluble collagen per unit of dry matter was increased, but this increase was much smaller than that of the free hydroxyproline. When cortisone was administered daily from the 8th to 15th day, the content of soluble collagen on the 16th day was below that of the controls even when expressed per unit of dry matter.

In all series the content of total hydroxyproline, expressed per unit of dry matter, increased after cortisone. Since even the smallest doses of cortisone used in this work slightly decreased the weight of the embryos, it seems possible, as suggested by Chvapil[7], that the increase is not real, but due to a decrease in the amount of substances other than collagen.

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PHYSIOLOGY

A Common Pathway for Uptake of Glycine and Proline in Various Living Cells

COMPETITION experiments with various amino-acids suggested several pathways for re-absorption of these compounds in dog kidney tubules, namely, concerned with: (*a*) arginine, histidine and lysine; (*b*) leucine and isoleucine; (*c*) glycine transport, respectively[1].

From the excessive excretion of the basic amino-acids, ornithine, lysine and arginine, as well as cystine in human cystinuria, a common pathway in the human kidney was suggested for these analogues[2]. This concept was confirmed by intravenous injection of lysine into non-cystinuric subjects when an 'artificial' cystinuria ensued[3]. Intravenous glycine had no such effect[3].

From similar infusion experiments a second re-absorption pathway, shared by glycine, proline and hydroxyproline, has been inferred for human renal tubules. This system has a higher preference for the two imino-acids than for glycine itself[4]. The excessive excretion of most of the protein amino-acids in Hartnup disease, with the exception of glycine and proline[5], is compatible with this hypothesis, that is, the glycine–proline pathway is relatively unaffected in this hereditary condition but the transport pathways for other amino-acids are faulty in the renal tubules[6].

The experiments reported here were designed to examine the competition of proline in glycine uptake in another type of mammalian epithelium, that is, mucosa of small intestine. The everted sac *in vitro* technique[6] was used with male albino rats. Uptake of glycine was measured spectrophotometrically with ninhydrin[7]. Proline did not interfere with the glycine estimations under the experimental conditions. The concentration ratio was calculated as the fraction:

$$\frac{\text{Concentration of glycine on the serosal side}}{\text{Concentration of glycine on the mucosal side}}$$

that is, a ratio greater than 1·0 indicates active transport against a concentration difference.

Table 1. GLYCINE AND PROLINE UPTAKE BY EVERTED INTESTINAL SACS

Test compound	No. of sacs	Concentration ratio of glycine	Rate of transference of glycine (µM/100 mg fat-free dry wt./h)
Glycine (20 mM)	10	1·36 ± 0·06	4·21 ± 0·03
Glycine (20 mM) + proline (20 mM)	12	1·19 ± 0·07	2·65 ± 1·16

Uptake of glycine was reduced by the presence of L-proline when present in equimolar amounts (Table 1). The values reported were statistically significant at the 0·1 per cent level. Inhibition of glycine uptake by proline has been demonstrated previously with hamster intestine[8]. It is of interest that L-proline has the greatest affinity for the glycine uptake system of Ehrlich ascites tumour cells of all the compounds tested[9]. Hence this competitive effect is seen in three very different systems, namely, the renal tubules, intestinal mucosa and free-living tumour cells. Therefore, it seems likely that glycine and proline share a fundamental and primitive transport pathway in living tissues. In kidney at least the glycine–proline pathway is apparently under genetic control, transmitting as a recessive character[4,10].

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Supersensitivity of Aqueous Outflow Resistance in Rabbits after Sympathetic Denervation

THE fall in intra-ocular pressure in patients with glaucoma simplex following topical epinephrine therapy has been noted repeatedly[1–3]. Recent tonographic analysis related this effect primarily to an increase in the facility of aqueous outflow although initially some small reduction in aqueous secretion may take place[4–6]. The mechanism by which the change in facility occurs is not known. Since tissues chronically deprived of their effector nerves develop an increased sensitivity to the neurohumoral transmitter, denervation experiments were performed to investigate the specificity of the response of outflow resistance to adrenergic influences.

Forty New Zealand albino male rabbits, weighing 2·0–2·5 kg, were subjected to right superior cervical ganglionectomy under thiopental sodium (Abbott) anæsthesia. One week later the animals were divided into three groups. The first group consisted of animals the eyes of which were given a 10-µl. injection of lævo-noradrenaline bitartrate in varying concentrations into each anterior chamber. The second group received a 10-µl. injection of lævo-noradrenaline bitartrate into each vitreous cavity. The third group consisted of saline-injected control animals. Injections were made with a calibrated Hamilton precision ground syringe and a 30-gauge needle. 2–3 h after injection, the intra-ocular pressure and outflow facility were measured after the technique of Sears[7] and Sears and Barany[8]. The animals were first anæsthetized with intravenous urethane (ethyl carbamate, Merck) and the eyes cannulated by means of a spring and trigger device[9]. Intra-ocular pressures were recorded using a Sanborn electromanometer system and the facilities of aqueous outflow were determined by measuring the steady-state increase in intra-ocular pressure caused by infusion of 2·0 µl./min of normal saline simultaneously into the anterior chamber of both eyes.

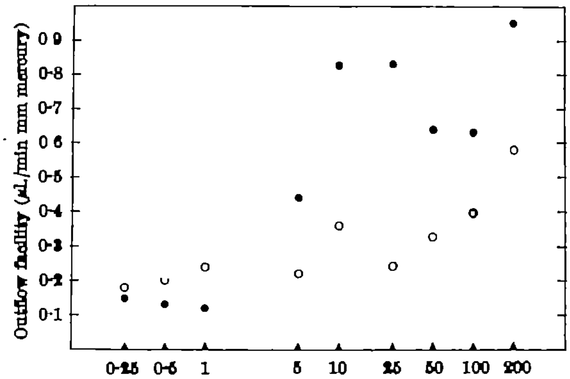

Fig. 1. Response of outflow resistance to adrenergic agents. ●, *RE*, denervates; ○, *LE*, normal

The effects of intra-vitreal injections of 1-noradrenaline on outflow resistance are indicated in the log dose response curve (Fig. 1). 3–6 animals were examined at each dose-level. In response to 1-noradrenaline there was a marked increase in outflow facility on the denervated side. A progressive increase in outflow facility occurred on the normally innervated left side. The latter effect was appreciable only at levels greater than 25 µg. On a molar basis, slightly higher doses were required to produce similar effects with 1-adrenaline. Isoproterenol was more effective (1·5 times) than either 1-noradrenaline or 1-adrenaline at lower doses. At higher levels of isoproterenol, the resistance-lowering effect was less marked on the denervated side, although greater effects were achieved on the normal side than with either 1-adrenaline or 1-noradrenaline. Intra-vitreal injections of 1·0 µg 1-noradrenaline bitartrate and less did not significantly affect the outflow resistance. At these levels the resistance on the denervated side was higher than on the normal left side as in the control saline injected animals (*RE* 0·16 ± 0·02, *LE* 0·24 ± 0·03 µl./min, mm mercury, $n = 6$, $p = 0·05$). Since no catecholamines could be recovered from the denervated eyes, while in control eyes the total catecholamines averaged 0·74 µg/g (ref. 10) iris and ciliary body, it is probable that the homolateral increase in outflow resistance noted one week after ganglionectomy is related to a decrease in (or absence of) the continuous release of adrenergic substances acting on the trabecular meshwork. Studies testing this hypothesis are in progress.

Anterior chamber injections could produce effects equal to those after vitreous injection in approximately 1/500 the intra-vitreous concentration. No definite resistance lowering effects were noted below 5·0 mµg with intra-cameral injection.

This work complements the studies of Barany[11], who noted that pretreatment with systemic reserpine prevented the resistance lowering effect resulting 24 h after superior cervical ganglionectomy. Further, α blockade accomplished by anterior chamber injection of phentolamine and phenoxybenzamine similarly reduced or prevented the 'ganglionectomy effect'. This finding suggested that the locus of action for 'α' active adrenergic material is on the trabecular meshwork. In the work reported here no effect was noted after an intra-vitreous injection of 1·0 μg of 1-noradrenaline bitartrate. By contrast, marked effects were noted with only 20 mμg after anterior chamber injection. Considerations of diffusion, protein binding, and degradation will of course necessitate high intra-vitreous dosage. The great sensitivity demonstrated with anterior chamber injection, however, would tend to exclude ciliary muscle and/or vascular effects to explain the resistance changes, and place the site of action in the trabecular meshwork.

Evidence for β or inhibitory adrenergic effects on the trabecular meshwork are inconclusive at the present time. Attempts to reproduce the elevated outflow resistance found one week after ganglionectomy using isoproterenol, a high β active material, showed that at low dose-levels isoproterenol acted more like noradrenaline, a strong α and a weak β stimulator. It is possible that at higher dose-levels, isoproterenol did have both β and α activity since the drop in outflow resistance was less than that which occurred with noradrenaline.

Parallel clinical studies of outflow resistance in patients with Horner's syndrome[12] also show a greater outflow facility on the homolateral side. The outflow resistance is further decreased after topical epinephrine bitartrate. Essentially similar results have already been reported by Krishna[13], who made a pharmacological denervation with topical guanethidine and noted a further reduction in intra-ocular pressure and outflow resistance when topical epinephrine was added.

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Distribution of Precorticotrophin in Blood

IN previous communications[1–3] the existence of precorticotrophin—a precursor of corticotrophin—was demonstrated in the anterior pituitary tissue of the ox. Later, I[4] reported the presence of a material behaving like precorticotrophin in normal rabbit blood serum. Young[5] observed that the insulin-like activity of the serum or plasma from arterial blood, or from blood oxygenated *in vitro*, was higher than that of serum or plasma from comparable venous blood, or blood deoxygenated *in vitro*. In view of this, it appeared worth while to investigate the presence of precorticotrophin-like material in sera obtained individually from arterial and venous blood.

Blood collected from carotid artery and from jugular vein of normal male rabbit under 'Nembutal' anaesthesia was allowed to stand overnight in a refrigerator. Next day sera were collected and freeze-dried. Just before the experiment each serum sample was dissolved in distilled water such that 1 ml. of the solution corresponds to 1 mg of the sample. A portion of such solution was next treated with urea in such an amount that its concentration was 6 M with respect to its final volume. Corticotrophic activity of both portions of solutions, untreated and urea treated, was determined by the Sayers method as used in the previous investigation[4].

Table 1. MEAN SAYERS ACTIVITY OF ARTERIAL AND VENOUS BLOOD SERUM OF NORMAL RABBIT

Group	Treatment	Mean adrenal ascorbic acid depletion (mg/100 g) ± *S.E.*
A	Saline only	35·8 ± 10·45 (15)*
B	Standard ACTH† solution (10 mμ/ml.)	77·2 ± 15·91 (6)
C	Arterial serum	37·0 ± 10·08 (20)
D	Arterial serum urea treated	75·1 ± 8·74 (20)
E	Venous serum	62·7 ± 9·81 (12)
F	Venous serum urea treated	53·3 ± 14·22 (12)

* Figures in parentheses indicate total number of observations.
† Gift of standard ACTH from Dr. D. B. Ghosh, director, Central Drug Laboratory, Calcutta, is acknowledged.

Table 1 shows the results obtained in the Sayers test when the test animals were injected intravenously with different test solutions at a dose of 0·25 ml./100 g body-weight. It is found that the response produced in the Sayers test by the arterial serum (Group *C*) is of the same order as that produced by saline only (Group *A*). But the response produced by the urea-treated arterial serum (Group *D*) is significantly higher ($0·001 < P < 0·01$) than that produced by untreated arterial serum (Group *C*). This indicates the presence of a precorticotrophin-like material in the arterial serum. On the other hand, venous serum untreated (Group *E*) compared with the group on saline only (Group *A*) appears to possess slight, although not significant ($P < 0·40$), activity in the Sayers test; such activity is not enhanced further after treatment of the venous serum with urea (Group *F*). It therefore appears that the precorticotrophin-like material which was reported earlier to be present in circulation[4] is demonstrable only in arterial but not in venous blood serum. Further, it explains, at least partly, the controversy that exists regarding the presence of corticotrophin in blood.

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Preparation of Antisera to Human Follicle-stimulating Hormone

WE have previously reported the preparation of antisera to fractions of human pituitary follicle-stimulating hormone which inhibited the activity of homologous antigens in mice and agglutinated red cells coated with the antigens, but they were ineffective against human chorionic gonadotrophin in rats[1]. The antisera have since been found to cross-react with human chorionic gonadotrophin in the red cell technique and when absorbed with human chorionic gonadotrophin they no longer agglutinated red cells coated with follicle-stimulating hormone. The antibodies, therefore, are not specific for follicle-stimulating hormone and we have now prepared antisera to new fractions.

The method of fractionation was by starch-gel electrophoresis, which is described fully elsewhere[2]. The segment of gel containing the follicle-stimulating hormone activity was eluted in saline, mixed with bentonite and given intravenously to rabbits or intraperitoneally to mice. The total dose given to rabbits was equivalent in biological activity to about 12,000 mg of the reference preparation *HMG*24; the dose given to mice was about 1,500 mg equivalents.

In vivo experiments showed that doses down to 0·025 ml. of these antisera inhibited the activity of homologous antigens in mice. *In vitro* tests were performed by the hæmagglutination method using tanned sheep red cells coated with follicle-stimulating hormone. The titres of the antisera varied from 1/200 to 1/1,600 and were reduced only slightly after absorption with human chorionic gonadotrophin. This was in striking contrast to the behaviour of antisera prepared to the follicle-stimulating hormone before electrophoresis. The reaction between the absorbed antisera and red cells was now inhibited by preparations of follicle-stimulating hormone (down to 0·3 μg/ml., equivalent to 1 mg *HMG*24/ml.) but not by human chorionic gonadotrophin (highest level tried was 100 μg/ml. containing about 100 I.U./ml.).

It thus appears possible to produce antisera to follicle-stimulating hormone suitable for immunological assay. The correlation of results obtained by this method and by bioassay will be reported elsewhere.

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Cholinesterase and Glutamic Decarboxylase Levels in the Brain of the Hibernating Hamster

LITTLE information is available concerning the biochemistry of the brain of the hibernating animal although a considerable amount of data has been reported concerning the electrophysiology. Hibernating animals respond to stimuli with cries, positional changes and arousal[1]; but conspicuous activity was reported to be absent in the electrocorticogram of the golden hamster (*Mesocricetus auratus*) during arousal until body temperatures reached 19°–21° (ref. 2). Recordings from sub-cortical areas showed electrical activity at lower temperatures to be largely confined to the limbic system[3], while in the awakening European hamster (*Cricetus cricetus*) there was a sequence of increasing electrical activity with rising body temperature from mesencephalon to neocortex[4]. Cortical and sub-cortical neuronal activity has been recorded in the ground squirrel during deep hibernation, but only at 10 per cent of the amplitude found in the awakened brain[1]. It therefore seemed of interest to determine whether changes in enzyme systems of particular significance to the nervous system, such as cholinesterase and glutamic decarboxylase, occurred in the hibernating brain concomitantly with these electrophysiological phenomena.

In the work reported here adult male golden hamsters were housed at 5° in individual cages with food and water *ad libitum*. After entering deep hibernation for at least the second time the hamsters were killed by decapitation, the brains removed and homogenized, and the enzyme assays performed. Control animals which had never hibernated were housed at 21°.

Enzyme assays were performed in duplicate on the homogenates. Cholinesterase was measured by Albers's modification[5] of Gal's acetylthiocholine method[6] in which the homogenate was incubated with acetylthiocholine chloride, the reaction stopped by the addition of four volumes of 6 per cent perchloric acid, the sample centrifuged, and the absorbancy of the supernatant solution measured at 227 mμ. Results are expressed as the mean of the differences between these values and non-incubated controls per mg of tissue protein after a standard dilution of the incubation mixtures. Glutamic decarboxylase was measured according to Albers and Brady[7] with the addition of 2×10^{-3} M arsenite to block catabolism via α-ketoglutarate. In this procedure the formation of $^{14}CO_2$ from glutamate-1-^{14}C was measured. Data are presented in terms of mean value of radioactivity in the recovered $^{14}CO_2$ above non-incubated controls per mg of protein. Protein was measured according to Lowry *et al.*[8]. Significance was determined by the Fisher *t* test.

Preliminary studies indicated that measured activity in a given preparation was proportional to the protein content of the homogenate within the range used in these investigations and that the rate of the reactions was roughly constant during the incubation periods.

Table 1. CHOLINESTERASE ACTIVITY IN BRAIN HOMOGENATES FROM HIBERNATING AND NON-HIBERNATING GOLDEN HAMSTERS

Condition of animals (and No.)	Incubation conditions Temperature	Duration (min)	Mean decrease in absorbancy at 227 mμ/mg protein	Significance
Hibernating (5)	30°	20	0·757 ± 0·112	$P < 0.05$
Non-hibernating (5)			0·863 ± 0·035	
Hibernating (5)	21°	45	0·990 ± 0·102	$P < 0.15$
Non-hibernating (5)			1·136 ± 0·144	
Hibernating (5)	10°	45	0·520 ± 0·104	$P < 0.01$
Non-hibernating (5)			0·712 ± 0·138	

Table 2. GLUTAMIC DECARBOXYLASE ACTIVITY IN BRAIN HOMOGENATES FROM HIBERNATING AND NON-HIBERNATING GOLDEN HAMSTERS

Condition of animals (and No.)	Incubation conditions Temperature	Duration (min)	Mean radio-activity of $^{14}CO_2$ produced/mg protein (c.p.m.)	Significance
Hibernating (5)	30°	20	164 ± 18	$P < 0.01$
Non-hibernating (7)			123 ± 22	
Hibernating (5)	21°	30	131 ± 42	$P < 0.05$
Non-hibernating (7)			86 ± 23	

Cholinesterase activity, assayed at 30°, was depressed 14 per cent in the hibernating brain (Table 1) while determinations at lower temperatures, where the scatter of the data was greater, gave roughly similar results. The decrease in activity, although small, is consistent with the relative electrical silence of the hibernating cortex[2], an area with a high concentration of cholinesterase in the non-hibernating animal[11]. Adaptive changes in brain cholinesterase activity have been demonstrated under other experimental situations[12,13]. Further, a recent study has shown a marked increase in sensitivity of hibernating hamsters to a cholinesterase inhibitor[14].

Glutamic decarboxylase activity, however, was 30 per cent higher in the hibernating brain (Table 2). The data were insufficient to determine whether this change was due merely to variation in enzyme concentration or to alteration of the energy of activation of the enzyme, as has been demonstrated with preparations from hibernating heart[9,10]. The product of glutamic decarboxylase, γ-aminobutyrate, has been implicated in the nervous system as an inhibitory agent and as a participant in energy metabolism through the γ-aminobutyrate shunt[15]. Examination of oxygen consumption and anaerobic glycolysis have shown little difference between brain slices from hibernating and non-hibernating golden hamsters[9], although conflicting results have been reported from other species[16,17]. The postulated role of γ-aminobutyrate as a regulator of neuronal activity[18], and its ability when administered topically to the cortex to augment certain electrical activity while repressing others[19], is compatible with the present findings.

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Effects of Intracarotid Injection of Hypertonic Saline on Spinal Reflex Excitability

DURING the past few years[1] we have been able to demonstrate that increasing the extracellular osmotic pressure by subcutaneous injection of hypertonic saline not only induces thirst and drinking in the rat, which has been extensively studied by others[2], but also produces an increase in the responsiveness of the animal and a relatively intense facilitation of previously learned thirst-associated simple motor acts[3] like running through an enclosed alley. The purpose of the present series of experiments was to measure this facilitatory effect in terms of an electrophysiological change in the final common path involved in the expression of this behaviour.

Changes in the amplitude of the monosynaptic spike in the cut fifth lumbar ventral root reflex discharge in response to appropriate low threshold, fast conducting afferent stimulation in cut muscle nerves of the hind leg served as a measure of this effect as a function of time following intracarotid injection of small amounts of hypertonic saline. Rats were prepared under ether anaesthesia, fixed to a rigid supporting apparatus, and were then switched to 'Flaxedil', 2 mg/kg administered intravenously, to prevent movements and were maintained on artificial respiration. The experiments were carried out 2½–3 h later. A large portion of the back and the hind limbs were deafferented by cutting the lumbar spinal roots. Reflex discharges were recorded through silver wire electrodes under paraffin oil at a cord temperature 37·5° C in response to rectangular pulses, 0·01 msec in duration, applied to the appropriate afferents. Afferent input was monitored by recording from the dorsal root close to the spinal cord. Potentials were amplified and recorded by means of Tektronix 122 pre-amplifiers, a 532 four trace oscilloscope, and a Grass C4D kymograph camera. Blood pressure was monitored in the femoral artery by means of a Statham *P23 AC* pressure transducer and a Grass pre-amplifier and polygraph.

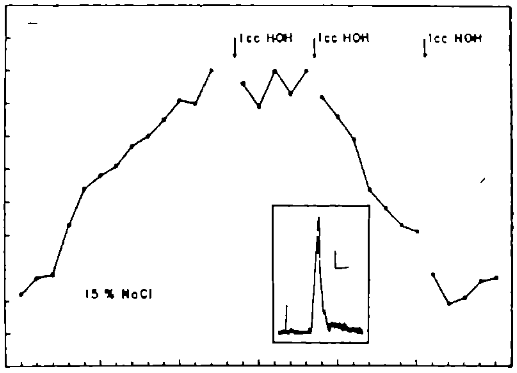

Fig. 1. Percentage change in lumbar (5) ventral root reflex discharges relative to the pre-injection mean value following a 1·0 c.c. intracarotid injection of 15 per cent sodium chloride, and successive 1·0 c.c. injections of distilled water. The lower tibial was stimulated ¼ sec and each point in the figure is the mean amplitude of 15 successive responses. The inset illustrates a typical monosynaptic reflex discharge. Calibration: 0·2 mV vertical, 1 0 msec horizontal

Results are presented in Fig. 1 where the percentage change in amplitude of the monosynaptic reflex discharge (relative to the pre-injection mean value) following a 1·0 c.c. intracarotid injection of 15 per cent sodium chloride, and successive 1·0 c.c. injections of distilled water, are plotted as a function of time in min. Solutions were injected at the rate of 0·02 c.c./sec. Monosynaptic reflex discharges were elicited by stimulating the lower tibial nerve at a frequency of 1/4 sec. Each point in Fig. 1 is the mean amplitude of 15 successive responses. The effect is quite pronounced and reversed by the injection of distilled water. The inset in Fig. 1 illustrates a typical monosynaptic reflex discharge. Within limits the intensity and duration of the effect depend on the amount of sodium chloride injected. It also occurs when other small nerves like the lateral and medial gastrocnemius are stimulated. The threshold is approximately 0·05 c.c. of a 3 per cent sodium chloride solution. A 0·15 c.c. of 9 per cent sodium chloride produces a facilitation which endures for 25 min. The effect occurs in adrenalectomized rats and cannot be attributed to changes in blood pressure, heart rate, or to an increase in the afferent input. It does not occur in spinalized animals. The effect occurs very rapidly, almost immediately, even with small volumes and weak concentrations, and is mediated centrally through a supraspinal mechanism. Small volumes of isosmotic concentrations of glucose and urea produce a very slight facilitation and increase the variability in amplitude of the reflex discharges. Distilled water depresses reflex excitability and at times increases the variability. Rat Ringer's solution produces a slight facilitation. Serum produces a slight facilitation and then depresses excitability. Because of the possibility of a significant difference in diencephalic temperature (the diencephalon is known to contain thermoreceptors which respond to changes in blood temperature[4]) and rectal temperature to which the injected solutions were adjusted, the effects of small volumes of serum at 5°, 10°, and 15° C above and below rectal temperature were studied. There were no significant effects of varying temperatures of the small volumes of serum injected; if anything, cold serum produced a slight facilitation. Observations confirming these results have been made in 56 different animals.

These results are significant because they demonstrate an electro-physiological change in spinal motorneurone excitability which is correlated with the facilitation of a previously learned thirst-associated simple motor act. They also provide a possible theoretical context for evaluating the recent work[5–7] on environmental induced (changes in blood composition) reactions of neurones in the supraoptic nuclei and the area postrema in terms of an animal's behaviour.

The results of a more detailed analysis of this effect and correlated changes in the electrical activity of the central pathway involved in its mediation which is in progress will be reported in the future.

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PHARMACOLOGY

6β-Hydroxycortisol (4-pregnane-6β,11β,17α,21-tetrol-3-20 dione) in Liquor Amnii

6β-HYDROXYCORTISOL was originally isolated from human urine by Bernstein *et al.*[1].

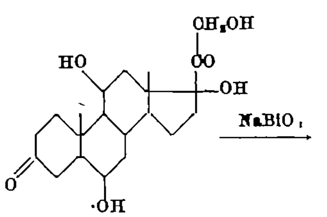
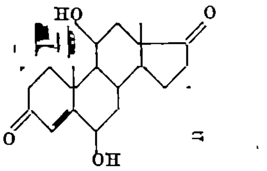
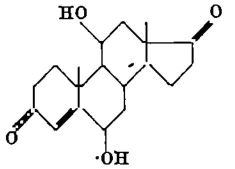
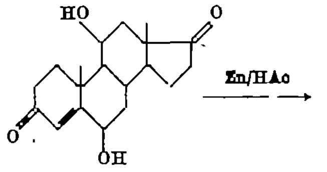
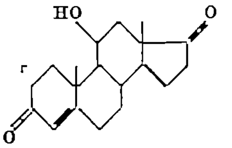
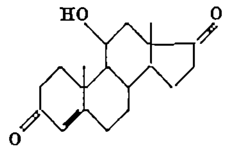
The possibility that 6β-hydroxycortisol might be present in liquor amnii was suggested by the work of Ulstrom *et al.*[2], who found that the major pathway for the metabolism of cortisol in the newborn was by 6β-hydroxylation.

Liquor amnii was initially extracted with ethyl acetate using a modification of the methods described by Katz[3]. The extract was washed with N/10 sodium hydroxide in 20 per cent sodium sulphate, and then on two occasions with 20 per cent sodium sulphate. It was then dried over anhydrous sodium sulphate and filtered after the addition of two drops of glacial acetic acid. The filtrate was evaporated to dryness at 40° C using a rotary evaporator, the residue being transferred to a small wide-necked glass bottle, with five 1-ml. portions of a 1 : 1 chloroform/methanol mixture. This was taken down to dryness in a stream of nitrogen gas at 40° C, taken up in the chloroform/methanol mixture, and then transferred to a Whatman No. 3 *MM* paper. After equilibration for 3 h the spot was run for 7 h at 30° C in a *B*/50 solvent system[4]. The polar steroids remained at the base line, and were eluted with about 4 ml. of an ethyl acetate/methanol (2 : 1) mixture[5]. The extract was taken down to dryness in a stream of nitrogen gas at 40° C and the residue taken up in the chloroform/methanol solvent (1 : 1) and spotted on to Whatman No. 2 paper.

After equilibration overnight the spot was allowed to run for 12 h in a modified *BuC* system[6] at room temperature.

The paper was then dried, and examined under ultra-violet light. A clearly visible spot was seen, with an approximate R_F-value of 0·05. An elongated strip of paper from baseline to solvent front was removed and dipped into a 2 : 1 solution of 10 per cent aqueous sodium hydroxide in 50 per cent methanol and 0·02 per cent blue tetrazolium in ethyl alcohol[8]. The strip was dried in an oven at 60° C for 10 min and again viewed under ultra-violet light, using a minus blue micro 4 gelatine filter (Ilford).

Table 1. PROPERTIES OF 6β-HYDROXYCORTISOL

		Isolated	Reference*
1	Ultra-violet absorption in alcohol	238 mμ	238 mμ
2	Reaction with blue tetrazolium	+	+
3	Fluorescence with NaOH	+	+
4	Mobility in two chromatographic systems (a) Mod. *BuC* (Dixon[6]) (b) Chloroform/formamide (Zaffaroni[7])	 R_F, 0·06 R_F, 0·01	 R_F, 0·06 R_F, 0·01
5	Absorption spectrum in conc. sulphuric acid (maxima) (Bernstein[8])	— 265, 290 350, 370 — 470, 515 mμ	240, 265, 285 350, 365, 390, 475, 525 mμ
6	Mobility after acetylation with acetic anhydride chromatography using 'LT 21/80' (Bush[9]) system	R_F, 0 06	R_F, 0 06
7	Mobility after reduction using sod. bismuthate (Bush[10]) 	 R_F value in 'LB 21/80' chromatographic system (Bush[9]) = 0 01	 R_F value in 'LB 21/80' chromatographic system (Bush[9]) = 0·01
8	Mobility after further reduction using Zn/HAC (Bush[11]) 	 R_F value in 'L.T. 21/85' chromatographic system (Bush[9]) = 0 24	 R_F value in 'L.T. 21/85' chromatographic system (Bush[9]) = 0·24

* The 6β-hydroxycortisol was kindly supplied by Dr. Seymour Bernstein, Lederle Laboratories, Pearl River, New York.

Three additional spots showing a brilliant yellow fluorescence were seen, two more polar and the third slightly less polar than the ultra-violet absorbing spot. All spots were eluted with a 2 : 1 ethyl acetate/methanol solvent. The less polar of the four spots (compound 1) has been tentatively identified as 6β OH cortisol.

Identification of the isolated 6β-hydroxycortisol was based on the properties as listed in Table 1, the results being consistent with those obtained by the reference 6β-hydroxycortisol. The quantity of 6β-hydroxycortisol isolated from liquor amnii was so small that it was impossible to subject the compound to infra-red analysis.

Identification of the other polar steroids isolated from liquor amnii is being actively pursued.

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HÆMATOLOGY

Staphylococcal Isocoagulases

Staphylocoagulase has been assumed to be a single enzyme elaborated by pathogenic *Staphylococci*. Investigators working with purified highly concentrated staphylocoagulase have described their product as either homogeneous[1] or non-homogeneous[2,3] when studied by free boundary electrophoresis, ultracentrifugation, or starch column chromatography.

We have now been able to show that *Staphylococci* in broth culture liberate into the medium a family of staphylocoagulases. A potent coagulase-producing strain of *Staphylococcus aureus* (*S*–58) was grown for 48 h at 37° C in tryptose broth containing 5 mg/l. of thiamine. After centrifugation, staphylocoagulase was precipitated from the supernatant by addition of 3 vol. of 95 per cent ethanol, slowly and at ice-bath temperature. The collected sediment, redissolved in distilled water, clotted oxalated 1 : 10 human plasma in 2 min and contained 50,000 clotting units/mg of protein. This preparation was subjected to electrophoresis on starch gel according to the method of Smithies[4]. Conditions of electrophoresis were: *tris* buffer *p*H 8·6, 4 V/cm, 18 h. The gel was then sliced horizontally and the cut surface placed on sterile fibrinogen agar (3 per cent bovine fibrinogen and 2 per cent rabbit serum[5]).

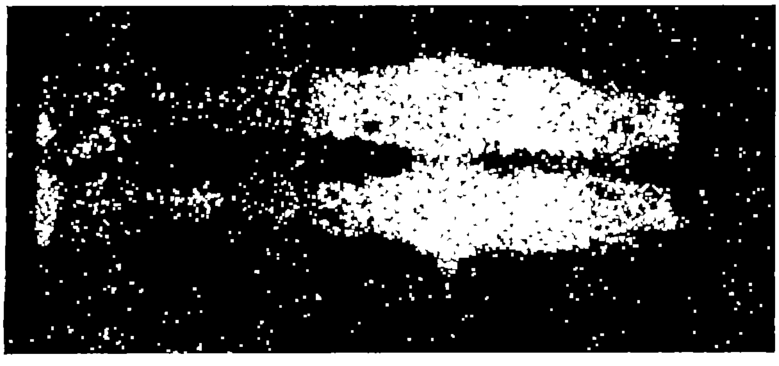

Fig. 1 Staphylococcal isocoagulases. The site of staphylocoagulase isoenzymes separated by starch-gel electrophoresis is marked by precipitation of the fibrinogen in the fibrinogen-agar. They appear as five grey opaque zones

After 24 h at 37° C, it was found that fibrinogen precipitates at several sites (Fig. 1). The reaction extending from the origin to the first band represents trailing. If a very powerful staphylocoagulase preparation is used we find trailing will obscure the individual bands. Since the reaction at the gel and agar surface is specific, it would seem that five isocoagulases are present. This experiment has been repeated with staphylocoagulase prepared from other strains and, although as many as eight distinct bands have been found at times, we have not found less than five.

Staphylocoagulase thus joins other enzyme systems, such as lactic dehydrogenase, characterized by iso-enzymes. Work is in progress to determine whether there exists differences between various coagulase-positive strains and among the several isocoagulases. Preliminary observations show that the more intensive the 'purification' procedure the fewer the isoenzymes which can be detected. Until it can be shown that the behaviour of each is like that of the others, particularly with regard to co-factor specificity and requirements, caution must be exercised in the design of studies with the staphylocoagulase system. Finally, it is not clear at this time whether the isocoagulases are entirely extracellular or whether one or more represent intracellular enzymes[4].

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Bivalent Nature of Incomplete Anti-D(Rho)

Erythrocyte antibodies were first demonstrated by their ability to agglutinate erythrocytes suspended in saline; these have been termed complete antibodies. In 1944, Wiener[1] and Race[2] independently showed that certain erythrocyte antibodies were incomplete, possessing the ability to fix to the erythrocyte surface without leading to agglutination. It has been postulated[1], and generally accepted, that the complete saline agglutination antibody is bivalent, and the incomplete coating antibody univalent. Recent investigations in our laboratory using antibodies labelled with iodine-131 have led us to question this hypothesis[3,4]. Our results could best be explained by assuming a bivalent nature of incomplete erythrocyte antibodies.

The assumption of a bivalent nature of incomplete antibodies introduces the problem of explaining the inability of these substances to induce agglutination of saline suspended erythrocytes. A possible explanation would appear to be the unavailability of one or more reactive sites. It may further be postulated that the unavailability of reactive sites is due to the molecular conformation of the incomplete antibody. The experiments described here have been carried out to test this concept. The objective was to change the molecular conformation of incomplete erythrocyte antibodies. If the postulate were correct, it would be possible to make available the non-reactive site by this change, thereby converting incomplete erythrocyte antibodies into a saline acting, agglutinating type.

Incomplete anti-D(Rho) sera of high titre, without saline-agglutinating activity, were used. Titres were determined with standard bromelin and antiglobulin sera procedures. 2-Mercaptoethanol at 0·01 M, 0·1 M and 1·0 M concentrations in isotonic saline were used. Equal volumes of incomplete antibody sera and 2-mercaptoethanol were incubated for 15, 30 and 60 min at 4° C, 22° C and 37° C. The mercaptoethanol-treated incomplete antibody sera were then tested for saline agglutinating ability by adding one drop of a 4 per cent saline suspension of erythrocytes and incubating at room temperature for 5–15 min. Agglutination was determined macroscopically after centrifuging for 1 min at 1,000 r.p.m. At 4° C no saline agglutination was noted after mercaptoethanol treatment. At 37° C and 2-mercaptoethanol concentrations of 1·0 M and 0·1 M, denaturation of the sera occurred. Active mixtures were obtained with 2-mercaptoethanol concentrations of 1·0 M and 0·1 M, and an incubation time of 15–30 min at 22° C. Incomplete anti-D(Rho) sera so treated possessed the ability to agglutinate saline suspended D(Rho) positive erythrocytes. Table 1 summarizes some representative values. D(Rho) negative erythrocytes were not agglutinated by the mercaptoethanol-treated sera. In addition, antiglobulin and bromelin testing of the mercaptoethanol-treated incomplete anti-D(Rho) sera did not reveal any loss of titre of the antisera.

Table 1. Effect of 2-Mercaptoethanol on the Saline-agglutinating Ability of Incomplete Anti-D(Rho)

		Antisera titre					
Anti-D(Rho) No. 1	Test	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32
Untreated	Antiglobulin	4+	3+	3+	3+	2+	2+
	Saline	–	–	–	–	–	–
2-Mercaptoethanol							
1·0 M	Saline	2+	tr+	–	–	–	–
0·1 M	Saline	2+	1+	tr+	–	–	–
Anti-D(Rho) No. 2							
Untreated	Antiglobulin	4+	4+	4+	4+	3+	3+
	Saline	–	–	–	–	–	–
2-Mercaptoethanol							
1·0 M	Saline	4+	3+	2+	1+	–	–
0·1 M	Saline	3+	2+	1+	–	–	–

In order to rule out the possibility that the saline-agglutinating effect was due to mercaptoethanol action on the antigen site, experiments were performed using erythrocytes pretreated with 2-mercaptoethanol at concentrations, temperatures and incubation times similar to those used here. After washing the mercaptoethanol-treated erythrocytes, they were exposed to untreated incomplete anti-D(Rho) sera. With this procedure, 2-mercaptoethanol was inactive in converting incomplete anti-D(Rho) into a saline-agglutinating form.

There is general agreement that erythrocyte antibody sera contain a mixture of both incomplete and saline acting antibodies. Although no saline-agglutinating activity could be demonstrated in the incomplete anti-D(Rho) sera used in this study, the possibility that mercaptoethanol potentiated the action of minute quantities of saline acting anti-D(Rho) was considered. This appeared unlikely in view of prior experiments showing that the agglutinating activity of 19*S* antibodies is destroyed, rather than potentiated, with mercaptoethanol treatment[5]. To test this, various saline acting anti-D(Rho) sera were treated with 2-mercaptoethanol in a fashion similar to that used with incomplete anti-D(Rho). This study revealed the inability of 2-mercaptoethanol to potentiate the action of saline acting anti-D(Rho). Representative results are summarized in Table 2.

The ability of 2-mercaptoethanol to convert incomplete anti-D(Rho) into a saline-agglutinating antibody would appear to offer experimental confirmation of the postulate that incomplete antibody is of a bivalent nature. A working hypothesis would be to assume a molecular structure with one reactive group exposed and one or more reactive groups unavailable because of the molecular conformation of the antibody. Changing the tertiary and possibly the secondary structure of the molecule by breaking disulphide linkages with 2-mercaptoethanol would appear to make available the reactive group which had been hidden. The relatively low titre of saline-agglutinating activity obtained by 2-mercaptoethanol treatment, without loss of antiglobulin or bromelin activity, indicates that the crude techniques reported in this communication have only succeeded in exposing relatively few of the postulated hidden reactive sites.

Table 2. Effect of 2-Mercaptoethanol on the Saline-agglutinating Ability of Complete Anti-D(Rho)

		Antisera titre					
Anti-D(Rho) No. 1	Test	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32
Untreated	Saline	3+	3+	2+	2+	1+	–
1·0 M 2-Mercaptoethanol	Saline	1+	–	–	–	–	–
Anti-D(Rho) No. 2							
Untreated	Saline	3+	2+	2+	1+	tr+	–
1·0 M 2-Mercaptoethanol	Saline	2+	2+	2+	–	–	–

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An Uncommon Blood Group Isoantibody (Anti-M) in Neoplastic Disease

The first case in this series of anti-M sensitized patients was reported in 1958 because it presented a unique exception to the generalization that cold agglutinins are without significance in transfusion practice. The patient had received no M stimulus from either previous transfusion or pregnancy. We commented: "The possibility of a relation between the abnormal isoantibody and the patient's carcinoma of the cecum is being investigated"[1].

At that time we could find records of only 47 instances of anti-M sensitization in the entire literature. We have since encountered 9 additional patients with anti-M antibodies, and what seems remarkable is that 9 of our total of 10 cases had neoplastic disease. Six patients had carcinoma, 3 showed uterine leiomyoma. The single non-neoplastic patient had acute gastritis with bleeding duodenal ulcer. There was no particular bias in favour of neoplastic disease in the material examined; namely, cross-match specimens in 5 large general hospitals. Even if 50 per cent of all samples tested had been from patients with neoplasms, the odds against a purely chance ratio of 9 neoplasms to 1 non-neoplasm would be 102 : 1. The actual incidence of neoplasm in the surveyed population was unknown, but surely was much less than 50 per cent.

Table 1 summarizes the clinical and laboratory findings on these 10 cases. All were blood group NN and showed anti-M in the circulating plasma. We have begun screening M-negative cancer cases for anti-M and thus far have encountered 15 M-negative patients among 173 tested. These 15 were tested for anti-M with negative findings in thirteen. The two patients who were positive for anti-M (patients C. D., D. B.) are included in Table 1.

In two M-negative patients with anti-M, we tested cancer tissue obtained at the time of surgery for possible

Table 1. Neoplastic Disease with Anti-M

		Date	Sex	Age	Blood group	Diagnosis	Anti-M titre	Transfusions	Pregnancies	Comment
1.	J. B.	7/57	F	69	O Rh+NN	Ca. cæcum and breast	1/16 5° 1/8 20°	0	1NN	Almost complete absorp. by Ca. cæcum
2.	C. D.	9/58	M	76	A Rh+NN	Adenoca. colon and prostate	1/4 5° 1/1 25°	4		1-tube absorp. Ca. node, colon
3.	H. S.	3/62	M	68	O Rh+NN	Adenoca. prostate	1/2 5° 1/2 room	0		37° active vs. MM cells
4.	R. B.	4/62	F	49	B Rh+NN	Ca. breast c̄ metastases	Active 37° and room			Memorial Hosp. Long Beach
5.	B. M.	8/59	F	54	B Rh+NN	Leiomyoma, uterus	Active 37° and room	1MN	2	Women's Hosp. Detroit
6	C. B.	11/57	F	44	O Rh+NN	Leiomyoma, uterus	Active 5° and 37°	3	5	Los Angeles Co. Gen. Hosp.
7	E. Y.	/55	F	41	A Rh+NN	Leiomyoma, uterus	1/256 37°	0	2	Los Angeles, Co. Gen. Hosp.
8.	M. S. S.	5/62	F	75	O Rh+NN	Ca. tongue	Active 37° and room			Memorial Hosp. Long Beach
9.	E. F.	3/62	F	65	O Rh+NN	Acute gastritis, bleeding duodenal ulcer	Active room	0	2	Memorial Hosp. Long Beach
10.	D. B.	8/62	M	23	O Rh+NN	Embryonal Ca. testicle	1/16 5° 1/4 37°	0		37° antibody incomplete

presence of antigen M. Cancerous and non-cancerous sections of formalinized tissue (cæcum, colon, prostate, breast) were washed, ground and resuspended to 30 per cent by weight in saline and tested for ability to remove patient's anti-M from the autologous serum. The data suggested that some specific absorption of anti-M was obtained. Cancer tissue repeatedly showed complete or almost complete absorption of anti-M in case J. B., but only a 1-tube drop in titre (50 per cent reduction) was obtained in case C. D. Non-cancerous segments from the same tissue pieces showed little or no absorption of anti-M. Nevertheless, the specificity of absorption by cancer tissue remains dubious. We never succeeded in recovering anti-M in 37° and 56° C eluates[2] from these 'coated' tissues.

Detection of M-like antigen in tissues of an M-negative blood group individual would present an exciting and perhaps pathogenetically significant anomaly. Therefore, we plan to repeat these absorption experiments with fresh cancerous tissue rather than formalinized tissue, when the opportunity presents itself.

We feel it is more important at this time to seek confirmation of the association of anti-M and neoplastic disease, rather than to attempt any explanatory hypotheses. *A priori*, there seems little point in seeking anti-M among neoplasms, but it may prove worth while to seek for neoplasm in patients who present anti-M—especially in the absence of known M-antigen stimulus from transfusion or pregnancy.

We thank Dr. Elmer R. Jennings and Miss Corinne Hindmarsh, Memorial Hospital, Long Beach, for permission to include their 4 cases of anti-M (patients E. F., R. B., M. S. S. and B. M.), and Dr. Paul R. Thompson, Los Angeles County General Hospital, for his 2 cases (C. B. and E. Y.).

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PATHOLOGY

Topographic Distribution of Hepatic Necrosis in Bromobenzene, Thioacetamide, Tannic Acid Poisoning, and Inhibition by Aminoacetonitrile of the Necrosis Induced by Bromobenzene

AMINOACETONITRILE inhibits necrosis induced by carbon tetrachloride in the liver of the rat[1]. Necrosis by carbon tetrachloride affects the parenchymal cells surrounding all the tributaries of hepatic veins, from the smallest (centrolobular veins) to the largest. This localization can only be explained by an increased permeability of the wall of the hepatic veins causing the onset of necrosis[2]. (Necrosis of the cells of the central zone of the lobule can be caused by increase in permeability not only of the centrolobular veins but also of the last tract of the sinusoids.) The fact that such necrosis occurs only around the efferent vessels of the liver and not around the periportal areas and the first tract of the sinusoids indicates that this increased permeability is not the result of direct action by carbon tetrachloride but is due to some substances liberated in the blood by the parenchymal cells under the action of this poison[2].

Probably other kinds of hepatic necrosis experimentally induced by toxic compounds and described as centrolobular ones, as well as the central hepatic necrosis produced by toxic substances and recorded in human pathology, are actually localized around all the tributaries of hepatic veins, including the largest ones, and are, therefore, induced by the same mechanism as that of carbon tetrachloride.

The experiments described here were aimed at examining the localization of the necrosis which appears in the liver of rat following poisoning by bromobenzene, thioacetamide and tannic acid, such compounds being known to induce centrolobular necrosis[3–5]. At the same time the action of aminoacetonitrile on these lesions has been investigated.

For the experiments with bromobenzene 40 male albino rats weighing about 200 g were used, each of them receiving an intraperitoneal injection of 0·12 ml. of this substance mixed up with 0·38 ml. olive oil. Half the animals received, both simultaneously and 24 h later, a subcutaneous injection of 20 mg aminoacetonitrile hydrosulphate dissolved in 0·2 ml. water and neutralized with sodium hydroxide. Three rats treated with bromobenzene alone died within 48 h after administration of the poison. No histological examination of their livers was made. None of the rats treated with bromobenzene and aminoacetonitrile died in the same period. 48 h after bromobenzene administration the animals were killed; liver fragments were excised from near the hilus, fixed in Davidson fluid and embedded in paraffin. The sections were stained with hæmatoxylin and eosin.

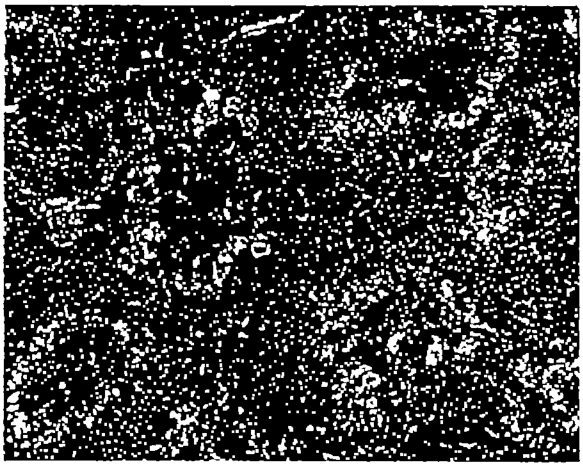

Fig. 1. Liver of rat treated with bromobenzene. (Stained with hæmatoxylin and eosin, × c. 40)

For the experiments with thioacetamide 14 male albino rats weighing about 200 g were used, each of them receiving a subcutaneous injection of 40 mg of this substance dissolved in 1·6 ml. water. Half the animals received, both simultaneously and 24 h before thioacetamide administration, a subcutaneous injection of 20 mg aminoacetonitrile. 24 h after the administration of thioacetamide the animals were killed and the experiment was completed with the same method used for bromobenzene.

Experiments with tannic acid were carried out by using 14 male albino rats weighing about 200 g. The animals were injected subcutaneously with 2 ml. of a 10 per cent aqueous solution of this acid. Half of them received, both simultaneously and 24 h before tannic acid administration, a subcutaneous injection of 20 mg aminoacetonitrile. 24 h after the administration of tannic acid the animals were killed and the experiment was completed with the same method used for bromobenzene.

Of the 17 rats treated with bromobenzene alone, 2 showed microscopically normal livers, whereas the livers of the remaining 15 rats presented a widespread necrosis and hydropic degeneration around all the tributaries, including the largest ones, of the hepatic veins (Fig. 1).

The liver of all the 20 rats treated with bromobenzene and aminoacetonitrile had a microscopically normal aspect (Fig. 2).

The liver of all the rats treated with either thioacetamide or tannic acid showed necrosis of the parenchymal cells around all the tributaries of the hepatic veins, including the largest ones.

The same result and the same severity of lesions was observed in the liver of all the animals, which together with these two substances, had received aminoacetonitrile.

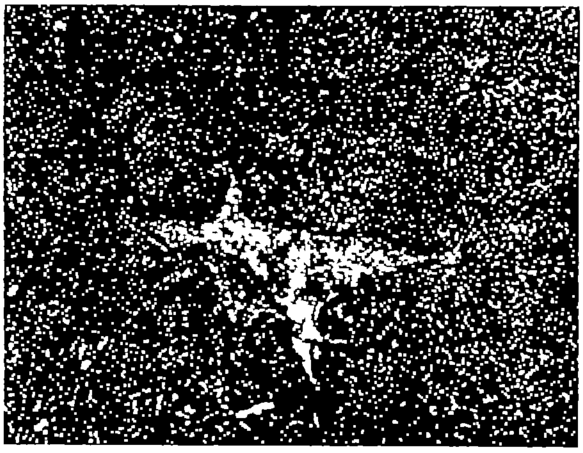

Fig. 2. Liver of rat treated with bromobenzene and aminoacetonitrile. (Hæmatoxylin and eosin, × c. 40)

In conclusion, in poisoning by bromobenzene, thioacetamide, and tannic acid, the localization of hepatic necrosis occurs not only around the centrolobular veins, but also around all the tributaries of the hepatic veins, including the largest ones. It follows that with all probability an essential condition for the appearance of these lesions, as for that promoted by carbon tetrachloride, is an increase in permeability of the wall of the hepatic veins, which is induced by products liberated in the blood by parenchymal cells under the damaging action of these poisons.

Aminoacetonitrile entirely inhibits necrosis by bromobenzene, whereas it does not exert any inhibition on the necrosis induced by either thioacetamide or tannic acid.

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In vivo, Extracellular, Collagen Fibrillogenesis

In vitro collagen fibrillogenesis has been demonstrated by investigators[1–3] from solutions of collagen obtained by various means. This communication presents what we believe to be the first evidence for extracellular, *in vivo*, aggregation of collagen fibrils.

In an investigation of the utilization of tritiated proline in healing wounds in scorbutic guinea pigs, we have found the sequence of incorporation of proline to be similar to that in normal animals[4,5]. This proceeds as follows: the radioactivity appears first in fibroblasts and next appears in the extracellular material. In scorbutic wounds, the extracellular material is fibrillar but lacks the characteristic banding of collagen seen in the electron microscope as well as the characteristic staining reactions of collagen. Autoradiographically this fibrillar material was found to retain the label after the isotope had disappeared from the fibroblasts. This investigation is concerned with the fate of both proline and the extracellular material following ascorbic acid administration, as seen via electron microscopy and autoradiography.

Female guinea pigs weighing about 300 g were placed on a scorbutigenic diet for 8 days. Eight linear incisions were then made in the dorsal skin and the wounds were allowed to 'heal' by first intention for 6 days. At this time, each animal was given 1-proline-3,4,^{3}H (12 μc./g body-weight) intraperitoneally. Six days following administration of tritiated proline a wound was removed from every animal, fixed and embedded as for electron microscopy[4]. Each animal was afterwards given 100 mg ascorbic acid intraperitoneally and placed on a diet containing 20 mg ascorbic acid per 20 g diet. The remainder of the wounds were removed at 1, 4, 12, 16, 24 h, 2, 3, and 6 days following administration of ascorbic acid. All the tissues were fixed and embedded in epoxy resin as for electron microscopy. Tissue sections were cut at 1μ, coated with nuclear track emulsion, exposed for 5 weeks, and stained[7]. Five random areas of two sections from each block were photographed, as well as areas adjacent to the sections, for background determinations. Grains overlying cells, extracellular material, etc., were counted using a modified form of Chalkley's method[8], and the counts were corrected for background.

The results of the grain counts demonstrated that the intensity of label over the cells remained low at all the time periods examined, while the grain density over the extracellular fibrillar material remained at the same high level present prior to ascorbic acid administration. Electron microscopic examination of the wounds prior to giving ascorbic acid showed the presence of the characteristic non-banded extracellular fibrils previously described[8]. Within 24 h after ascorbic acid administration, this fibrillar material was entirely replaced by characteristically banded collagen fibrils, most of which were of similar diameter.

Thus the extracellular material which is labelled 24 h after ascorbic acid administration can be clearly identified as collagen, and the intensity of labelling is similar to that seen in the fibrillar material which could not previously be identified as collagen. These results are consistent, therefore, with the conversion, extracellularly, of the non-collagenous fibrillar material present in scurvy, to collagen, following ascorbic acid administration.

We are aware that these results again raise the possibility of the presence of an extracellular collagen precursor in scurvy in which *in situ* hydroxylation of proline and lysine might occur. Since this remains a controversial issue, we are attempting to extract this material and identify its amino-acid content, in the hope of increasing our understanding of this complex situation.

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Prediction of Spontaneous Hereditary Diabetes Mellitus in Chinese Hamsters by Means of Elevated Alpha-2 Serum-levels

CLINICAL and pathological observations on Chinese hamsters (*Cricetulus griseus*) with spontaneous hereditary diabetes mellitus have been reported previously in several papers[1–7]. Generally speaking, the disease in Chinese hamsters is primarily pancreatogenic, with degranulation, hydropic degeneration and deficiency of β-cells. Other forms of pathological expression noted in symptomatic animals are glomerulosclerosis, arteriosclerosis, increased periodontal breakdown, and proneness toward the formation of adenocarcinomas of the pancreas.

Elevated serum α-2 levels several times greater than normal (3–8 per cent) have been reported[8] in diabetic hamsters, or in families with a high incidence of spontaneous diabetes. When symptomatic members of these high incidence families were randomly hybridized by single or double crosses involving two or four grandparents of diabetic background, normal α-2 values were re-established. At the time when this investigation was reported, many animals had high α-2 values with no clinical evidence of diabetes. In this communication we shall show that with but a few exceptions these non-symptomatic high α-2 hamsters afterwards developed chemical diabetes while the low α-animals generally remained symptom-free.

Both diabetic and non-diabetic inbred animals were obtained from the colony maintained at the Children's Cancer Research Foundation. The methods in breeding and maintaining the animals have already been described[9]. Animals were observed for a period of at least 250 days, and in some cases longer, for evidence of diabetes. Only animals showing prolonged glycosuria were considered as confirmed diabetics.

Blood was obtained by cardiac puncture and the α-2 serum-levels were determined by the Spinco model *R* system of paper electrophoresis, using their recommended procedures.

For purposes of analysis, the hamsters were divided into three groups consisting of: (1) those which were never diabetic (within 250 days); (2) those which had no initial symptoms, but later developed diabetes; and (3) those which were diabetics or showed initial glycosuria, and later were confirmed as diabetics. The condition of the animals at the time when the electrophoretic analysis was run was the point of reference for the above categories. These results are given in Table 1.

Table 1. RELATIONSHIP OF SERUM PAPER ELECTROPHORETIC α-2 LEVELS WITH THE DIABETIC STATE IN 89 CHINESE HAMSTERS*

Serum α-2 (%)	No diabetes	Confirmed diabetes No initial symptoms	 Initial symptoms and/or established diabetes
3–5	8	0	0
6–10	18	5	6
11–15	1	7	10
16–20	1	6	7
21–25	1	2	10
26–30	0	2	4
31–35	0	0	1
Total	29	22	38

* All animals observed for at least 250 days.

It will be observed that out of the 29 which did not develop diabetes, 26 animals had α-2 levels of less than 10 per cent, the majority (18 animals) giving levels between 6 and 10 per cent. Only three animals gave levels above 10 per cent. It is conceivable that if these latter animals were observed for a longer period—up to a year—diabetic symptoms might have developed.

In the 22 animals showing no initial symptoms, none was observed in the 3–5 per cent group, in contrast to the previous group where 8 animals were found to have the lowest α-2 levels. There were 13 initially non-symptomatic animals in the 11–20 per cent α-2 interval.

Similarly, the 38 animals with established diabetes or initial symptoms, such as glycosuria, and later confirmed as diabetics, showed no members in the 3–5 per cent α-2 group. There were 27 animals in the 11–25 per cent α-2 interval.

These results show a high degree of association between the active diabetic state in Chinese hamsters and the serum α-2 level. Some overlap occurred in the 6–10 per cent α-2 interval between the non-diabetics, the initially non-symptomatic, and the symptomatic groups. Most of the initially non-symptomatic high α-2 animals (above 10 per cent) were later confirmed as diabetics when observed for a period of 9 months.

The possible prognostic implications of elevated α-2 levels in selected groups of high diabetic risk, such as overweight new-borns, is being investigated by a variety of techniques.

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IMMUNOLOGY

Non-specific Precipitation Patterns obtained in Double Diffusion between Serum and Extracts of Normal Organs and Tumours

It has been shown that the antigenic structure of tumours and of the corresponding normal tissues is different[1]. Specific antigens were demonstrated in spontaneous, induced and transplanted tumours of mice, rat, rabbits and chickens, as well as in various human tumours by the method of anaphylactic reaction following desensitization. By means of the gel diffusion of Ouchterlony, Zilber *et al.*[2] were able to show an antigen in extracts from a mice hepatoma lacking in normal liver. We were able to confirm this work in a tumour of the rat; we obtained a specific antigen present in the granular fraction of a transplantable hepatoma.

With this different antigenic structure in mind, we tried to elicit some immune response in rats treated with extracts of liver and hepatoma. Until now we failed to induce a response in the animals treated by tumour extracts, but in the course of this work we obtained curious lines between serum and tissue extracts, analysed by double diffusion in agar. Those lines simulate a true antigen–antibody system, and it is the aim of this communication to describe the precipitation lines and to compare them with the characteristics of true antigen–antibody systems.

We injected five groups of rats with total liver extract, with the microsomal fraction of the liver, with normal kidney, and with extracts of a transplantable hepatoma, and, finally, with the microsomal fraction of this hepatoma. The organ extracts were made in a Waring blendor, and the microsomal fractions were isolated by differential centrifugation. The animals received three injections, one of them in Freund's adjuvant. Ten days after the last injection the animals were bled.

Table 1 shows the results of this experiment analysing the serum of each animal against the five antigens studied.

Table 1. PRECIPITATION PATTERN OBTAINED BETWEEN THE SERUM OF RATS IMMUNIZED WITH NORMAL AND TUMOUR EXTRACTS AND THE EXTRACTS

Animals previously injected with	Precipitation lines present with*				
	Normal liver		Hepatoma		Kidney
	Total extract	Microsomal	Total extract	Microsomal	Total extract
Total liver extract	3/4	0/4	0/4	0/4	2/4
Microsomal liver	3/4	0/4	0/4	0/4	3/4
Kidney	1/4	0/4	0/4	0/4	4/4
Total hepatoma extract	3/4	0/4	0/4	0/4	2/4
Microsomal hepatoma	3/4	0/4	0/4	0/4	3/4
Normal serum (without previous injury)	3/4	0/4	0/4	0/4	3/4

* Results expressed as $\frac{\text{No. of animals with precipitation lines in their serum}}{\text{Total No. of animals analysed}}$

It is clear that practically all the animals present a line between their own serum and the extracts of liver and kidney, without any specificity regarding the immunization procedure. It was surprising to find analogous precipitation lines towards normal serum; it was only necessary to work with concentrated extracts to obtain the lines. We analysed also serum of rats carrying a hepatoma graft and found precipitation lines: hepatoma extracts and normal extracts provided precipitation patterns, if the reactants were sufficiently concentrated.

The using up of serum by corresponding antigens is possible. The factor present in serum is destroyed by boiling; but not the factor present in the organ extracts. On the contrary, after boiling of the organ extracts, the lines are even clearer.

The mobility of the factor present in the serum is that of an α_2-globulin; the organ factor has a β-globulin mobility.

In this experiment we did not succeed in demonstrating the presence of true anti-tumour immunity, but we discovered a factor present even in normal serum, and in serum of tumour-bearing rats, reacting with a component present not only in extracts of normal organs (liver, spleen, kidney) but also in some tumour preparations. This serum factor seems not to increase after immunization of the animals with normal organ extracts or with tumour extracts. In our case it is not a reaction between hæmoglobin present in the tumour or organ extracts and some serum protein, as described by Leonardi[4], Ambrosino *et al.*[5], Peetom *et al.*[6] or by Metzgar and Grace[7]. It is also different from the precipitation described by Tomasi[8].

This precipitation pattern looks very similar to a true antigen–antibody system, but can be differentiated in our mind by some points. Similar to an antigen–antibody system is the fact that a state of equivalence exists for this system, and that it is possible to obtain pictures similar to antigen and antibody excess. It is also possible to exhaust the serum factor by addition of small quantities of tissue extracts.

But in other aspects this system is quite different from a true antigen–antibody system. The mobility of the factor found in the serum is situated in the region of α_2-globulin, and not in the region of the antibodies, which are β- and/or γ-globulins. The tissue factor is not destroyed by boiling. The precipitation line appears only in concentrated extracts. Those three factors enabled us to distinguish between precipitation lines and a true antigen–antibody system, in which the antibody has always a β-γ-globulin mobility and is destroyed by boiling.

It is possible that the lines described here are due to electrostatic forces[3] or to chemical affinity between the components. It is necessary for everybody working with double diffusion in agar to have in mind these non-specific precipitation patterns, to avoid false interpretation of the agar plates[7]. Some controls of precipitation lines can be made very easily, as, for example, boiling of the serum and immunoelectrophoresis. In this way specific precipitation patterns can be differentiated from non-specific ones.

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RADIOBIOLOGY

Isolation of a Radiation-Mortality Reducing Factor from Spleen

The radiation-mortality reducing action of cell-free extracts obtained from spleens of mice, guinea pigs and dogs has been demonstrated in a series of previous investigations[1,2]. The extraction procedure used for preparing spleen extracts has been described elsewhere[2,3]. The observation that homologous, as well as the heterologous, spleen extracts were equally effective suggests that a common chemical entity (or entities) may be the therapeutic agent[4]. This is a preliminary report of the isolation and characterization of fractions obtained from dog spleen extracts which contain this factor.

The reagents were of the highest purity available and the solutions were made with distilled water. The spleen extracts were prepared from mongrel dogs of either sex weighing 40–62 lb. The spleens were surgically exposed and the hilus vessels clamped prior to removal. The weighed organs were cut into small pieces, placed on dry

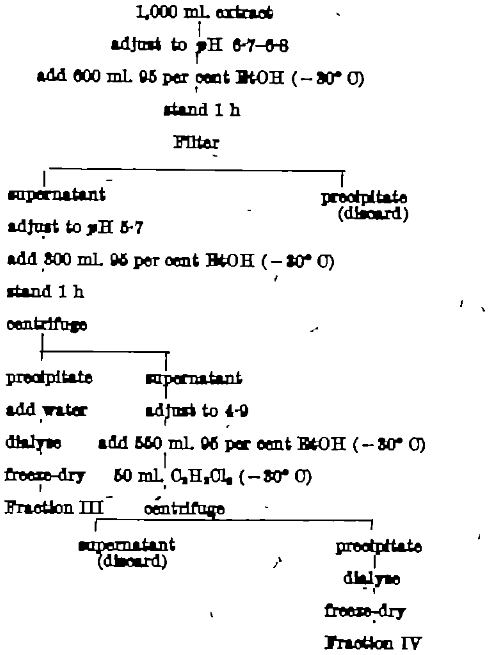

Fig. 1. A procedure for the alcohol fractionation of dog spleen extract

ice for 2 h prior to grinding in the frozen state. The powder was leached with 0·9 per cent sodium chloride for 24 h in a refrigerator. The supernatant was then centrifuged, filtered, freeze-dried, and tested for sterility in accordance with the standard procedure[1].

Evidence that this factor is associated with, or is, a macromolecule was afforded by several approaches. Sequential quantitative dialysis utilizing Visking No. 27 'Cellophane' membrane localized the bioactivity in the non-dialysable fraction. Gel chromatographic analysis utilizing 'Sephadex *G*-50' as the stationary phase and water as the eluant yielded four fractions all of which possessed substantial degrees of bioactivity.

A fraction isolated from spleen extract at 60–85 per cent relative saturation of ammonium sulphate possessed a considerable degree of protective action. However, the bio-assays were somewhat equivocal due to interference by the ammonium sulphate present in spite of dialysis for 72 h against 10 changes of distilled water. With alcohol fractionation four fractions were obtained. The first two fractions were ineffective, while Fractions III and IV exhibited considerable bio-activity. This procedure is described in the flow-sheet in Fig. 1. The fractionation was carried out in a cold room with suitable precautions to maintain the correct temperature and rate of addition of the pre-cooled reagents to minimize denaturation. The pH was adjusted with either 0·1 M dipotassium hydrogen phosphate or 0·25 N acetic acid.

The results of several fractionation experiments are summarized in Table 1. The dosage used corresponded to the relative amount of each fraction isolated from the crude extract. The procedure for the bio-assays is described in the table. A χ^2 test analysis indicates that the data are statistically significant falling within the $0·02 > P > 0·01$ level for the individual fractions and $P < 0·001$ for the extract[2,3]. Thus it may be concluded that these materials exhibit a decided radiation ameliorating action. A measure of the relative effectiveness of the fractions is gained by noting that 30 and 5 mg of Fractions III and IV are almost equivalent to 200 mg of the original spleen extract.

Table 1. Comparative Study of the Radiation-Mortality Reducing Effect of Various Fractions isolated from Spleen*

Fraction	Mean dose (mg/day†)	Survivors after 20 days‡	Survival (%)	χ^2 computed relative to control	Probability
Extract	200	40/60	66·7	14·7	< 0·001
Fraction III	30	32/60	53·3	5·91	$0·02 > P > 0·01$
Fraction IV	5	22/40	55·0	6·24	$0·02 > P > 0·01$
Saline control	—	19/60	31·6	—	—

* Male guinea pigs, mean weight 425 ± 50 g, exposed to a 2,400 c. cobalt-60 source for an exposure of 650 r. in air (refs. 2 and 3).

† Treatment consisted of five injections daily of 0·4 ml. starting 24 h after exposure to radiation. The weight of sample varied with the preparation used; however, the range was about 20 per cent.

‡ These data represent the number of survivors relative to the total number of animals tested, being the summation of several experiments over a 6-month period.

Preliminary studies indicate that a simplified procedure is achieved by pre-heating the extract, adjusted to pH 4·9 ± 0·1 at 60° C for 10 min prior to fractionation. This removes about 70 per cent of the protein, resulting in an almost quantitative precipitation of hæmoglobin. The alcohol fractionation is then performed as described. Fraction III-*H* recovery and activity is in the same order as Fraction IV; however, Fraction IV-*H* exhibits little or no protective action.

Some of the properties of the active principle may be deduced from these investigations. The boiling of spleen extract at pH 6·8 for 20 min caused a considerable loss of activity. Spectroscopic analysis revealed not only a maximum at 278 mμ but also that the ratio of absorbances at 278 mμ to 260 mμ ranged from 1·35 to 1·70. This supports the thesis that the fractions contained little or no nucleic acids or their derivatives. The presence of additional absorption maxima at 410, 545, and 575 mμ were due to the presence of hæmoglobin. Since the 410 mμ absorbance is about 10-fold greater than that of the 280 mμ absorption maximum of the other proteins in this extract this facilitates the calculation of the hæmoglobin content of the various fractions. Evidence that hæmoglobin was not the active principle was derived from the observation that the hæmoglobin content of the more active preparations was about 10 per cent and by experiments using hæmoglobin as such.

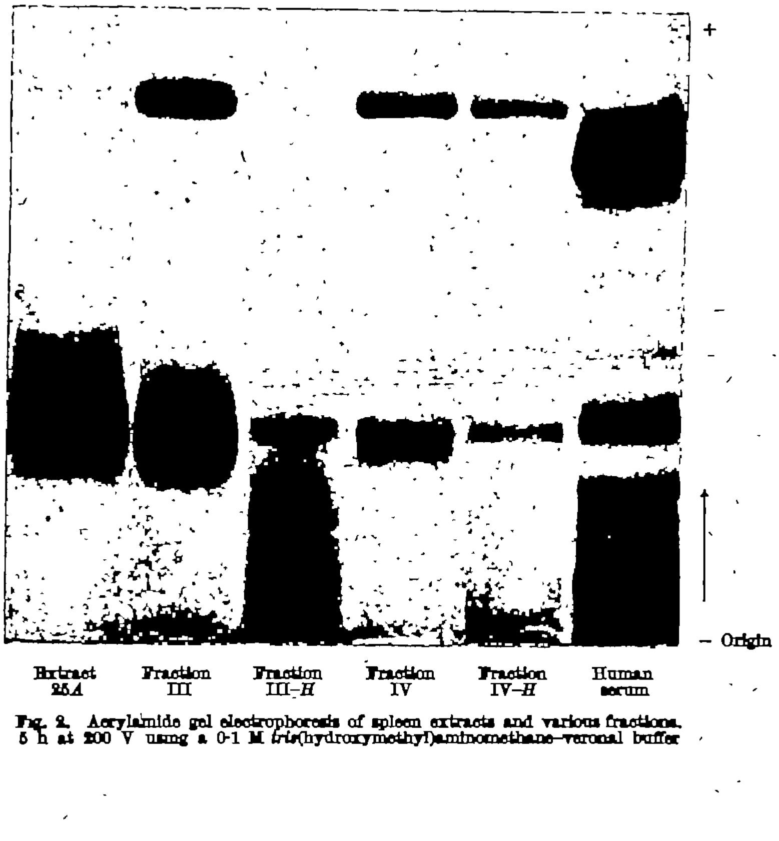

Fig. 2. Acrylamide gel electrophoresis of spleen extracts and various fractions. 5 h at 200 V using a 0·1 M *tris*(hydroxymethyl)aminomethane–veronal buffer

Electrophoretic studies were made with acrylamide gels using 0·1 M *tris*(hydroxymethyl)aminomethane: veronal buffer, *p*H 8·6 (ref. 6). Typical electrophoretic patterns are presented in Fig. 2 in which Fractions III and IV obtained by alcohol fractionation are compared with Fractions III-*H* and IV-*H* obtained by the pre-heating procedure. Human serum served as the reference. All the fractions, with the exception of III-*H*, contained a fast component the mobility of which is similar to that of albumin and another group of proteins the mobility of which falls in the globulin range. Since Fraction III-*H*, a high-potency fraction, contains little if any of the albumin-like material this suggests that the active component falls in category characterized by a lower mobility. Furthermore, Fraction IV-*H*, which possesses little or no protective action, contains a large amount of the fast component relative to the globulin-like species.

The previous data coupled with the results of semimicro Kjeldahl, biuret, and dry-weight analysis suggest that this principle is associated with or is a polypeptide or protein. More definitive conclusions about the nature of this factor must await the isolation of more active and homogeneous fractions.

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Radiation Protection by Cyanide of both Rats and Mice

MANY investigators have found[1-4] that the rat, unlike the mouse, is afforded little or no protection to irradiation damage by the prior administration of cyanide salts. In all their experiments, a limited dose-range of cyanide was tested. The frequently cited results of Dowdy *et al.*[1], for example, were obtained using a single dose (3 mg/kg) of sodium cyanide. A recent theory[5] states that compounds such as cyanide and azide owe their radiobiological action to the property of stabilization of cuprous (Cu I) ion involved in oxidative processes. The proposal suggests that the dose required to achieve protection would be sharply defined and, if not limited by chemical toxicity of cyanide, it should be possible to demonstrate protection in the rat using the proper dose. Accordingly, survival was compared in rats and mice given a wide range of doses of sodium cyanide prior to X-irradiation.

The animals used were female *CF* No. 1 mice, 70 days old and weighing 25 ± 2 g, and female Sprague–Dawley rats, 7–8 months old and weighing 270 ± 15 g. X-ray exposures were delivered with a General Electric Maximar unit operated at 250 kVp and 15 m.amp., using 0·5 mm copper and 1·0 mm aluminium filters. The mice were irradiated on a revolving wheel equipped with individual ventilated plastic capsules, at a dose rate of 38·4 r./min. Rats were placed in individual ventilated aluminium boxes, which were shifted through 180° once during the exposure; the dose rate was 21·7 r./min. Sodium cyanide solutions were freshly prepared before injection in concentrations of 0·02 and 0·03 per cent and the *p*H was adjusted to 7·0 with sodium cyanide. All treatment injections were given intraperitoneally 3–6 min before irradiation. Control animals were given no injections.

Preliminary toxicity data on sodium cyanide given intraperitoneally were obtained. For mice, the 30-day LD_{50} was 6·3 mg/kg and the maximum safe dose was 5·5 mg/kg. For rats, the 30-day LD_{50} was 4·0 mg/kg and the maximum safe dose was 3·0 mg/kg.

The irradiation experiments were carried out at two levels of X-irradiation for each species. The total whole-body doses delivered were 850 r. (LD_{90}) and 800 r. (LD_{75}) for the rats and 750 r. (LD_{90}) and 650 r. (LD_{50}) for the mice. The results of the experiments carried out at the higher irradiation levels are shown in Fig. 1. Significant protection was obtained in both rats and mice. In the case of mice given 750 r. the degree of protection increases with increasing dose of cyanide, beginning at a level of about 2 mg/kg. However, in the rat given 850 r., cyanide exerts no appreciable protection until a dose of about 3 mg/kg is reached where a sharp rise in protection ensues. This rise was checked in two separate experiments. In one, 50 per cent survival was observed and in the other 67 per cent. Considering the abrupt response these results show good agreement.

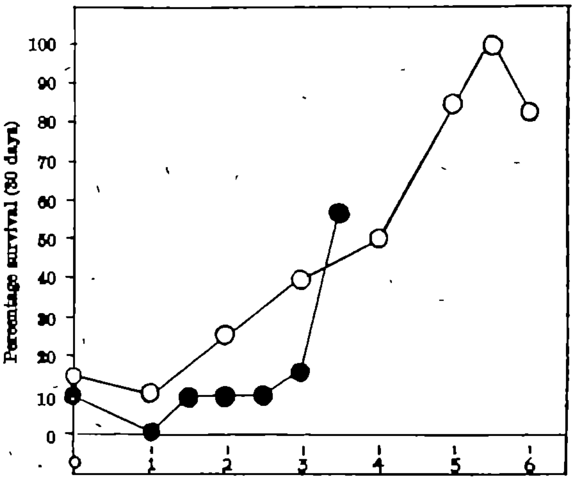

Fig. 1. Effect of treatment with sodium cyanide on the survival of X-irradiated rats and mice. Sodium cyanide was given intraperitoneally 3–6 min before irradiation. Black circles represent the results obtained on rats given 850 r.; each point is based on 20 rats except for the controls where 30 were used and the 1 0 mg/kg level where there were only 10. Open circles show the results from mice irradiated at 750 r.; each point represents 20 mice except for the 5·5 and 6 0 mg/kg levels where only 9 and 6 were used, respectively

Experiments at a lower level of irradiation indicated that for both the mouse and the rat the minimum amount of cyanide required to bring about significant protection was less than that needed for higher irradiation doses. The lower cyanide doses were about 1·0–2·0 mg/kg for mice given 650 r. (survival = 80 per cent for treated and 40 per cent for controls) and 2·0–2·5 mg/kg for rats given 800 r. (survival = 40 per cent for treated and 10 per cent for controls).

The observation that the radioprotective dose of sodium cyanide increases with larger doses of irradiation is noteworthy both from theoretical and practical points of view. Since cellular permeability generally increases with increasing irradiation dose[4] the explanation probably lies elsewhere. It is tempting to speculate that a contributing factor to the cyanide-radiation dose relations arises from the fact that cyanide ion reacts with peroxy radicals[4] and hydrogen peroxide[6]. Consequently, since the concentration of peroxy radicals produced *in vivo* presumably increases with increasing radiation dose, the dose of sodium cyanide needed to reach critical intracellular sites would necessarily increase. An elucidation of the mechanism involved would entail a study of the degree of protection as a function of the time elapsed between the administration of cyanide and onset of irradiation.

The previously mentioned theory[5] suggests that cyanide prevents a radiation-induced oxidation of the cuprous component of 4-electron transfer oxidases such as cytochrome oxidase through the selective formation of a cuprous cyanide complex which eventually dissociates. Accordingly, the cytochrome-*c* oxidase activity of the livers of mice injected with sodium cyanide was measured by the method of Hogeboom and Schneider[7]. The observed inhibitions of the enzyme were 10 per cent at a dose of 2 mg/kg of sodium cyanide and 40 per cent at 5 mg/kg. These preliminary results are suggestive inasmuch as they approximate the dosages needed for protection. We were unable, unfortunately, to demonstrate radiation-induced inhibition of cytochrome oxidase in mice or rat tissues. The difficulty is presumably associated with the sensitization of the enzyme (actually reduction of Cu^{++} to Cu^{+}) by the sulphydryl groups of the protein which are released on homogenization of the tissue[8,9].

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Alterations in Skeletal Muscle after X-Irradiation and their Similarity to Changes in Muscular Dystrophy

ALTHOUGH muscle constitutes almost half the body-weight in mammals, it is generally thought to be very radioresistant and to play an insignificant part in the bodily changes which follow irradiation. In excised frog muscle, changes in the resting transmembrane potential and electrolyte composition were found only after massive doses of X-rays[1,4]. However, transient weakness and increased fatigability have been observed in man and animals after modest doses of radiation[2,4]. Caster and Armstrong[5] found a large negative potassium balance in rats after an LD_{50} of X-rays. The lost potassium appeared to originate principally in muscle. A transient post-irradiation aldolasæmia[6] also occurs as well as an amino-aciduria and creatinuria[7-9] which appears to be linearly dependent on the dose of radiation[10]. It seems likely that the creatine and other amino-acids and the aldolase were lost, in large part, by skeletal muscle and it occurred to us that subtle *in vivo* changes in muscle after irradiation might have been overlooked.

Male Sprague–Dawley rats weighing about 250 g were anæsthetized by the intraperitoneal injection of 33 mg/kg body-weight ammonium pentobarbital, placed in cylindrical lead chambers which shielded all but the hind quarters by 1-in. thickness of lead, and irradiated with 3,000 r. 250 kV X-rays filtered through 1·0 mm aluminium and 0·5 mm copper at a rate of 200 r./min. Groups of four animals were killed after 6, 12, 24, 48, 72 and 96 h; control animals were sham irradiated. The animals were bled and plasma electrolytes, aldolase and lactic dehydrogenase as well as erythrocyte electrolytes were determined by methods described previously[11]. Muscle from the irradiated limbs was analysed for electrolytes[11].

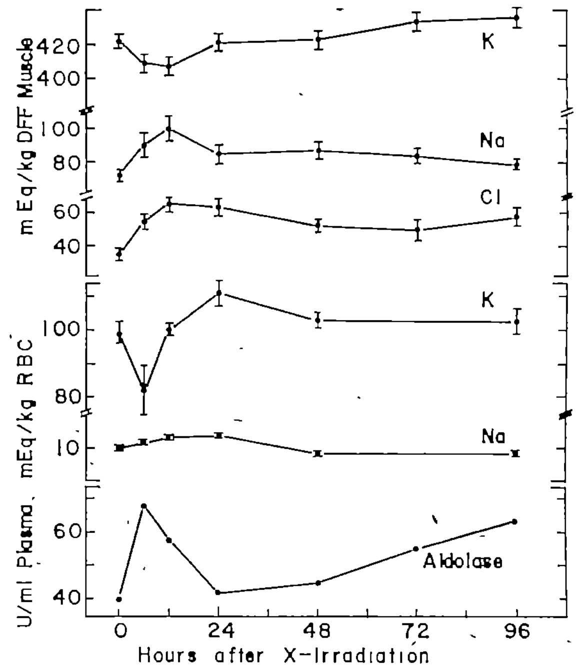

Fig. 1. Muscle and erythrocyte electrolytes and plasma aldolase after irradiation of the hindquarters of rats with 3,000 r. RBC, Red blood cells; DFF, dry, fat-free

The results are depicted in Fig. 1. Muscle potassium fell from a control value of 422 ± 4·1 m.equiv./kg dry, fat-free muscle to 407 ± 7·2 m.equiv./kg dry, fat-free muscle at 12 h but by 24 h returned to 423 ± 3·1 m.equiv./kg dry, fat-free muscle. Muscle sodium rose from a control value of 72·3 ± 2·0 m.equiv./kg dry, fat-free muscle to a peak of 98·1 ± 2·2 m.equiv./kg dry, fat-free muscle at 12 h and returned slowly to a level not significantly different from that of the controls. Muscle chloride also rose from a control value of 35·2 ± 2·2 m.equiv./kg dry, fat-free muscle to a peak of 65·7 ± 6·2 m.equiv./kg dry, fat-free muscle at 12 h. Erythrocyte potassium showed a diphasic pattern, first dropping significantly from control values at 6 h and at 24 h increasing above control values. Erythrocyte sodium rose from a control value of 9·90 ± 0·66 m.equiv./kg to a peak of 14·16 ± 0·72 m.equiv./kg at 24 h. The plasma electrolytes after irradiation were not significantly different from the values in controls.

The rise in plasma aldolase-levels from a mean in control animals of 40·2 U/ml. to 68·2 U/ml. 6 h after irradiation, followed by a rapid return to control levels, was like the aldolasæmia which has been reported previously[6]. A similar transient increase in plasma lactic dehydrogenase-levels was found. The ratio of muscle wet weight to dry, fat-free muscle in muscle from irradiated groups was not significantly different from the mean of 4·23 in controls.

The apparent lowering of muscle potassium concentration and increase in sodium and chloride concentrations may be due, in part, to a relative increase in the extracellular fluid as a result of the hyperæmia and œdema which is known to follow irradiation[12]. In view of the coincident loss of aldolase, creatine and the negative potassium balance which must be attributed largely to losses from skeletal muscle, we feel that our findings are best explained by a transient increase in cellular permeability of muscle.

In addition to weakness, a low muscle potassium, high muscle sodium and chloride concentrations as well as creatinuria and aldolasaemia are found in hereditary muscular dystrophy and have been attributed to altered cellular permeability[11]. Such changes in permeability of muscle cells may be a common reaction to certain kinds of injury and disturbances in metabolism.

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BIOLOGY

Thermogenic Effect of Stilbœstrol on Ewes

At œstrus the cow's body temperature is elevated without detriment to subsequent fertilization[1,2]. Increased locomotory activity may partly account for the thermal response, but attention has also been directed to other metabolic changes at œstrus. Some evidence has been obtained that œstrogens may influence the cow's body temperature (Fallon, unpublished). It was decided to examine the effect of stilbœstrol on the rectal temperature of the ewe.

Eight mature Merino ewes were ovariectomized; mean live-weight was 33 kg. Eight weeks later they were divided at random within four groups and accommodated in individual metabolism cages to restrict exercise. During experimental periods the sheep were denied access to food and water.

Aqueous preparations were made to provide 2 ml. doses containing 0, 25, 50 and 100 μg stilbœstrol. The preparations were stored at room temperature 2–3 weeks before use in experiment *A*; and in experiment *B* similar solutions were prepared within 2 h before use. Intra-jugular injections were performed according to the schedule in Table 1.

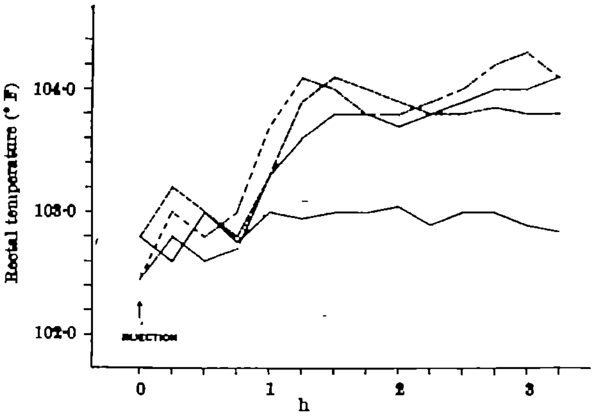

Fig. 1. Rectal temperatures in spayed ewes following intra-jugular injection of aqueous stilbœstrol solutions after 2–3 weeks' storage at room temperature. ·······, 100 μg; —·—, 50 μg, – – –, 25 μg stilbœstrol; ——, control

Table 1. PROGRAMME OF INTRA-JUGULAR INJECTIONS WITH AQUEOUS STILBŒSTROL SOLUTIONS

	Exp. *A*					Exp. *B*		
Age of solution	2–6 weeks					< 2 h		
Date	17/5	24/5	7/6	21/6	14/6	28/6	26/7	6/8
Ewe	(μg stilbœstrol)							
247	0	50	100	25	25	0	50	100
248	0	50	100	25	25	0	50	100
242	25	100	50	0	0	25	100	50
243	25	100	50	0	0	25	100	50
241	50	25	0	100	100	50	25	0
249	50	25	0	100	100	50	25	0
245	100	0	25	50	50	100	0	25
250	100	0	25	50	50	100	0	25

Table 2. RECTAL TEMPERATURES 1½–3½ H AFTER INTRA-JUGULAR ADMINISTRATION OF STILBŒSTROL IN OVARIECTOMIZED EWES

Stilbœstrol (μg)	No. of records	Mean rectal temperature (° F.)	*S.E.* (° F.)
		Exp. *A*	
0	72	102·99	0·05
25	72	103·91	0·06
50	72	104·06	0·08
100	72	103·89	0·09
		Exp. *B*	
0	72	103·34	0·11
25	72	103·20	0·12
50	72	103·38	0·10
100	72	103·24	0·11

Rectal temperatures were recorded about mid-day, immediately before injections, and thereafter at 15-min intervals. After a delay of about 45 min, the older solutions of stilbœstrol used in experiment *A* (Fig. 1) caused the spayed ewe's rectal temperature to rise by about 1° F. Records 1½ – 3½ h after injection (Table 2) indicate that each dose-level of stilbœstrol was thermogenic in relation to the control injections with water ($P < 0{\cdot}001$) and that the three dose-levels produced comparable effects ($F = 1{\cdot}78$). Fresh preparations of stilbœstrol used in experiment *B* were ineffective in eliciting a consistent thermal response within 3½ h after injection.

The role of storage in the development of thermogenic properties of aqueous stilbœstrol solutions is of interest since such preparations also lose œstrogenic potency[3,4]. However, the oxidative inactivation of the œstrogen molecule (*in vitro* or *in vivo*) has been shown to reverse its pituitary function from inhibition to stimulation[5]. Thus, the thermal response of the spayed ewe to stilbœstrol may involve profound neuro endocrine interactions.

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Body Temperature of Birds in Relation to Nesting Habits

The body temperature of birds has been examined by various workers[1–5,7,8]. Temperature has been shown to fluctuate in response to various internal and external influences[1,3,4,8]; there is a difference of the order of 2° C between active birds and those at rest[3,4], and a difference of 1·5° C has been demonstrated between day and night body temperatures in quails[2]. Previous work[7] has shown that the night body temperature of the masked weaver (*Ploceus velatus*) is 1·6° C lower than the day temperature.

The day body temperatures of 80 species of land birds from southern Africa have been recorded during routine collection of museum specimens. A fast-registering rectal thermometer was inserted through the cloaca to the centre of the body-cavity immediately after the bird had been shot. The temperatures of wounded birds were not taken[5,6]. Where more than one reading for a species was available, the mean was used.

Table 1 indicates the rate of decrease in body temperature after the bird is shot. The readings were taken at 30-sec intervals for 3 consecutive min.

Table 1

Species	Wt. (g)	Temperature (° C) 30	60	90	120	150	180 sec
Sacred ibis, *Threskiornis aethiopicus*	964	42·1	42·1	42·1	42·1	42·1	42·1
Laughing dove, *Stigmatopelia senegalensis*	100	43·0	43·0	43·0	43·0	42·9	42·8
Fork-tailed drongo, *Dicrurus adsimilis*	57	42·6	42·6	42·5	42·2	42·1	42·0
Golden-breasted bunting, *Emberiza flaviventris*	23	42·8	42·8	42·7	42·0	42·3	41·9
Blue waxbill, *Uraeginthus angolensis*	14	42·1	42·1	41·0	41·6	41·1	40·8

In no instance did the temperature start to drop before 1 min had elapsed after death. As all readings were taken during this 60-sec period, post-mortem decrease in body temperature can therefore be ignored.

The species under discussion are divided into two main groups, the 'nest builders' and the 'nest scrapers'. The first-mentioned use nesting material, thereby insulating their eggs to some extent, while the second group deposit their eggs on the bare ground or rock where they are not insulated from the substratum. The nesters are subdivided into tree-, hole- and ground-nesters.

Mean body temperatures of the three nesting groups are as follows:

	M	*S.D.*	*N*	$S.E._m$
Tree	41·78	0·7564	39	0·1211
Hole	41·85	0·7612	20	0·1702
Ground	41·98	0·4566	10	0·1444

The differences between these means are not statistically significant.

Mean body temperatures of nest builders compared with nest scrapers are as follows:

	M	*S.D.*	*N*	$S.E._m$
Nest builders	41·80	0·7170	69	0·0863
Nest scrapers	40·20	1·2809	11	0·3862

These mean values are significantly different at the 1 per cent level. ($t = 9{\cdot}627$, 78 d.f., $P < 0{\cdot}01$.)

No definite conclusions can as yet be drawn as to why the nest scrapers should have a lower body temperature than nest builders. However, most nest scrapers have relatively long bare legs, for example, red-crested korhaan (*Lophotis ruficrista ruficrista*), crowned plover (*Stephanibyx coronatus coronatus*), blacksmith plover (*Hoplopterus armatus*), Kittlitz's sandplover (*Charadrius pecuarius*), Cape dikkop (*Burhinus capensis*), etc., although there are exceptions such as rufous-cheeked nightjar (*Caprimulgus rufigena*) and Namaqua sandgrouse (*Pterocles namaqua*).

During the incubation of nest builders it has been shown[4,8] that there is a 'negative' temperature gradient between the upper and the lower surface of the egg, that is, the upper surface in close contact with the body of the incubating bird has a higher temperature than the lower which is in contact with the nesting material. At the Austin Roberts Bird Sanctuary, Pretoria, this gradient was found to be of the order of 4°–5° C for the Egyptian goose (*Alopochen aegyptiacus*), and 5° C for the yellowbill (*Anas undulata*).

In the rufous-cheeked nightjar in the Kalahari Desert, for 4 h (12 noon–4 p.m.) during the hottest part of the day, the temperature gradient between the upper and lower egg surfaces is 'positive' and of the order of 2° C. This reversal of the temperature gradient, where the lower surface temperature of the egg is now higher than that of the upper, is due to the fact that the heat from the substratum (reaching a temperature of 61° C) is conducted to the eggs. It has been shown[9] that certain birds keep their eggs cool during part of the day by shading them. In the nightjar and possibly in other nest scrapers, cooling appears to be achieved by incubation instead[10]. The body of the bird thus acts as a conductor removing excess heat. In this respect the lower body temperature of scrape-nesting birds may be of importance. The long bare legs of some scrapers also fit into this picture as they increase the uninsulated surface of the body, thus enabling the bird to disperse the excess heat more readily.

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Urea-Levels in Blood and Tissues of Hibernating and Non-Hibernating Hedgehogs

ANALYSES were carried out during the summer from active hedgehogs (*Erinaceus europaeus*), which were fed on a 'high protein diet', after fasting for one day. Hibernating animals in winter were kept in a constant-temperature cold room (+ 4·0 ± 0·5° C) at least one week before analyses were made.

Urea analyses were performed using the manometric method developed by Krebs and Henseleit[1] as presented by Umbreit, Burris and Stauffer[2]. Besides this, the standardized method of Biochemica 'Boehringer *TC–U*' (C. F. Boehringer and Soehne G.m.b.H., Mannheim) was used. Tissue analyses were performed from tissue homogenates. Control determinations were carried out using well-fed white laboratory rats. Means from analyses of rat blood are: 31·6 mg urea/100 ml. serum and 36·0 mg urea/100 ml. whole blood. These are comparable with those previously reported[3].

Results are summarized in Table 1. Values from whole blood and blood serum are expressed in mg of urea/100 ml., those from tissues in mg urea/100 g fresh weight. In parentheses is the number of animals (sample size) from which the means presented were calculated.

It is significant that the normal urea-level in blood of awake hedgehogs is very high when compared with those of other species analysed[3]. This high urea-level has been previously[4] reported in another hibernating animal, *Citellus parryi barrowensis*.

Table 1

	Awake animals	Hibernating animals	Significance of differences
Whole blood	96·6 ± 8·2 (10)	121·0 ± 5·3 (27)	$P > 0{\cdot}02$
Blood serum	101·5 ± 3·5 (20)	118·2 ± 3·8 (11)	$P > 0{\cdot}005$
Muscle*	104·3 ± 8·6 (7)	152·0 ± 7·9 (10)	$P > 0{\cdot}001$
Small intestine	80·7 ± 5·9 (10)	133·0 ± 9·1 (10)	$P > 0{\cdot}001$

* Diaphragma was used

Hedgehogs show a marked increase in blood (and serum) urea-level in their hibernating state. Because urea is a readily diffusible compound, this increase is also found in tissues. Klar[5] has previously reported increased non-protein nitrogen-levels in hibernating hedgehogs. He claims that the non-protein nitrogen-level increases parallel with the duration of hibernation. The main cause for azotemia demonstrated by Klar is evidently the increased urea-level reported in this communication. Failure to demonstrate gradually increasing urea-levels during hibernation in this investigation probably results from periodic awakenings during hibernation[6].

The increase of urea-levels in hibernating animals depends evidently on the markedly diminished ability of kidneys to remove nitrogen-containing catabolites from the body. The main cause for this renal insufficiency is, according to Hong[7], decrease in glomerular filtration rate which follows the diminished renal blood flow during hypothermia.

In normal conditions the amount of nitrogen-containing compounds excreted in urine is qualitatively and quantitatively held as a criterion of protein metabolism. With reference to the protein metabolism during hibernation, analyses of urine have been performed[8,9], and from the results it was deduced that the protein metabolism is greatly reduced, even more than the general metabolism. These generalized deductions based on urine analyses cannot be considered as quite valid, because the retention of catabolites of protein metabolism during hibernation is evident.

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Response of the Testes of Purple Sea Urchins to Variations in Temperature and Light

STUDIES on the reproductive activity of the purple sea urchin, *S. purpuratus*, made during the past eight years at Pacific Grove, California, indicate the presence of an annual breeding cycle, with maximum gonadal activity occurring during the winter months. In the course of the present investigation, designed to examine the effect of latitude on the breeding activity of the sea urchin, it was observed that the sea urchin populations distributed over a 20° latitudinal range have a uniform spawning period. These observations suggest that the reproductive activity of the purple sea urchin might be independent of temperature over a physiological range represented by this distribution. Therefore a photoperiodic response was considered as a possible explanation for the breeding cycles observed.

An experiment designed to test the role of light and temperature on gonadal activity of sea urchins was consequently prepared. The equipment was built and the experiment undertaken in the marine laboratory of the University of California, Los Angeles, where a 28,000-gallon circulating closed sea-water system is maintained. Additional experiments were performed in the laboratory of Marineland of the Pacific, Palos Verdes, California. Two tanks were constructed; one was designed to have 14 h of controlled illumination, the other, 6 h. The sea-water was maintained at 15° ± 0·5° C. Twenty-five sea urchins were placed in individual plastic containers with numerous holes for proper circulation of sea water. Each animal was removed and a 0·25-in. hole was made in the ambitus of the interadius. The sex of the animals was determined, and the gametogenic state assessed by observation of gonadal biopsies. Males were selected for this experiment. The hole in the test was then sealed with 'Plasticine' clay and treated with penicillin.

Once the spermatogenic state has been ascertained, the twenty-five selected animals were placed in the 14-h lighted tank in their plastic containers. An abundant supply of food was maintained. When examining the sea urchins for gametogenic activity the clay plug was removed and a small biopsy of the testes taken and examined under a phase-contrast microscope. Weekly checks were made on each animal over a period of 12–15 weeks. Immediately following this period, the 25 urchins were transferred to the 6-h lighted tank and were examined similarly.

The results obtained so far indicate that at 15° ± 0·5° C the 14-h period primarily stimulates the production of gonial cells. Occasionally, spermatocytes, spermatids, and spermatozoa were observed. However, when the day-length was reduced to 6 h the gonial cells, which were predominant during the long-day stimulus, were reduced in number and the spermatids, spermatocytes and spermatozoa were produced during the 6-h artificial day.

In order to assess the significance of temperature changes alone on gonadal activity, experiments are at present being made at 10° C. Eventually it is hoped to carry out experiments at 5° C, 20° C, and 30° C.

The foregoing temperatures in combination with various photoperiodic stimuli should provide an adequate means for testing gonadal activity of the sea urchin as affected by the two primary environmental variables, temperature and light.

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Demonstration of Humoral and Cellular Fixed Antibodies against Homologous Tissue Antigens

THE question whether circulating antibodies appear during sensitization with homologous tissue antigens and whether they have an effect on the homograft reaction has often been discussed. Ever since the work of Mitchison[1], there is no doubt that the mononuclear cells play an important part in the homograft reaction; however, the theory that they are carriers of sessile antibodies was not unchallenged, especially since up to this date exact methods for demonstrating cellular fixed antibodies were not known.

The aim of our investigation was first to examine serological phenomena during homograft reaction and second to detect cellular fixed antibodies against homologous tissue antigens using *in vivo* and *in vitro* methods. For donors we used healthy male albino rabbits (Weisse Wiener), for recipients healthy male rabbits of the Belgian Giant strain. For sensitization we applied tissue antigens differently prepared from the spleen and kidney of the donor. To guarantee an individual specific sensitization, the antigenic material of one donor was applied to one recipient only. To one group of rabbits (group *A*) we injected a mixture of spleen and kidney cells at a concentration of 10^8 cells, to the second group (*B*) an extract prepared according to the technique described by Lejeune-Ledant[2] and to the third group (*C*) an extract prepared according to the same technique but lyophilized in a concentration corresponding to 10^8 cells. All antigens were applied intravenously. After 7 days, skin grafting according to Billingham and Medawar's method[3] was performed in order to detect transplantation immunity. 14 days after the beginning of sensitization, a series of 5 injections of lyophilized extract was applied to each recipient at intervals of 2 days at a concentration of 2×10^7 cells.

The sera for the examination of serological phenomena[4] were obtained before sensitization and afterwards at intervals of 7 days (5 blood samples altogether). Alterations in the protein content of the sera could not be demonstrated during the sensitization period. In the first two weeks the paper electrophoretic analyses of serum protein fractions showed an increase in α_1-globulins in all three groups and later on a slow increase in γ-globulins which, however, was not present in group *A*. Whereas it is suggested that the increase of the α_1-globulin fraction is a sign of an inflammatory reaction, the increase of γ-globulins could be interpreted as related to antibody formation. Experiments to detect precipitating antibodies gave negative results without exception. On the contrary, with the hæmagglutination technique of Boyden[5] individual specific antibodies could be demonstrated, but only of a low titre. It is interesting that these antibodies were present as early as 7 days after the beginning of sensitization. In our investigation the properdin content of the sera determined by an inulin adsorption method[6] rose significantly 14 days after the first sensitization and 7 days after the grafting of homologous skin, respectively. Later on, the properdin values returned to normal level.

To demonstrate transplantation immunity, a gross and microscopic examination of the homografts on the sixth day after grafting was performed. Whereas in group *A* (sensitization by cells injected intravenously) no sign of graft breakdown could be observed, the grafts in group *B* and *C* (sensitization by fresh and lyophilized tissue extracts by the intravenous route) were almost or completely destroyed at this date.

For the detection of sessile antibodies we used the regional lymph nodes of the grafting site and the spleen of rabbits of group *B* (25 specimens altogether) obtained on the fourteenth day after beginning of sensitization and on the seventh day after skin grafting, respectively. The organs were pressed through a stainless-steel sieve and, so isolated, the cells were tested for the existence of sessile antibodies following three different methods: (1) The cells were transferred to untreated animals[7]. In no case could transplantation immunity be shown. These observations are in contrast to the findings of Mitchison. An explanation might be the genetic difference of the rabbits causing the destruction of the transferred lymph cells by homograft reaction, whereas Mitchison used inbred strains of mice.

(2) Investigations of the passive cutaneous hypersensitivity reaction[7] were performed. Here the cells were injected intracutaneously into untreated rabbits and 12 h later the corresponding antigen was given. On the average 24–48 h later a positive reaction with induration, erythema in every case and sometimes even necrosis could be observed. Controls with a non-corresponding antigen or with saline showed mostly a negative result. Experiments with non-sensitized lymph cells were also negative.

(3) In order to detect sessile antibodies by an *in vitro* method we utilized the technique described by Warnatz *et al.* and Steffen[7,8]. Here lymph node and spleen cells of sensitized rabbits were incubated with the corresponding antigen *in vitro* and the washed cells mixed with a heterologous antiserum (dog, rat) of a known titre and specifically directed against that antigen. The decrease in the titre of the antiserum after incubation was determined. It is assumed that the antigen reacts with the antibodies which are fixed at the investigated cells. The decrease in titre of the heterologous antiserum after incubation with cells so prepared indicates the existence of cellular fixed antibodies. In our investigation the titre decreased 1–3 steps of an antiserum dilution row. As compared with results of the controls these findings suggest that cellular fixed antibodies exist.

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β-Carotene and Sexuality in the Mucoraceae

THERE have been frequent reports of an association between β-carotene and reproduction in the Phycomycetes[1]. Recently, a stimulation of β-carotene production in mixed + and − cultures of four heteromictic[2] species of the Choanephoraceae has been reported[3,4]. It is not known whether this is a general or an isolated phenomenon in the Mucorales.

The following strains of heteromictic species were tested for β-carotene accumulation in intra- and inter-specific mixed cultures:

1 *Mucor hiemalis* +1
2 *M. hiemalis* −1
3 *Phycomyces nitens* +*H*102
4 *P. blakesleeanus* +1
5 *P. blakesleeanus* −1
6 *P. blakesleeanus* −6
7 *P. blakesleeanus* −*E*45
8 *Helicostylum pyriforme*
9 *Cunninghamella elegans* +1
10 *C. elegans* −1
11 *Absidia glauca* +1
12 *A. glauca* −1
13 *Mucor ramannianus*
14 *Circinella* sp.
15 *Rhizopus nigricans* +1
16 *Pilobolus sphaerospora*
17 *Conidiobolus villosus*
18 *Thamnidium elegans*

In addition, three homomictic species were tested:

19 *Rhizopus sexualis*
20 *Zygorhynchus moelleri*
21 *Z. vuilleminii*

All were originally obtained from the Centraalbureau voor Schimmelculture, except 13, which was isolated in this laboratory from soil. The homomictic species formed abundant zygospores on solid medium, as did the heteromictic strains in the appropriate combinations.

An inoculum of 10^7 asexual spores, which was found to be optimal, was added to 25 ml. of modified Schopfer's[5] medium in 150-ml. conical flasks plugged with gauze-covered cotton wool. The flasks were mounted on an illuminated reciprocating shaker at 25° C and the submerged shake cultures grown for up to four weeks. Cultures were triplicated at least.

The washed and partially dried mycelium was extracted with acetone and the principal pigment, β-carotene, characterized and determined spectrophotometrically[6,7].

Strains 1–8 grown singly did accumulate β-carotene to varying degrees. However, in no mixed culture was the amount of β-carotene significantly different from the expected mean of the values accumulated by the component strains grown alone (Table 1). Moreover, none of strains 9–21 accumulated β-carotene to a detectable degree, either in isolation or in mixed cultures although the homomictic cultures formed zygospores.

Table 1. β-CAROTENE ACCUMULATION BY MIXED CULTURES.
(Values are in μg β-carotene/g dry weight of mycelium)

Strains	1 × 2	3 × 5	3 × 6	3 × 7	4 × 5	4 × 6	4 × 7	5 × 6	6 × 7	6 × 7
Expected	406	148	110	147	175	137	174	112	149	111
Observed	457	137	133	152	166	128	102	120	152	123
S.D.	30	10	12	13	15	11	12	12	4	13

These results confirm and extend those of Reichel and Wallis, who found no stimulation of 'carotene' accumulation in mixed cultures of their strains of *P. blakesleeanus*[8]. Such a stimulation appears at present, therefore, to be confined to the Choanephoraceae.

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Effect of Gibberellic Acid on Flowering of Apple Trees

It has been shown by Sironval *et al.*[1] that gibberellic acid (GA_3) affects growth of the main shoots in apple trees (var. Golden Delicious) and apical dominance. Apple-trees treated with 300 p.p.m. GA_3 (1–2 ml. given twice a week on the apical bud and young leaves) throughout the growing season are greater and form twice as many lateral shoots as control trees,

On the other hand, it has long been claimed by gardeners that, in apple trees, growth of the shoots and flowering are antagonist processes. Indeed, Wareing and Nasr[2], investigating the effects of gravity on growth and flower formation, have shown that apple trees (apple rootstock No. 3436) grown horizontally have a reduced growth, but initiate more flowers than apple trees grown vertically (gravimorphism). We found it interesting to investigate flower formation of apple trees treated with gibberellic acid (GA_3).

Apple trees (var. Golden Delicious) were treated with GA_3 (100 and 300 p.p.m.) in 1961 according to the method of Sironval *et al.* At the end of the growing season, the plants treated with 300 p.p.m. GA_3 were greater than the control plants (increase of about 15 cm); the plants treated with 100 p.p.m. GA_3 were also a little higher than the controls. The treated plants bore 9–11 lateral shoots, while the controls bore only 4–5 lateral shoots. In the spring of 1962 the apple trees were re-examined for flowering.

Table 1 gives the absolute number of flower buds found on the whole plant and also on the main and lateral shoots. It is seen that treated plants formed more flower buds than control plants. The increase in the absolute number of flower buds resulted chiefly from the lateral shoots, which were more numerous in treated plants; the main shoots gave a similar absolute number of flower buds in treated and control plants.

Fig. 1 gives the percentages of the different categories of buds found on main and lateral shoots, and on the whole plants: dormant buds, vegetative and flower buds.

(1) On main shoots there was nearly the same percentage of dormant buds in treated and control plants. The percentage of flower buds was, however, lower in treated than in control plants.

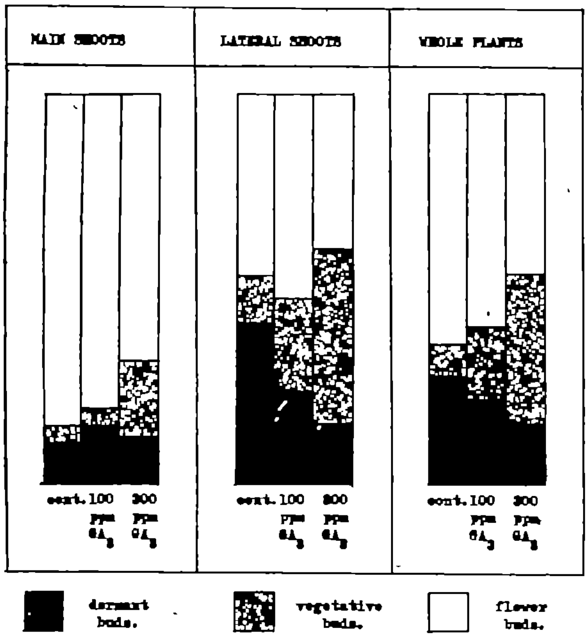

Fig. 1. Effect of gibberellic acid applied to apple trees (var. Golden Delicious) during the growing season 1961 on the proportion of dormant, vegetative and flower buds found in the spring of 1962 (relative number of the different kinds of buds expressed in percentage of the total number of buds)

Table 1. Effect of Gibberellic Acid on the Absolute Number of Flower Buds per Tree

Series	Whole plant	Main shoots	Lateral shoots
Controls	38	24	14
100 p.p.m. GA_3	58	21	37
300 p.p.m. GA_3	56	19	37

(2) On lateral shoots, the percentage of dormant buds was lower in treated plants than in control plants. The percentage of flower buds was again lower in treated than in control plants, except for the 100 p.p.m. treatment.

(3) For the whole plant, we found a decrease of the percentage of dormant buds and a decrease of the percentage of flower buds in treated trees when compared with the control ones.

This means that if, in absolute value, the number of flower buds is higher in GA_3-treated apple trees, owing to the great increase of the number of buds present on the lateral shoots, the percentage of flower buds is decreased when GA_3 is applied.

It may be concluded that the enhancement of the growth of the shoots (both main shoots and lateral shoots) by gibberellic acid treatment corresponds to a relative decrease in the capacity of the buds to form flowers. The result is similar to that obtained by Wareing and Nasr using gravimorphism; it corresponds to the relation generally claimed in practice between rate of growth of the shoots and flower formation in apple trees.

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ENTOMOLOGY

Feeding Stimulants required by a Polyphagous Insect, *Schistocerca gregaria*

It has been shown by Hamamura *et al.*[1] that food selection in the silkworm larvæ (*Bombyx mori*) is controlled by three chemical factors which they have called the attractant, the biting and the swallowing factors.

The desert locust (*Schistocerca gregaria*), which is a polyphagous insect, appears to require only the attractant and the swallowing factor to evoke the normal feeding behaviour. This behaviour pattern begins with the locust approaching the food and then lowering the tips of the antennæ and palps on to the food surface and biting. Sustained feeding then continues without further testing of the food with the antennæ; the palps alone are used to test the food as the insect feeds.

The experiments described here were performed to determine whether a few widely dispersed plant compounds would stimulate feeding by *Schistocerca*, or whether a number of compounds each present in a limited range of plants would be required. A series of extracts were made from plants on which the locust normally feeds, and these extracts were then run singly on to filter paper circles. The solvent was removed from the papers, and these were then placed in cages containing locusts. It was observed that the ether extract alone stimulated a marked testing behaviour in the locusts, and this testing always terminated with a single bite after which the locust would test the surface of the filter paper elsewhere. It was therefore assumed that the ether extract contained the attractant factor, and possibly a biting factor.

To determine whether this stimulant was the same substance or substances in the plants that were tested, a series of paper chromatograms were made using the ether extract and a variety of solvents and solvent mixtures. The chromatograms were then dried and placed in cages with locusts. Certain solvents (phenol, acetic acid) were found to be retained on the chromatograms sufficiently to make the entire chromatogram repellent to the locusts. Other solvents (petrol, ether, butanol, carbon tetrachloride, acetone) did not have this effect, and in these instances the locusts responded to one zone on each chromatogram with their complete testing behaviour which included the final bite. From these experiments it seemed unlikely that *Schistocerca* required an individual biting factor that Hamamura *et al.* have found necessary for satisfactory feeding by *Bombyx*.

Table 1. CLASSIFICATION OF FEEDING STIMULANTS BASED ON THE BEHAVIOUR OF *Schistocerca* TOWARDS PLANTS

Behaviour of locust towards the plants	Olfactory stimulant	Gustatory stimulant	Classification of plant	Examples of plants in each category
Tests surface of plant and feeds	Positive attractant	Positive acceptant	Acceptable	*Dactylis* *Plantago* *Lamium*
Tests surface of plant, bites but does not feed	Positive attractant	Negative rejectant	Non-acceptable	*Zea* *Tagetes*
Locust turns away from plant	Negative repellent	Negative rejectant		*Myrica* *Lavandula* *Cupressus*

The R_F value of the active zone was not the same in each plant for equivalent solvent systems, so it was concluded that *Schistocerca* will respond to a number of attractants.

As Hamamura *et al.* have reported for *Bombyx*, the feeding stimulant is also found in the acetone extract, and filter paper circles with an acetone extract of an acceptable plant plus the ether extract of *Dactylis* induced the locust to feed (the ether extract was required to induce the locust to bite the substrate and so bring its oral receptors into contact with the surface of the filter paper). A comparison of the acetone extracts of a series of plants showed that there was more than one acceptant substance in some plants (*Dactylis* and *Trifolium* both had two), and in certain instances (*Lavendula*) a plant may have positive feeding stimulants present in its tissues although the effect of these substances is masked by negative feeding stimulants.

A classification of the feeding stimulants proposed to account for the observed feeding behaviour of *Schistocerca* towards possible food material is given in Table 1. (Note: Thorsteinson[2] adopted this classification after discussion with me.)

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MICROBIOLOGY

In vitro Species (or Type) Transformation among Strains of *Brucella*

EXAMPLES reported in the literature concerning mutation in *Brucella* organisms have been received with strong criticism[1,2] arising, apart from other reasons, from the scarce number of cases involved or based on the negative results of other workers[3].

This communication presents the results of my work in this field, fundamentally related to the taxonomy of this bacterial genus, with the purpose of offering support to one of the opinions in conflict. The observations seem to prove that *Brucella* is a group of micro-organisms of particularly labile character and not infrequently affected by transformation.

Early in 1958 it was unexpectedly noticed that three *Br. melitensis* recently isolated appeared to have changed their serological behaviour only by reacting with the monospecific *abortus* serum; when tested biochemically these strains reacted like *Br. abortus*. This observation was taken at first with great reserve as it could have obviously been caused by accidental error; but a similar finding, in cultures of old storage, was soon found in the German literature as reported by Roots and Sprockhoff[4]. Decisive evidence was, however, obtained from a group of five other *Br. melitensis*. These strains, while changing antigenically to *abortus*, retained their original biochemical characteristics, in this way assuming the pattern of Renoux's *Br. intermedia*. A search for variation was therefore decided: care was taken to prevent any labelling faults to take place during the transfers of the strains in stock and the whole collection of cultures was checked at least once or twice a year by the following tests: (*a*) acriflavine test ($S \rightarrow R$ variation); (*b*) urease test; (*c*) slide agglutination test (using monospecific sera diluted to end-titre according to my technique[5]). For a final identification the inhibition dye test was also applied using basic fuchsin and thionin at concentrations of 1/25,000 and 1/50,000 and also safranin at 1/10,000. This dye was found to be a very useful tool for differentiating *Br. suis* from any other *Brucella*.

During 1959 it was found that a group of 26 strains had changed their previous classification.

When recently my collection of cultures was typed by the four known bacteriophages—10/I, 24/II, 212/XV and 371/XXIX—isolated and kindly supplied by Prof. J. Parnas (to whom I am indebted) an unexpectedly high number of discrepant results were registered. I admitted that mutation could explain them. In fact when the cultures concerned were again characterized biochemically and serologically a second group of 38 converted strains emerged from: (*a*) the whole of (27) *Br. melitensis* showing phage-sensitivity; (*b*) part of the *Br. abortus* showing phage-resistance; (*c*) other strains.

Altogether 64 stock cultures, out of a collection of 300, have suffered species or type reversion. The changes were as follows:

(*a*) 35 *Br. melitensis* changed into: 12 *Br. abortus*; 9 *Br. intermedia*; 11 *Br. suis*; 3 *Br.* 'strain AM' (*Br.* 'strain AM' is a strain with the biochemical characteristics of *Br. abortus* but reacting serologically with the *melitensis* serum).

(*b*) The variation of 12 *Br. abortus* occurred thus: 4 strains (types I and III) into *Br. suis*; 1 strain type III into *Br.* 'strain AM'; 1 strain type I became biochemically atypical and was taken as variant '*abortus-suis*'; 6 strains suffered inter-type variation: 4 strains type III changed into type I, 1 strain type II into type I and 1 type I into type III.

(*c*) All the 16 *Br. intermedia* of my collection suffered variation and they were now: 10 *Br. suis*; 5 *Br. abortus*; 1 *Br. melitensis* (atypical).

(*d*) 1 *Br.* 'strain AM' changed into *Br. abortus*.

Multiple or successive mutation was also observed in some cultures.

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A Biochemical Test for Crown Gall Bacteria

Agrobacterium tumefaciens is the agent for crown gall disease of several plants, thus causing serious economic losses in many countries. Except for its phytopathogenic action, it does not display any characteristic physiological or morphological properties. Its final identification is established by experimentally induced tumours, a procedure which often demands too much time for quick intervention in an epidemic. A quick, easy and specific identification test would be very useful. We wish, therefore, to report that *Agrobacterium tumefaciens* and *A. radiobacter* produce 3-ketoglycosides from the corresponding disaccharides and bionic acids. We propose a test based on this property, which is very simple, can be carried out in any phytopathological laboratory with ordinary glassware and chemicals, gives a result after 1–2 days and is, so far as we can judge from our present experience, limited to members of the genus *Agrobacterium*.

In previous papers[1–4] we described a new type of microbiological sugar oxidation which so far is unique in its mechanism because it consists in the oxidation at carbon 3 of the glycosyl moiety of disaccharides and bionic acids. Other compounds are not attacked. The following 3-ketoglycosides have been prepared: 3-ketolactose (3-ketogalactopyranosyl-glucose), 3-ketomaltose, 3-ketosucrose, 3-ketolactobionic acid and 3-ketomaltobionic acid. These compounds can easily be detected because they quickly reduce the usual sugar reagents (Fehling, Benedict, Luff, etc.) at room temperature. It was difficult to decide the exact taxonomic position of our bacteria, which carried out the foregoing oxidation (small, motile, Gram-negative rods, tentatively classified as *Alcaligenes*) due to the lack of typical characteristics and the fact that 3-ketoglycosides had never been described before.

Therefore, a systematic search among several bacterial genera was undertaken, which showed that only *Agrobacterium tumefaciens* and *A. radiobacter* produced the same 3-ketoglycosides as our strains. The formation of the latter compounds was studied with aerated, growing cultures in the medium previously described[2] and was followed either by reduction at room temperature of the Benedict reagent or by reaction with semicarbazide[4]. Ten different strains of *A. tumefaciens* and 4 strains of *A. radiobacter* (from the American Type Culture Collection, Washington; the National Collection of Industrial Bacteria, Aberdeen; Dr. J. la Rivière, Laboratory for Microbiology, Delft, The Netherlands, and Dr. Ch. Bonnier, Institut Agronomique, Gembloux, Belgium) were positive. Lactose was a particularly good substrate and with most strains a very rapid and nearly quantitative yield of 3-ketolactose was obtained. Only *A. tumefaciens*, strain No. 11.095 (A.T.C.C.), never produced 3-ketoglycosides. We therefore doubt its correct labelling, even more so since we found no phytopathogenicity. *Agrobacterium rhizogenes* (strain 11.325 from A.T.C.C. and 8.196 from N.C.I.B.) was physiologically quite different from the other two species and did not produce 3-ketoglycosides. A total of 80 strains representing at least 33 species of the following genera did not produce 3-ketoglycosides: *Acetobacter*, *Aerobacter*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Escherichia*, *Gluconobacter*, *Nocardia*, *Mycobacterium*, *Paracolobactrum*, *Pseudomonas*, *Rhizobium*, *Streptomyces* and *Xanthomonas*. It seems thus quite likely that our original strains also belong to the genus *Agrobacterium*. Electron microscopy showed them to possess 2–6 lateral flagella. They were not phytopathogenic for *Chrysanthemum frutescens*. Based on the foregoing information the following test for the quick detection of *A. tumefaciens* and *radiobacter* is proposed. A pure culture of the bacteria investigated, isolated from a plant tumour or any other source, is grown for 1–2 days at 25°–30° on slanted agar containing (in per cent w/v): 1, yeast extract (Difco); 2, glucose; 2, calcium carbonate; and 1·5–2 agar. A loopful of the bacteria is deposited on a solid medium (1 per cent lactose, 0·1 per cent yeast extract Difco and 2 per cent agar) in a Petri dish as a small heap, about 0·5 cm or less in diameter. 4–6 strains can be applied on the same plate. The latter is incubated at 28° for 1–2 days, afterwards flooded with a shallow layer of Benedict reagent and left at room temperature. If 3-ketolactose is present a yellow ring of Cu_2O becomes visible around the cell mass; after one hour it is maximal in diameter (about 2–3 cm), its yellow colour contrasting strongly with the blue reagent solution. Calcium lactobionate, when used in the test medium instead of lactose, yields the same results due to 3-ketolactobionate formation, but it is less readily available for routine analysis. Since the yield of 3-ketoglycosides from maltose, maltobionate and sucrose is much smaller, the rings are weaker and less distinct. Practical application of this test is being investigated.

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Effect of Bromouracil-labelled Transforming Deoxyribonucleic Acid of *Bacillus subtilis* on Cell Growth

BROMOURACIL inhibits bacterial growth through its incorporation into the deoxyribonucleic acids in place of thymine[1]; however, infectivity of bacteriophage[2] and transforming activity[3] remain even though the DNA is

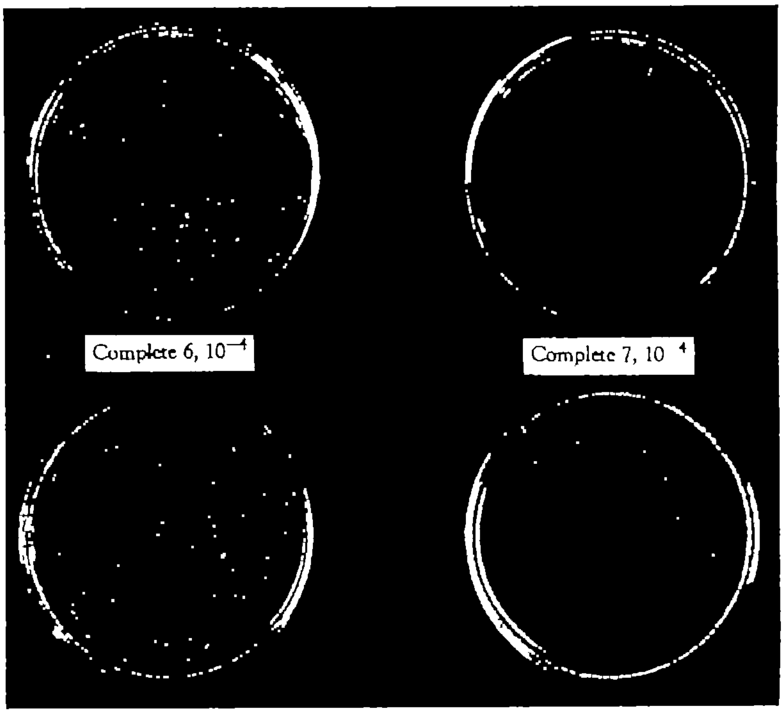

Fig. 1. Effect of incorporation of bromouracil labelled DNA on the growth of *Bacillus subtilis* strain 168 on the enumeration medium. Competent *B. subtilis* cells were exposed to wild-type DNA and to wild-type DNA labelled with bromouracil. The plates on the left are a dilution of the cells exposed to the normal DNA, while those on the right are the same dilution of cells exposed to labelled DNA. In both cases the same original concentration of cells was used

heavily labelled with bromouracil. In this communication we wish to report on the incorporation of bromouracil into *Bacillus subtilis* wild-type DNA and its effects on cell growth when the organisms are plated on the medium used for enumeration of the total cell number in transformation experience.

Ephrati-Elizur and Zamenhof[3] as well as Szybalski *et al.*[4] have reported failure to obtain measurable bromouracil incorporation into wild-type DNA. The wild-type *B. subtilis* cells were grown on the minimal medium described by Spizizen[5]; the bromouracil at a concentration of 20 μg per ml. was added 3 h prior to collecting the cells by centrifugation. DNA was isolated by the procedure of McDonald[6]. Paper chromatography of acid hydrolysed DNA by the procedure of Djordjevic and Szybalski[7] revealed the presence of bromouracil in the isolated DNA.

Transformation of strain 168 (indole- to independence) was obtained with bromouracil·labelled DNA as well as with normal DNA. The labelled DNA was slightly less effective. Transformations were done by the procedure of Young and Spizizen[8].

There was a reduction by about 10[2] of the number of cells growing on the enumeration (minimal plus tryptophan) medium of total cell number. Fig. 1 shows photographs of Petri dishes containing dilutions of the cells exposed both to normal and bromouracil containing DNA cells plated on the control medium. This effect occurred when normal and bromouracil labelled DNA's were mixed in equal proportions; however, the normal DNA antagonizes the bromouracil DNA. Acid hydrolysis of the bromouracil labelled DNA completely abolished the effect of growth inhibition. Addition of free bromouracil to the transformation medium did not inhibit growth on the control medium.

Although our results agree with those of Ephrati-Elizur and Zamenhof[3] and Szybalski *et al.*[4,9] in that the incorporation of bromouracil into DNA allowed retention of transforming activity, we find that incorporation of bromouracil into DNA causes an apparent decrease in the number of viable cells which are able to grow on control medium when these cells have been incubated under transforming conditions with bromouracil labelled DNA. The possibility that the incorporation of bromouracil is mutagenic under these conditions is being investigated.

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VIROLOGY

Potentiating Effect of 5-Fluorouracil on the *In vitro* Antipoliovirus Activity of Guanidine

PREVIOUSLY[1,2] we have shown that guanidine possesses a strong and highly specific inhibitory action against enteroviruses. This antivirus activity of guanidine seemed to us of interest due to: (1) its specificity; (2) the simplicity of guanidine molecule; (3) the ability of guanidine to stop the cytotoxic effect of poliovirus even when added to cell cultures which were infected several hours previously.

However, we were unable to determine the mechanism(s) of the *in vitro* inhibiting activity of guanidine against the poliovirus. Guanidine inhibition of poliovirus replication was not counteracted by a number of biologically active substances such as amino-acids, purine and pyrimidine bases and vitamins. In addition guanidine did not seem to act directly either on the virus or on the cell.

In order to elucidate the mechanism involved in the antiviral action of guanidine, we have investigated how this action might be influenced by chloramphenicol and 5-fluorouracil. It is known that the former inhibits protein synthesis and the latter interferes with nucleic acid synthesis.

Table 1. *In vitro* INHIBITION OF POLIOVIRUS CPE BY THE ACTION OF GUANIDINE HYDROCHLORIDE ACTING SINGLY OR IN COMBINATION WITH CHLORAMPHENICOL OR WITH 5-FLUOROURACIL

Guanidine hydrochloride (mg/ml.)	Chloramphenicol (mg/ml.)	5-Fluorouracil (mg/ml.)	CPU inhibited*
0·25	—	—	1,000,000 (4)
0·125	—	—	10,000 (1)
0·082	—	—	1,000 (4)
0·066	—	—	600 (9)
0·05	—	—	100 (9)
0·04	—	—	40 (3)
—	0·05†	—	<10 (2)
0·082	0·05	—	1,000 (2)
0·05	0·05	—	100 (2)
—	—	0·033†	20 (7)
—	—	0·02	5 (9)
—	—	0·01	<5 (4)
—	—	0·0033	0 (1)
0·125	—	0·02	1,000,000 (1)
0·082	—	0·02	100,000 (3)
0·082	—	0·0033	10,000 (1)
0·066	—	0·033†	300,000 (3)
0·066	—	0·02	160,000 (5)
0·05	—	0·011	10,000 (2)
0·05	—	0·033†	40,000 (3)
0·05	—	0·02	10,000 (3)
0·05	—	0·01	1,000 (1)
0·04	—	0·02	5,000 (3)

* Mean values. In parentheses the number of proofs effected.
† Maximum dose tolerated by cultured cells.

Table 2. INFLUENCE OF 5-FLUOROURACIL ON THE *in vitro* INHIBITION OF POLIOVIRUS REPLICATION BY GUANIDINE HYDROCHLORIDE

Viral inoculum (CPU)	Guanidine hydrochloride (γ/ml.)	5-Fluorouracil* (γ/ml.)	CPU present into 1 ml. of medium of whole culture after days: 1	2	3	4
10^5	—	—	10^6	10^8 †		
10^4	—	—	10^5	10^7 †		
10^5	—	20	10^7	10^7 †		
10^5	—	25	10^5	10^6 †		
10^5	66	—	10^6	10^6 †	10^7 †	
10^6	66	25	10^3	10^5	10^6 †	
10^3	66	—	10^4	10^6	10^6 †	
10^5	66	25	10^6	10^4	10^5	10^5 †
10^4	66	—	10^4	10^4	10^7	10^6 †
10^4	66	25	<10	10^3	10^6	10^6 †
10^2	66	—	10	10^2	10^4	10^6 †
10^2	66	25	<10	<10	10	10^6 †
10^4	50	—	10^6	10^5	10^7 †	
10^4	50	20	10^4	10^4	10^5	10^6 †

* HeLa cell tube cultures infected with different inocula of polio-1-virus (0·1 ml.). These were incubated at 20° C × 60′, washed four times with 5 ml. of Hanks's BSS which was added with maintenance medium (with or without guanidine and 5-fluorouracil). The cultures were then incubated at 36° C. Titration of CPU present in the culture media (or in whole cultures after cryolysis) was performed by the end-point method, at several time-intervals from viral inoculum.
† Titrations performed with the whole culture after its cryolysis.

Polio-1-virus (strain Brunhenders) was propagated in stationary cultures of HeLa cells, maintained in a medium containing Hanks's balanced salt solution (BSS), lactalbumin hydrolysate and calf serum. The substances to be tested were added to the culture media in the proper concentrations. The poliovirus cytopathogenic effect (CPE) inhibition was evaluated after 5 days of incubation at 36° C. The inhibition of the poliovirus replication was evaluated by counting the cytopathogenic units (CPU) present at various time-intervals in the culture media. The results are summarized in Tables 1 and 2. They show that chloramphenicol, in doses tolerated by the cells, does not inhibit the poliovirus CPE, nor does it reinforce the antipolio activity of guanidine. The antiviral activity[3] of 5-fluorouracil is confirmed by our experiments. Further, the results demonstrate that inactive or very slight active doses of 5-fluorouracil potentiate the antipolio-activity of guanidine.

We do not know the mechanism of this potentiating effect. It can be postulated that guanidine and 5-fluorouracil both interfere with the ribonucleic acid synthesis, or that 5-fluorouracil makes the cells less suitable for supporting the virus replication. However, Kovacs[4] has observed that HeLa cells treated with large doses of colchicine are able to support poliovirus replication, as do the untreated cells.

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Intranuclear Inclusions produced by Bovine *para*-Influenza

Myxoviruses generally produce cytoplasmic but not nuclear inclusions in infected tissues. Reports on the cytopathology of human strains of the *para*-influenza viruses[1–4] in stained tissue culture preparations record only the finding of intracytoplasmic inclusions, although in *FL* cells specific intranuclear immunofluorescent staining has been demonstrated with *para*-influenza 3 (ref. 5). Reisinger *et al.*[7] reported both intranuclear and intracytoplasmic inclusions in calf kidney tissue culture infected with the *SF* 4 bovine strain of *para*-influenza 3.

We have passaged the *SF* 4 strain in primary monkey kidney tissue and *Hep* 2 cell cultures and have been able to demonstrate nuclear and cytoplasmic inclusions in both systems. Intranuclear inclusions have been most readily demonstrated in infected *Hep* 2 cell cultures. Cultures were grown on a medium consisting of Earle's BSS + 15 per cent horse serum + 0·1 per cent glucose + 1 per cent yeast extract + 0·2 per cent sodium bicarbonate with antibiotics. Before infection, cultures were changed on to a maintenance medium of the same composition as the growth medium, except that the horse-serum content was reduced to 1 per cent. Tube cultures inoculated with approximately 100 $TCID_{50}$ were fixed with Bouin's fluid at 2, 3, 4 and 6 days post-infection and were stained with Ehrlich's hæmatoxylin and eosin. Eosinophilic nuclear inclusions were first detected at 3 days, and at this stage were only in the nuclei, contained within syncytial areas. By four days the inclusions were demonstrable in all the nuclei within syncytial areas. Multinucleated cells containing nuclear inclusions always contained also cytoplasmic inclusions. One to nine inclusions were found scattered within the nucleus, and were sometimes of such a shape as to suggest that two or three had become fused together. Fig. 1 shows an infected *Hep* 2 cell sheet fixed and stained four days after infection. The clear halo surrounding the inclusions is probably a product of the method of fixation.

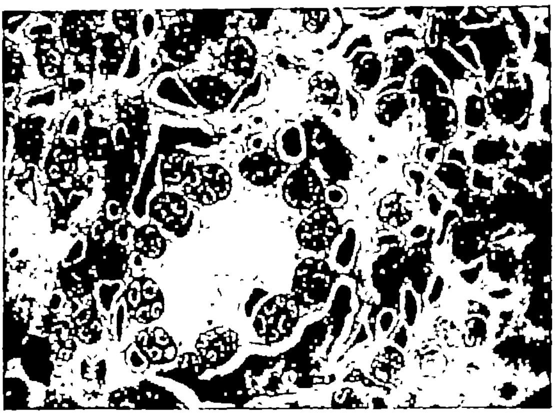

Fig. 1. *Hep* 2 cell monolayer 4 days after infection with *SF* 4 virus (stained with hæmatoxylin and eosin, × c. 233). Individual cells have lost their identity, becoming fused into a large syncytium. Nuclear and cytoplasmic inclusions are numerous and are surrounded in each case by a halo

I have been unable to demonstrate similar nuclear inclusions in *Hep* 2 cells infected with a human strain of para-influenza 3 under the same conditions. These findings suggest a biological, as well as a serological[8–10], distinction between human and bovine strains, although further work is needed to demonstrate that nuclear inclusion formation is a constant distinguishing feature. Waterson[11,12] has suggested that the measles, distemper and rinderpest viruses be placed in the Newcastle disease virus group of myxoviruses. The production of nuclear inclusions by *SF* 4 virus, an accepted myxovirus, provides another link in this association. It also means that not every virus growing in monkey kidney and *Hep* 2 cells producing syncytia and nuclear inclusions is measles as is sometimes assumed. It might be easy to confuse the two without serological or hæmagglutination tests.

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Demonstration of Leptospiral and Viral Antigens in Formalin-fixed Tissues

During the past few years the fluorescent antibody procedure has been applied with increasing success in the demonstration of microbial antigens. Naturally occurring cases of leptospirosis, venereal infections, influenza, rabies, and other diseases have been diagnosed[1] and further investigations will undoubtedly extend the application of this new diagnostic tool.

The application of the fluorescent antibody procedure is nevertheless limited by the lability of some of the antigens to be demonstrated. The quick freezing of tissue specimens, which is the best method for retaining the immunological activity of the antigens, is often not practicable under field conditions. Both fixation in 10 per cent formalin and storage at room temperature progressively decrease the property of the antigens contained in the tissues to bind with the fluorescent conjugate.

Application of the fluorescent antibody procedure to the detection of leptospiral[2] and viral[3] antigens in formalin-fixed tissues has been attempted with negative results. Its feasibility was considered worth investigating, and several chemical substances were taken under consideration to determine their capacity in neutralizing the action of the formalin on the antigens.

Immune sera, globulins, fluorescent conjugates and tissue extracts were prepared according to a procedure previously reported[4]. Antileptospira sera were prepared in rabbits; anti-canine distemper and infectious hepatitis sera in dogs; anti-Newcastle disease sera in rabbits and in chickens; anti-infectious bronchitis, laryngo-tracheitis and fowl pox sera in chickens. To obtain a high antibody-level it was sometimes necessary to concentrate a conjugate by ammonium sulphate precipitation[5]. Acetone tissue extracts, for absorption of the conjugates, were prepared from fresh and formalin-fixed tissues of several animal species. Four micron sections of both fresh and formalin-fixed tissues were obtained by means of a cryostat (Harris). A Fluorex (Reichert) apparatus and the filter combination *UG*–1 (primary), *W*–2*A* (secondary) and *S–GB* 12, *GG*–9, respectively, were used for microscopical observation.

Leptospiral smears which had been kept in 10 per cent formalin for two months and were no longer stainable by means of the conventional fluorescent antibody procedure were blotted dry and fixed in acetone at room temperature for 5 min. After drying at 37° C they were placed for 5 min in one of a series of ten-fold dilutions of sodium phosphate monobasic, dibasic and tribasic, sodium bisulphite, sodium carbonate and bicarbonate, sodium hydroxide, potassium phosphate monobasic, potassium hydroxide, ammonium sulphate, ammonium chloride, ammonium oxalate and ammonium hydroxide. Afterward the slides were washed in pH 7·2 buffered saline containing 3 per cent 'Tween 80', blotted dry and reacted with the immune conjugate at 18° C (or at 37° C). After washing in buffered saline the slides were counterstained with rhodamine aspecific conjugate (Difco) diluted 1 : 10, washed in buffered saline, and mounted with buffered glycerine. Specificity of the positive results was determined in every case; immune non-labelled globulin applied to the section before the fluorescent conjugate inhibited the specific fluorescent stain. It was seen that of all the chemicals tested only the contact for 3–5 min with 1 per cent ammonium hydroxide or for 5 min with 25 per cent sodium bisulphite allowed a bright staining of the microbial bodies. A similar effect was obtained by exposing the smears to ammonium hydroxide vapours for 48 h at 37° C. Leptospira could be detected by application of ammonium hydroxide or sodium bisulphite to sections of formalin-fixed tissues of experimentally and naturally infected animals. Tissues gave identical results when submitted to fluorescent antibody diagnosis either after quick freezing or after formalin fixation for several months, and slight shrinking of the leptospira was the only morphological alteration observed. A good correlation was found between the results obtained by the fluorescent antibody procedure applied to formalin-fixed tissues and those obtained from the same cases by serological, isolation and silver impregnation techniques.

Formalin-fixed post-mortem tissues from cases of canine distemper and infectious hepatitis were submitted to the action of ammonium hydroxide and sodium bisulphite. These cases had been diagnosed by the clinical symptoms, the presence of inclusion bodies, and the demonstration of viral antigen with the fluorescent antibody procedure in sections of quickly frozen tissues. The viral antigen detected in formalin-fixed tissues by the fluorescent antibody technique was visible with equal clarity and in the same intracellular location as in the tissues which were frozen immediately after collection. The only observed difference was a marked shrinkage of the fluorescent areas.

Viral antigen was likewise clearly visible in formalin-fixed tissues of chicken embryos and chickens experimentally infected with Newcastle disease, infectious bronchitis, laryngo-tracheitis and fowl pox. The results obtained compared very favourably with those obtained from egg inoculation and from the application of fluorescent antibody procedure to quickly frozen tissues.

No cross-reaction was noticed between the different conjugates used in this study; normal non-infected tissue did not stain. 'Tween 80' applied before the fluorescent conjugate increased the brightness of the specific fluorescent areas two- to four-fold, allowing a considerable reduction in staining time. The non-specific fluorescein staining which results from the use of conjugates treated with heterologous tissue extracts can be quenched by the use of rhodamine counterstain. This finding was helpful in the diagnosis of diseases affecting different animal species because it eliminated treatment of the conjugates with species-specific tissue extracts. The striking contrast between the red background and the green antigen facilitated the detection of a considerable number of doubtful reactions.

These modifications of the fluorescent antibody procedure warrant further investigation because of the following practical implications: (*a*) elimination of the hazard of handling infection material; (*b*) simplification of collection, shipment and storage of suspected specimens; (*c*) investigation by means of the fluorescent antibody procedure of specimens submitted for routine histopathological examination; (*d*) possibility of a more extensive use of this relatively new tool.

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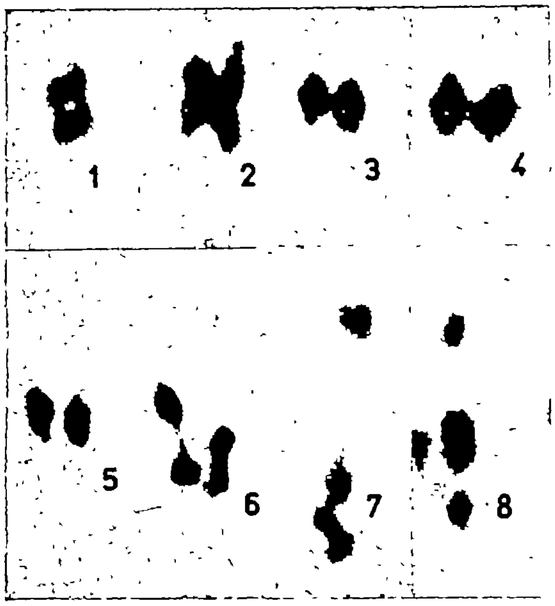
CYTOLOGY

Pseudo-Quadrivalents in Autotetraploid Rice

In the course of a statistical examination of chromosome pairing in PMC's of various induced tetraploid forms of *Oryza sativa*, I have found a regular decrease of the quadrivalent frequency from diakinesis to metaphase I. For 533 cells observed at diakinesis and 741 at metaphase, the mean numbers of quadrivalents per cell were respectively 7·61 and 6·25 : this reduction (18 per cent) is very significant. Together with the reduction of quadrivalent frequency, a complementary increase in the number of bivalents (from 8·63 to 11·01) was noticed. A few uni- and tri-valents were also present at diakinesis (on an average 0·15 and 0·05 per cell) and their number increased up to metaphase (respectively 0·47 and 0·17). These observations showed that some quadrivalents became dissociated before anaphase; they generally gave rise to two bivalents, but sometimes to one trivalent and one univalent or one bivalent and two univalents.

During diakinesis and, more frequently, during prometaphase and metaphase, some chromosomes appeared to be less closely associated than in typical quadrivalents: they were separated by a partly achromatic zone or a more or less elongated thread. Some groups of four chromosomes appearing as quadrivalents, were thus formed by a loose association between two bivalents or, less frequently between one bivalent and two univalents or one trivalent and one univalent. During metaphase, the bivalents associated in this way were both regularly oriented in the spindle, just as two independent bivalents, the achromatic zone being thus parallel to the spindle fibres. I found many intermediate figures between apparently typical quadrivalents and pairs of bivalents which seemed almost free (Figs. 1–6). I also found some stages of dissociation of quadrivalents giving one trivalent and one univalent



Figs. 1–8

(Fig. 7) or one bivalent and two univalents (Fig. 8). At metaphase, the location of some tri-, bi- and uni-valents showed that these chromosomes were proceeding from a quadrivalent.

If it is assumed that in every quadrivalent observed during prophase the four chromosomes are bound by chiasmata, it is difficult to explain their separation before anaphase. During metaphase, the chromosomes are stretched to the spindle poles, which indicates the direction of the forces operating on them; but the partition of the quadrivalent in two bivalents does not occur in that way. The chiasma frequency is not high in rice: Soriano[1] found a mean of 1·6 per bivalent for five diploid varieties. In spite of that rather low chiasma frequency, 63·4 per cent of the chromosomes of the tetraploid are associated in quadrivalents at diakinesis: this proportion is high, in comparison with the tetraploids induced in many other species. The four homologous chromosomes certainly gather at zygotene, but chiasmata are not always formed between all of them, so that a number of uni-, bi- and tri-valents may occur. It is possible that some chromosomes which are not bound by chiasmata remain nevertheless together up to diakinesis or metaphase, due to an adherence between them. Thus, some groups of four chromosomes are not true quadrivalents and may be called pseudo-quadrivalents.

These pseudo-quadrivalents are to be compared with the pseudo-bivalents observed in some haploid, asynaptic or hybrid plants, where homologous chromosomes do not form chiasmata. Several hypotheses have been proposed to explain the origin of the pseudo-bivalents, but the most frequently suggested is a fusion of the chromosome matrix[2,3]. This hypothesis may be admitted as a tentative interpretation of the nature of the adherence between some chromosomes which are not bound by chiasmata in autotetraploid rice. Thus, the decreasing number, after diakinesis, of the chromosome associations appearing as quadrivalents, and the increasing frequency of the uni- and tri-valents are interpreted as resulting from a more or less precocious slackening of some inter-chromosomal connexions in pseudo-quadrivalents.

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GENETICS

Differences in Interactions between Selective Forces acting in the Wild on Certain Pleiotropic Genes of *Cepaea*

The snail *Cepaea nemoralis* is highly polymorphic for colour and banding varieties of its shell. The genetic basis of this polymorphism is fairly well known, and involves several loci, linked and unlinked, with alleles controlling clear-cut characters. The snail is also highly sedentary. It therefore offers a favourable opportunity for investigating local differences in the interaction of selective forces affecting the polymorphism. The colour and banding varieties of the shell have been shown already[1] to be subject to visual selection by predators, the more conspicuous shells being preferentially removed, so that the intercolony variation, which is considerable, of the phenotype frequencies is largely determined in relation to the visual properties of the backgrounds of the colonies. Since the polymorphism is stable, some non-visual selection, probably acting as heterozygote advantage, must maintain the balance, while visual selection sways it to different extents in different places. The majority of Cain and Sheppard's observations were made in the Oxford district; they pointed out that elsewhere a different general balance might be found because of regional differences in the overall non-visual selective values[2].

Such effects have now been found by us on a far greater scale than was anticipated, and involve both great predominances (sometimes up to saturation) of particular genes over areas large compared with that of a panmictic unit of this snail, and the abutment of such areas on others with very different frequencies, producing extremely steep morph-ratio clines. Moreover, in these areas we find a virtual annihilation of the effects of visual selection, although there is evidence that this class of selection is acting.

In the Oxford district and adjacent districts off the Chalk, intercolony variation accords well with variation in background. But on the Marlborough Downs, only 35 miles from Oxford, the situation is quite different. These are fairly typical chalk downs with large expanses of grass, and a few beech and other deciduous woods. The district investigated is roughly a rectangle of approximately 5 by 4 miles and has been searched intensively, about 7,500 snails from 148 localities having been examined. The populations are often very large and widespread here, as is usual in calcareous districts. Over almost all the district there is no correspondence of phenotype frequencies with backgrounds although habitats with sharply contrasting backgrounds are occupied by the snails, predating thrushes are almost everywhere, and in one locality at least visual selection has been demonstrated by a comparison of predated and unpredated shells.

In a compact area of about 8 square miles there is an almost complete absence of the five-banded form, the most usual of the banding forms in most colonies of *Cepaea*. This is caused by a fixation of the modifier *M*[3] converting the five-banded to the mid-banded form. In the north-east of the district investigated is an area of about one square mile which, on the contrary, has a very high percentage of five-banded snails (75–100 per cent). This heavily five-banded area approaches very close to the non-five-banded area in the extreme north of the district, and here the percentage of the five-banded form rises from 34 to 98 per cent over only 500 yards. Enclosed within the non-five-banded area there is a sub-area in which brown forms are extremely abundant, reaching 93–98 per cent on some stretches of open downland. This brown sub-area abuts on another in which yellow forms predominate, and at one place a change from 93 per cent brown forms and 0 per cent yellow forms to 12 per cent brown forms and 80 per cent yellow forms occurs over a distance of at most 180 yards. (Pink forms remain at about 8 per cent.) Finally, a long narrow sub-area, cutting right across the colour sub-areas, has a remarkably high proportion of the spread-banded form, very rare elsewhere.

Subsequent investigations in other districts of downland have shown similar areas, characterized by high frequencies, often approaching saturation, of yellow, pink, mid-banded, spread-banded, and five-banded forms; the frequencies do not vary in relation to the available types of background, and the common morphs are often visually inappropriate. In all these areas, including those of the Marlborough Downs, the sizes of the areas and of the populations enclosed are so great that the possibility of genetic drift being an important factor can be neglected. Selective forces, certainly not visual, must be acting to produce these *area effects*, and calculation suggests that quite slight changes of selective value of the homozygotes relative to the heterozygotes, from those present in the Oxford district, would suffice to produce a very marked area effect. The nature of the non-visual selective forces needs investigation.

Several workers have shown a variation in gene-frequency from place to place because of the variation in action of one selective force or in interaction of two[3-6]. The results are analogous with ours for the Oxford district, in which, although non-visual selection maintains the polymorphism, the variance between colonies is caused by variation in direction and pressure of visual selection. But we find in other districts that, with the same classes of selection acting, the variance between colonies is as great as or greater than in the Oxford district, yet is determined scarcely at all by visual selection but almost entirely by non-visual, in such a way that large areas are characterized by rather constant and often extreme gene-frequencies which may change abruptly at the edges of the areas. With suitable material, such differences in action and interaction of different selective forces will no doubt be shown to occur widely.

A full statement of this investigation will be published elsewhere[7].

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Transpositions of Modulator in Maize into Divided and Undivided Chromosome Segments

Variegated pericarp ($\overline{P^{rr}Mp}$), a highly unstable allele in maize, comprises two distinct and separable components, symbolized as P^{rr} and *Mp*. The P^{rr} gene which, when unaccompanied locally by *Mp*, conditions uniformly pigmented (self-coloured) pericarp and cob is fixed in position at the *P* locus in the short arm of chromosome 1. In contrast, modulator (*Mp*) is an element which is transposable independently in somatic cells from this position to a wide variety of other chromosome sites, and preferentially to those nearby. *Mp*, when immediately associated at the *P* locus with P^{rr}, suppresses pigment-producing action of the latter, giving colourless pericarp and cob. The composite, variegated pericarp phenotype is the outcome of numerous *Mp* transpositions during ear development, resulting in P^{rr} expression in the form of irregularly distributed coloured pericarp sectors of various sizes on a colourless background[1,2].

The results summarized here show that during a mitosis in which a transposition from the *P* locus occurs a state of division at the recipient locus is not a necessary condition of re-incorporation of *Mp* into a chromosome. Critical evidence on this point was afforded by the genetic analysis of twinned mutations of medium variegated to self-coloured and light variegated pericarp.

Twinned self-coloured and light variegated mutants evidently originate in a somatic mitosis which results in daughter cells of two kinds with respect to *Mp*. These cells then become progenitors of a pair of adjacent, but unlike, cell lineages. *Tr–Mp* composition of the light variegated component can be inferred directly from the resulting pericarp phenotype. Medium variegated plants are heterozygous for variegated and a stable colourless allele ($\overline{P^{rr}Mp}/P^{wr}$). Light variegateds carry a transposed modulator (*tr–Mp*) in addition. Self-coloured mutants are P^{rr}/P^{wr} at the *P* locus, and may or may not carry a transposed modulator. The presence or absence of *tr–Mp* in the self-coloured co-twin can be determined in crosses of this mutant with a suitably constituted strain possessing the dissociation (*Ds*) factor described by McClintock[3].

Assay by the *Ds* technique of 80 independently occurring twinned mutations showed that in 52, or about two-thirds of them, the self-coloured mutant was *tr–Mp* positive and was *tr–Mp* negative in the remaining 28. Thus the twinned mutations, although phenotypically alike, proved to be of two genotypes.

It may be assumed in the case of the minority class of twins that when division of the chromosome carrying the variegated allele ($\overline{P^{rr}Mp}$) occurs at the *P* locus *Mp* is lost from one, but not the other, of the two resulting chromatids, and that one daughter cell (light variegated) acquires directly, but in a different location, the *Mp* unit which the other daughter cell (self-coloured) loses.

Two *tr–Mp* units, one in each co-twin, are to be accounted for in the majority class of twinned mutations even though, as in the other category, there is a single donor variegated allele in the parent medium variegated cell ($\overline{P^{rr}Mp}/P^{wr}$). Evidently *Mp* is reduplicated twice during a single mitotic cycle in these cases, once at the *P* locus and again elsewhere after transposition.

It was postulated that *tr–Mp* in the majority class of twinned mutations is incorporated at a new site during a differential mitosis before the chromosome at that level has split into chromatids. Reduplication of *tr–Mp* then ensues in phase with division of the chromosome segment in question. A prediction that follows from this hypothesis is that *tr–Mp* will be located in the same position in both members of such a twin.

Three-point linkage tests between *tr–Mp* and markers on the short arm of chromosome 1 were made with 13 twinned mutations of the majority class. The observed amounts of recombination for the seven twinned mutations in which *tr–Mp* was found to reside on chromosome 1 are given in Table 1. Estimates of recombination in this material are affected by secondary transposition of *tr–Mp*, as well as by sampling. In 11 of the 14 paired entries in Table 1 the recombination value is less in the self-coloured than in the light variegated class. This bias is attributable to the fact, established in a previous investigation, that when more than one *Mp* unit is present, as in light variegated

Table 1. Recombination Frequencies between Transposed Modulator and Chromosome 1 Markers in Co-twin Mutants

Twin mutant	Member	No. of plants scored	Percentage recombination of *tr–Mp* with			
			P	*T*1–5*b* (1S.17)	*T*1–2*b* (1S.43)	*T*1–7*c* (1S.79)
1	Self-coloured	764	3·7		5·6	
	Light variegated	542	2·2		4·6	
4	Self-coloured	633	3·2		9·0	
	Light variegated	402	6·4		9·4	
6	Self-coloured	137	13·0		19·7	
	Light variegated	581	9·3		13·1	
9	Self-coloured	466	7·1		10 7	
	Light variegated	121	4·1		4·1	
11	Self-coloured	323	12·4		11 2	
	Light variegated	228	4·4		4·4	
12	Self-coloured	967	10·3			3·4
	Light variegated	473	21·1			5·1
13	Self-coloured	136	20·4	19·1		
	Light variegated	480	32·3	19 0		

plants the frequency of transposition of each *Mp* is sharply reduced[4].

When allowance is made for secondary transposition of *tr–Mp* and sampling effects, two conclusions become apparent from the results in Table 1. First, the amounts of recombination observed between *tr–Mp* and the markers within co-twins correspond closely enough to suggest that the sites of *tr–Mp* in the two respective mutants are identical. Each of the seven twinned mutations affords an independent test of this relation, and conformity to the rule is apparent in six of them. Twin 11 is possibly an exception. The second conclusion is that the position to which *Mp* is transposed differs from twin to twin.

The coincidence in chromosome position of *tr–Mp* in the members of a twin pair is in agreement with the hypothesis that *Mp* may be transferred from the *P* locus to a new site, and then re-established there, in advance of division of the chromosome into chromatids at that level.

A fuller account of these experiments has been published in *Genetics*[5].

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Mutation and Recombination of Flagellar Antigen *i* of *Salmonella typhimurium*

CHANGES in the flagellar antigenic constitution of many *Salmonella* species have been reported[1]; but their genetical basis is unknown. As the flagellar antigen is a protein, flagellin, the commonest kind of mutational change in it is presumably alteration of a single amino-acid, which would perhaps cause only a minor change in its serological character. We now report a serological and genetical investigation of mutants of *S. typhimurium* with slightly altered forms of the phase 1 flagellar antigen *i*.

S. typhimurium strain *LT2 adeC*-7 *proA*-46[2] was inoculated into plates of semi-solid medium containing a just immobilizing concentration of rabbit anti-*i* serum and an excess of serum against the alternative, phase 2, flagellar antigen. Swarms spreading faster than the parent strain yielded eight mutants, each with a phase 1 antigen slightly different from the wild-type antigen *i*. A further mutant antigen was obtained in a strain of *S. typhi* previously given antigen *i* by transduction from *S. typhimurium*. Serological analysis of the nine mutant forms of *i* (*iM*5 to *iM*13) showed that two of them were antigenically indistinguishable; these two mutants arose in different clones, presumably from identical mutations. No reversion to wild-type *i* was detectable, even by the sensitive test of selection in semi-solid medium with mutant-specific serum. All the mutants were normally motile in phase 1 (cf. 'curly' mutants[3]) and their phase 2 antigens were unchanged. All, like the parent, had ε-*N*-methyl lysine in their flagellins[4]. However, antigens *iM*6 and *iM*9 differed from wild-type *i* and the other mutant forms by their reaction with non-specific reagents; bacteria with these flagellar antigens were agglutinated, to titres of 2–100, by sera from non-immunized rabbits and rabbits immunized with serologically unrelated *Salmonella*, and even by several anti-*i* sera completely absorbed with the antigen used for immunization. Suspensions of *iM*6 and *iM*9 flagella, but of no others, caused flocculation of Indian ink particles in 0·5 per cent sodium chloride. The method used for isolation of mutants would detect only those which lacked some serological specificity of wild-type *i*. As was to be expected the anti-*i* serum used to select mutants agglutinated the mutants to lower titres than it did wild-type *i*. Several other rabbit anti-*i* sera agglutinated all the mutants and the wild-type to about the same titre; but these sera after complete absorption with any one mutant still agglutinated wild-type *i*, in most cases to a substantial titre. This shows that, as expected, each mutant form of *i* fails to combine with one or more kinds of antibody molecule which combine with, and agglutinate, wild-type *i*; that is, that each mutant has lost some of the antigenic specificity of wild-type *i*. Anti-*i* (wild-type) sera fully adsorbed with any one mutant agglutinated some other mutants, as well as wild-type; sera from three different rabbits gave the same complex pattern of residual activity. These results, and those with one serum absorbed with all possible pairs of mutants, indicate the existence of at least thirteen sub-factors in antigen *i*, each mutant lacking a different sub-factor or combination of sub-factors.

Unabsorbed rabbit anti-mutant sera had equal titres (1,000–20,000) with the homologous mutant antigen, wild-type *i* and the rest of the mutant forms of *i*; after complete absorption with wild-type *i* each serum still agglutinated its homologous mutant antigen, to titres of 200–6,000. Thus all the mutants (except perhaps the non-specifically agglutinable *iM*6 and *iM*9) possess antigenic specificities or sub-factors absent in wild-type *i*. Antisera against *iM*7, *iM*10 and *iM*12, fully absorbed with wild-type *i*, agglutinated all three mutant antigens; their relationship as analysed by cross-absorption is:

*iM*7	a, b, c
*iM*10	a, b, d, e
*iM*12	a, d, f

the letters representing antigenic sub-factors absent from wild-type *i*.

To test for recombination between pairs of mutant *i* antigens we first obtained from each serological mutant one or more non-motile (because non-flagellated) variants resulting from mutation at *fla* sites closely linked to *H*1, the locus determining the phase 1 flagellar antigen[5,6]. A fla^- variant of one *i* mutant (recipient) treated with phage *P*22 grown on the fla^+ form of some other *i* mutant (donor) was inoculated on to semi-solid medium containing sera against the donor and against the recipient mutant *i* antigens, each fully absorbed with wild-type *i* antigen, and anti-1,2 serum, to immobilize bacteria expressing the phase 2 antigen; motile (fla^+) transductants with wild-type (that is, recombinant) *i* antigen, but no others, can spread in this medium. Antigen *iM*8 could not be tested because it was available only in *S. typhi*. No wild-type recombinants were found in crosses of *iM*9 with other mutants; it may be a double mutant. Wild-type *i* recombinants formed 1–30 per cent of the total of fla^+ transductants in crosses of *iM*6 with *iM*5, *iM*7, *iM*10, *iM*11 and *iM*12; and a very small proportion in crosses of *iM*10 with *iM*5, *iM*11 and *iM*12 and of *iM*5 with *iM*11. Thus the sites of the *iM*6, *iM*10, *iM*5 and *iM*11 mutations at least do not overlap. The order of the sites of mutation within the $H1^i$ gene and the linked fla^- sites was inferred from experiments of this sort, and others involving fla^- donors, on the premise that fla^+ transductants with *H*1 antigens requiring four cross-overs would be much less common than those requiring only two cross-overs. For example, with a $fla^-{}_D$ donor and a $fla^-{}_R$ recipient a high proportion of fla^+ transductants with the mutant *i* antigen of the donor excluded the order fla_R–fla_D–$H1$, which would necessitate four cross-overs; and with a fla^+ donor and fla^- recipient an abundance of fla^+ transductants with wild-type (that is, recombinant) *i* excluded the order *fla*—*D*–*R*–, where *D* and *R* are the sites of mutation within $H1^i$ of the donor and the recipient, respectively. The inferred order of the mapped sites is *fla* 50–*fla* 58–*fla* 57—*M*10–(*M*5, *M*12)–*M*11–*M*6—*fla* 52–*fla* 55. Iino[3] attributed the locomotor deficiency and short wave-length of the 'curly' phase 1 flagella of *S. typhimurium* strain *SW* 577 to mutation within the *H*1 locus; crosses of strain *SW* 577 with *fla*- forms of our serological mutants confirmed this, and indicated that the 'curly' site is probably between the *M*11 and the (*M*5, *M*12) sites.

Thus mutation within $H1^i$, the gene determining the structure of flagellin *i*, may cause a change in the serological character of antigen *i* (and in some mutants a non-specific agglutinability also) without impairment of locomotor function; or a loss of locomotor function without antigenic change[4]; and the sites of mutation can be mapped linearly within the gene. Some of our mutant *i* flagellins have now been shown to give tryptic-digest peptide maps different from that given by wild-type flagellin[7]; the amino-acid sequence in flagellin being unknown, it is not possible to compare the genetic map with the order of the corresponding amino-acid changes along the polypeptide chain.

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MISCELLANEOUS

Tsetse in the Environment of Ancient Man in Southern Rhodesia

SUMMERS[1] in Southern Rhodesia has examined the distribution of prehistoric sites (that is, containing traces of ancient man), and the historic routes followed by invaders and pioneers since 1823, and concluded that there was throughout the whole prehistoric and historic periods "a marked aversion to Mopane woodland" (that is, deciduous woodland dominated by *Colophormum mopane* and now occurring especially in the Zambezi and Limpopo valleys). He suggests that this aversion was because of the presence of the tsetse *Glossina morsitans* in *mopane* areas.

It is generally agreed (for example, Hornby[2]) that tsetse, before modern drugs came into use, kept out all kinds of domestic stock except poultry from their areas, and that consequently they excluded any men who could not or would not live without their stock. Historical examples of this exclusion embrace the alignment of the roads used by the ox-drawn wagons of European pioneers, as noted by Summers[1]. However, Summers's hypothesis requires that tsetse should exclude also Stone Age men who had not yet acquired any domestic animals. If tsetse had any effect on the distribution of such primitive men, it must have been wrought through the human disease, sleeping sickness, and of course Rhodesia is the type-locality of the organism, *Trypanosoma rhodesiense*, which causes the acute form of sleeping sickness. It should be noted, however, that sleeping sickness as we know it to-day does not exclude hunters and food gatherers. In the first place, although sleeping sickness cannot occur in the absence of tsetse, tsetse can occur in the absence of sleeping sickness; in fact sleeping sickness is very localized, and the areas in which it is found are but a small fraction of the areas occupied by its vectors. Secondly, even in places where sleeping sickness occurs, hunting and food gathering go on. A well-known instance is the Malagarasi River system in the Western Province of Tanganyika, which is almost uninhabited during the wet season, but is visited by hunters, fishermen and collectors of honey during the dry season. During 1953, 1954 and 1955 this area produced more than half the cases of *T. rhodesiense* sleeping sickness diagnosed in all Africa (Ashcroft[3]). It is, of course, possible that in the past *T. rhodesiense* sleeping sickness was a more widespread and more easily contracted disease than it is to-day, and that it totally excluded man in the same way that animal trypanosomiasis (nagana) totally excluded cattle from all tsetse areas in the recent past. But it is also possible that *T. rhodesiense* sleeping sickness is a modern disease which did not exist at all in prehistoric times. In the absence of any evidence either way it does not seen legitimate to postulate alterations in the properties of a trypanosome to explain archæological observations.

It is suggested that, while the alignment of the European pioneers' roads to avoid tsetse areas is a historical fact, an alternative explanation must be sought for the aversion to *mopane* areas of peoples not known to have domesticated animals.

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A Radioactive Marking Ink

WHEN autoradiography is used to detect labelled compounds on paper chromatograms, a radioactive ink is a useful adjunct. With this ink, a visible mark can be made on both the paper and the X-ray film in order to assist their re-alignment after the film has been developed. For radioactive components of such ink, non-volatile compounds of carbon-14 or sulphur-35 have been suggested, but suitable materials are expensive so that a cheaper alternative was sought.

A satisfactory ink has now been made incorporating promethium-147, which emits β-particles (0·27 MeV) and has a half-life of 2·6 years. I thank Dr. J. C. Charlton of the Radiochemical Centre, Amersham, for the suggestion that this inexpensive isotope might be useful.

The promethium-147 was supplied as the chloride 1 mc. in 0·25 ml. N hydrochloric acid. This solution was diluted with 1·5 ml. water and N ammonia solution was added to make it neutral to phenol red; it was then added dropwise to 56 ml. 'Mandarin' indian ink (Winsor and Newton) with thorough mixing after each addition. If the isotope is supplied in a larger volume of acid, or for other varieties of ink, it would be advisable to check first that the ink will tolerate the addition of the calculated amount of ammonium chloride.

Marks made with the ink mixture gave intense images on Ilford 'Ilfex' film after overnight contact. For use on chromatograms which required some weeks' contact to detect compounds of low activity, the mixture was further diluted 1 : 20 with indian ink.

The radioactivity in the ink marks on ordinary filter paper or on filter paper impregnated with silicic acid was not removed by repeated washing with water; it was, however, leached out by N hydrochloric acid.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 28

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, .C.2), at 2.30 p.m.—Discussion on "Electricity as a Measure of the Rising andard of Material Prosperity", opened by the President.

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burl-gton House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. Ju Chin Chu olytechnic Institute, Brooklyn): "The Role of the Chemical Engineer the Space Age".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING ROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—iscussion on the Government White Paper: "Industrial Training: Govern-ent Proposals".

UNIVERSITY OF LONDON (in the Chemistry Lecture Theatre, Birkbeck llege, Malet Street, London, W.C.1), at 6 p.m.—Prof. D. J. G. Ives: ome Reflections on Water"* (Inaugural Lecture).

Tuesday, January 29

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, ower Street, London, W.C.1), at 1.15 p.m.—Dr. H. Kalmus: "Axioms d Theorems in Biology".*

PLASTICS INSTITUTE, REINFORCED PLASTICS GROUP (at the Wellcome uilding, Euston Road, London, N.W.1), at 4 p.m.—Symposium on "Cost-g".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in mmittee Room 9, House of Commons, London, S.W.1), at 5.30 p.m.—r Frederick Brundrett, K.C.B., K.B.E., M.A., and Sir Owen Wansbrough-nes, K.B.E., C.B.: "Science and Government".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, ndon, S.W.7), at 5.30 p.m.—Sir Patrick Linstead, F.R.S.: "Ten Years fter—The Growth of the Imperial College 1953–63"* (Decennial Address).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical edicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—r. R. L. Turner: "Molecular Aspects of Clot Lysis".* (Sixth of twelve ctures on "The Scientific Basis of Medicine" organized by the British stgraduate Medical Federation.)

INSTITUTION OF ELECTRICAL ENGINEERS AND ROYAL AERONAUTICAL CIETY, LONDON JOINT GROUP (at the Royal Aeronautical Society, 4 amilton Place, London, W.1), at 6 p.m.—Discussion on "Connectors in rcraft".

SOCIETY OF INSTRUMENT TECHNOLOGY, SYSTEMS ENGINEERING AND UTOMATION SECTIONS (at Manson House, 26 Portland Place, London, .1), at 7 p.m.—Mr. D. A. Mackintosh: "The Application of Automation the Pulp and Paper Industry".

Wednesday, January 30

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, ndon, W.1), at 5 p.m.—"The Eruption of Kilauea 1959–60" (colour und film); Dr. P. E. Kent and Dr. R. Casey: "A Kimmeridge Sandstone North Lincolnshire".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy ace, London, W.C.2), at 5.30 p.m.—Mr. J. F. Eastham and Dr. E. R. ithwaite: "Pole-Change Motors using Phase-Mixing Techniques".

CHALLENGER SOCIETY (in the Rooms of the Linnean Society, Burlington ouse, Piccadilly, London, W.1), at 5.45 p.m.—Mr. B. D. Loncarevic: The First Results from the International Indian Ocean Expedition".

BRITISH INSTITUTION OF RADIO ENGINEERS, TELEVISION GROUP (at the ondon School of Hygiene and Tropical Medicine, Keppel Street, Gower reet, London, W.C.1), at 6 p.m.—Mr. F. J. D. Taylor: "Propagation of levision Signals by Satellite".

ROYAL SOCIETY OF ARTS (at John Adam Street, London, W.C.2), at p.m.—The Right Hon. Lord Robens, P.C.: "Coal and its Future" (Cad-an Memorial Lecture).

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Mr. J. Farradane: bstracting and Information Retrieval".*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with e SOUTH-WEST ESSEX COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, the South-West Essex College of Technology, Forest Road, London, .17), at 7 p.m.—Film Show.

Thursday, January 31

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, ower Street, London, W.C.1), at 1.15 p.m.—Mr. P. J. Syrett: "Photo-nthesis: the Basis of Life".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 0 p.m.—Sir Lindor Brown, F.R.S., Mr. H. M. Powell, F.R.S., Sir Gordon therland, F.R.S., Dr. H. W. Thompson, F.R.S., and Prof. C. H. Wadd-gton, F.R.S.: "Some Aspects of Science in China To-day".

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, ndon, W.C.1), at 5 p.m.—Dr. J. D. Judah (Wistar Institute, Philadelphia): The Biochemistry of Ion Transport".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at voy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Resistance twork Analogues as Aids to Solving the Laplace Equation".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION G.88 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on 'sychological Tests—Are They Any Use ?" opened by Mr. A. G. P. Elliott.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical dicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—. R. D. Keynes, F.R.S.: "The Nerve Impulse".* (Seventh of twelve tures on "The Scientific Basis of Medicine" organized by the British stgraduate Medical Federation.)

Thursday, January 31—Friday, February 1

CHALLENGER SOCIETY and REPRESENTATIVES FROM THE MARINE LABORATORIES (Development Commissioners' Scheme) (at the Zoological Society of London, Regent's Park, London, N.W.1), at 10 a.m. daily—Meetings on "Productivity" and "The Effects of Warm Water Effluents"; Dr. L. H. N. Cooper: "An Approach to Historical Oceanography"; Dr. J. Wickstead: "Food and Feeding in Pelagic Copepods".

Friday, February 1

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the SOCIETY OF DYERS AND COLOURISTS, at the Royal Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Mr. W. H. Cliffe: "An Historic Approach to Colour".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), from 6.30 p.m. to 10 p.m.—Conversazione.

Saturday, February 2

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. Robinson: "The Recreations of a Naturalist".*

Monday, February 4

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. Donald W. Tull: Presidential Address.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. L. F. Boyd: "Celestial Laboratories".*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. M. Stacey, F.R.S.: "Recent Investigations on Immuno-Polysaccharides".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned;

ASSISTANT EXPERIMENTAL OFFICER (male, with a pass degree, or its equivalent, in agriculture, botany or biology) AT THE WELSH PLANT BREEDING STATION, to assist in the classification, recording and maintenance of the Station's collection of plants introduced to this country—The Secretary, Welsh Plant Breeding Station (University College of Wales), Plas Gogerddan, near Aberystwyth (January 31).

SENIOR BIOCHEMIST (with a science degree, preferably with honours, or the A.R.I.C. and experience in clinical biochemistry or a closely related field) IN THE DEPARTMENT OF PATHOLOGICAL BIOCHEMISTRY, Glasgow Royal Infirmary—The Secretary, Board of Management for Glasgow Royal Infirmary, 84 Castle Street, Glasgow, C.4 (January 31).

HEAD (with a higher degree and substantial teaching and industrial experience) OF THE DEPARTMENT OF ELECTRICAL ENGINEERING (Grade IV), Enfield College of Technology, Enfield, Middlesex—Chief Education Officer (F.3), Middlesex County Council, 10 Great George Street, Westminster, London, S.W.1 (February 1).

PROFESSOR AND HEAD OF THE DEPARTMENT OF BIOCHEMISTRY, NUTRITION AND FOOD SCIENCE, University of Ghana—The Registrar, University of Ghana, P.O. Box 25, Legon, Accra; or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (February 4).

LECTURER IN THE HISTORY OF SCIENCE—The Registrar, The University, Leicester (February 7).

LECTURER (with competence in physiological psychology and/or experimental psychology) IN PSYCHOLOGY—The Registrar, The University, Leicester (February 7).

LECTURER and ASSISTANT LECTURER IN PURE or APPLIED MATHEMATICS; LECTURERS and ASSISTANT LECTURERS (with a special interest in teaching microbial cytology, microbial metabolism, or general biology of micro-organisms) IN THE MICROBIOLOGY DEPARTMENT; an ASSISTANT LECTURER IN PHYSICS; and an ASSISTANT LECTURER IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (February 8).

LECTURER (with a special interest in geochemistry) IN GEOLOGY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (February 8).

PROFESSOR AND HEAD OF THE DEPARTMENT OF PHYSICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 8).

PROFESSOR OF PSYCHOLOGY (to be Head of the new Department of Psychology)—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 8).

LECTURER or ASSISTANT LECTURER IN SOCIOLOGY (Social Anthropology)—The Secretary, The University, Northcote House, The Queen's Drive, Exeter (February 9).

ASSISTANT LECTURER (with special qualifications in applied mathematics) IN THE DEPARTMENT OF MATHEMATICS—The Assistant Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (February 11).

LECTURER or ASSISTANT LECTURER (with qualifications in either pure or applied mathematics) IN MATHEMATICS—The Registrar (Room 22, O.R.B.), The University, Reading (February 11).

BIOCHEMIST (with an M.Sc. or Ph.D. in biochemistry or physiological chemistry, or equivalent, and at least three years satisfactory experience of biochemistry or chemical pathology) with the Cork Health Authority—The Secretary, Local Appointments Commission, 43 Upper O'Connell Street, Dublin 1, Republic of Ireland (February 15).

CHAIR OF ZOOLOGY in the University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, February 15).

LECTURER (preferably with qualifications in statistics or numerical analysis) IN THE DEPARTMENT OF MATHEMATICS, University of Ghana—The Registrar, The University of Ghana, P.O. Box 25, Legon, Accra; or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (February 15).

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Sheffield (February 16).

OFFICIAL FELLOWS (2), one as TUTOR IN INORGANIC CHEMISTRY and the other as TUTOR IN BIOCHEMISTRY or PHYSIOLOGY—The College Secretary, Balliol College, Oxford (February 18).

LECTURER or ASSISTANT LECTURER (preferably with research interests in solid-state physics, particularly the Mossbauer effect, low temperatures and N.M.R.) IN PHYSICS—The Registrar, The University, Manchester 13 (February 20).

HEAD (with good academic qualifications and preferably industrial or research and teaching experience) OF THE DEPARTMENT OF PURE AND APPLIED PHYSICS—The Secretary, Royal College of Advanced Technology, Salford 5, Lancashire (February 22).

CHAIR OF ELECTRONICS—The Secretary and Registrar, The University, Southampton (February 23).

LECTURERS or ASSISTANT LECTURERS (2) IN PHYSICS—The Registrar, The University, Hull (February 25).

CHAIR OF VETERINARY MEDICINE tenable at the Royal Veterinary College —The Academic Registrar (University of London), Senate House, London, W.C.1 (February 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY (including Agricultural Chemistry), PHYSICS, PHARMACY, ENGINEERING, METALLURGY, and related subjects—The Registrar, The University, Nottingham (February 28).

CHAIR OF CHEMICAL PATHOLOGY at the Postgraduate Medical School of London—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 1).

LECTURER/ASSISTANT LECTURER (preferably with experience in teaching and research) IN THE DEPARTMENT OF BIOCHEMISTRY in the newly established Faculty of Medicine, University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Malaya, Pantai Valley, Kuala Lumpur, Federation of Malaya (London and Malaya, March 15).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY or PHYSICS—The Academic Registrar, University of London, Senate House, London. W.C.1 (April 1).

TURNER AND NEWALL RESEARCH FELLOWS IN ENGINEERING, INORGANIC CHEMISTRY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, COLLOID SCIENCE, BIOCHEMISTRY, ENGINEERING, METALLURGY, PHARMACOLOGY, CHEMOTHERAPY, or related subjects—The Secretary-General of the Faculties, The Old Schools, The University, Cambridge (April 30).

ASTROPHYSICIST (with a Ph.D. degree and some observatory experience), to take charge of a new 15-in. reflector with photoelectric and spectrographic attachments, to teach, and to undertake a research programme in close relation with an active radio astronomy group based in the Departments of Physics, Electrical Engineering, and Mathematics—Head of the Department of Physics, Queen's University, Kingston, Ontario, Canada.

BIOLOGIST, for cancer research involving tissue culture and electron microscopy—Prof. K. R. Hill, Pathology Department, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

LECTURER (with a good honours degree in mathematics and experience of teaching in schools) IN THE DEPARTMENT OF EDUCATION—The Registrar, The University, Liverpool, quoting Ref. No. CV/219.

NATIONAL RESEARCH COUNCIL and other POSTDOCTORAL FELLOWSHIPS IN THE DEPARTMENT OF PHYSICS—Prof. M. W. Johns, Chairman, Department of Physics, McMaster University, Hamilton, Ontario, Canada.

POSTDOCTORAL RESEARCH ASSISTANT, for work on nuclear magnetic double resonance techniques—Dr. D. W. Turner, Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

PRINCIPAL LECTURER (well qualified with sound teaching and research experience) IN CHEMISTRY—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

RESEARCH ASSISTANTS and GRADUATE TEACHING ASSISTANTS (students studying for degrees or Ph.D. and M.Sc. in Chemistry) at the Edmonton or Calgary Campus—Dr. H. B. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH PHYSICIST, to carry out a programme of absorption cross-section measurements in the vacuum ultra-violet—Prof. R. W. Ditchburn, F.R.S., J. J. Thomson Physical Laboratory, The University, Whiteknights Park, Reading.

SCIENTIFIC INFORMATION OFFICER (Abstractor) (with a degree in agriculture or natural science and able to translate from German and/or Russian) —The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

TECHNICIAN or JUNIOR TECHNICIAN (preferably experienced in bacteriological, immunological and serological techniques) IN THE DEPARTMENT OF ANIMAL HUSBANDRY AND VETERINARY HYGIENE—The Assistant Secretary, Royal Veterinary College (University of London), Boltons Park, Hawkshead Lane, Potters Bar, Middx.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Ciba (A.R.L.), Ltd. Technical Notes, No. 239 (November, 1962): Heavy Electrical Insulation. Pp. 8. (Duxford. Ciba (A.R.L.), Ltd., 1962.) [1012

The Royal Institute of Chemistry. Qualifications in Chemistry. (Information Leaflet.) Pp. 7. (London: The Royal Institute of Chemistry, 1962.) [1112

Ministry of Education. Managements Studies in Technical Colleges: First Report of the United Kingdom Advisory Council of Education for Management, 1962. Pp. viii+19. (London: H.M. Stationery Office, 1962.) 2s. net. [1112

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The Institute of Navigation. Traffic Regulation in the Dover Strait: The Report of a Working Group. Pp. 32. (London: The Institute of Navigation, 1962.) [1112

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Bulletin of the British Museum (Natural History). Geology. Vol. 7, No. 6: The English Cretaceous Turritellidae and Mathildidae (Gastropoda). By H. L. Abbass. Pp. 173–196+plates 28–32. 14s. Vol. 7, No. 7: The Morphology of *Tubicaulis africanus* sp. nov., a Fossil Fern from Tanganyika. By Henry Smith Holden and the late William N. Croft. Pp. 197–211+plates 33–36. 11s. (London: British Museum (Natural History). 1962.) [12

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Department of Scientific and Industrial Research: Building Resear Station. National Building Studies: Special Report, No. 32: Simplifi Tables of External Loads on Buried Pipelines. By N. W. B. Clarke. P iv+11. (London: H.M. Stationery Office, 1962.) 1s. 6d. net. [12

Trans-Antarctic Expedition, 1955–1958. Scientific Reports. No. Geology, 2. Fossil Floras of Antarctica. By Edna P. Plumstead. With Appendix on Antarctic Fossil Wood by Richard Kräusel. Pp. 154+ plates. 75s. net. No. 11: Geology 4: Geology of Victoria Land Between t Mawson and Mulock Glaciers, Antarctica. By B. M. Gunn and G. Warre Pp. 157+2 maps. 60s. net. (London: Trans-Antarctic Expedition Co mittee, 1962.) [12

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United States Department of the Interior: Geological Survey. Bullet 1085-D: Botanical Prospecting for Uranium on South Elk Ridge, San Ju County, Utah. By Frank J. Kleinhampl. Pp. v+105–188+plate 9. Bullet 1166-A: Geophysical Abstracts No. 188, January–March, 1962. By James Clarke, Dorothy Vitaliano, Virginia S. Neuschel and others. Pp. iii+15 40 cents. (Washington, D.C.: Government Printing Office, 1962.) [4

United States Department of the Interior: Geological Survey. Pr fessional Paper 374-I: Yampa Canyon in the Uinta Mountains, Colorad By Julian D. Sears. Pp. iii+33+1 plate. Professional Paper 450-C: Sho Papers in Geology and Hydrology, Articles 60–119 (Geological Surv Research, 1962). Pp. vi+146. (Washington, D.C.: Government Printi Office, 1962.) [4

United States Department of the Interior: Fish and Wildlife Servic Bureau of Sport Fisheries and Wildlife. Circular 140: Your Stake in We lands: What Wetlands Are—Their Importance to You—What's Happe ing to Them. Pp. 12. (Washington, D.C.: Government Printing Offic 1962.) 15 cents. [4

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Canada: Department of Mines and Technical Surveys. Geological Surv of Canada. Geological Maps—Preliminary Series. Map 10–1962: Millik Lake, Saskatchewan (Sheet 3). Map 30–1962: Radoile Lake, Manito (Sheet 64J). Map 31–1962: Kasmere Lake, Manitoba (Sheet 64N). M 33–1962: Lake George, Ontario (Sheet 41 K8). (Ottawa: Director, Ge logical Survey of Canada, 1962.) [10

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Annals of the New York Academy of Sciences. Vol. 98, Art. 4: Rhythm Functions in the Living System. By William Wolf and 56 other autho Pp. 753–1226. (New York: New York Academy of Sciences, 1962.) [10

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Canada: Department of Mines and Technical Surveys. Annual Repo for the calendar year 1961. Pp. vii+75+12 pages of photographs. (Ottaw Queen's Printer, 1962.) 1 dollar.]10

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THE BRITISH MUSEUM

THE British Museum Bill, which received its second reading in the House of Commons on December 20, is an important first step towards the projected National Reference Library for Science and Invention—that essential and overdue counterpart of the new National Lending Library for Science and Technology. It provides, in particular, means by which the transfer can be effected, in due course, of books and other objects to this projected Reference Library as well as to the new library building to be erected at Bloomsbury. As the chief secretary to the Treasury and Paymaster-General, Mr. J. Boyd-Carpenter, explained in moving the second reading, the British Museum is the oldest of Britain's major national cultural institutions, and its age alone has meant that its legal foundations are tied up in a great mass of largely archaic legislation. The Bill proposes to repeal *in toto* some sixteen statutes, two of which go back to the eighteenth century.

While this is one of the great libraries in the world, the capacity of which is vastly overtaxed, under the present law it is possible, apart from the newspaper repository at Colindale, to store this material only in the Bloomsbury building. Although erection of a new library building on a site adjoining the Museum has been proposed for some years, the present law would not permit the use of such a building for the books of the library. Fresh legal powers would first be necessary, as they are required to permit lending the contents of the Museum for exhibition or disposing of its property. Mr Boyd-Carpenter also referred to the need to revise the constitution of the trustee body and the desirability of giving a separate legal entity to the present British Museum (Natural History) at South Kensington with its out-station at Tring, which had, in effect, for many years been a separate institution.

Taking these four major matters in succession, Mr. Boyd-Carpenter said that, under the present law, the collections could only be housed for exhibition in "authorized repositories" as defined by statute. Under Clauses 3 and 9 of the Bill, it would be possible to designate further places, such as the proposed library building at Bloomsbury, and the National Reference Library for Science and Invention, to be built, as a part of the Museum, on the South Bank in buildings to be shared with the new Patent Office and including the existing Patent Office library. It is hoped that work on this will start in 1963 and that the Reference Library will function not later than 1966.

This is the aspect of the whole project with which scientists and technologists are probably most familiar. However, it received little attention in the debate, although Mr. W. R. Rees-Davies stressed the importance of completing as soon as possible the building of the National Reference Library, and Mr. Austin Albu dealt with this in greater detail. Mr. Albu thought that there now seemed to be general agreement that the plans produced for the new Reference Library under Sir Frank Francis were developing satisfactorily, but that there was some concern as to whether what was proposed would prove a satisfactory permanent arrangement. There was reason for strengthening the scientific and technological side of the trustees, and Mr. Albu thought that the Research Councils had an interest in this matter. He was not convinced that such a specialized library as the new Reference Library had its place in the British Museum, and there was still some anxiety over the separation of the current books and periodicals, to be situated on the South Bank, and the non-current books, which would be located at Bloomsbury. Quick reference to these was also frequently important, and, while Mr. Albu gave a qualified welcome to the Bill, it was not unreasonable that he should look for some assurance that the constitution of the Trustees was such as to ensure that the special interests of science and technology and the contribution which an efficient reference library of this character could make to industry were fully safeguarded.

That alone puts into perspective the importance of the proposals of the Bill with regard to Trustees. Mr. Boyd-Carpenter did not suggest that the present system was not functioning reasonably well, though he pointed out that this was due largely to the development of the non-statutory system of the Standing Committees which carried out most of the day-to-day control of the Museum. Nevertheless, the present body of fifty-one people, twenty-three *ex-officio*, was by modern standards too large, and it seemed highly desirable to put this body on a similar basis to the trustees of other major national institutions.

The British Museum Act, 1753, specified the Archbishop of Canterbury, the Lord Chancellor and the Speaker of the House of Commons as Principal Trustees. Others specified at the time included the Secretaries of State, of whom there were then only a few, the Lord Chief Justice and the Bishop of London. It has been inevitable that all these could not be present at all meetings concerning the Museums. In fact, the running of the two Museums has been in the hands of the Standing Committee of twenty, mostly Trustees elected with the view of such service, but including one previous *ex-officio* trustee, in Mr. Chuter Ede, in view of the interest he had taken as Home Secretary. It has been useful to have had on call in the Principal Trustees men whose wisdom and experience have been a great source of strength in difficulty, but one of the main disadvantages has derived from the fact that the Standing Committee has had to serve the two Museums.

This disadvantage will be removed by the formal separation of the two Museums, and a proposed new trustee body of twenty-five for the British Museum, Bloomsbury, fifteen appointed by the Prime Minister on the same basis as for other national institutions, will remove the basis for some superficial criticism. Four of the new Trustees will be appointed on the nominations of the Presidents of the Royal Society, the Royal Academy, the British Academy and the Society of Antiquaries of London, respectively, and five by the Trustees of the British Museum. Professional expertise is not sought in the Trustees but rather sympathy with the work, a wide knowledge of affairs and of Government administration, and the capacity to encourage the staff. The new Trustees will appoint their own chairman.

On the third point, Mr. Boyd-Carpenter said that the Bill would give the Trustees the power to lend abroad on the same basis as at home, and would regularize the lending power at home distributed over a number of statutes—subject to the interests of students visiting the Museum. He assured the House that the words of the proviso gave the Trustees sufficient protection against any pressure which the Government might exercise to make loans for purposes of broad Government policy which the Trustees might not wish to make. Related to this was the question of disposal covered by the following Clause 5 of the Bill. These powers were at present scattered in a number of statutes which it was proposed to repeal. Mr. Boyd-Carpenter thought that the powers now proposed represented a fair balance between reasonable restraint of reckless disposal of articles which subsequent Trustees might consider should be retained, and compelling a responsible body to retain objects which no sane person would wish to retain. Special provision was made in respect of unwanted books and periodicals so as to permit the advantages of microfilming being obtained, and tentatively the year 1850 had been taken as the line of demarcation.

The fourth point was the establishment, under Clause 7, of a separate body of Trustees for the present British Museum (Natural History). As already noted, this gives formal expression to a separation which has long existed in actual practice. Of the twelve Trustees for this Museum, eight will be appointed by the Prime Minister, one by the Treasury, on the nomination of the President of the Royal Society, and three by the Trustees of the British Museum (Natural History). Mr. Boyd-Carpenter thought that the Prime Minister would probably consult the Minister for Science in making his nominations.

Although from what Mr. Chuter Ede and Dr. Barnett Stross said in the debate it appears that the separation of the two Museums is regarded with anxiety in some quarters, from a practical point of view it is long overdue, and in spite of the surprising smoothness with which the previous arrangements have worked it should be possible to improve the effectiveness of the administration of both bodies without encouraging the division between the arts and the sciences to which Mr. Ede referred. It is clearly of advantage to remove the basis of some carping criticism, for the new arrangements for Trustees will permit the removal of some who were not even aware of their trusteeship and provide the opportunity for seeing that scientific interests, for example, are adequately represented on both bodies. It should be correspondingly easier to secure the services of men of the experience and judgment desired when it is known that the trusteeship is no sinecure but offers opportunities of genuine public service.

This is the more important, not only in respect of the new National Reference Library, if it is to render its full contribution to the service of science and technology, but also in respect of the service which the two Museums are to render to research. As Mr. J. Dugdale pointed out in the debate, the British Museum is both an international collection and a centre of learning and research. If the same institution is to serve both the scholar and the layman, wise judgment as well as skilful administration will be imperative, and this is equally true of the British Museum (Natural History). Dr. Barnett Stross was obviously somewhat concerned on this point and commented on the bearing which the hours of opening had on this question of the use of the Museum for research. Mr. Albu suggested that the British Museum (Natural History) now functioned to the extent of 90 per cent as a research institute for natural history and biology, in which fields it had an international reputation, and its staff included leaders in their special subjects.

On the question of staff, Clause 6 provides that the salaries and conditions of service of the director and other staff shall be determined by the Treasury, and in the debate Sir Cyril Black urged that the staff should be on a parity with the Scientific Civil Service in this respect. It was essential that the conditions of service should be adequate to retain the services of men who were leading authorities in their line of work. Mr. Ede also thought that no doubt should be left regarding the status of the Museum staff as Civil Servants.

The wide and enlightened interest taken in the functions of the two Museums was a most encouraging feature of the debate, and if those functions are to be satisfactorily discharged and the potentialities of the new National Reference Library realized the quality of the staff is of paramount importance. On this, the economic secretary to the Treasury, Mr. E. du Cann, who replied on the debate, said no more than that the staff had increased by 50 per cent in the past ten years, and it is to be hoped that all these points will be carefully watched as the Bill proceeds through the House of Commons and then through the House of Lords. If this first debate is any guide, there seems little reason to fear that the scrutiny in Parliament will be one-sided. There was no excessive preoccupation with the modernization of the display functions of the two Museums, and if their research functions received rather more attention than the contribution which a national reference library can make to technological research and industrial progress, the presence of the Minister for Science should ensure that the balance is redressed when the Bill reaches the House of Lords. The Advisory Council on Scientific Policy should make sure that he is adequately briefed on all the vital points.

It is indeed essential that the question of comprehensiveness should not be overlooked in this connexion, particularly in view of the location of the National Lending Library for Science and Technology outside London. That is a factor which needs to be weighed the more carefully in view of the proposed division of the contents of the Reference Library between the South Bank and Bloomsbury. It is only too easy under such conditions for careless or unenlightened administration, not to mention parsimony in finance, to put endless frustration in the way of the genuine scholar and investigator. There is no need to look beyond the Post Office to find endless examples of advances in technique and ease of communication repeatedly offset by human perversity.

How far, in fact, it is desirable to separate the library activities generally from those of the Museum proper may be for inquiry and consideration. In this connexion, however, it should be remembered that the present director of the British Museum, Bloomsbury, has shown himself well aware of the dynamic potentialities of the new Reference Library and imaginatively alive to what is required to realize such possibilities, as well as assuring that his staff would play a full part in the co-ordination of national library activities generally. He recognizes that no library can hope to be self-sufficient to-day and that effective co-operation is an essential condition of real economy of national resources. Equally, he recognizes that sufficient financial and material resources must be available to secure the buildings, equipment and staff of the quality required, and it is important that this factor should be impressed on the Government as cogently as possible in the forthcoming debates.

These and other requirements have been clearly set forth by Dr. R. S. Hutton in a paper contributed to a symposium in the *Journal of Documentation* for March 1961, and they will bear re-examination in the discussions on the new Bill. Useful as it may be to remove obsolete procedures and to free the British Museum, Bloomsbury, from restrictions of the past, including its close linking with the British Museum (Natural History), all this is only the first stage. It clears the way and gives the opportunity. Whether the opportunities are fully used depends on the vision and determination which are brought to bear, on the resources which the Government make available, and on the extent of the support and co-operation which is forthcoming from other libraries and institutions and from a wide range of scientists, technologists and scholars and their professional institutions.

ALFRED NOBEL

Nobel: The Man and His Prizes.

By H. Schuck, R. Sohlman, A. Osterling, G. Liljestrand, A. Westgren, M. Siegbahn, A. Schou and N. K. Stahle. Edited by the Nobel Foundation. Pp. x + 690. (Amsterdam and London: Elsevier Publishing Company, 1962.) 60*s*.

THIS huge volume, published under the auspices of the Nobel Foundation, gives an account of the life of Alfred Nobel and a full list of the prizewinners with comments on the achievements for which they received the award.

It is intended to issue within the next two years nine other volumes giving in full the Nobel Lectures in English up to 1961 with the address on the presentation of the awards and a biographical account of the prizewinner. Beginning with 1962, this full account of the awards will be given in annual year-books.

The story of the life of Alfred Nobel, based on all available documents, letters and information and impressions gained from his associates, is the most authentic account which has so far been written. It depicts a rather sensitive, lonely man of quite extraordinary ability and a high appreciation of the spiritual values in life. Though he had only a fragmentary education which ended when he was sixteen, he spoke and wrote fluently in English, French and German in addition to Russian and Swedish. His researches and inventions entitled him to the highest rank of scientists. He had also great financial ability. After losing his fortune through the fraudulence of his partners he built up another fortune which enabled him to establish the Nobel Foundation.

Probably the most interesting aspect of his life was his high idealism. Though he repudiated the Christian religion, which he thought had brought persecution and suffering to so many, and could not believe in a God who would inflict punishment for all eternity on suffering mortals, he was filled with awe and reverence for the mystery of life and a love for his fellow men, with an infinite compassion for unnecessary poverty and suffering.

He predicted that the science in which he was engaged would produce weapons of such destructive power that war would become impossible. He wrote: "When two army corps can annihilate each other in a second, all civilized nations, it is to be hoped, will recoil from war and discharge their troops". Modern nuclear and biological weapons have proved the truth of his prediction so far as the results in war are now concerned, but unfortunately civilized nations have not yet recoiled and agreed to settle their differences by peaceful means.

The rest of the book consists of the list of all the awards given since the Foundation was established in 1901 with an account of the achievements for which the awards were given. This interesting account affords a review of the great advance in science made since the beginning of the present century.

The Peace Prizes given for efforts made to realize Nobel's ideal of the end of war give interesting glimpses of international politics in the present century.

Such is the account of the life and achievements of a great idealist who devoted his life and his fortune to the promotion of human welfare. BOYD ORR

THE STUDY OF MAN

They Studied Man

By Abram Kardiner and Edward Preble. Pp. 287. (London: Secker and Warburg, Ltd., 1962.) 25*s*. net.

ONE of the authors of this book is a psychoanalyst who worked with Freud and has taught both anthropology and sociology; the other is an anthropologist in a school of psychology. The result is that this set of biographical essays comprises studies not only of the founder of psychoanalysis but also of six anthropologists: Tylor, Frazer, Boas, Malinowski, Kroeber and Ruth Benedict; a biologist, Charles Darwin; and two sociologists, Herbert Spencer and Durkheim. The essays combine useful biographical details with a certain amount of gossip and some shrewd observations on the scientific achievements of each of the subjects. Despite the rather arch titles of the chapters, for example, "Boas: Icy Enthusiasm"; "Freud: Chimney Sweeping", and the triviality of some of the material, each essay does help to throw some new light on the way in which the man concerned did his work.

The idea of the book is not to give a history of anthropology but to relate the "seminal hypotheses" of the "few great innovators" in the development of the science of man to the events of their lives and the spirit of the times. The main theme throughout the book is human adaptation, and the birth and growth of the scientific tradition of the study of human culture is presented within this framework. The hypotheses, theories and techniques of the scientists examined are represented as being essentially responses to the collective interests and needs of their time. Idiosyncracy and genius are not denied, but are treated as being on the individual level, not on the cultural level.

The book is an interesting argument for the importance of the study of the impact of institutions and cultural change on the human mind in modern times, and for the need for the development of techniques for measuring the effect of these changes on man. From this point of view it is an attempt to bring psychology and anthropology more closely together. But the final chapters on the significance of psycho-dynamics for the investigation of culture are not very convincing. The attempt to illustrate the usefulness of psycho-dynamics by explaining how scientific interest in man and culture "happened to develop in the nineteenth century" is not much more than a rather weak piece of social history.

R. FIRTH

HUSSERL'S PSYCHOLOGY

Phänomenologische Psychologie

Vorlesungen Sommersemester 1925. Von Edmund Husserl. Herausgegeben von Walter Biemel. (Husserliana, Band 9.) Pp. xxviii + 650. (The Hague: Martinus Nijhoff, 1962.) 31 guilders, paper; 37 guilders, cloth.

THE appearance of Volume 9 of *Husserliana* under the editorship of Dr. Walter Biemel is an occasion of special significance. He is now laying down his task after some fifteen years of close association with the

Archiv at Louvain. Besides the present assemblage of papers, he has been responsible for Volumes 2, 3 and 6. Therefore, now may be the appropriate moment to offer him an expression of gratitude on behalf of those who are scholars of Husserl, both for shouldering a heavy burden, and no less for achieving such a high level of meticulous accuracy and comprehensiveness in the treatment of his most difficult material.

The collection now before us comprises Husserl's lectures during the summer of 1925 on phenomenological psychology, together with some very important supplementary material, namely, his article in the *Encyclopædia Britannica*, (fourteenth edition, 1929, and subsequent issues), and the course he gave in Amsterdam on the same subject. In addition, there are a number of appendixes and comments on the text. It is characteristic of the editor's care that whenever it has been desirable to modify Husserl's original titles, the fact is noted in special < > brackets.

In general, these pages can be taken to cover what is essentially the whole of the master's thought up to that period on psychological and transcendental themes within the general conspectus of phenomenology.

Much of Husserl's labour consisted in an attempt (and in his view a triumphant one) to overcome the evils of psychologism, that is, the intrusion of psychological elements into what should be purely logical processes. He sees the methods of natural science and of psychology as quite distinct, except that both stand in need of some rationalization of the essential, if they are to become exact. Furthermore, it is clear that, on such a basis, phenomenological psychology and transcendental phenomenology together should improve the prospect that some day the ideal of Leibniz for an all-embracing science may materialize. In recent years, the rapid development of symbolic logic points to its possible conquest eventually of ethics and æsthetics. Even if this should prove to be the case, it would not invalidate Husserl's inclusion of metaphysics within his circle, nor yet of at least an element of teleology which seems to be inherent in some aspects of contemporary biological theory.

The importance of all this for present-day science lies perhaps in Husserl's profound grasp of the subjective. It may be that natural philosophy, in the widest sense, is moving outwards a little from its purely impersonal position into one in which mankind becomes more closely interwoven with the object of contemplation.

Husserl does not claim that adequate, apodeictic, fundamental knowledge follows of necessity from the use of the phenomenological method, but only that it can do so if the 'bracketing' or reduction—that is, suspension of judgment—and observation have been made with sufficient care. The divergent streams—historically speaking, transcendental for Descartes, psychological for Locke, Berkeley and Hume—are brought together again in phenomenology as the true successor of Platonic concepts and of all that is of the *esse* of Greek philosophy.

F. I. G. RAWLINS

THE SCIENCE OF PREHISTORY

The Idea of Prehistory

(The New Thinker's Library.) By Dr. Glyn Daniel. Pp. viii + 171 + 8 plates. (London: C. A. Watts and Co., Ltd., 1962.) 15*s*.

THE eight lectures here published were delivered in 1956 as the second Josiah Mason Lectures in Archæology in the University of Birmingham. The material of prehistory is defined by the author as "the unwritten remains of the early past of man". It thus covers all those ages of human history which precede the literate cultures of the ancient world, as well as the non-literate cultures which continued to subsist alongside the peoples which possessed written records. The predominant role of archæology in producing the material of prehistory is well brought out, though archæology itself is correctly defined as covering a wider field extending right down to the recent past.

Prehistory is a newcomer in the learned world. The term itself was first used in 1851. Dr. Daniel records its gradual development, showing how the study drew on the older activities of the collectors and the dilettante, how it profited from the triumph of stratigraphical geology and how it was influenced by the evolutionary concepts of the nineteenth century. Finally, he sets out the most recent stage that lies within our own memory, when the study became firmly based on the scientific method, on the abundant material provided by archæology and on contacts with the natural sciences. Much of the material in the four lectures devoted to this development is well known; but the lucid and logical presentation gives it a new force and vigour.

The next three chapters discuss the relations, not always happy, of prehistory with other branches of study. Here the author is often treading on delicate ground. His account of the diffusionist theory and his unequivocal condemnation of the extravagances of the hyper-diffusionist heresy of the recent past is a model of balanced analysis and judgment. His condemnation of the racist uses of prehistory will find general assent. The discussion of the relations between prehistory and ethnographic and sociological studies is perhaps unduly pessimistic. The summary of the Russian approach (p. 120) was written before the availability in English of Mongait's survey, and does not represent the present position. On the other hand, his desire to see a closer integration of prehistory into the general historical perspective is most welcome. History in the narrower sense (that is, written history) has much to gain from a wider use of archæology; prehistory, which depends so largely on archæological material, should provide the methodological basis for the association.

The final chapter, "Prehistory and the Public", sits rather loosely on what has gone before. It shows clearly that public interest in early history and prehistory is no modern development and offers certain explanations of the popular interest in the subject. Dr. Daniel concludes that the "real justification of prehistory is not in origins or techniques or in perspective, but in pleasure; the pleasure of prehistory is in the recovery of the treasures of man's prehistoric past that have been dropped on the way and lost".

C. A. RALEGH RADFORD

PROGRESS AND PROPHECY IN SPACE

Advances in Space Science and Technology

Vol. 4. Edited by Frederick I. Ordway III. Pp. xiv + 431. (New York and London: Academic Press, Inc., Publishers, 1962.) 100*s*.

THIS fourth volume follows the same pattern as its predecessors: six separate topics are reviewed in articles which average seventy pages each in length. In the first contribution, J. Mass and E. Vassy deal with the Doppler effect of artificial satellites. Starting from first principles, they show the manner in which the frequency of the received signal changes as a transmitting satellite passes by, and how this variation can be analysed for the purposes of orbital determination, navigation and scientific studies of the ionosphere. R. A. MacGowan is the author of the second contribution, a brilliant though inevitably somewhat speculative one on the possibilities of extra-terrestrial intelligence: he reviews the many theories of the origin of planetary systems (concluding that such systems are numerous) and discusses the origin of life; he tries to define the process of thinking, describes how 'thinking automata' might come to dominate the Earth and points out that such forms of 'life' would be the most likely emissaries from superior communities.

The third contribution, on multiple staging in carrier rockets, by H. E. Nylander and F. W. Hopper, is a competent and yet somewhat unsatisfactory survey, not because of any fault on the part of the authors but rather because of the subject, which although omnipresent in space technology is difficult to treat as a separate abstract topic. In the fourth article M. Tucker presents a thorough review of spacecraft entry and landing in planetary atmospheres. He lays most emphasis on re-entry into the Earth's atmosphere, but also discusses landings on Mars, Venus and Jupiter. Next, S. J. Gerathewohl gives an excellent account, running to more than a hundred pages, of the development of manned artificial satellites and space stations. But he evades the brutal truth that, if the space stations he describes so fully are outside the Earth's atmosphere, their crews are likely to be killed when bursts of high-energy protons are emitted from the Sun at times of major solar disturbances: he merely remarks that adequate radiation protection must be provided, without saying how. Finally, J. J. Barré provides a very detailed account of the possible role of radioactive elements as sources of energy for spacecraft propulsion, with copious data on the properties of the elements in question.

These admirable individual contributions have been well edited and printed; together, this volume and its three predecessors serve as a valuable, though as yet incomplete, encyclopædia of space science and technology.

D. G. KING-HELE

RADIOACTIVITY AND MAN'S ENVIRONMENT

Living with the Atom

By Prof. Ritchie Calder. Pp. ix + 275 + 16 plates. (Chicago and London: University of Chicago Press, 1962.) 5.95 dollars; 42*s*.

THIS book reports on two colloquia organized by the Section of Nuclear Medicine of the Pharmacology Department of the University of Chicago in 1960. They dealt with radioisotopes in man's environment, and with public health and the problem of disposal of radioactive waste. Both discussed the present state of knowledge and uncertainty about the hazards and benefits attending the use of radioactive materials, and also how this knowledge can be translated into public information.

The author gives his own and other people's contributions to a discussion on responsible reporting in Chapter 2. It is difficult for the reporter to steer a course between the attractive liveliness that approaches the sensational, and the dry factual report that few will read; and it seems that the value of good science articles is slowly breaking in on proprietors. He feels that there is a fear and distrust of scientists, as people who ought to be above human fallibility but unforgivably err like everyone else; and he points out the dangers also of the responsibility for major decisions resting in the hands of men who do not know sufficient about science to be able to challenge with any confidence the advice that comes to them from their experts. There is something in this, although one would feel that a knowledge of men and a flair for consulting the right experts is what brings men to high office. (What keeps them there, however, is often a shrewd calculation of the limitations of the populace; and, in an ideal democracy, every voter would as a duty be reasonably knowledgeable on every issue.) It is true, certainly, that there is less understanding of scientific matters than there is of really difficult subjects like economics or bringing up children; and that this is largely due to communications.

The book itself, a really excellent piece of reporting, illustrates the problems that the journalist has to face. It describes the uses of radioisotopes, and also their effect in changing the whole of man's environment. The accounts of the ways of disposing of radioactive wastes—burying, running out to sea, hopefully incorporating them in glasses, faithfully moving mountains by extruding acres of cement into underground faults, and ruefully counting the cost of using outer space as a dustbin—between them offer so much information that I found them quite exacting reading. A layman could be pardoned for wondering whether already there are curies at the bottom of his garden. Which method is the safest as regards long-term interference with the environment? What is the most practicable now? Which is the most economical? These are the questions that everyone ought to know enough at least to ask. The book includes accounts of the hazards of uranium mining, and of life in a nuclear submarine. It should serve to spread the author's sound common-sense attitude towards the problems of nuclear radiation, as well as the facts brought out in the colloquia.

G. R. NOAKES

PROGRESS IN OCEANOGRAPHY

The Sea

Ideas and Observations on Progress in the Study of the Seas. General Editor: M. N. Hill. Vol. 1: Physical Oceanography. Pp. xv + 864. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1962.) 188*s*.

THIS is the first of three volumes reviewing the achievements of the past twenty years in understanding the physical, chemical and geological processes in and below the oceans. The first volume, covering the major fields of physical oceanography, illustrates the vast expansion which has taken place in these subjects since the publication of *The Oceans* by Sverdrup, Johnson and Fleming in 1942.

Articles by thirty-three contributors cover the physical properties and equations of motion of sea-water, small-scale and large-scale interactions between the sea and the atmosphere, dynamics of currents, transmission of sound, light and other electromagnetic radiation, various types of wave motion, and turbulence. Wave motions are reviewed in articles on ripples, statistics and dynamics of wind waves, microseisms, internal waves, long waves, tides, surges and long-term variations in sea-level.

The editors are to be commended on achieving a satisfactory balance between the many rival claims on the space available. In spite of this wide coverage there remain some topics that could usefully have been expanded such as recent work on current systems, wind-wave generation, coastal processes and ship-wave interactions. It must be remembered that significant progress has been made in some of these fields, such as non-linear interactions, since the time of writing (December 1959–October 1960).

Some contributors succeed in providing particularly timely and valuable reviews of their subjects. Among these must be mentioned Groen and Groves on "Surges", Hansen on "Tides", including recent storm surge computations for the North Sea, Lafond and Cox on "Internal Waves", reviewing the latest measuring techniques such as the isotherm follower and providing much new information on internal waves and their correlation with surface slicks.

The most notable contribution (and longest, 214 pages) is the review of large-scale air–sea interactions by Joanne

Malkus which provides a detailed and coherent picture of the present position in this field. Interaction problems are classified into: (1) formally tractable dynamical problems such as wind-driven ocean currents: (2) conservation studies of sources and transport of energy and solutes, for particular ocean basins, for example; (3) statistical analysis of observational data; (4) descriptive studies relying largely on physical analogues. Many recent investigations of the heat and water exchange between the atmosphere and oceans are discussed and related to climatological and general circulation studies.

Of particular interest to all workers in geophysical subjects will be the stimulating and critical concluding remarks by Dr. Malkus concerning the direction which our future effort must take. In particular, attention is directed to the necessity for "growth in the fundamental physics of turbulent, heat-driven fluids" and the need to pose questions which "... isolate tractable features of the complexity which can be treated under relatively controlled conditions". The scientific and economic background to all geophysical investigations, including oceanography, depends on the answer to her final question "... concerning *what* do we need more and better data the most?". It would have been most refreshing if more contributors had taken advantage of the sub-title of the book to take such a critical look at their branches of the subject.

The reader is certain to be impressed by the growing stature of physical oceanography as a scientific discipline. Although basically an observational science, the growing numbers of theoreticians and experimenters which it has attracted are responsible for many new and valuable fields of investigation. The editors and publishers must be congratulated on this worthwhile enterprise; the remaining volumes will be eagerly awaited. J. N. HUNT

MATHEMATICAL ANALYSIS OF TRANSIENT CIRCUITS

Transient Circuit Analysis

By Prof. Y. H. Ku. (The Van Nostrand Series in Electronics and Communications.) Pp. xiv+441. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 79s.

THE importance of circuit analysis is reflected in the great number of books on this subject, especially those emanating from the United States. It is of some interest to compare the present book with another book on the same subject, also by an American professor, written about a decade ago.

Both books cover roughly the same ground, including Maxwell's and Kirchhoff's rules, Fourier series, integral and transform, Laplace and Heaviside transforms, complex variable and inverse transform, Bessel, gamma and error functions. There is also in both books a fairly full treatment of the transmission line.

The great difference between the two books lies in the authors' approach to the subject. In the earlier book the author is concerned to demonstrate to electrical engineers the importance of mastering advanced mathematical techniques in order to solve engineering problems. The book is well supplied with practical problems, the initial approach is more elementary and some of the more difficult mathematical proofs are omitted, the reader being given references to mathematical works. In the present book the author's approach is frankly mathematical, and he clearly has supreme confidence in the mathematical outlook and training of the modern electrical engineer. In an introductory chapter the author appears concerned to show that engineering problems do exist to which the mathematical techniques may be applied. Thereafter the work is mainly mathematical and one almost expects an asterisk to the first chapter with a footnote saying that this chapter may be omitted on a first reading. References are given throughout the book to other works, but the author has aimed at making his book as self-contained as possible and has included in appendixes reprints from original papers.

The symbols used are generally well chosen, but the British Standards Institution might be somewhat distressed at the apparent dimensional error in the equation:

$$L\frac{di}{dt} + R_0 i^2 + S\int i\,dt = e$$

The author states not very far away that $(R_0 i)$ is a resistance, but his demonstration of a non-linear equation could have been made without this unsatisfactory symbolism by writing the equation as:

$$L\frac{di}{dt} + \left(\frac{R_0}{I}\right) i^2 + S\int i\,dt = e$$

where I is a constant, having the dimensions of a current.

The author's treatment of the solution of differential equations is satisfying both in its adequacy and freedom from unnecessary complications. The inclusion of methods of solving cubic, quartic and quintic equations is an attractive feature.

The square wave is in the forefront of the Fourier analysis of periodic waves, but although the author gives the Dirichlet conditions for satisfactory analysis, he makes no reference to Gibbs's spike and presumably considers that since the spike does not affect the standard deviation it can safely be ignored. This possibly illustrates a difference between the mathematical engineer of to-day and the more practical-minded engineer of yesterday, but it seems regrettable that a defect in Fourier analysis, exposed more than sixty years ago, should be in danger of being forgotten by our present-day mathematicians. Gibbs's spike can, of course, be eliminated quite easily by changing the shape of the periodic wave from a square wave, which cannot be realized in practice, to any approximation to a square wave which has no discontinuity.

This minor criticism apart, the book is an excellent text-book on a difficult subject. It is intended for graduates and would be too advanced for most undergraduates.

A. H. M. ARNOLD

SCIENCE FOR THE MODERN WORLD

Chemistry, Physics and Biology for the Modern World

Chemistry. By Prof. George Porter. Pp. ix+116. Physics for the Modern World. By Prof. E. N. da C. Andrade. Pp. viii+100. Biology for the Modern World. By Prof. C. H. Waddington. Pp. ix+120. (Science for the Modern World Series, Vols. 1, 2 and 3.) (London: George G. Harrap and Co., Ltd., 1962.) 4s. 6d. each volume.

THERE are severe problems in writing a chemistry, physics or biology book which in the span of some hundred pages must cover the basic theories, techniques and technologies of the subject. Accepting the necessity to solve the problem, these three books are regarded as a fine attempt.

Their presentation is attractive, their English is direct, their diagrams clear and useful, and they are served by a very full index.

The chemistry book deals with atomic theory, the characteristics of common elements, their atomic structure and the shape of the molecules they form. The kinetic theory is introduced and used for explaining the states of matter. A number of important chemicals are then interpreted according to this theoretical background.

The physics book includes information on Newton's laws of motion, the absolute zero, the basic ideas of wave motion and the passage of electricity through matter. It also includes an interesting chapter on atomic structure which reviews the progress made and indicates the possibilities and the danger arising from the use of atomic energy.

The biology book progresses from the structure of cells and the functions of their parts to their association to form tissues and organs. It considers the integration of organs in an organism and then of organisms in societies and communities; finally, binding the whole sweep of living activity in the chains of time to arrive at the theory of evolution.

A reader coming to the subject for the first time will probably find the degree of compression in these books far too much for him. Ideas flow too quickly, one after another, and there is not sufficient space given to descriptions or diagrams which might illustrate them. They might well stimulate many towards further reading in the subjects, but the short book lists for physics and chemistry do not seem to be appropriate. Some of the recommendations are formal text-books and demand of their readers some mathematical ability and some laboratory experience.

The Series presents good reading for those with some experience of the subjects. They are not 'bread and butter' books, but should be regarded as readers and have a place in the library. A potential sixth-former might read them to real advantage, whatever his intended specialization, for he will gain from them not only a welter of information but also an overall picture of the subjects. Each member of the Series is complete in itself, and may be read without reference to the others.

PROTEIN CHEMISTRY

Advances in Protein Chemistry

Vol. 15. Edited by C. B. Anfinsen, Jr., M. L. Anson, Kenneth Bailey and John T. Edsall. Pp. x + 447. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 13 dollars; 93s.

FOR many biologists and chemists, *Advances in Protein Chemistry* is an institution rather than a phenomenon, but this volume may interest a wider audience. That the authority, originality and editing of the reviews are first-class goes without saying; but the subject-matter represents, I suspect, a conscious effort by the editors to look away from the present busy centre of protein research back to the origins and outward to the periphery where protein chemists are illuminating many ancient biological problems.

Thus the late Kurt Felix, who himself has spanned almost the whole history of protein chemistry, opens this volume with his final survey of protamines. This recalls the empirical cookery which began the science (and from which we can still learn so much), but jumps to the frontiers of fashion when discussing combination of protamines with nuclear deoxyribonucleic acid.

The same approach is reflected in two other chapters in this book. Czok and Bucher, in their review of crystalline enzymes from rabbit muscle myogen, take us back to the original source of many of the purified enzymes which are at present providing useful employment for enzymologists and protein chemists. Even biophysics has its neglected corners: D. W. Kupke quotes Linderstrøm-Lang as saying, "Osmotic pressure has simply become unfashionable", but manages to persuade us that a little digging into these particular archives would be very profitable.

Two other articles indicate that the editors are determined to expand the horizons of protein chemistry. How do metabolites go in and out of cells? Halvor Christensen asks all the right questions, refuses to supply any glib answers, but suggests that protein chemists could provide some useful facts.

Finally, one chapter threatens to bring us out of our laboratories altogether. A scholarly article by Waterlow, Cravioto and Stephens dispassionately discusses human protein malnutrition; but we are left feeling that this is one field of research which ought rapidly to be abolished for lack of starting material.

BRIAN S. HARTLEY

ENERGY METABOLISM IN THE RUMINANT

The Energy Metabolism of Ruminants

By K. L. Blaxter. Pp. 329 (4 plates). (London: Hutchinson and Co., Ltd., 1962.) 63s. net.

THE revival of interest in calorimetric investigations with farm ruminants owes much to the work done by Dr. K. L. Blaxter at the Hannah Dairy Research Institute. Since 1950, he and his colleagues—notably Dr. D. G. Armstrong and Dr. N. McC. Graham—have designed respiration chambers for calves, sheep and goats, and more recently mature cattle, and used them for investigations of energy metabolism with notable success. Outstanding have been the measurements of the efficiencies with which the lower fatty acids, produced in quantity in the fore-stomach of the ruminant, are used in maintaining body processes and producing body fat.

In the winter of 1960, Dr. Blaxter gave the Clive Behrens lectures in the University of Leeds in which he discussed the energy metabolism of ruminants in the light of recent knowledge, particularly in relation to the formulation of rations to meet the energy needs of domestic ruminants. The lectures have now been expanded to make a book which is undoubtedly a classic in its own sphere, as in that of animal nutrition in general.

The book is in four sections: the theory and technique of direct and indirect measurements of heat production; the expenditure of energy by ruminants, as affected by level of feeding, activity, environment and function; the utilization of the energy of the end-products of the digestion of food; and the application of experimental findings in the development of feeding systems. The treatment of subjects is comprehensive and in general of a high standard. One might mention in particular the description of the apparatus and technique of animal calorimetry; or the account of the utilization of the energy of the end-products of digestion of food for maintenance and in the processes of growth and fattening. The short account of energy metabolism in relation to lactation, however, is by comparison superficial, and in part misleading. On occasion, especially in the chapters on the effect of environment on heat loss, unnecessary and often tedious detail has been included: this could well discourage the reading of the book in its entirety, an unfortunate consequence since the author's logical development of his subject gives an excellent perspective.

The last section of the book, however, can be recommended without reservation. It includes a brilliant appraisal of present-day systems of evaluating foods and feed requirements, and of their historical development, written in a most stimulating manner. In the final chapter the framework of a new feeding system is described. It is based on the metabolizable energy of foods determined at the maintenance-level of nutrition, and in the calculation of feed requirements adjustments are made according to the type of ration and the function of the animal. This represents an important departure from existing systems which attribute to individual foods specific values for a particular function and assume that such values are additive and that the same relative values apply to all functions. The new scheme has many merits and is worthy of careful consideration.

The book is well produced, with very clear, but occasionally slightly imperfect, figures. It will provide useful reading for a wide audience, from the progressive farmer who wishes to be well informed to the research worker in animal physiology. It is, of course, also an excellent book for students.

J. A. F. ROOK

SELECTIVELY INDUCED CALCIFICATION

Calciphylaxis

By Dr. Hans Selye. Pp. xxxi + 552 + 13 plates. (Chicago and London: University of Chicago Press, 1962.) 25 dollars; 189*s*.

It is the opinion of Dr. Selye that the mammalian body contains a defensive or phylactic system which enables the body to produce calcareous deposits at localized sites. By this means the effects of a pathogen can be reduced and local inflammatory responses initiated. This idea forms the basis for the title of this book and for the long chapter (120 pages) devoted to a catalogue of the abnormal mineralizations known to medical science. The implication is that as with other defence systems calciphylaxis may become pathogenic in certain circumstances. It is important to realize, however, that there is no proof that any disease in man depends on calciphylaxis. Dr. Selye has reached his conclusions on the basis of some very exciting experiments and the phenomenon of calciphylaxis is best described by considering one of these.

A 100-g rat was given an oral dose of 1 mg of dihydrotachysterol. The following day 25 μg of ferric chloride were injected beneath the skin. A local deposit of calcium salts occurred during the next 2–3 days at the site of the injection. A different type of effect could be produced if ferric oxide saccharate was injected instead of ferric chloride. In this case it was found that after 2–3 days the heart, bile duct, duodenum and renal cortex were mineralized. Similarly, injecting egg albumen intravenously led to the calcification of the pancreas, while egg yolk produced a mineralization of the spleen and part of the liver.

Dr. Selye rationalizes these phenomena by talking first about the sensitizing agent. This is usually dihydrotachysterol although other substances that act like vitamin D or parathyroid hormones have the same effect. These substances normally mobilize calcium in the body, but there is no information as to what effects these very large doses have in these experiments except the idea that they sensitize the body. The second feature in these experiments is the critical time of 24–48 h between the two injections. Without this critical time-lapse the calciphylactic response is not usually obtained. This critical time is much greater than is necessary for the substances administered to produce their usual responses. Finally, there is the injection of the second substance, called the challenger or vital mordant. This is thought to act by increasing the ability of the target organ to calcify, although it does not appear to be responsible for the direct precipitation of calcium salts.

By developing these concepts Dr. Selye provides himself with a systematic basis for the discussion of the phenomenon of calciphylaxis. What is needed most in this investigation, however, is a background of biochemical data for at the moment the phenomenon is completely mysterious. This absence of analytical data also extends to other parts of the investigation. In a book of this length one is surely entitled to know which calcium salts are being deposited, but again this information is virtually absent. This complete absence of chemical data suggests that this book may be slightly premature, but this will be justified if it fulfils the wish of Dr. Selye that it attracts many more scientists to investigate this most interesting phenomenon and to use this powerful experimental tool.

K. SIMKISS

GEMSTONES

Gems

Their Sources, Descriptions and Identification. By Robert Webster. Vol. 1: Pp. xii + 1–464 + plates 1–17. Vol. 2: Pp. vii + 465–792 + plates 18–20. (London: Butterworth and Co. (Publishers), Ltd., 1962.) 190*s*. the complete set of two volumes.

The two volumes of this work form a comprehensive account covering the history, descriptive mineralogy and technology of gemstones (both natural and synthetic), and the special techniques which have been developed, especially since the Second World War, to test them.

The first volume begins with a short chapter on the geological origins of gemstones and the methods used for their mining and recovery. The main descriptive chapters follow, with diamond, ruby and sapphire, and emerald treated first, and other well-known gemstones next. A long chapter of nearly sixty pages, arranged alphabetically, then follows in which almost every mineral ever fashioned as a gemstone is described. Synthetic and various types of imitation gems are then discussed, and a section on the cutting and polishing of gemstones is inserted before a concluding chapter on organic gem materials. In this first volume the emphasis is largely on the history, distribution and mode of occurrence of gemstones; but adequate space is devoted to crystal form and chemical composition, and inclusions are given generous treatment. Very full descriptions are given of the absorption spectra and luminescent characters of the various species under X-rays and ultra-violet light. A most useful feature in this volume, of considerable assistance to the practising gemmologist, is the gathering together of all the descriptive and diagnostic characters for the individual species in the one chapter; in this respect the book differs from many other texts on the subject, which describe the various physical properties of gemstones in separate chapters.

The second volume deals with crystallography of gemstones and the determination of their physical properties. In describing the instruments and techniques used the approach is essentially practical, and there is a wealth of experimental detail. The theoretical background is by no means neglected, however, and explanations are usually developed along historical lines and from first principles. A number of useful identification tables (showing refractive indices/specific gravities, hardness, dispersion, luminescent characters/absorption spectra and other properties) and a glossary of alternative gem names, misnomers and trade names conclude the second volume.

A few minor errors were noticed in the text, and should be eliminated in any future edition. On p. 716 the heading of Table 2 should read "Colour Dispersions of Gemstones" and not "dispensions". Initial capitals are omitted from the names of the geological systems on several occasions and also from the generic names of fossils.

These volumes will form a most useful work of reference for practising gemmologists and for others seeking information on gemstones and gem testing. They are well produced on good quality paper, and a notable feature is the abundance of up-to-date black-and-white photographs, although some of the colour plates are of indifferent quality for modern colour printing—a disappointing feature in such an expensive work.

E. A. JOBBINS

John Anderson, Viscount Waverley

By Sir John W. Wheeler-Bennett. Pp. xv+430+16 plates. (London: Macmillan & Co., Ltd.; New York: St. Martin's Press, Inc., 1962.) 45s. net.

FOR most scientists Viscount Waverley's achievements, and more especially his contributions to science, have been so admirably summarized by Lord Bridges in his biographical memoir for the Royal Society and its addendum by Sir Henry Dale that there is little for Sir John Wheeler-Bennett's volume to add. Nevertheless, it is of real interest, particularly at the present time, for its demonstration of the importance of the scientist being sometimes 'on top' and not merely 'on tap' and as illustrating the value of a scientific training and outlook in the higher levels of the Civil Service and in the Cabinet itself. Sir John Wheeler-Bennett gives full weight to this aspect of Lord Waverley's career as well as to his outstanding gifts as an administrator. This is particularly notable in respect of Lord Waverley's part in the Anglo-American relations in the field of nuclear fission, of which Lord Bridges has commented that it is very difficult to say how this all-important development could have been carried out so successfully under the stress of war had there not been a Minister of the Crown with his very special qualifications for the work. If the Americans were impressed by the fact that a British Minister and member of the War Cabinet knew enough about science to understand what the scientists were discussing, scientists in Britain were equally impressed, as Sir Henry Dale has testified, by the value of having in a position of authority such as the Lord President of the Council, a man with so natural an understanding of the meaning of science, and so instinctive a knowledge of, and sympathy for, the possibilities and the difficulties of research. Apart altogether from the general interest of the book it has on these two counts an assured place in the literature on the relations of Government and science, if not of administration generally.

R. BRIGHTMAN

Nuclear Reactor Fuel Elements

Metallurgy and Fabrication. Edited by Albert R. Kaufmann. (Prepared under the auspices of the Division of Technical Information, United States Atomic Energy Commission.) Pp. xii+739. (New York and London: Interscience Publishers, a division of John Wiley and Sons, Inc., 1962.) 203s.

CONTRIBUTIONS from twenty-five authors make up the present book, which covers a wide range of topics from reactor physics to the processing of irradiated fuel elements. The book provides much which should be of general interest to reactor designers and to metallurgists engaged in the field of nuclear energy. The original intention to describe something of the art of making fuel elements is fulfilled in only three of the 18 chapters of the book, namely 11–13, followed by a useful catalogue of testing and inspection methods. These chapters describe in detail the manufacture of uranium bar and strip and the fabrication of clad fuel elements and bundles. The treatment is necessarily empirical but authoritative, and should appeal to the fabrication specialist. The fabrication of ceramic fuels is only briefly described. Seven chapters of the book are in the form of excellent short introductory essays on subjects including reactor physics, fuel element problems, cladding materials, heat transfer, processing of irradiated fuel elements, fluid fuel systems and fuel element economics. The remaining chapters are probably too long and detailed to appeal to the beginner and too superficial to appeal to the specialist for general reading; nevertheless, they contain very useful tabulated data on the alloy systems of uranium, plutonium and thorium, and a survey of operational types of fuel element with the performance statistics and main types of failure experienced. Unfortunately throughout the book there is very little reference to data published after 1958.

S. F. PUGH

Design and Function at the Threshold of Life

The Viruses. By Prof. Heinz Fraenkel-Conrat. Pp. vii+117. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 1.95 dollars; 14s.

THE chemical study of viruses is now recognized as important by even the most reactionary virologists, and is now contributing to our knowledge of biological systems of all kinds, largely because the fundamental mechanisms utilized by the viruses are essentially much the same as those found in the higher organisms, but the viruses are simpler and often easier to manage because of this simplicity.

Heinz Fraenkel-Conrat has contributed much to the advances which have been made in this field over the past few years, and a book from his pen is very welcome at this moment. Naturally, in dealing with the viruses, the author has concentrated very largely on the chemical aspects of the smaller members of this group, and in particular with the tobacco mosaic virus, which is certainly the most completely studied, if scarcely the most representative, of the small viruses.

A large part of the text is concerned with a detailed discussion of the chemistry of the proteins and of the nucleic acids, and the parts which they play, and therefore much of the book is of interest to biologists in general. The reader will also gain an interesting, if unintended, insight into the author himself.

Naturally, in a short book of this type it is not possible to do more than skim over the general subject of virology, although as the author himself states: "This little book contains a fair amount of information". Some excellent illustrations are included, but undue space has been given to pictures of models of tobacco mosaic virus "reconstituting", which, although fun, are not very informative.

It is surprising to note that, although the major theme of the text concerns the nucleic acid of the tobacco mosaic virus, the names of its discoverers are not mentioned anywhere.

ROY MARKHAM

The Nature of Biochemistry

By Prof. Ernest Baldwin. Pp. xiii+111. (Cambridge: At the University Press, 1962.) 13s. 6d. net; 2.75 dollars.

AN author who has previously written two highly successful and widely read books about biochemistry might perhaps be excused for feeling apprehensive about the merit and timeliness of his third effort. Prof. Baldwin need have no doubts on either score. Biochemistry is still very much of an unknown subject to the staff and sixth-form students of our schools, and there can be little doubt that many potentially good scientists are lost to biochemistry because of lack of adequate information at school-level. This book should be of considerable help in correcting this situation, for its purpose is to explain to those leaving school what biochemistry is about.

It is perhaps not surprising that the thread of Baldwin's own interest, comparative biochemistry, should run through the book, and this thread is particularly strong in the opening sections. However, this aspect usually has its own special attractions for sixth-form students, and the author uses it to good effect in illustrating the essential simplicity and unity underlying the complexity of biochemical processes.

Through being essentially readable, there is sufficient depth in the book to test the inquiring mind and indeed some passages will require considerable concentration on the part of the young reader. However, for the most part, the book flows in characteristic Baldwin style. Extremely well produced to the high standards usually associated with the publishers, this book can be strongly recommended to all who wish to learn something of the life-process.

K. S. DODGSON

LEARNED SOCIETIES AS PUBLISHERS

THE Scientific Publications Council met at the Ciba Foundation, London, on December 13, to discuss the "Publication of Journals by Scientific Societies" with Prof. A. S. Parkes (Physiological Laboratory, Cambridge) in the chair. Dr. P. N. Campbell (Courtauld Institute of Biochemistry, London) introduced the subject with which he had been much concerned in so far as it affects the Biochemical Society. Societies exist to advance their respective sciences; since publication is one of the chief means to this end, the production of a journal may be regarded as a most important activity for a scientific society, which must adopt a strict editorial policy to set a standard in the field.

Old-established journals produced by commercial publishers generally adopt the same high standards, but as publication in science grows more profitable, the proliferation of specialized journals leads not only to the fragmentation of science, but also to higher costs to subscribers and, in some instances, to lowering of editorial standards.

Societies can be associated with journals in one of three ways.

In the first arrangement the society owns and publishes the journal itself. This ensures it complete control, the greatest financial benefit and, provided the society is large enough (for example, the Chemical Society), it seems the most desirable scheme.

Secondly, the society may own the journal, but employ a publisher. This arrangement is adopted by many smaller societies with no permanent editorial staff, often to secure financial backing when starting a new journal. The publisher prints, promotes, sells and distributes the journal for a commission. The Cambridge University Press does this for a number of societies, treating all exactly alike on a *pro rata* basis. It does an excellent and reliable job of printing at low cost; but the commission charged for its publishing activities, Dr. Campbell considered, is high. However, if a society has no professional staff, such liaison with a publisher may be a reasonable compromise.

Thirdly, the journal may be owned by a publisher though the society appoints the editors. The society runs no financial risk, but it also stands to gain least if the journal succeeds, so that this arrangement is rather unsatisfactory. However, a society wishing to operate in this way might do well to consider the Company of Biologists, a non-profit-making organization which owns and publishes several journals on this basis.

What can be done to help scientific societies regain the initiative ?

Dr. Campbell thought that responsible scientists should be urged to consider carefully before accepting requests to serve as honorary editors of a new commercial journal. The time had come, he proposed, for societies to collaborate, taking advantage of the best commercial methods, in the production, promotion and distribution of their journals. A source of funds is required to tide societies over the early stages of publication; also advice is needed on printing and distribution costs, and accommodation must be found for editorial offices. A joint distributing organization should be created to enable societies to collaborate over subscriptions and the storage and despatch of their journals; the same organization might undertake promotion of sales.

In reply to a question, Dr. Campbell said that he advocated increasing the size of existing journals rather than a multiplicity of small journals published on science, although, as Dr. T. R. E. Southwood (Imperial College of Science and Technology, London) suggested, societies start new journals not only to cover new fields but also because the editors of existing publications have neither time nor space for expansion. The question of sales promotion restrains many societies from severing their connexion with publishers, since large institutional sales are needed to subsidize the free distribution of journals to societies' members.

Mr. A. L. Bacharach (*British Journal of Nutrition*) perceived the horns of a dilemma: one could support small specialist journals, intelligible and interesting throughout, which, because of their small circulations, are expensive; or one could support large unspecialized journals, which are to a considerable extent unintelligible and costly in time to read selectively.

Dr. D. Richter (Medical Research Council Neuropsychiatric Unit, Carshalton) thought national journals are tending to give way to international periodicals with world-wide circulations such as can best be provided by the great publishing houses.

Mr. Donn Casey (Reproduction Research Information Service) described his experiences in starting the *Bibliography of Reproduction*, a new title-list with fourteen issues annually of eighty pages each. He is compiling, promoting and distributing this with the aid of one full-time assistant with some part-time help. It is printed by lithography, the plates for which are prepared in the office with an I.B.M. typewriter, no photographic process being used. The quality is quite good, while the process is fast and costs a fraction of normal type-setting.

Mr. R. W. David (Cambridge University Press) said that he hoped he could speak with uncommercial impartiality, although this did not mean that the Cambridge University Press was not as competitive and efficient as any commercial publisher. A society should first satisfy itself that its publisher is really necessary. The publisher provides (*a*) finance and (*b*) a distribution service. The former is much easier than many societies believe; it is financial assistance at the early stages that is so valuable. Once a journal is established the subscriptions come in before the bills have to be paid. Through his distribution facilities, a publisher can promote sales to a marginal public; but a specialist journal for a well-defined clientele might need no publisher at all. The publisher's commission normally covers promotion, warehousing and distributing costs. However, as Dr. H. J. Rogers (National Institute for Medical Research, Mill Hill) pointed out, a society undertaking these tasks for itself would not necessarily realize the whole of the publisher's commission, for it would not only lose the benefit of the university press, whose printing costs are low, but it might also have to employ extra staff.

Dr. A. C. Stickland (Institute of Physics and the Physical Society) said that many printers and binders will distribute journals directly. Mr. J. C. Graddon (Royal Society) agreed, adding that the Royal Society now publishes by itself and employs a firm of packers and distributors. Many Fellows have waived their right to copies of all the Society's publications in favour of receiving, on request, free reprints of particular papers that interest them. Dr. Campbell's scheme was reminiscent of the science centre proposals for collaboration between the societies in shared premises, with their own publishing house and printer in the basement.

Dr. W. R. S. Garton (Imperial College of Science and Technology, London) emphasized the distinction between reputable publishers and a less-scrupulous minority that act as *entrepreneurs*—it is the latter that start most of the new journals in order to 'sell' science as a commodity. It is wishful thinking to suppose that in the absence of a genuine need such journals would fail for lack of support: librarians dare not ignore them. A puzzling feature is the list of illustrious names on the titulary boards of these journals—names that not even death can always delete from the roll, as Dr. R. K. Callow (National Institute for Medical Research, Mill Hill) interjected. New journals should in general only be supported if they bear the imprint of a society. Mr. A. V. S. de Reuck (Ciba Foundation, London) remarked that if economic selection did not eliminate poor journals, perhaps some way should be found whereby journals could be reviewed like books and some machinery for explicit selection set up for the benefit of librarians. Mr. J. A. Rivers (J. and A. Churchill, Ltd., London) replied that reviews may sell good books but they do not necessarily condemn bad ones, and he doubted whether they would be effective with periodicals. While they are profitable, new journals will continue to arise, Dr. Rogers believed, and it would be a tragic mistake for scientific societies to lower their standards in order to compete with the less-desirable of these. The societies must set the standards for all, including the commercial publishers.

A. V. S. de Reuck

THE TEACHING OF BIOLOGY

By Prof. D. G. CATCHESIDE, F.R.S.
Department of Microbiology, University of Birmingham

There is at present much discussion concerning the reform of biological teaching. The reasons are varied. There are impressions that university entrants in biology are poorer than formerly and worse prepared for the study of their subject, so biology must be made more attractive for brighter pupils. There are all the newer studies for which space must somehow be made, giving rise to questions as to which of the older material, if any, is fundamental and what relative adjustments could be made. There is the recognition that very much in the living world is common to plants and animals and micro-organisms, and that therefore the cleavage into botany and zoology, as well as the separation from medicine and agriculture, goes deeper, far deeper, than it should. This is especially true of the structure and function of cells, studied by the methods of physiology, biochemistry and genetics. However, the common ground extends well beyond cell biology, and it is possible to write truly biological curricula which, with quite minor changes, may be studied with reference to either plants or animals, or to a combination of the two or, even, to a very considerable extent, with micro-organisms only. As well as the immense growth of biochemistry, there is an important new field of biophysics to take into account. The less important, though more obvious, aspect of this concerns the specialized machines with which modern physics has supplemented the microscope; the more important aspect is the way in which individual physicists, shying from the anonymity of teams grouped around huge machines, are bringing physical ways of thought to bear on biological problems.

There are therefore several adjustments required in biological education, without further delay, partly to prevent continued fragmentation of biology, partly so that biologists may understand the newer biochemical and biophysical knowledge which is sweeping their subject, and partly so that biochemists and biophysicists may be given a broad and fundamental view of biology. Only in this way is there a chance of making the spectrum of biological knowledge continuous. No one person can hope to encompass the whole in any detail. Most will be specialists in some fairly narrow range, though a few may absorb to differing extents in several regions.

The solution to teaching at university-level lies in the devising of new curricula which are not mere chimeras of existing courses, for biology is not merely a mixture or synthesis of zoology and botany. The courses should permit a biology student to specialize to a greater or lesser extent and attain an honours standard in three years from entry into a university. The specialisms possible may be expected to be more varied than at present, recognizing that none can properly cover the whole of botany or of zoology. Indeed, what university course really does so even at the present time? The first year of a biology student should include a general course in biology and a general course in physical sciences; between them these should provide the whole of the specialist or vocational study.

Most authorities seem to agree that more understanding of the principles of physical sciences is needed by most biologists. It is fairly easy to see what these should be, and there is a reasonable prospect that physicists and chemists will be sufficiently interested to plan educative courses which will achieve the main objectives. These should be to fit the developing biologist with an understanding of the principles of the physical sciences, especially in the context of their relevance to biological systems. The significant topics, several of which are not exclusively chemical or physical, ought to include:

(1) Elementary particles and atomic structure, isotopes and their application to biology.

(2) Dynamics, mass and gravity; the biological effects and applications of high and low gravity.

(3) Chemistry of inorganic and organic substances, with special reference to those of biological importance.

(4) Macromolecules.

(5) Rates of reaction, catalysis and the properties of membranes.

(6) Electricity, light and other radiations; biological applications, for example, X-ray crystallography.

(7) Thermodynamics; work in biological systems; high-energy bonds.

(8) Stellar and planetary evolution.

(9) Earth sciences, climatology and meteorology.

The general course in biology, which should be suitable also for biochemists and biophysicists, must be planned to give a perspective of the subject, both as regards the different levels of integration which it has and which cut right across the division into plants and animals and, indeed, micro-organisms, and the relationship of these levels to one another. Broadly, the topics may be arranged in the following way:

(1) The cell as the basic unit of organisms, its constituent materials and their acquisition and its metabolism. Emphasis needs to be placed on the general similarity in constituent compounds and processes, on the means of energy transport and utilization, and the control of metabolic processes.

(2) The organization of cells to form tissues and more-or-less complexly differentiated organisms, with the consequent problems of procurement and processing of materials and the organization and integration of functions.

(3) Reproduction, ranging from the cellular and subcellular levels to the organismic and dealing with chromosome theory, the structure and function of genes and the problems of development and differentiation.

(4) Evolution, including the elementary processes of change in heredity, the evolution of adaptation, the nature of genetic systems and the consequences of these for variation and the supposed nature of species and speciation.

(5) Diversity and the problems of classification, with special reference to the principles and purposes, both as regards the classification of plants and animals as a whole and also at the finest levels of distinction. Suitable illustrative material should be drawn from plants, animals and micro-organisms, though not exhaustively.

(6) The behaviour of populations, as regards the interdependence of the individuals for energy and materials, their interactions and consequent change. The relation of organisms to environment, noting the relation of ecology to evolution.

(7) Behaviour, as a special aspect of relationship between individuals or of individuals with environment.

It is on this basis that one may take issue with Prof. R. D. Preston[1] that above the level of "cell biology", defined by him as "the physics and chemistry of individual cells", botany and zoology are too different to teach together in one department and that they are "at least as different as physics and chemistry". The reality of biology as a subject lies in the unity inherent in the apparently universal similarity in the constitution of cells, of their mode of reproduction and of their integration, differentiation and evolution to more complex levels of organization.

One may agree, in principle, with Preston that no one can be expected to have the capacity to contain the whole of botany or of zoology and at the same time have the mathematical and physical knowledge and skill needed to cope with certain of the work commonly referred to as 'molecular biology'. Nevertheless, every biologist, in the full sense of the term, should have a general understanding of the principles behind such work and of the results and their significance. This is essential if the co-operation of biologists with physicists, specialized in various ways, is to be achieved. Likewise, it is essential to design courses in biology suitable for the biological education of those physicists and chemists who aspire to be biophysicists and biochemists.

I would probably agree, also, that 'classical' biologists, presumably anatomists, morphologists and taxonomists, generally dealing with material that is not alive, are supremely necessary and probably have no need for an extensive knowledge of the physical sciences. In the realm of anatomy, as revealed by the electron microscope, we are surely back to the position of lack of serious distinction between plant and animal material. No one can fail to be impressed by the similarity of structure shown by sperm tail and the cilia of fern spermatozoa. In electron microscopy, no botanist can afford to ignore work on animals, or to abandon the field to the biophysicist.

In the case of taxonomy, also, I would like to be sufficiently revolutionary, or perhaps atavistic, to suggest that training in this 'specialism' should fit the taxonomist to turn to plants or animals. The principles and methods are the same, only the details are different; a competent taxonomist could surely acquire a good working knowledge of a new group in a year or two, if he put his mind to it, in the presence of proper collections and library and laboratory facilities. The more modern aspects, the new systematics, embraces experimental methods and concepts in cytology, genetics and evolution which are, common to plants and animals. These have been studied over many years by geneticists, sometimes with plants and sometimes with animals, but always with a broad knowledge of the field. Here the 'gap' is illusory.

I do dispute that the work of taxonomists, anatomists and morphologists is the foundation on which all the rest stands. A modest knowledge of genetics, for example of the bacteria or of certain viruses, would dispose of this notion. The whole deductive and inferential structure of biology as a science, largely derived by the methods of genetics, physics and chemistry, is independent of descriptive biology in its development. Of the methods, only the first is characteristically biological and biological alone.

After the first year at the university, during which one may hope for a common course for biologists, we may expect that many students will wish to specialize. It should be possible to do this to different extents and into fields which are not co-extensive with those of the historical divisions of botany and zoology. In neither of these has teaching ever been truly comprehensive, simply owing to the exigencies of time and the limitations of staffing. It would be realistic to recognize this and to plan second- and third-year courses leading to suitable specialization in, for example, taxonomy and ecology, genetics, plant or animal physiology and biochemistry and microbiology, as well as more general courses or combinations of courses for those who may need them. Clearly, the possible range, as now, would be determined by the staffing at any particular university.

In common with Prof. R. A. Morton[2], in commenting on the 'training' of biochemists, I believe it is essential that any course of biology should be educational, intellectually stimulating and contain aspects hard enough to stretch the mind of the student. The quality of education is to be judged mainly by the extent to which it kindles a true spirit of continued inquiry and study. The precise content of the course matters little; the spirit and the manner of its study do. These should constantly lead to inquiry and discovery by the student. It is unnecessary to make believe that a course in any way covers the whole of a subject. A student, well educated as a student, will be able to go on and learn more of his subject and even to branch out into new fields after graduation.

My purpose has been to indicate how the teaching of biology at a university can be unified and what most biologists' education in the physical sciences should encompass. Likewise, I have desired to indicate what minimum view of biology as a whole the biochemist and biophysicist should have.

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[1] Preston, R. D., *Nature*, 193, 131 (1962).

[2] Dodgson, K. S., *Nature*, 191, 955 (1961).

COLOUR AND LIFE

> I sometimes think that never blows so red
> The Rose, as where some buried Cæsar bled . . .
>
> *Omar Khayyám.*

IT is a far cry from bleeding bread and medieval massacres of Jews to interior decoration and eye-shadow; from photosynthesis to photography, respiration to Rembrandt, ultra-violet honeylines to tattooing and television. All these and other topics, more chaste perhaps, were compassed in and between the sessions (during September 20–21, at Birkbeck College, London) of the thirteenth annual symposium of the Institute of Biology, the title of which epitomizes the dual link, the unsaturated bond, between them all: "Colour and Life".

The first morning's session, under the chairmanship of Prof. O. V. S. Heath, dealt with structural colours (Prof. W. D. Wright, Imperial College, London) and chemical colours (Dr. J. B. Harborne, John Innes Institution, for plants; Prof. T. W. Goodwin, Aberystwyth, for animals). The effects of surface, of transparency and of background on specular and diffuse reflexion include structural colour when there is differential reflexion due to scattering, interference or diffraction, though the last is of doubtful significance in living systems. Tyndall scattering (macaw) and interference (peacock, beetles, *Morpho* butterflies) were illustrated and analysed, using spectral reflexion factor methods (for a first general idea) and spectral luminance factor methods (which take account of direction and therefore discriminate specular and diffuse components and do justice to iridescent hues). Though coloured transparent non-metals, unlike metals, have not a coloured specular reflexion, the metallic effect can be produced from a multilayer of such material.

The first requirement for a pigment is a conjugated system of alternating double and single carbon–carbon bonds. Carotenoids, all based on a tetraterpenoid skeleton (8 five-carbon isoprene units), are widely distributed in most plant organs. Animals can only synthesize to the sterol (triterpenoid) level; their carotenoids are direct diet derivatives. Incidence and colour function (flowers, fruit, skin, feathers) in both kingdoms was reviewed.

Flavonoids (anthocyanins and allies) are mainly of higher plants. Progressive colour changes occur as phenolic hydroxyls are multiplied (for example, scarlet to mauve) or for other simple changes. Many are indicators and perhaps stimulated the Rubáiyát couplet felicitously quoted by Harborne. Odd cults like blue roses will vainly seek delphinidin in Rosaceae; blues must here be engineered—if engineered they must be—otherwise.

Quinonoids (*p*-benzoquinone nucleus) are again mainly plant pigments, often mysteriously hidden in roots or under bark. They colour fungi and occur in echinoids and scale insects—ancient and modern providers of dyestuffs.

Nitrogenous pigments are the most diverse. Allied to chlorophylls and blood pigments (*vide infra*) based on the four-pyrrol porphyrin molecule is prodigiosin of bleeding bread. The polymeric-indole-group melanins mainly belong to animals (possibly fungi, too), but their distribution is confused by looseness of definition (formerly, anything black was a melanin), and the difficulty of detaching their proteins. Other nitrogenous pigments were reviewed.

In the general discussion, there was substantial agreement on the need to measure colour of whole organisms; ripening of tomatoes, lures in ethology and ultra-violet honey guides being quoted.

The afternoon session (under Prof. Helen Porter) included colour and classification in lower plants (Prof. G. E. Fogg, Westfield College, London), photosynthetic pigments (Prof. C. P. Whittingham, Queen Mary College, London), respiratory pigments (Prof. H. Munro Fox, Queen Mary College) and colour change in animals (Dr. D. B. Carlisle, Anti-Locust Research Organization).

Clandestine consultation of coloured Floras apart, pigment specificity and a largely biochemical taxonomy make thallophytes amenable to colour diagnosis; 'misfits' (*Prototheca*, photosynthetic bacteria) are few. The by-product type of pigment in fungi and lichens is, not surprisingly, too variable to use above genus-level, and its use may be frankly artificial (for example, spore colour); but photosynthetic pigments are fundamental and conservative, and validly diagnose major groups in bacteria and algae (conditioned masking, as in red *Hæmatococcus*, apart)—though broader-based evidence could be desired. Difficult cases were reviewed and the legitimacy of phylogenetic speculation assessed.

Photosynthetic efficiency is measured by comparing action spectra (oxygen yield against wave-length) with absorption spectra. In brown algae, fucoxanthin contributes as efficiently as chlorophyll; phycocyanin and phycoerythrin are similar, carotenoids less efficient. The energy is transferred to chlorophyll with low loss by inductive resonance. Derivative-spectrum studies, comparison with the adenosine triphosphate and electron sequences in animal respiration, and Witt's short-flash experiments correlating types of absorption-change with the electron-transport sequence, lead to the now generally accepted concept of two photosynthetic processes using different pigments and linked by a thermal dark reaction (Whittingham and Bishop) which involves loss of free energy probably coupled with phosphorylation.

From the succinct review of the structure and occurrence of respiratory pigments, cytochrome was omitted as having little colouring effect on tissues and none on whole animals. Hæmoglobin, hæmerythrin and chlorocruorin contribute little direct, hæmocyanin nothing; but non-respiratory hæmoglobin derivatives (porphyrins in the earthworm, certain shells, touraco birds) do. In a few marine worms, green is due to dietary chlorophyll; but the green of insects and *Actinia*, like the greens and blues of birds' eggs, blue wrasse and blue coral, is due to bilins (based on a pyrrol chain instead of a ring)—much commoner contributors than porphyrins to animal colour.

Changes in animal colour are morphogenetic (gradual metabolic substitution through heat (*Carausius*, *Schistocerca* hoppers), light, diet (*Flamingo*, *Carcinus*), background (isolated *Locusta* hoppers), crowding (gregarious *Locusta* hoppers), courtship (mallards, prawns), etc.)—or kinetic (chromatophoral darkening of prawns in unwelcome light; breeding-dress of some fish; aposematic-cryptic transformation of *Sepia*, etc.). The simplest responses, of either type, are directly stimulated, but more often the effector is under central control, either nervous (beak in cephalopods, with their special brainlobe) or endocrine (Crustacea, sexual colour-change of fish); or both.

In a lively but unresolved discussion, the 'aposematic' function of striping in the (highly palatable) *gregaria* hoppers was challenged in favour of disruptive crypsis (still) combined with episematic signal function (moving).

The general discussion which followed brought out the dangers of the effects of colour contrast on description. Relevant charts produced by the Technical Optics Section, Imperial College of Science and Technology, were demonstrated.

The theme of the second morning, at which Dr. K. Mellanby took the chair, was colour perception and behaviour. Dr. J. D. Carthy (Queen Mary College) (invertebrate perception) opened with the caveat: sense-organ discrimination need not imply colour vision, which can only be tested by whole behaviour. *Pieris brassicae*, neutral to green while feeding but highly attracted when egg-laying (Ilse), shows the need for the comprehensive approach, as does the history of bee-training to colour. An extensive review followed of visual pigments, evidence of all types for spectral discrimination and sites thereof, and double receptor systems, in such groups as have been investigated.

Dr. U. Weidmann (Birkbeck College), speaking on colour and animal behaviour, reviewed the whole vast field of releasers, their interaction with one another and the background, and the conflicting selection between camouflage and signalling: to trace the mode of evolution of the colour patterns that play such a prominent part in this behaviour. The special case of symbiont signalling was illustrated from Manning's experiments with honey-guides for bumble-bees and Daumer's demonstration of ultra-violet honey-guides in (to us) plain red flowers (bees seeing the ultra-violet pattern). Finally, evidence of some rudimentary æsthetic appreciation in certain groups was presented.

Dr. Katharine Tansley (Institute of Ophthalmology), speaking on human colour perception, briefly reviewed the accepted facts about rods and cones, and the evidence for and against the two principal theories of colour vision (the trichromatic and Hering's). In the face of the complex interconnexions of retinal ganglion cells, it is not reasonable to expect clear-cut sensitivity curves. Simultaneous contrast may arise, on the other hand, not through adjacent pigmentary synthesis and breakdown (Hering) but through adjacent nervous excitation and inhibition, for which these interconnexions are relevant. If such opponent mechanisms are present in each of three pigment systems, both theories stand to some degree confirmed. Discussion on all three papers in the morning session continued vigorously into the lunch-hour.

The final session, at which Sir Gerard Thornton, president of the Institute, took the chair, was centred around the psychology of colour. Dr. R. W. G. Hunt

(Kodak Research Laboratories), dealing with the acceptability of colour reproduction, started with the faults of even a purely optical image (flare, contrasting background, in telescope and camera obscura), and showed how they were added to in transparency-viewing and television by reduced luminance (and therefore colour-saturation) and subtense, before we even start to match colours. This again results in further loss of colour-saturation because the process-colour mixture stimulates more than one of the retinal receptor types. In general, acceptability depends on a scene's looking as it might have looked.

Miss Kathleen A. Battersby (studio director, British Colour Council) discussed, as an artist, colour in everyday life. She traced the evolution of the new technology and the parallel growth of dictionaries of colour standards it has exacted, covering hue (subjective counterpart of wave-length), tone (depth of colour), intensity (degree of brilliance), and texture (a range appropriate to different materials). Gay and beautiful colours are now available to the humblest; background and lighting studies show that it pays to beautify workplaces, a doctrine illustrated by a striking series of 'before-and-after' lantern slides.

Finally, Dr. H. Gwynne Vevers (Zoological Society of London) reviewed adornment in man and other animals. Among invertebrates camouflaged by debris, there is some evidence of systematic choice. Active adornment by transfer of coloured secretions is known in birds, though in mammals, living in an olfactory world, it is rare until man. The speaker discussed tattooing and analogous practices, body paints of plant (woad, henna) and mineral origin (ochres) and the evolution of cosmetics from ancient Egyptian 'kohl' through medieval lip-rouges to the phantasmagoria of current, mainly synthetic, products.

W. B. BROUGHTON

RIBOSOMAL AGGREGATE ENGAGED IN PROTEIN SYNTHESIS: CHARACTERIZATION OF THE ERGOSOME

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RIBONUCLEOPROTEIN particles (ribosomes) are essential components in protein synthesis. In all cells so far examined, the basic ribosomal units are particles of uniform size characterized by a sedimentation constant of 70–85 *S* and composed of two relatively stable sub-units of unequal size corresponding to about 50 *S* and 30 *S*. Dimers of 100 *S* have been isolated from *E. coli*[1], higher aggregates with sedimentation constants of up to 155 *S* have been observed in preparations from mammalian cells[2-4]. Less is known about the functional unit that is fully active in protein synthesis. From studies with *E. coli* ribosomes, Tissières *et al.* have concluded that both 70 *S* and 100 *S* particles are active when combined with a messenger RNA containing the genetic information[5,6]. Similar conclusions were drawn from experiments with mammalian reticulocytes believed to be endowed with a more stable RNA template[7]. However, in all these experiments only a small fraction (approximately 5 per cent) of the ribosomes were shown to be active *in vitro* and hence the proposed relations between the physical state and biological activity are subject to considerable uncertainty. Further difficulties arise from spatial considerations: it is not easy to imagine how a messenger RNA molecule, large enough to direct the synthesis of a protein sub-unit, may be accommodated on the surface of a 100 *S* (70 *S*) particle in a configuration that remains accessible to a sequential line-up of aminoacyl transfer-RNA molecules. Some of these difficulties would seem to disappear if it were postulated that in the native state a small number of 70 *S* particles are combined with the messenger RNA (*m*RNA) to form a larger structural unit. We postulate that about five 70 *S* ribosomal particles, engaged in protein synthesis, form a precise geometric structure providing a unique spatial arrangement for the messenger RNA to display its active functional surface. The results of experiments with rat liver ribosomes reported here give support to this hypothesis.

In an attempt to correlate particle structure and amino-acid incorporation activity, ribosomes were prepared by different methods and the sedimentation pattern in a sucrose gradient compared with the results of incorporation studies. Homogenization was carried out in Hoagland's[8] medium *A* (0·05 M *tris*, 0·025 M potassium chloride, 0·005 M magnesium chloride, 0·25 M sucrose). Standard ribosomes ('*A* ribosomes') were prepared according to Korner[9] by addition of deoxycholate (DOC) to the post-mitochondrial supernatant and subsequent centrifugation at 105,000*g* for 120 min. Zone centrifugation analysis in a sucrose gradient containing Med. *A* salt buffer (0·05 M *tris* buffer, 0·025 M potassium chloride, 0·005 M magnesium chloride) showed that in such preparations most of the ribosomes were present in the 73 *S* and 113 *S* state, the relative proportions contained in these two peak positions being subject to considerable variations (Fig. 1*a*, *b*). Similar variations are found in published data obtained both with bacterial and mammalian ribosomes[2,4-6,10,11]. We found that these variations are mainly determined by the temperature at which the manipulations were carried out. In particular, lowering of the temperature settings of the ultracentrifuge during the preparative runs produced a pronounced shift of the distribution toward the heavier particles. When temperature conditions close to 0° C were maintained throughout the isolation procedures, more than 50 per cent of the ribosomes sedimented at a rate corresponding to more than 100 *S* (Fig. 1*c*).

In order to determine the biological significance of the heavier aggregates, the isolation procedure was modified. The post-mitochondrial supernatant fraction containing 1·3 per cent DOC was added as a 5-ml. top zone to 13-ml. centrifuge tubes. The tubes had been previously filled with 4 ml. of 0·5 M sucrose layered over 3 ml. of a more concentrated sucrose solution. In three sets of tubes the sucrose concentration of this bottom layer was adjusted to 1·0, 1·5 and 1·8 M respectively. All tubes were then centrifuged at 105,000*g* for 4 h. The transparent pellets were rinsed, dissolved in medium *A* salt buffer and the solutions clarified by low-speed centrifugation.

The results of analytical tests, summarized in Table 1, show that increasing the density of the bottom sucrose layer resulted in an efficient purification of the dense nucleoprotein particles by eliminating most of the protein impurities and other contaminants of lower density or molecular weight. Thus, in the tubes containing 1·5 and 1·8 M sucrose, the metallo-proteins remained as an intensely yellow band just below the interface of the bottom layer; the pellets were nearly colourless and dissolved quickly to give perfectly clear solutions. In contrast, the pellets formed in 1·0 M sucrose were yellow and, when re-suspended, always gave turbid solutions. Table 1 also shows the amino-acid incorporation activity of the three preparations, both with respect to original input and the RNA content of the recovered ribosomes. It may be seen that in all three preparations, the ribosomes

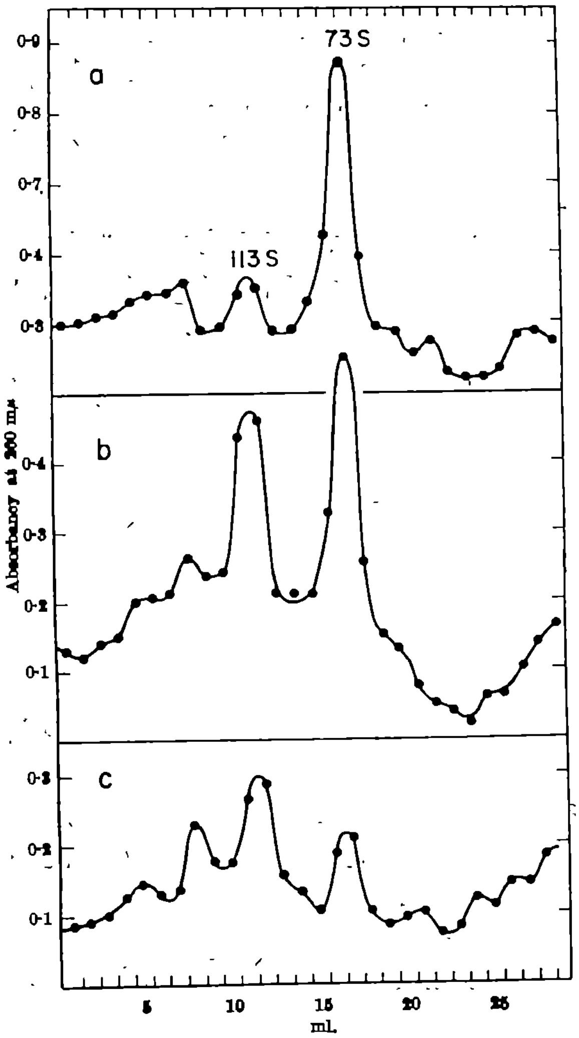

Fig. 1. Zone centrifugation analysis of different preparations of *A* ribosomes. 1-ml. samples were layered in form of an inverted gradient (12) over 27·5 ml. of a sucrose gradient (exponential 0·3–1·0 M) containing medium *A* salt-buffer (0·005 M Mg^{++}) and centrifuged in a Spinco *SW* 25 rotor for 4 h at 25,000 r.p.m. The bottom of the plastic tube was punctured with a hypodermic needle and 1·0 ml. fractions collected with a specially designed fraction collector

recovered from 1 g rat liver incorporated practically the same amount of ^{14}C-leucine into protein, whereas the recovery of RNA decreased from 1·85 to 1·16 mg/g liver as the density of the sucrose was raised. This suggested that the observed increase in specific activity resulted from the selective sedimentation of a heavy particle fraction responsible for biological activity. This conclusion is supported by the results of the sedimentation analysis shown in Fig. 2. From the observed change in particle size distribution it may be estimated that ribosomes with a sedimentation constant of less than about 200 *S* are considerably less active in protein synthesis than the heavier aggregates of the ribosome solutions. After incubation for 15 min at 37° C, most of the heavy aggregates had broken down into 113 *S* and 73 *S* particles, so that the sedimentation patterns of the three preparations became almost identical.

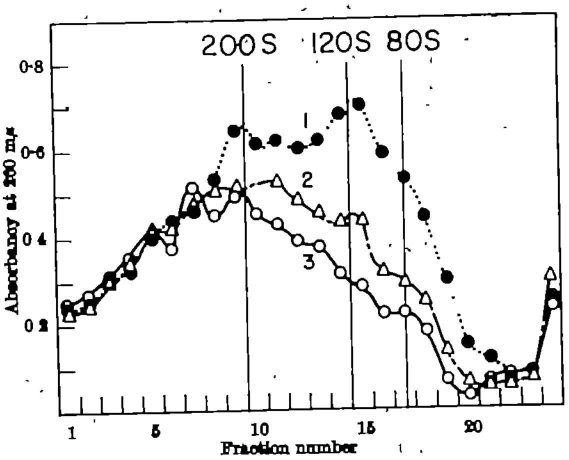

Fig. 2. Sedimentation patterns in a sucrose gradient of ribosomes purified by centrifugation through sucrose layers of different densities. Preparations 1, 2 and 3 were isolated as described in the text and 0·3 ml. samples, containing the ribosomes in form of an inverted gradient, layered over 4·4 ml. of a sucrose gradient (exponential 0·26–0·75 M). The tubes were centrifuged in a Spinco *SW* 39 rotor for 20 min at 39,000 r.p.m. and 4° C, and 24 × 0·20 ml. fractions collected as described in legend to Fig. 1

The physical nature of the active particles and the mechanism of dissociation during incubation were investigated in subsequent experiments. Ribosome preparations ('*C* ribosomes') containing more than 90 per cent of heavy particles ($S > 120$) were used. They were prepared by the procedure described here in which the sucrose concentration in the bottom layer of the preparative centrifuge tube was raised to 2·0 M. The purity of these preparations is reflected in their low protein content (weight ratio of protein/RNA $\leqslant$ 1) and their dependence on an external amino-acid supply for optimal protein synthesis (Fig. 3).

The structure of the heavy aggregates was studied by examining the size distribution of *C*-ribosomes after centrifugation in a 30-ml. sucrose gradient. For optimal resolution in the analysis of the sedimentation pattern, the fluid was collected by passage through a continuous-flow ultra-violet absorption cell attached to a strip chart

Table 1. CHEMICAL COMPOSITION AND AMINO-ACID INCORPORATION ACTIVITY OF RIBOSOMES ISOLATED BY ZONE CENTRIFUGATION THROUGH SUCROSE SOLUTIONS OF DIFFERENT DENSITIES

	Sucrose concentration in 3-ml. bottom layer (M)	Rat liver input, wet weight (g)*	Protein content of ribosomes recovered per 1·0 g liver (mg)†	RNA content of ribosomes recovered per 1·0 g liver (mg)‡	Approximate weight RNA/protein	^{14}C-leucine incorporated by ribosomes recovered per 1·0 g liver (c.p.m.)§	^{14}C-leucine incorporated per mg RNA (c.p.m.)
Preparation 1	1·0	12	3·04	1·85	0·6	7,600	4,150
2	1·5	12	1·10	1·42	1·3	7,400	5,150
3	1·8	12	0·77	1·16	1·5	7,200	6,200

*Homogenization was carried out after addition of 28 ml. medium *A* to 12 g rat liver. After sedimentation of the heavy cell elements, 20 ml. of the post-mitochondrial supernatant (ref. 8) were carefully removed with a syringe, avoiding contamination with sediment or lipid layer on top. In calculating the values shown in the table, only the recovered portion of the postmitochondrial supernatant per g liver was considered; this corresponds to 60–70 per cent of the actual yield.

†Assayed according to Lowry *et al.* (ref. 13) using crystalline bovine albumin as standard.

‡Estimated from the absorbancy at 260 mμ using the relation 20 absorbancy units = 1·0 mg RNA.

§Incorporation assays were carried out under standard conditions as described in the legend to Fig. 3. The ribosomes were prepared as described in the text and assayed after storage at −60° C. The incorporation tubes (total volume 1·0 ml.) contained the standard mixture described in the legend to Fig. 3 with the following additions: 0·2 ml. ribosomes equivalent to 0·6 g liver, appr. 4 mg. *p*H 5 enzyme protein (ref. 8), 0·1 μmoles each of 19 L-amino-acids excluding leucine and 0·04 μmole DL-leucine-1-^{14}C with a specific activity of 10·2 mc./mmole. The incubation time was 30 min.

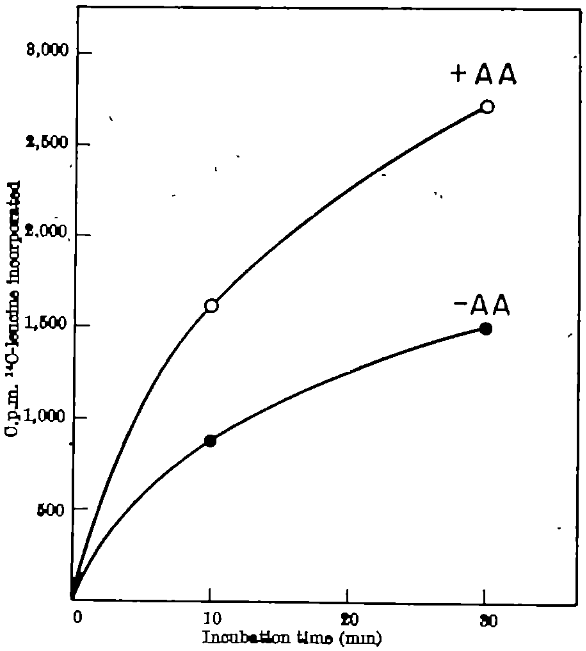

Fig. 3. Stimulation of amino-acid incorporation activity of *C* ribosomes by addition of amino-acid mixture. The standard incorporation mixture, adjusted to a final volume of 1·0 ml., contains the following reagents in μmoles: $MgCl_2$ (3·2), HCl–*tris* buffer *p*H 7·6 (30), β-mercaptoethanol (2·0), ATP (1·0), GTP (0·4), K-phosphoenolpyruvate (10), crystalline pyruvate kinase (0·01 mg). Ribosomes, soluble enzymes, radioactive leucine and non-radioactive amino-acid mixture are added as specified below. After incubation at 37°, the reaction mixture is stopped with 1 ml. 1 M sodium hydroxide. After standing at room temperature for 1 h, protein was precipitated by addition of 1 ml. 2·5 M perchloric acid containing 20 mg 'Hyflo Super-Cel'. (In the 0-time controls samples kept at 0° C are immediately subjected to this treatment.) The precipitate, collected on a filter disk, was washed successively with 0·5 M perchloric acid, propanol–ether (1 : 2) and propanol–ether–$CHCl_3$ (2 : 2 : 1) and dissolved in a hyamine (1 ml.)–toluene (15 ml.) scintillation mixture (ref. 14) for radioassay in a Packard 'Tri-Carb' liquid scintillation spectrometer, model 314 X. Maximal deviation between duplicate samples above 100 c.p.m. is < 10 per cent above 1,000 c.p.m. < 5 per cent of the mean. Counting efficiency is approximately 50 per cent. Zero time controls usually give about 50 c.p.m.
The assays represented in the graph contained the following additions. 0·04 μmoles DL-leucine-1-^{14}C (10·2 mc./mmoles), ribosomes equivalent to 0·6 g liver (corresponding to about 0·5 mg RNA) and postmicrosomal supernatant equivalent to 0·06 g liver. The postmicrosomal supernatant was prepared by centrifugation of the postmitochondrial supernatant at 105,000*g* for 2 h. and subsequent dialysis at 0° C for 24 h against a 60-fold volume of medium *A* salt buffer containing β-mercaptoethanol (0·005 M). A mixture of 19 L-amino-acids (0·1 μmoles each) excluding leucine was added only to the series represented by the upper curve

recorder. A constant flow rate was maintained with a precision pump. A typical recording, reproduced in Fig. 4*a*, shows a sequence of 7 peaks, spaced at decreasing intervals, ranging in position from about 70 to 240 *S*. The seventh peak is barely resolved from a large proportion of heavier material that appears polydisperse, presumably because the close spacings of the bands in this region prevents further resolution under these conditions.

Incubation of *C*-ribosomes at 37° C in medium *A* salt buffer solution causes some breakdown of the heavy aggregates resulting in a corresponding increase of the absorbancy in the satellite peaks (Fig. 4*b*). No optically detectable quantities of low molecular weight material absorbing in this region were released under these conditions. Addition of minute quantities of crystalline pancreatic ribonuclease, corresponding to a final concentration of 1·2 × 10^{-8} g/ml., greatly accelerates this breakdown process with the result that most of the particles are now obtained in the 73 *S* and 113 *S* state (Fig. 4*c*). Under these conditions, an increase of the absorbancy at 255 mμ, equivalent to about 3 per cent of the total input, was sometimes observed in the top layer of the gradient. The nature of this material and the mechanism of its release are now under investigation.

The regular spacing of the satellite peaks and the RNase-induced breakdown into 70–113 *S* particles indicates that the higher aggregates represent oligomers of the 73 *S* particles.

For molecules of similar shape and density, the sedimentation constant *S* is related to the particle weight *m* by the equation:

$$S^{3/2} = km$$

in which *k* is a constant. When the experimentally determined $S^{3/2}$ values corresponding to the satellite peaks were plotted against the relative particle weights, taken to be multiples of the 73 *S* mass, a straight line was obtained (Fig. 5). This suggests that the 73 *S* particles are assembled into larger aggregates of compact structure and similar shape so that transitions from one aggregate form to another will not result in drastic shape changes.

More precise information concerning the size of the active particle was gained from incorporation studies with isolated fractions taken directly from sucrose gradient tubes. *C* ribosomes were centrifuged in the 30-ml. swinging bucket rotor and 1·0-ml. fractions collected.

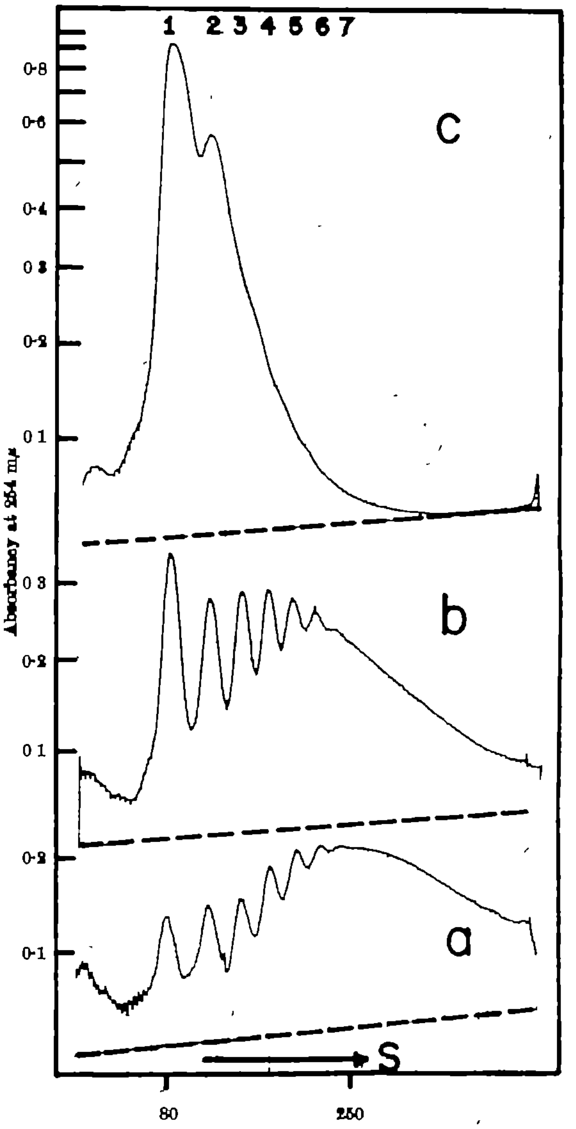

Fig. 4. Effect of RNase on particle size of *C* ribosomes. (*a*) *C* ribosomes (0·5 mg RNA) were suspended in medium *A* salt buffer and centrifuged in a sucrose gradient for 2 h at 25,000 r.p.m. as described under Fig. 1. The absorbancy of the effluent was scanned in a flow cell and recorded automatically. The pellet at the bottom of the tube contained 20 per cent of the input absorbancy. (*b*) *C* ribosomes (0·5 mg RNA) were incubated in 1·0 ml. of medium *A* salt buffer at 37° C for 5 min. and analysed as above. The pellet contained 4 per cent of the input absorbancy. (*c*) *C* ribosomes were treated as in (*b*), except that the incubation medium (1·0 ml.) contained 0·012 μg 5x-crystallized pancreatic ribonuclease. No pellet was observed. In all three samples the absorbancy near the top may be attributed to the presence of β-mercaptoethanol, added to the ribosome solutions before storage

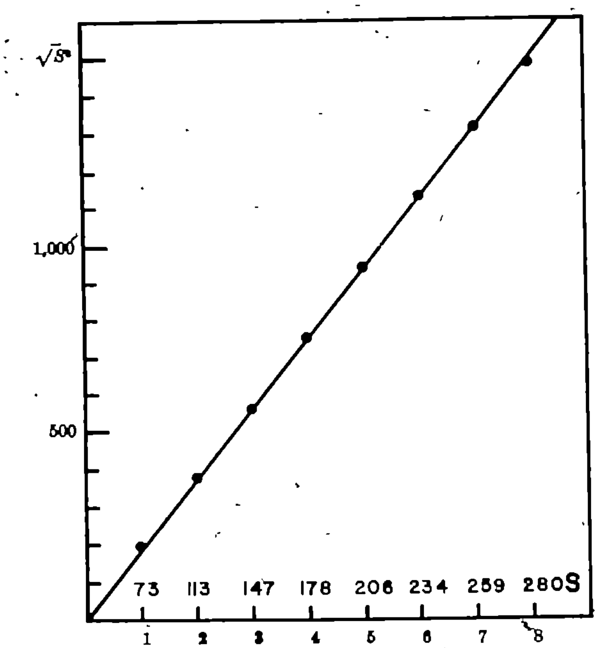

Fig. 5. Relationship between sedimentation rate and particle mass of satellite peaks. The sedimentation constants were estimated by comparison of the peak positions with an experimental reference curve obtained by calibration of the sucrose gradient with the monomeric particle as standard. The numerical S values were computed on the basis of 73 S for the monomer, representing an average of published values

Aliquots were removed to determine the absorbancy distribution pattern (Fig. 6*a*). Every three consecutive 1·0-ml. fractions were then pooled in ascending order, so as to give 8 cuts (pool fractions 1–8) covering the range of particle sizes from about 50–500 S. The ribosomes of each pool fraction were then sedimented into a pellet and redissolved in medium A salt buffer. The pellet from the original sucrose gradient was included in this series. In order to check whether the physical state of the resuspended particles had changed during the concentration procedures preceding the incorporation assay, aliquots were removed from the concentrated pool fractions 2, 4 and 7 and subjected separately to zone centrifugation analysis in a sucrose gradient. The resulting patterns are shown in Fig. 6*b*, *c* and *d*. The amino-acid incorporation activity of each pool fraction was measured, using duplicate samples and different ribosome concentrations ranging from 0·15 to 0·60 mg RNA per 1-ml. incorporation mixture. An excess of rat liver supernatant was present in all incorporation tubes, so that the amount of protein formed was always proportional to the concentration of the ribosomes in the incubation mixtures. All samples were treated with alkali to remove radioactivity bound to transfer RNA.

In Fig. 6*a*, the specific incorporation activity (c.p.m. ^{14}C-leucine incorporated/0·15 mg RNA) has been plotted against the effluent volume corresponding to the various pool fractions. The range of particle sizes contained in these fractions is indicated on the abscissa. The recording of a calibration run is included for comparison. It is evident from the curve in Fig. 6*a* that the specific activity increases with aggregate size over a range corresponding to 100–250 S, reaching a constant level for particles sedimenting between about 250–600 S. This range included 80–90 per cent of the total C ribosome population, including the particles obtained from the pellet of the original gradient. The results show clearly that the 73 S monomers are inactive. Partial activity is perhaps associated with the dimers $(73\ S)_2$, certainly with the trimers $(73\ S)_3$; it continues to rise rapidly through the following two stages and then becomes constant. It is difficult, however, to pinpoint the state of aggregation at which full activity is reached. The data shown in Fig. 6 suggest the pentamers $(73\ S)_5$ as lower and the heptamers $(73\ S)_7$ as upper limit.

Of particular interest is the redistribution of particle sizes observed after concentration and recentrifugation of the three samples taken from different regions of the original gradient (Fig. 6*b*-*d*). Although in each case a main distribution peak is centred near the position of the parent fraction in the original gradient, a large proportion of the input has been dispersed into both heavier and lighter fractions. It should be noted that the resulting spectrum of particle sizes is not continuous but contains several peaks in the seemingly polydisperse region (250–500 S). The apparent periodicity of the heavier peaks may reflect unspecific aggregation of the most frequent and/or stable particle size contained in the parent fraction, for example, $(73\ S)_5$ in Fig. 6*c*.

The foregoing experiments have shown that: (1) fully active ribosomes exist in the form of aggregates consisting

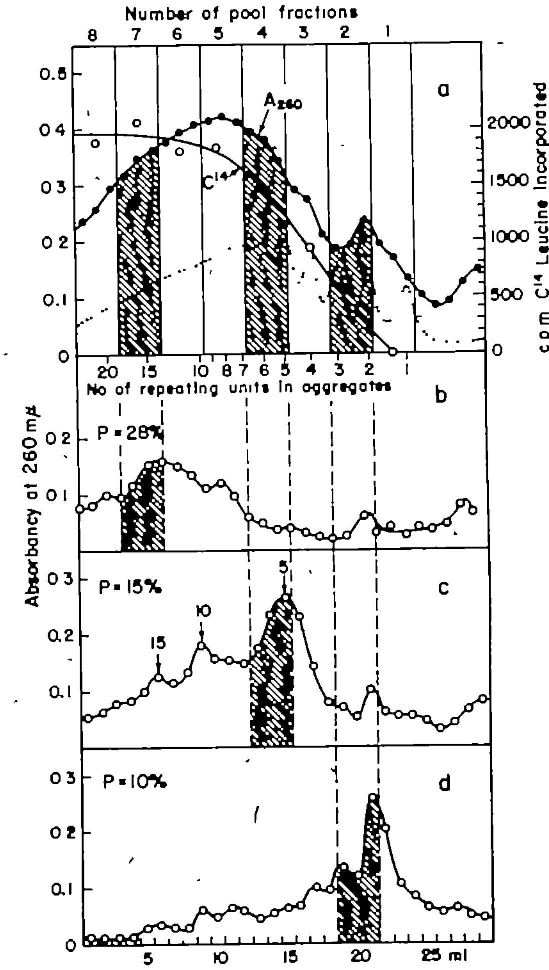

Fig. 6. Amino-acid incorporation activity of C ribosomes as a function of particle weight. (*a*) C ribosomes (4 mg RNA input per tube, three tubes per run) were fractionated in a sucrose gradient as described under Fig. 4. The resulting sedimentation pattern, measured spectrophotometrically, is shown in the curve marked $A260$. The pellets contained 40 per cent of the input. The fractions were pooled as indicated in the graph (pool fractions 1–8), the particles spun down and the pellets from each pool resuspended in 1·1 ml. medium A salt buffer. Aliquots from these concentrates, containing 0·15–0·60 mg RNA, were used for incorporation assays. The incorporation mixtures contained 0·02 μmoles L-leucine-1-^{14}C (25 mc./m.mole) and non-dialysed post-microsomal supernatant equivalent to 0·12 g liver. Addition of 19 L-amino-acids was omitted. The incubation time was 30 min, all other conditions as described under Fig. 3. The specific activity, expressed as alkali stable, acid-insoluble radioactivity incorporated per 0·15 mg RNA has been plotted against the corresponding pool fraction (curve marked ^{14}C). The specific activity of the pooled pellets from the original gradient tubes was 1,900 c.p.m./0·15 mg RNA. The dotted curve represents the sedimentation patterns of a similar run determined by the continuous recording method. The abscissa is calibrated in multiples of the 73 S sub-unit on basis of the data in Fig. 5. (*b*–*d*) Sedimentation patterns obtained with aliquots from pool fractions 2, 4 and 7. P indicates the percentage values of the total input recovered in the pellets

of at least 5–7 73 *S* particles held together by an RNase sensitive structure; (2) during incubation of *C* ribosomes at 37° C, a slow degradation of heavy aggregates into smaller particles is taking place, apparently because the preparations are still contaminated with traces of RNase; (3) the amino-acid incorporation activity of a given ribosome preparation is directly related to the proportion of heavy aggregates present in the population. Viewed together, these results suggested another approach for determining the size of the basic unit of protein synthesis. Thus, it was to be expected that the amino-acid incorporation, measured after various periods of pre-incubation, should be proportional to the surviving fraction of active particles, namely, those aggregates which are equal to or larger than the critical size required for full biological activity. In an experimental test of this relationship, *C* ribosomes were incubated at 37° C in medium *A* salt buffer for 0, 5, 10 and 30 min. At the end of incubation aliquots were removed for determination of the particle size distribution in a sucrose gradient, and the remaining samples incubated for an additional 30-min period in the presence of the complete amino-acid incorporation mixture.

From sedimentation diagrams similar to those shown in Fig. 4, the relative decrease in the population of heavy aggregates, observed after various preincubation periods, was computed for several cut-off points covering the critical range from $\geqslant (73\ S)_4$ to $\geqslant (73\ S)_7$. These values are listed in Table 2 together with the relative decrease of the ^{14}C-leucine incorporation. It is evident that the breakdown rate of the aggregate populations defined by the cut-off points $\geqslant (73\ S)_5$ and $\geqslant (73\ S)_6$ roughly parallels the rate at which the incorporation yield is reduced. Some marked deviations, however, are noted. Thus, the rate at which the incorporation yield decreases is rapid at the beginning, then levels off between 10 and 30 min, whereas the breakdown rate of heavy particles lags behind at first and continues with little change after the decrease in incorporation yield has nearly ceased. The initial lag of aggregate breakdown is especially pronounced for populations $\geqslant (73\ S)_4$–$(73\ S)_6$. Apparently, the rate of reduction in the amount of protein synthesized after various intervals of preincubation reflects the rate at which the trace of RNase originally present in the preparation is inactivated.

Table 2. EFFECT OF PREINCUBATION ON AGGREGATE SIZE AND AMINO-ACID INCORPORATION ACTIVITY OF *C* RIBOSOMES

Pre-incubation time (min)	Amount of ^{14}C-leucine incorporated into protein: C.p.m.	Amount of ^{14}C-leucine incorporated into protein: Decrease (per cent)	Decrease in fraction of particles with aggregate size *n* (per cent), *n* = $(73\ S)_4$	$(73\ S)_5$	$(73\ S)_6$	$(73\ S)_7$
0	5,110	0	0	0	0	0
5	4,140	19	5·5	6·5	8·5	14
10	3,680	28	10	12	16	23
30	3,210	37	27	32·5	39	48

A concentrated solution of *C* ribosomes (1·75 mg RNA/ml.) in medium *A* salt buffer containing β-mercaptoethanol (0·005 M) was preincubated at 37° C and aliquots removed, at the time-intervals indicated in the table, for sedimentation analysis in a sucrose gradient (0·6 ml.) and for amino-acid incorporation (0·2 ml.). The incorporation mixtures contained 0·02 μmoles L-leucine-1-^{14}C (25 mc./mmole) and dialysed postmicrosomal supernatant equivalent to 0·18 g liver. The incubation time was 30 min, all other conditions as described under Fig. 3. The particle size distribution was computed from the appropriate integrated areas in the automatically recorded sedimentation patterns.

The apparent delay in the shift of the particle size distribution, on the other hand, may be due to an unspecific attachment to the heavy aggregates of the small, biologically relatively inactive, particles formed in the beginning of the incubation period. This interpretation is favoured by the results of the previous experiment shown in Fig. 6. On the basis of this interpretation, we conclude from these results that the size of the basic active particle is either $(73\ S)_5$ or $(73\ S)_6$. An unambiguous decision that the basic unit is the pentamer $(73\ S)_5$ was possible by a more direct method, the details of which will be reported elsewhere. In these experiments the specific activity of the nascent protein, expressed as ^{14}C-leucine incorporated per mg ribosomal RNA, was determined as a function of particle size after short incubation pulses followed by fractionation in a sucrose gradient. It was found that the specific activity increased through the satellite peaks, reaching a maximum in the peak corresponding to the pentamers; it declined somewhat in the peak containing the hexamers and increased again to the original level near the position of the decamers, remaining nearly constant throughout the region of the heavier aggregates.

Discussion

In this article we have described a novel ribosomal particle which appears to be the functional unit of protein synthesis and which we therefore propose to call 'ergosome'. In the following we have made an attempt to combine our observations into a model of the ergosome. Although this model contains many speculative features, we believe it nevertheless to be useful, both as a working hypothesis and a basis for discussion.

We visualize the ergosome as a roughly spherical or doughnut-shaped particle with a sedimentation constant of about 200 *S* corresponding to a molecular weight of about 20 million. It is composed of five 73 *S* particles whose 30 *S* sub-units, oriented toward the centre of the aggregate, are adjacent to each other. The five 73 *S* monomers are held together by one messenger-RNA (*m*RNA) molecule which may be pictured as running through the groove between the 30 *S* and 50 *S* sub-units. The spaces between the 73 *S* monomers expose single stranded regions which are extremely sensitive to enzymatic cleavage. Single breaks in these regions result in a stepwise fragmentation of the ergosome into smaller oligomers with a final liberation of free 73 *S* particles.

The rapid fragmentation of the ergosome by RNase indicates that Mg^{++} ions are not primarily responsible for its structural integrity. This also applies to the larger aggregates which we regard as resulting from an unspecific aggregation of ergosomes with each other and with their smaller breakdown products. For gradual removal of Mg^{++} does not result in a significant shift to smaller aggregate sizes. However, when the Mg^{++} concentration is reduced to less than 10^{-3} M, 50 *S* and 30 *S* particles appear in addition to the entire spectrum of heavy aggregates, indicating that Mg^{++} ions are specifically required only for the integrity of the 73 *S* particle as first demonstrated by Tissières and Watson[1]. Rapid removal of Mg^{++} by addition of ethylenediamine tetraacetic acid causes immediate dissociation of the entire aggregate population into 50 *S* and 30 *S* sub-units.

More detailed information concerning the attachment of *m*RNA to the ribosomal sub-units and its structural relationship to the growing peptide chain has been obtained from studies on the mechanism of breakdown at low Mg^{++} concentrations of ergosomes containing labelled *m*RNA and nascent protein. A preliminary account of such studies has been presented previously[15], a more detailed report will be given in a forthcoming paper. On the basis of these results we may further elaborate our hypothetical model: The stretches of *m*RNA that we have pictured as being embedded in the groove between the two sub-units of the 73 *S* particle are attached to the 30 *S* sub-units. During protein synthesis the information of the *m*RNA is read by transfer RNA molecules moving successively through this groove. The end of the growing peptide chain is in contact with the 50 *S* sub-unit lining the groove along the side opposing the 30 *S* particle. The 50 *S* face of the groove may also play an important part as surface for the attachment of the enzymes participating in the amino-acid polymerization. Near the end of the growing peptide chain, the gap between the two sub-units of the 73 *S* particle is thus bridged by one or several transfer RNA molecules, resulting in an increased stability of the particles containing the nascent protein against dissociation at low Mg^{++} concentrations. Such an effect has indeed been observed with *E. coli* ribosomes by Tissières *et al.*[5] and with rat liver ribosomes in our own experiments[15,16]. In addition, this picture would explain the observation that on complete dissociation of the 73 *S* particles, brought about by further reduction of the Mg^{++} concentration, most of the nascent protein moves with the heavy shoulder of the 50 *S* peak in a sucrose gradient, whereas the rapidly labelled *m*RNA coincides with the 30 *S* peak[4,15]. Our model also predicts that under these conditions of dissociation the transfer RNA molecule at the growing end of the peptide chain should remain attached to the 50 *S* particle carrying the nascent protein, since it is linked to the carboxy group of the terminal amino-acid through a covalent bond. This interpretation is supported by findings of Elson[17].

In our hypothetical model of the ergosome, the functional unit of protein synthesis is represented as a structure of uniform size consisting of five 73 *S* particles held together by one *m*RNA molecule, which is assumed to direct the synthesis of one protein sub-unit. Such a model seems to conflict with the apparent heterogeneity with respect to the size of the protein sub-units and their messengers that have been encountered[18]. This difficulty may be overcome by postulating that ergosomes of constant size, for example, the pentamer $(73\ S)_5$ considered in our model, may contain either one large or several smaller molecules of *m*RNA, depending on the size of the protein sub-unit to be synthesized. Alternatively, the ergosomes themselves may vary in size according to the size of their messenger. A decision between these two possibilities would require information about the size of the protein molecule synthesized by our smallest fully active unit $(73\ S)_5$. From the specific activity incorporated in our experiments after a 30-min incubation period we obtain a molecular weight of about 20,000, corresponding to the addition of 30 amino-acids per 73 *S* particle (mol. wt. 4×10^6). This calculation assumes that the ergosome fraction accounts for all of the activity and represents 66 per cent of the total ribosomal population present in our incubation mixtures. Since in our incorporation system about 80 per cent of the nascent protein remains attached to the ribosomes, we must further assume that at the beginning of the incubation the ribosomes are on the average half-filled with non-radioactive peptides. Hence, each ergosome would be capable of synthesizing one protein molecule corresponding to a molecular weight of at least 40,000. This value is probably still too low, since no correction has been made for the breakdown of active aggregates taking place during incubation. It is reasonable to assume, therefore, that an ergosome of the postulated size would be capable of synthesizing protein molecules of molecular weights as large as 50,000–100,000. If we assume a coding ratio of 3 (ref. 19), the ergosome would be large enough to accommodate a messenger of approximately 500,000 molecular weight, which is well within the range of values suggested in the literature[6,18].

If, on the other hand, the ergosome is engaged in the synthesis of several identical protein sub-units, our model further predicts that for structural reasons the sizes of these sub-units and their corresponding messengers would have to follow the series *M*/1, *M*/2, *M*/3, etc., in which *M* represents the largest polymer size that can be accommodated by the active aggregate. Again, it would seem that the different sedimentation constants reported for isolated *m*RNA are not incompatible with this interpretation, although some of these preparations may have been considerably contaminated by breakdown products resulting from RNase action[5,6,11,20–27].

Of particular interest is our finding that aggregates that are smaller than the ergosome exhibit partial activity when their incorporation yield is computed per particle mass. Since these smaller aggregates have been shown to represent breakdown products of the ergosome, we assume that they contain only a fragment of the original template. Consequently, we would conclude that only those of the smaller aggregates would be active that carry unfinished peptide chains attached to a partially filled fragment of the template. This interpretation is in harmony with other evidence indicating that peptide synthesis must proceed in one direction from a fixed starting-point on the template[19]. It is likely that the ribosome preparations described in the literature consist largely of such breakdown products. This interpretation would explain both the small proportion and more rapid sedimentation of the so-called 'active' particles[6]. An indication of the biological significance of ribosomal aggregates may be found in the observations of Arnstein with rabbit reticulocyte ribosomes[3] and, most recently, in studies with bacterial ribosomes and synthetic messengers, published after this work had been concluded[28,29].

In conclusion, we wish to emphasize that the model which we propose is tentative. We are aware that it presents a number of difficulties which could not be discussed within this limited space. It is our hope that they will be resolved by further experiments which are now in progress.

We thank Dr. G. Attardi for kindly giving us a copy of his manuscript before publication. This work was supported by grant *IN58B* from the American Cancer Society Institutional Research Grant Committee and by research grants *E*–1500 and *E*–4303 from the U.S. Public Health Service. In addition, one of us (F. O. W.) was supported by training grant 2*E*–80 and another (H. N.) by senior research fellowship *SF*–274 from the U.S. Public Health Service.

Note added in proof. After this manuscript went to press (December 12, 1962), the general structure of the ergosome proposed here has been confirmed by electron-micrographs of ribonucleoprotein particles from rabbit reticulocytes (Warner *et al.*, *Science*, 138, 1399 ; 1962).

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NEWS and VIEWS

Instruments and Electrical Engineering at the Royal Aircraft Establishment : Mr. W. J. Charnley

MR. W. J. CHARNLEY, who was recently promoted to deputy chief scientific officer, has been appointed head of Instruments and Electrical Engineering Department, the Royal Aircraft Establishment, Farnborough. Mr. Charnley graduated in civil engineering at the University of London, qualifying with honours, B.Eng. in 1940. In 1943 he joined the Aerodynamics Department of the Royal Aircraft Establishment, Farnborough, and after a brief period in the Low-Speed Wind Tunnel Division was moved to the Flight Division, where he was engaged on all aspects of aerodynamic research in flight on high-speed aircraft. In 1953 he was placed in charge of the High-Speed Section of the Department. He was transferred in 1955 to the Blind Landing Experimental Unit at Martlesham Heath as superintendent. This Unit is the department of the Royal Aircraft Establishment responsible for research and development of systems to enable aircraft to land in bad weather, and Mr. Charnley was directly concerned with the successful development of the Blind Landing Experimental Unit system of automatic landing. Mr. Charnley was awarded M.Eng. in 1945, and in 1949 became an Associate Fellow of the Royal Aeronautical Society. He was awarded the Bronze Medal of the Institution of Navigation in 1959 for a paper on blind landing. Mr. Charnley recently completed the 1962 course at the Imperial Defence College.

Military and Civil Systems Department, the Radar Research Establishment: Dr. E. V. D. Glazier

DR. E. V. D. GLAZIER, chief scientific officer, has been appointed head of Military and Civil Systems Department of the Radar Research Establishment. Dr. Glazier gained both his B.Sc. and Ph.D. degrees in electrical engineering at the University of London. His early training was in general electrical and mechanical engineering in industry followed by appointments in the engineer-in-chief's office of the General Post Office, where he was engaged on the development of telephone systems and defence communications. In 1942 Dr. Glazier was transferred, under war-time arrangements, to the Signals Research and Development Establishment, where he was responsible for the development of a wide range of signals equipment for the Army. After a short period in Headquarters at the end of the War, he was appointed as head of the Line and Acoustic Group at the Signals Research and Development Establishment, and in 1952 promoted to lead the Research Division of that Establishment, where he was responsible for research into a number of problems in Army radio and electronics. In 1957 he returned to Headquarters of the Ministry of Aviation as director of scientific research (electronics and guided weapons), where he was responsible for advising in research programmes and organizing the early Ministry of Aviation programme for satellite observations. During this period he was secretary of the Radar and Signals Board of the Scientific Advisory Council. In 1959 Dr. Glazier transferred to the Radar Research Establishment as head of the Ground Radar Department, where he was responsible for the development of ground radar and data handling equipment and for collaboration with the French Air Ministry on the development of radar systems. In 1962 he was transferred to head of Airborne Radar Department. He is at present a member of the Institution of Electrical Engineers' Electronics Divisional Board.

The Harrison Memorial Prize, 1962 : Dr. A. Carrington

DR. ALAN CARRINGTON has been awarded the Harrison Memorial Prize of the Chemical Society for 1962 in consideration of his contribution to the study of theoretical chemistry, especially his work on electron spin resonance. Dr. Carrington is a graduate of the University of Southampton. He obtained the B.Sc. degree in 1955 and has since undertaken postgraduate research at the Universities of Southampton, Minnesota and Cambridge, where he now holds a research fellowship at Downing College. The Harrison Memorial Prize is awarded for outstanding merit in any branch of pure or applied chemistry. It was created in 1922 to commemorate the services of the late Colonel Edward Frank Harrison, formerly deputy controller of the Chemical Warfare Department, for the protection of the British Forces from poison gas in the First World War. It is awarded to the British chemist, under thirty years of age, who, in the opinion of the Selection Committee, has during the previous five years conducted the most meritorious and promising original investigations in chemistry and published the results.

Ninetieth Birthday of Prof. Hans K. A. S. von Euler

PROF. HANS K. A. S. VON EULER, one of the pioneers of modern biochemistry, in particular enzymology, celebrates his ninetieth birthday in Stockholm on February 15. He was born in Augsberg, Bavaria, to a German aristocratic family. Although at first he had intended to be a painter, he went to Sweden as a young graduate to study under the great physicochemist Svante Arrhenius, and thus began his scientific career. In 1909 he became professor of general and organic chemistry at the Stockholm Royal University and founded the well-known Biochemical Institute there. Dominant among the research carried out there on enzyme chemistry was alcoholic fermentation, for which Euler received the 1929 Nobel Prize for Chemistry together with A. Harden. The immense amount of work carried out in the enzymological field by Euler is crowned by his great "Chemistry of Enzymes" (*Allgemeine Chemie der Enzyme*, 1910–34), the first modern monograph on this field. On one hand, the research of the codehydrase I joins Euler's enzymological work with his numerous studies on the biochemistry of vitamins, and, on the other hand, with his later studies of the biochemistry of tumours. Concerning the latter, he especially undertook investigations of the turnover of nucleic acids in tumours by means of labelled compounds—a technique which he had initiated together with G. v. Hevesy. Von Euler's work dealing with tumour research includes two important monographs, the "Biochemistry of Tumours" (*Biochemie der Tumoren*, 1942) and the "Chemotherapy and Prophylaxis of Cancer" (*Chemotherapie und Prophylaxe des Krebses*, 1962). Von Euler retired from teaching in 1941 but continued to carry out research work at the Stockholm Institute. He has been responsible for inspiring and teaching a great number of scientists now working in the field of biochemistry in all parts of the world. All have found him a careful teacher, an excellent example and a fatherly friend.

The Institution of Chemical Engineers : Committee in Ireland

THE Institution of Chemical Engineers has established a Committee in Ireland, constituted as follows: *Chairman*, Prof. J. P. O'Donnell, University College, Dublin; *Honorary Secretary/Treasurer*, Mr. J. Kelleher, Shamrock Fertilizers, Wicklow; *Assistant Honorary Secretary*, Mr. R. K. Sinnott, Du Pont (U.K.) Ltd., Londonderry; Mr. E. V. Casburn, Seaweed Ltd., Galway; Dr. F. L. Kafka, Du Pont (U.K.) Ltd., Londonderry; Mr. I. McCallum, Irish Refining Co. Ltd., Whitegate, Co. Cork; Mr. P. H. Whitaker, Chemstrand Ltd., Coleraine, Co. Derry; Mr. W. A. D. Windham, Arthur Guinness Son and Co. Ltd., Dublin. The objects of the Committee are "(*a*) to promote an interest in chemical engineering in Ireland, including interest in the schools; (*b*) to bring chemical engineers together for discussions on matters of technical interest; (*c*) to provide a representative opinion of chemical engineers in Ireland". Further information can be obtained from: Mr. J. Kelleher, "Fernhurst", Hillside Road, Greystones, Co. Wicklow, or the General Secretary, the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

Science and the Public Mind

A MEMORANDUM by W. Schramm, *Science and the Public Mind*, based on various studies in the United States of public knowledge of science and scientists, supports the view that knowledge of science is widely, but not deeply, distributed in the United States (American Association for the Advancement of Science. Miscellaneous Publication No. 62–3: *Science and the Public Mind: A Memorandum for B. G. Sherburne, Jr., from Wilbur Schramm*. Pp. 20. Washington, D.C.: American Association for the Advancement of Science, 1962). There are still large areas of ignorance and an individual's education is the chief predictor of his knowledge of science, followed by use of mass media; after leaving school, mass media supply most of the increment in knowledge. Where such information is sought depends largely on the topic and on the characteristics of the individual. The more personalized and/or sensational is a scientific article the larger is its audience, and mass media tend to use these techniques in reporting science and to select material that can be so treated. It is to be regretted that they do not apply to material for entertainment the same standards of scientific accuracy that they apply to advice and reporting. Public attitudes towards science and scientists are generally favourable, although not very accurately informed. The public is interested in getting more scientific information, but the acquisition of new scientific information does not necessarily result in a logical chain of belief, attitude and behaviour. Besides noting some negative indicators as to what information on science should be provided for the public, Mr. Schramm suggests ways in which scientists themselves can help, for example, by paying closer attention to school curricula in science and to the level of science teaching, contributing to the training of science writers, facilitating the dissemination of information about their work and encouraging the study of the processes by which scientific knowledge is conveyed and applied.

Incomes Policy and the Professional Employee in Britain

IN a pamphlet, *Incomes Policy and the Professional Employee*, issued by the National Union of Teachers, Mr. M. Shanks, reviewing critically the Government's attempt to operate an incomes policy, suggests that the National Incomes Commission should be made a representative body (Pp. 15. London: National Union of Teachers, 1962). He argues that the National Economic Development Council is the most logical agent to formulate an incomes policy, and that under such a policy the salaries of professional employees would be determined by assessing the nation's need for such services and a formula designed to keep these salaries in a proper relation to industrial wages. This relation needs first of all to be agreed, and Mr. Shanks refers to the belief that in Western democracies there is a built-in distortion in favour of the private sector as against the public sector.

The British Hat and Allied Feltmakers Research Association

THE fifteenth annual report of the Director of Research of the British Hat and Allied Feltmakers Research Association, covering the year ended August 1962, refers to a survey of the storage cellars of members which established the general pattern of bacterial infection and showed a highly significant relation between the degree of infection and the season of delivery (Pp. 22. Droylsden, Manchester: British Hat and Allied Feltmakers Research Association, 1962). This programme limited progress on the carrotting of fur, but the Blankenburg and Zahn method of assessing the felting ability of wool was examined. Work continued on dyeing felt with acid milling dyes for fully-planked fur felt and half-planked wool felt and a study was made of the incidence of gas-fume fading of dyed fur and wool felts, while the problem of hat sizes has been further investigated and the geometrical properties of hatters' ovals have been summarized in a technical report. Factors likely to influence the development of static electricity in the forming machines were examined, and anomalous results of the coefficients of friction of felt have been attributed to contamination of the contact services with grease, while the programme comparing other possible materials with felt as padding in protective headgear was completed. A list of publications is appended.

Trade Associations and Professional Bodies of the United Kingdom

THE directory *Trade Associations and Professional Bodies of the United Kingdom*, compiled by Patricia Millard, is designed to provide a ready means of finding the address and telephone number of any nationally important trade, professional or technical association in the United Kingdom and of identifying the associations with any specific field of activity. With few exceptions, only national organizations are included, but trade unions, livery companies and chambers of commerce are excluded. The directory is in two parts: an alphabetical directory and a classified index with cross-headings (Pp. viii+128. London: C.B.D. Research, Ltd., 24 Holborn Viaduct, 1962. 30*s*.).

A Bibliography of Personnel Management

A BIBLIOGRAPHY recently published by the Institute of Personnel Management replaces that published by the Institute in 1950 and its subsequent supplements (*Personnel Management: a Bibliography*. Pp. 39. London: Institute of Personnel Management, 1962. 7*s*. 6*d*.). The books have been selected as helpful to the personnel manager who wishes to broaden his knowledge of the background of his job, and those included in the Institute's examination book list for students are marked. The books are arranged in eleven sections, the last dealing with sources of information, and the selection appears to be well-balanced and representative within the broad headings.

Dogs and Stilboestrol

DOGS (18 female, 4 male) were given subcutaneous injections of diethylstilboestrol at intervals of 4–6 weeks by Dr. Anne G. Jabara, of the Department of Pathology, University of Melbourne, and developed alopecia after 11 months (*Austral. J. Exp. Biol.*, 40; 1962). All areas except the head and distal parts of the legs were affected and the denuded skin became dry, scaly and excoriated. Microscopic changes were observed in all elements of the skin, and the hair follicles were atrophic. The hair grew again 3–7 months after injections were stopped, and at necropsy 11–14·5 months later the skin was histologically normal. Other findings included testicular atrophy, a high incidence of inguinal hernia, and endometrial hyperplasia which progressed to a cystic stage and eventually to pyometra. When injections were stopped, the endometrium regenerated after an interval the duration of which was independent of the duration of treatment or of total dose administered, but may have been related to the time taken to deplete the residual depot. Treated dogs also showed an increase of pituitary acidophils and a reduction in basophils and chromophobes; these changes were reversed on hormone withdrawal. Generalized or focal atrophy of the pancreas occurred in four of the longer surviving dogs; this was regarded as a toxic effect of stilbœstrol.

The American Society of Pharmacognosy

THE following have been elected officers of the American Society of Pharmacognosy: *President*, Dr. Gordon H. Svoboda, Eli Lilly and Co.; *Vice-President*, Dr. Melvin R. Gibson, professor of pharmacognosy, Washington State University. Dr. Varro E. Tyler, jun., professor of pharmacognosy, University of Washington, was elected to the Executive Committee. The Society's secretary and treasurer are elected for three years; neither term expires in 1963. Mr. Rolf Westby of Eli Lilly and Co. is secretary, and Dr. Frank A. Crane of the University of Illinois is treasurer.

The Iron and Steel Institute: Indian Elections and Awards

THE following elections and awards have been announced in connexion with the Iron and Steel Institute's special meeting in India during January 21–February 12 (see *Nature*, 196, 816; 1962): *Honorary Members*, Sir Jehangir J. Ghandy, director-in-charge, the Tata Iron and Steel Co., Ltd.; Sir Biren Mookerjee, chairman, the Indian Iron and Steel Co., Ltd., and governing director, Martin Burn, Ltd.; Mr. J. R. D. Tata, chairman, the Tata Iron and Steel Co., Ltd., and chairman of the Tata Group. *Honorary Vice-presidents*, Dr. D. P. Antia, president, the Indian Institute of Metals, and general manager, Industrial Projects Division, Union Carbide, India, Ltd.; Dr. B. R. Nijhawan, director, National Metallurgical Laboratory, Jamshedpur, and vice-president, the Indian Institute of Metals. *Award of a Scroll of Honour*, Mr. R. D. Lalkaka, honorary secretary, the Indian Institute of Metals.

The Institute of Metals: Awards

THE following awards of the Institute of Metals for 1963 have been announced: the Institute of Metals (Platinum) Medal, to Mr. G. L. Bailey, director of the British Non-Ferrous Metals Research Association, London, and a past-president of the Institute, in recognition of his outstanding services to the non-ferrous metal industries; the Rosenhain Medal, to Dr. B. A. Bailey, professor of theoretical metallurgy at the University of Sheffield, in recognition of his outstanding contributions in the field of physical metallurgy.

Electron Microscopy of Thin Crystals

A SUMMER school in electron microscopy of thin crystals will be held in the Department of Metallurgy, University of Cambridge, during June 23–July 1. The school is intended for research workers from industry and universities who already have some basic knowledge and operational experience of electron microscopy, and will include lectures and demonstrations on the specialized operation of electron microscopes, the theory of electron diffraction and image contrast and the interpretation of electron diffraction patterns and micrographs from metal and ceramic specimens. The lecturers will be Dr. P. B. Hirsch, Dr. A. Howie, Dr. R. B. Nicholson, Dr. D. W. Pashley and Dr. M. J. Whelan. The fee for the course will be £24, and college accommodation will be provided at an inclusive cost of £21. Further information can be obtained from the Board of Extra-Mural Studies, Stuart House, Mill Lane, Cambridge.

Lady Tata Scholarships and Fellowships in Leukæmia

THE Trustees of the Lady Tata Memorial Trust invite applications for fellowships, scholarships and grants for research on leukæmia, in the academic year beginning October 1. In view of the affinity between leukæmia and other forms of neoplastic disease, candidates with programmes of research on any aspects of malignant disease which may throw light on problems of leukæmia will be eligible for consideration, and especially those studying leukæmogenic viruses in mammals, the epidemiology and natural history of leukæmia, and the genetical and cytological aspects. Applications should be submitted before March 31. Further information can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3.

Announcements

DR. T. E. ALLIBONE, director of the Associated Electrical Industries Research Laboratory, Aldermaston, Berkshire, and director for Research and Education, Associated Electrical Industries (Woolwich) Ltd., has been appointed Rutherford Memorial Lecturer during a period to be arranged in the winter of 1963–64 in India and Pakistan, under the terms of the scheme to commemorate the late Lord Rutherford of Nelson.

MR. A. A. PART, deputy secretary in the Ministry of Education, has also been appointed deputy secretary in the newly created Ministry of Public Buildings and Works (see *Nature*, 196, 724, 937; 1962). Mr. Part will be responsible for policy matters affecting programmes of work carried out by the Ministry and, in particular, for matters arising from the forthcoming amalgamation with the Ministry of Works Directorates of the three Service Departments.

As from January 1, Dr. P. R. Day became secretary of the British Mycological Society. All communications for the secretary should in future be sent to him at the John Innes Institute, Bayfordbury, Hertford.

"CLIMATIC Factors and Agricultural Productivity" is the title of a symposium to be held at the Department of Geography and Anthropology, University College of Wales, Aberystwyth, on March 13. Further information can be obtained from Mr. J. A. Taylor, Department of Geography and Anthropology, Alexander Road, Aberystwyth, Cards.

THE Institute of Fuel, in collaboration with the City and Guilds of London Institute, is organizing a conference, "Education and Training in Fuel Technology in Relation to National Needs", at Borough Polytechnic, Borough Road, London, S.E.1, on March 8. Dr. A. Parker, lately director of fuel research of the Department of Scientific and Industrial Research, will give an address, "The Importance of Fuel Technology to Productivity". Further information can be obtained from the Secretary, Institute of Fuel, 18 Devonshire Street, London, W.1.

AN Anglo-Japanese symposium on "Nuclear Power", jointly organized by the United Kingdom Atomic Energy Authority and the Japan Atomic Industrial Forum, Inc., will be held in Tokyo during March 26–28. The British delegation will include representatives of the Authority, the Central Electricity Generating Board and the British nuclear industry. The delegation will present a symposium on "British Nuclear Power Station Development". Further information can be obtained from the United Kingdom Atomic Energy Authority, 11 Charles II Street, London, S.W.1.

THE FERMI CHICAGO PILE

On December 2, 1942, at 3.25 p.m. Chicago time, the first self-sustaining nuclear chain reaction was initiated and controlled. The success of the experiment was mainly due to Enrico Fermi. He directed the construction and the operation of the pile situated in the West Stands, Stagg Field, University of Chicago, in what had been a squash-rackets court. To mark the twentieth anniversary of this historical occasion and as a tribute to the memory of Enrico Fermi the International Atomic Energy Agency has issued a special number of its *Bulletin* (December 2, 1962) containing an introductory article by S. Eklund, director general of the Agency, and thirteen other articles, by distinguished scientists, devoted to descriptions of the first atomic pile or developments in the field of nuclear energy in various countries. English, French, German, Russian and Spanish editions of the *Bulletin* are to be published.

In his introductory remarks, the director general comments that immediately after the Second World War when the basic knowledge of atomic energy spread all over the world it was thought that the new source of energy could be put to widespread practical use within a very short time. But now, twenty years after the first reactor was put into operation, we have learned that development in nuclear energy must follow the same sequence as in every other major technological advance and involve only small steps at a time. The introduction of nuclear power will not be a sudden event, but instead a gradual process. However, the benefits already accruing from radioisotopes, an important by-product of nuclear energy, and their wide use in research and industry, require especial mention.

All the articles, except the simple descriptive account of the first atomic pile entitled "The First Pile" by C. Allardice and E. R. Trapnell, which was written in 1946 and is reprinted in the *Bulletin*, are original contributions. They include a brief personal account by Laura Fermi of her husband during the period he was working on the so-called 'metallurgical project' and of the absolute secrecy he maintained, even from her, of the work he was doing. Lise Meitner writes about the right and wrong roads to the discovery of nuclear energy; Otto Hahn on "Enrico Fermi and Uranium Fission"; H. D. Smyth on the publication of the "Smyth Report"; S. K. Allison on the "Initiation of the Chain Reaction" and his own particular task, the search for pure materials required for the pile; and G. T. Seaborg on the production of plutonium and its chemical extraction. Sir John Cockcroft describes the early days of the Canadian and British atomic energy projects. B. Goldschmidt discusses France's contribution to the discovery of the chain reaction and shows that the work of French scientists, while it did not contribute directly to the success of December 2, 1942, did nevertheless back up the efforts of Great Britain to persuade the United States to tackle the uranium problem on an industrial scale, and played a large part in the birth of nuclear activities in Canada and in the development of heavy water reactors. V. S. Emelyanov gives some historical details of the study of radioactive materials, and uranium, and graphite, by Soviet scientists leading up to the production of uranium and graphite for the first atomic reactor in the U.S.S.R.

In his article entitled "Thoughts on the 20th Anniversary of *CP*–1" E. P. Wigner states that on the memorable occasion of December 2, 1942, the scientists involved in the nuclear project realized that both the economic and political world would be affected by their great achievement. Economically they thought that the main effect would be cheaper energy, but so far this objective has not been realized. In the political field they hoped and expected that the terrible nature of nuclear weapons would have such a sobering effect on the Governments of all nations that they would forsake their conflicting aspirations and submit to a larger community of law and order. This was based on a misunderstanding. The issues of war and peace are governed not by the nature of weapons but by the conflicting desires of Governments.

The remaining two articles are by J. A. Wheeler and G. de Hevesy, who discuss "Fission, Then and Now" and "The Reactor and the Production of Isotopes", respectively.

S. Weintroub

THE BRITISH COUNCIL

The annual report for 1961–62 of the British Council * is in two parts, the general survey of the year being accompanied by an article "Teaching Courses" which, besides describing the Council's part in recruiting British teachers to serve overseas, seeks to answer some questions asked by potential candidates for overseas teaching posts. In addition to filling teaching posts in its own permanent service the Council recruited 226 teachers on short-term contract, of which 140 were for schools and 45 for universities and training colleges, about 25 per cent of those in universities being at the professorial level and rather more than half in English departments. Most of the Council's educational work in developing countries is complementary to technical co-operation, and experience in Nigeria and Ghana has clearly indicated that the opportunities open to the Council for educational work—especially in English language teaching—increase as the number of expatriate civil servants decreases. Arrangements have already been made for collaboration with the Department of Technical Co-operation in Nigeria which may become the pattern for similar co-operation elsewhere in Africa.

The Council's English Teaching Information Centre, formed in 1960 to collect and co-ordinate experience and information about teaching English as a second or foreign language, now distributes about 1,000 copies of its *English-Teaching Abstracts and English-Teaching Bibliography* in more than ninety countries. Moreover, the Council has maintained close contact with the British Broadcasting Corporation and with the Centre for Educational Television Overseas in its investigations on the production of English language teaching films for television and for classroom work. A substantial financial contribution is being made towards the running costs over the next five years of the English schools in Nicosia; £34,000 was contributed for a library building in Port Harcourt, Eastern Nigeria; £12,000 towards one at Kaduna, Northern Nigeria; a grant of £35,000 was made to the Sierra Leone Library Board towards the capital cost of a Central Library and headquarters building in Freetown; late in 1961, £27,000 was granted towards the establishment of a central and headquarters library at Ibadan, Western Nigeria. In all, during two and a half years, grants totalling £175,000 have been allocated for library development in seven centres.

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* *The British Council.* Annual Report, 1961–1962. Pp. vii+107+12 plates. (London: The British Council, 1962.) 2*s*. 6*d*. net.

There are now estimated to be some 60,000 overseas students in Britain and during the year the Council met and assisted 10,407 on arrival, of whom 5,127 were from the Commonwealth and 3,601 were colonial. Permanent accommodation was arranged for 320 in Council residences, 874 in university and other hostels or halls of residence, and 6,301 in lodgings, as well as transit accommodation on first arrival for more than 4,500 and temporary accommodation when away from place of study for nearly 7,000. Hospitality was arranged for and accepted by 13,547, more than 20,000 attended week-end courses, study visits and surveys, nearly 2,500 holiday courses of 7–15 days, more than 12,000 Council centres and 4,000 introduction courses. With the capital fund of £3 million which the Council is administering for the Government, a target of an additional 5,000 hostel places has been set, wherever possible by voluntary societies and other organizations, and 37 projects for hostel development (providing an additional 1,382 places) have been approved. There are also plans for some expansion of the Council's social and cultural centres for overseas students and trainees. About half the overseas students and trainees in Britain are studying some branch of science, technology or medicine and about half the advisory visits arranged by the Council overseas are concerned with these subjects.

Of 7,522 overseas visitors in Britain assisted by the Council (including 646 Colombo Plan trainees, 633 United Nations Fellows and 378 Scholars and 416 Teacher-Bursars under the Commonwealth Educational Co-operation), 1,030 were in medicine, 1,717 in science and technology and 1,535 in social science. Besides 73 university interchange visits (54 to Britain) arranged under the Commonwealth Scheme, 134 short visits were arranged under the Foreign Scheme (65 to Britain) as well as 4 longer teaching visits by guest professors and lecturers to university departments, and 99 visits of younger research workers between British and foreign universities (53 to Britain). In all 358 specialist tours, advisory visits and delegations were arranged during the year, of which 52 were in science and technology, 51 in medicine and 25 in social science, and the Council assisted in the selection of candidates for 113 scholarships offered to British students in 21 centres.

THE NATURE CONSERVANCY

THE thirteenth annual report of the Nature Conservancy for the year ended September 30, 1962[*], records a total of 100 National Nature Reserves with an acreage exceeding 190,000, and the Conservancy has thus completed the greater part of its original acquisition programme of some 140 reserves and an area of about 250,000 acres. Difficult acquisition problems are presented by some of the proposed reserves and completion may still be considerably delayed. However, these figures do not include some nine local and eleven forest nature reserves or two wildfowl refuges, many more of which will be required, but it is already clear that the programme set forth in 1947 in the report of the Wild Life Conservation Special Committee was fundamentally sound.

With the substantial progress in acquisition, the focus of effort shifts progressively from the acquisition of reserves and their rehabilitation to the formulation of comprehensive plans of management and the development of research projects, although the report refers to progress during the year in making management plans as disappointing. On the other hand, encouraging progress is reported in working co-operative arrangements, particularly with the new county naturalists' trusts which are emerging on a national scale as effective organizations for acquiring reserves and furthering conservation. A much wider spread of informed public interest is, however, desirable, although the launching of the British national appeal for the World Wildlife Fund has encouraged hopes of placing the voluntary movement on a firmer basis. Conservation requires large financial and practical support from voluntary sources and this requires education, guidance and self-restraint by citizens, which are imbued preferably through voluntary and elected bodies.

Nevertheless, despite encouraging indications of growing co-operation and such signs of progress as the issue of the Conservancy's first monograph, *Plant Communities of the Scottish Highlands* (see *Nature*, 196, 206; 1962), the striking advance in the amount of advice on specific problems, such as toxic chemicals and wildlife, provided for authorities and organizations concerned, a careful reading of the report suggests that the national contribution to conservancy is too small. The report itself admits to a difficult year financially. The grant-in-aid was £535,000, of which £465,000 was for general purposes and £70,000 for capital expenditure. Receipts amounted to just more than £14,000, but it was necessary to transfer £14,000 from the capital expenditure to the general expenses account, and the increase of £55,000 in the income for 1962–63 is almost entirely absorbed by rising salaries and general expenses. Expenditure on research, training and special surveys amounted to no more than £76,626, of which £33,782 was for grants and research and £22,149 for studentships and training grants, figures which compare with £65,414, £28,965 and £18,428, respectively, in 1960–61.

In this connexion, a sub-committee under the chairmanship of Prof. W. H. Pearsall, after reviewing the responsibilities of the Conservancy for grant-aided research, including the present status of biological research and of support for all aspects of ecology, concluded that the Conservancy's responsibilities should be broadened. This would permit stronger liaison with the universities, the desirability of which was emphasized by the Select Committee on Estimates in its 1957–58 report, and would provide greater and more flexible support for research into natural biological resources and the fundamental aspects of biology underlying it. The Conservancy has accepted this recommendation in principle, but its implementation depends on the outcome of other current reviews of Government support for biological sciences. Eight new grants were approved during the year and ten existing grants renewed or extended. Provision for research studentships is also at present under review; fourteen awards were made during the year and thirteen applications for third-year extensions were approved. Six awards were again made for the one-year diploma course on conservation.

A feature of the report is the increasing attention given to international and overseas activities. The Conservancy is an active member of the International Union for the Conservation of Nature and Natural Resources, and besides reporting on meetings of the Commissions on Ecology and on Education of that body, the present report includes notes on the first world conference on national parks at Seattle, the International Council for Bird Preservation, the thirteenth International Ornithological Congress, the fifteenth Limnological Congress, and an international conference in May 1962 at which the feasibility was agreed of an International Biological Programme on the biological basis of productivity and human welfare.

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[*] *The Nature Conservancy.* Report of the Nature Conservancy for the year ended 30th September, 1962. Pp. viii+153+15 plates. (London: H.M.S.O., 1962.) 10s. net.

Some thirty pages of the report are now occupied by accounts of scientific research in progress at the Merlewood Research Station and Moor House Field Station, the Furzebrook Research Station, the Monks Wood Experimental Station, the Speyside Research Station, the Bangor Research Station and in Scotland. Botanical studies on low-level woodlands embrace studies of nutrient cycles in oak woodlands, those of the invertebrate fauna of woodlands, the nutrient and energy-cycles in woodlands and the effect of management on invertebrates. The establishment of trees on Pennine moorland, woodland mycology and chemical studies on organic matter all received attention. Other investigations were concerned with moorland plants, the ecology of southern heaths, coast physiography and ecology, climatology and hydrology, sheep-grazing and vegetation, the productivity of mountain grasslands and biometrics. In Scotland, besides botanical research on woodlands, the assessment of the vegetational productivity of Rhum continued, as did zoological work on the red grouse, a study of the food of the red deer and detailed studies of the grey seal breeding colonies in Orkney and North Rona.

The Scientific Advisory Services were concerned with the factors to be taken into account in afforestation of national parks, and, besides the monograph already noted and a second which has now been sent to press, they were also concerned with publication of a new edition of the *Flora of the British Isles* and the *Atlas of the British Flora*. The Conservancy is represented on the Committee on Hydrological Research by Dr. E. B. Worthington, and in the planning and execution stage of the proposed catchment area research a member of the Conservancy's staff will be attached to the Hydrological Research Unit. The Conservancy has also co-operated in the West Midlands Inland Water Survey, and in April 1962 it issued an important statement of policy on the introduction of species, affirming the importance of wider public recognition of the basic principle that in any area the integrity of the natural fauna and flora is in itself a vital natural resource, the conservation of which ought to be an object of national policy.

Nearly all the National Nature Reserves in Scotland, more than half of those in England, and a third of those in Wales are now being managed under approved plans, and about half a dozen of those prepared more than five years ago are being revised for the next quinquennium. The Conservancy is convinced of the need for a concerted approach by land-using interests to the problems involved in making the best use of the hill ground of Scotland—the need for much more fundamental and applied scientific research is stressed. Steps have been initiated to review existing knowledge, work in progress, identify gaps and build up efforts into a comprehensive programme. Following a study of the status, amenities and scientific interest of about thirty-eight surviving named areas of the Norfolk Broads in a survey of the impact of contemporary developments, the Conservancy is anxious to initiate research into problems of pollution in the Broads and towards a better understanding of water movements, tidal effects and rate of mud deposition on water depths and marginal effects. It is seeking to organize a wardening service in Snowdonia based on voluntary agreement with safeguards for landowners and tenants, the National Park authorities, public access and the Conservancy, and it has also recently expressed concern about possible disturbances due to the use of 'hovercraft' over tidal flats, shallow lagoons and rivers and marshes. The Ministry of Transport and the Ministry of Aviation have agreed to keep the Conservancy informed regarding permits to fly hovercraft and proposals for legislation. Full agreement was also reached with the Post Office to find an alternative route for a submarine cable which it was proposed to bring ashore on the Norfolk coast and which threatened scientific interests. Some concern is again expressed regarding damage and litter on reserves and wildfowl refuges which present serious problems.

POSSIBLE APPLICATION OF THE MASS-ENERGY CYCLE ON A MICRO- AND MACRO-SCOPIC SCALE IN THE UNIVERSE

By J. L. HUGHES

Department of Physics, University College of North Wales, Bangor

ONE form of the mass-energy cycle is shown in Fig. 1. Experimental support for the cycle comes from the fact that all known particles of lower energy may be created from a 10-BeV electron. The only particle interactions allowed by the cycle which have not been observed experimentally are the build-up and decay of photons although these are not essential to the concept when applied on a macroscopic scale.

It will be assumed that the cyclic reactions occur in the positive Dirac states and since the negative states are normally filled the creation of antiparticles will be considered as abnormal events in the universe. (Dipping into the negative Dirac states could nevertheless provide an important and convenient means of approaching the ideal equilibrium ratio under abnormal conditions such as those existing in a cosmic ray air shower. For example:

primary particle→
x_1 secondary particles and antiparticles→
x_2 secondary particles ($x_2 > x_1$).)

The basic postulate of the present work is as follows.

In any region of the universe the ratio of mass to energy will always tend to a constant value. That is:

$$\frac{\text{Mass}}{\text{Energy}} \rightarrow \text{const.}$$

(This refers to the ratio of the amount of mass and energy in any region and not the Einstein equivalence ratio of $\frac{1}{c^2}$.)

If this constant be put equal to unity, then: (*a*) in any region of the universe where mass/energy > 1 the conversion of mass into energy will be favoured; (*b*) in any region of the universe where mass/energy < 1 the conversion of energy into mass will be favoured.

Due to the large velocity of photons and the mutual gravitational attraction of the mass $> o$ particles, it is unlikely that a stable equilibrium would ever be attained locally, the fluctuations about the ideal mass/energy ratio representing the mass-energy cycle.

Eddington[1] objected to the cyclic concept since it was not consistent with the second law of thermodynamics in an expanding universe. Applying the cycle on a macroscopic scale, however, it can be seen that the observed expansion of the universe turns out to be only an apparent one. It is true that clusters of galaxies move away from each other but this is so only for their mass content. The equally important photon content actually move towards each other into interclusteral space cancelling the observed expansion. The photons are then transformed in accordance with *b*, while the mass $> o$ particles in the clusters themselves condensing under mutual gravitational attraction transform according to *a*.

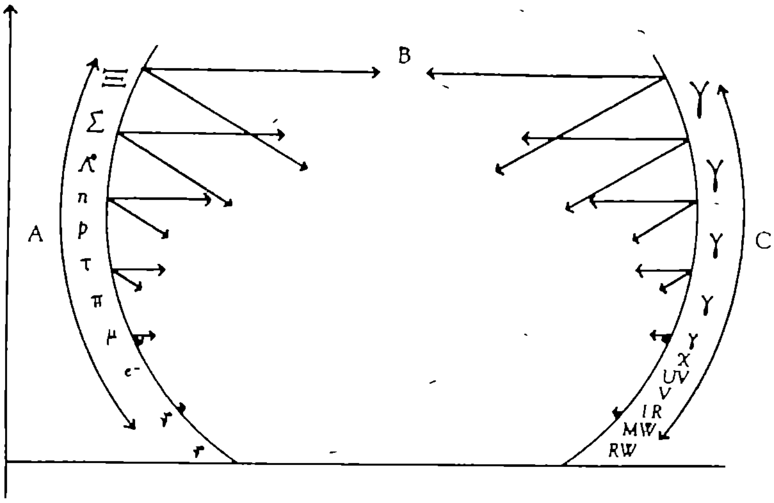

Fig. 1. One form of the mass–energy cycle. *A* and *C* represent the build-up and decay of mass and energy, respectively, and *B* their interconversion
Ξ, Σ, Λ°, hyperons; *n*, neutron; *p*, proton; τ, tau meson; π, pi meson; μ, mu meson; e^-, electron; ν^0, neutrino. Photons: *RW*, radio-wave; *MW*, micro-wave; *I.R.*, infra-red; *V*, visible; *UV*, ultra-violet; *X*, X-ray; γ, gamma-ray

Any sufficiently large volume *V* of the universe would lose particles by the outward flow of photons and clusters of galaxies but gain by the inward flow of particles from the surrounding space, these eventually forming new clusters in accordance with *b*, in the interclusteral regions of *V*.

The density of matter in *V* would on the average be constant both in space and time and it could be considered as an isolated system involving thermodynamically perfectly reversible reactions between mass and energy, basic postulate. The universe based on the mass-energy cycle would thus satisfy the second law of thermodynamics, its entropy remaining constant.

Possible experimental proof of the basic postulate. According to the present postulate the ratio of mass to energy in any region of the universe tends to a constant value. By analogy with other equilibrium systems it has been assumed that the greater the departure of the mass/energy ratio from the ideal value the more unstable does the matter in the region concerned become.

The electron offers a means of verifying the postulate experimentally since above 1 MeV it travels at essentially the velocity of light, any further gain appearing as mass. Under these relativistic conditions the dimensions of the electron decrease as its mass increases, hence its mass density becomes extremely large. Infinite mass is prohibited by the postulate so that the electron eventually would have to reach a critical density after which it becomes unstable and transforms in accordance with *a*. The critical density is likely to be attained sooner in an environment predominant in mass than one predominant in energy such as the envelope of a supernova. It is of interest to note that electrons of 10^{12} eV have been observed in supernovæ via their instability, while no electrons of primary origin greater than 10^{10} eV have been observed in cosmic rays striking the Earth. The linear accelerator at Stanford producing 50-BeV electrons will probe this apparent gap in the electron spectrum at a beam current of 100 microamps, representing close packing of electrons which may well have attained the critical density and become unstable.

The postulate results in the stability of a particle being dependent on its individual characteristics and those of its surroundings. Assuming all else to be constant with only a change in environment, differences should occur in the mean life-times of particles such as mesons and hyperons if the postulate is correct. A relatively large spread in mean life-times of such particles does exist which may be within experimental error.

The observational consequence of the cyclic concept would result in distant galaxies being visible only in the radio frequency range since the more energetic photons would have transformed *en route* (*b*), thus also satisfying Olbers' paradox.

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[1] Eddington, Sir Arthur, *The Nature of the Physical World*, 81 (Dent and Sons, 1938).

SLOWLY VARYING COMPONENT OF SOLAR RADIO EMISSION

By Dr. M. K. DAS GUPTA and D. BASU
Institute of Radio Physics and Electronics, University of Calcutta

IT is well established that the solar radio emission can be broadly classified as: (1) a fairly constant basic component (quiet sun emission—thermal origin); (2) highly variable components of different types (disturbed sun emission—non-thermal origin); (3) a distinct type of slowly varying radiation only at centimetre and lower decimetre wave-lengths. The last one shows a slow day-to-day variation in intensity closely correlated with sunspot numbers exhibiting roughly a 27-day recurrence period, and as such is usually called the slowly varying component of solar radio emission. The close association of the centimetre radiation from the Sun with the sunspots was first detected by Covington[1] at a frequency of 2,800 Mc/s. Subsequent observations by other workers[2–5] at different frequencies showed the same effect. It was revealed that this characteristic type of solar radio emission is limited to a certain range of frequencies. But as yet this range is not clearly defined, and the two limiting frequencies beyond which the slow variations are undetectable are not precisely known. However, according to Hachenberg[6], this component extends over the frequency-range of 500 Mc/s–30,000 Mc/s, while according to Pawsey and Smerd[7] the range is 500 Mc/s–10,000 Mc/s. Moreover, the frequency at which the correlation between the slowly varying radio emission and the relative sunspot numbers would be a maximum, if there be any, is not clearly known. We were thus prompted to examine these aspects of the slowly varying component of solar radio emission in relation to recent data.

In our analysis we first plotted the daily mean flux of solar radio emission at five different frequencies—9,400, 3,750, 2,800, 2,000 and 1,000 Mc/s for the International Geophysical Year period (July 1957–December 1958). Fig. 1 shows these plots at four different frequencies along with the plot of Zurich relative sunspot numbers R_Z for the same period. All the five curves show substantially similar variations and indicate pronounced maxima and minima following roughly the so-called 27-day cycle. The close correlation between the daily mean flux with the relative sunspot number is also quite clear. An additional striking feature exhibited by the daily mean flux curves is that the pronounced maximum to minimum amplitude of this variation changes rather systematically with frequency. This phenomenon led us to examine the matter

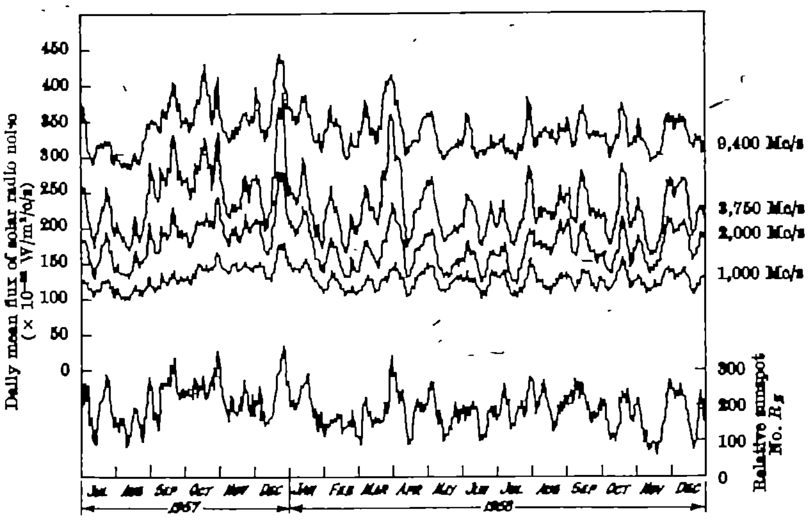

Fig. 1. Plots showing the variation with time of the daily mean flux of solar radio noise at frequencies 9,400, 3,750, 2,000 and 1,000 Mc/s together with that of the Zurich relative sunspot numbers for the International Geophysical Year period (July 1957–December 1958)

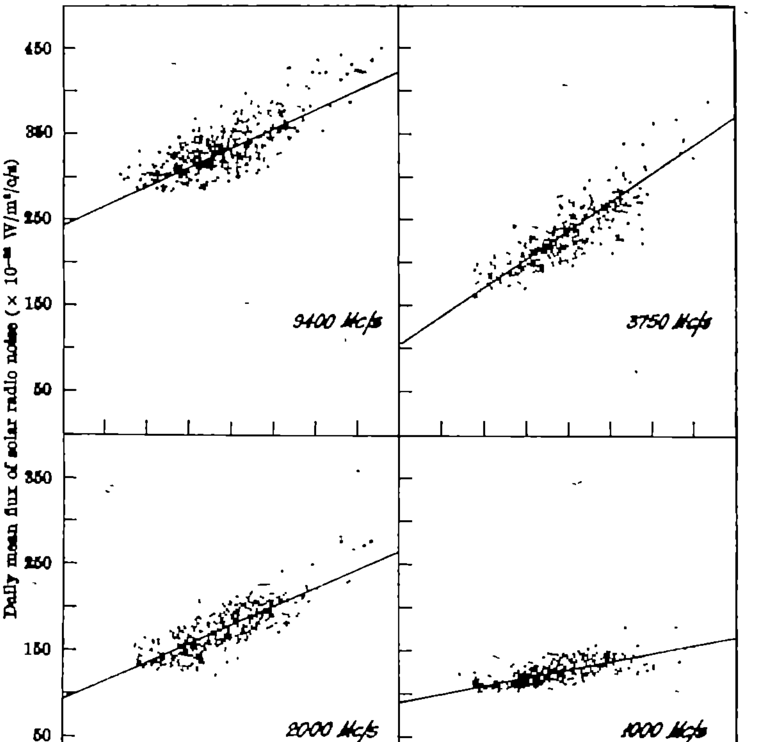

Fig. 2. Scatter diagrams showing the correlation between observed daily mean flux of solar radio noise, at frequencies 9,400, 3,750, 2,000 and 1,000 Mc/s and the Zurich relative sunspot numbers for the International Geophysical Year period. The corresponding regression lines are also indicated in the diagrams

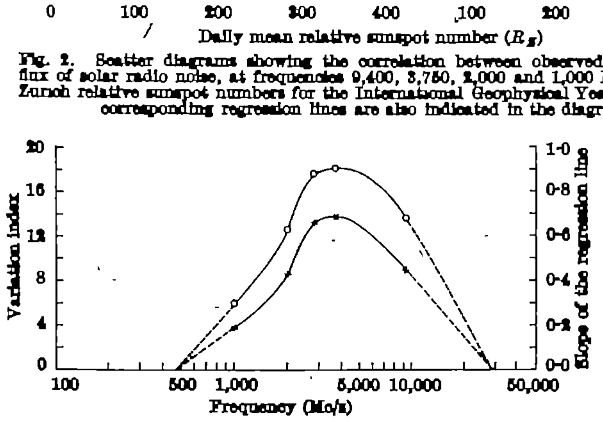

Fig. 3. The curves showing the relation of the variation index (○) and the slope of the regression line (×) with frequency

in some detail and as such a variation index had to be adopted. For any particular frequency, the changes in amplitude between the pronounced maxima and the corresponding minima showing roughly the 27-day cycle were first carefully measured from the daily mean flux curve for the entire period under study. The average value of these changes has for convenience been termed the 'variation index' for that frequency. The variation indices for the different frequencies as mentioned here were thus calculated separately.

Secondly, scatter diagrams showing the correlation between daily mean flux of solar radio emission and the relative sunspot numbers were drawn for all these different frequencies as shown in Fig. 2. (The corresponding diagram for 2,800 Mc/s drawn separately is not shown here.) The respective regression lines are also indicated in these diagrams. A very striking feature as revealed from these diagrams is that the slope of the regression line also changes systematically with frequency. The slopes in the different cases were calculated.

Fig. 3 shows a plot of the variation index as already mentioned (upper curve) and that of the slope of the regression line (lower curve) versus the frequency. It is interesting to note that both these curves are identical, showing a maximum near about 4,000 Mc/s. It may be logically assumed that both the variation indices as well as the slopes of the regression lines will have uniform distributions as shown by the continuous lines through the two sets of points. On extrapolation, the continuous curves in both the cases are found to intersect the frequency axis at 470 Mc/s on the low-frequency side and near about 29,000 Mc/s on the high-frequency side. Hence our analysis led us to draw the following conclusions regarding the slowly varying component of solar radio emission mainly in relation to the frequency:

(1) The two limiting frequencies for which both the variation index as well as the slope of the regression line will be zero are 470 Mc/s and 29,000 Mc/s. These are, therefore, the two frequency limits beyond which the characteristic slow variations of solar radio emission will ultimately subside.

(2) The correlation between the daily mean flux of solar radio emission and the relative sunspot numbers will be maximum at a frequency near about 4,000 Mc/s.

(3) The variation index as already mentioned will be roughly proportional to the slope of the regression line and hence to the correlation. The index, therefore, might conveniently be used as a measure of the correlation between the two phenomena.

It should be noted in connexion with the limiting frequencies as already mentioned that so far as the low-frequency side is concerned the experimental observations at 450 Mc/s at Boulder do not show the presence of the typical variations, but Lehany and Yabsley[3] observed these at 600 Mc/s. On the high-frequency side, however, the data are insufficient for any definite conclusion. Nevertheless, it appears from our analysis that the estimates of Hachenberg[4] as regards the high-frequency cut-off is a better approximation than that given by Pawsey and Smerd[7].

We thank Prof. S. K. Mitra and Prof. J. N. Bhar for helpful discussions. One of us (D. B.) thanks the C.S.I.R., Government of India, for financial assistance. Data used in our analysis were obtained from International Geophysical Year data published by the Science Council of Japan, and *CRPL-Solar Geophysical* data books.

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DEMONSTRATION OF THE PRODUCTION OF A NEW TYPE OF REMANENT MAGNETIZATION: INVERSED TYPE OF THERMO-REMANENT MAGNETIZATION

By TAKESI NAGATA, MINORU OZIMA and MITUKO YAMA-AI
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It is conventionally understood that thermo-remanent magnetization (TRM) is produced by cooling a ferromagnetic body from a certain high temperature in a magnetic field[1].

However, in the course of an investigation of the magnetic behaviour of magnetite at low temperatures (from liquid nitrogen temperature to room temperature), we found that a fairly intense and stable remanent magnetization was acquired by heating magnetite grains from − 150° C to room temperature in a moderate magnetic field (10 oe). This is quite striking, since a heating process is usually understood to loosen a magnetic domain configuration while magnetic domains are 'frozen in' in a cooling process. The remanent magnetization thus acquired is tentatively called here an inversed type of thermo-remanent magnetization or ITRM, as the direction of the temperature change in which the remanent magnetization is acquired is just opposite to the case of an ordinary type of thermo-remanent magnetization. This article presents some characteristics of the ITRM as well as the qualitative interpretation of the acquisition mechanism.

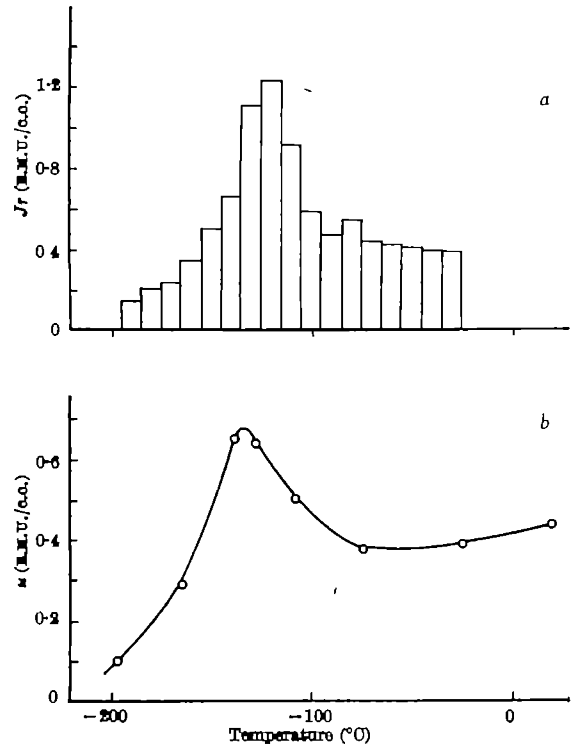

Fig. 1*a*, Intensity spectrum of partial ITRM's produced over a temperature range of 10° C at $h = 10$ oe; *b*, temperature change of susceptibility of magnetite measured at $h = 10$ oe

Table 1

Remanent magnetization	Temperature-range in which remanent magnetization is acquired	Direction of temperature change in which remanent magnetization is acquired	Intensity of the remanent magnetization measured at room temperature ($h = 10$ oe)
IRM	Isothermally at room temperature	Isothermal	0 51 E.M.U./c.c.
TRM	Curie point to room temperature	Cooling	7 27 E.M.U./c.c.
ITRM	−150° C to room temperature	Heating	3 61 E.M.U./c.c.

Experiments were carried out with synthesized stoichiometric magnetite (Fe_3O_4) of which grain sizes range over several tenths of a millimetre. Magnetic intensity measurements were made with a ballistic magnetometer. The magnetite grain were packed in a glass tube (diameter 0·2 cm; length 2 cm) for measurement. In Table 1 some characteristics of ITRM are compared with those of isothermal-remanent magnetization (IRM) and thermo-remanent magnetization. The intensity of ITRM is about one-half of TRM produced at the same magnetic field. Fig. 1*a* shows the intensities of partial ITRM's, each of which is produced by heating the sample over a temperature-range of 10° C at a d.-c. field of 10 oe and afterwards heating to room temperature in zero magnetic field. From the figure the maximum production of the partial ITRM is clearly seen to lie at about −130° C. In order to examine the stability, all the partial ITRM's were demagnetized by an a.-c. field (Fig. 2). In the same figure the a.c. demagnetization curves of an IRM and an ordinary type of TRM produced by field-cooling from the Curie point to room temperature at $h = 10$ oe are also shown for comparison. It is to be noted that the partial ITRM produced at the temperature-range of −125° C to −115° C is as stable against a.-c. demagnetization as TRM, while the stability is much poorer in the case of the ITRM's produced at higher temperature-ranges. Both experiments clearly show that a fairly intense and stable remanent magnetization is acquired by field-

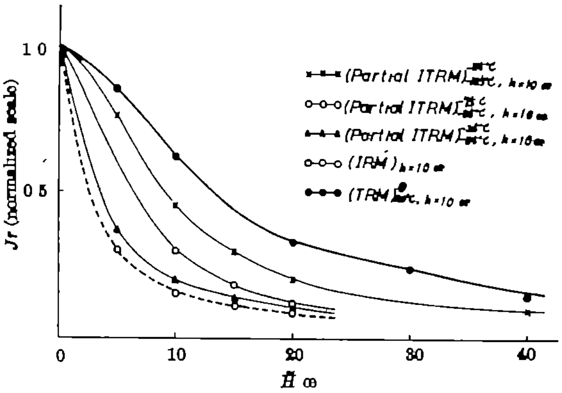

Fig. 2. a.-c. field demagnetization of partial ITRM's IRM and TRM

heating over a relatively narrow range of temperature, that is, from −150° C to −120° C.

The acquisition mechanism of ITRM can be explained if we take account of the marked change of the crystal anisotropy constants of magnetite at low temperature. It is well established that the crystal anisotropy constants of magnetite vanish at −143° C (ref. 2). Fig. 1*b* shows the temperature variation of the susceptibility of magnetite measured at $h = 10$ oe. The marked increase of the susceptibility at about −140° C is due to the sharp change of the crystal anisotropy constants from finite values to zero for this temperature. As inferred from the susceptibility–temperature curve the domain walls would be largely displaced under the influence of a magnetic field to result in the marked enhancement of the magnetization at this temperature. If the temperature is then raised while the magnetite grains are still subjected to the magnetic field, the rapid change of the crystal anisotropy energy with increase of temperature tends to constrain the domain walls to the displaced positions at lower temperatures. After the removal of the magnetic field at room temperature we observe a fairly intense and stable remanent magnetization, that is, the inversed type of thermoremanent magnetization.

It should be noted here that any ferromagnetic body of which coercivity is mainly governed by the crystal anisotropy energy may acquire a stable remanent magnetization in a process of a temperature change in a magnetic field, provided that the temperature change (irrespective of the direction) causes a sharp increase of the crystal anisotropy energy. This argument is strengthened by a further observation on cobalt chips. Cobalt (Curie point = 1,115° C) is known to have crystal anisotropy constants which vanish at about 250° C (ref. 3). A fairly intense and stable remanent magnetization was observed at room temperature by cooling cobalt chips from 250° C to 200° C at $h = 5$ oe and afterwards cooling to room temperature in zero magnetic field. A detailed report of this result will be given elsewhere.

Finally, in connexion with rock magnetism it is interesting to speculate that the ITRM might be responsible for the origin of the stable natural remanent magnetization observed in some stony meteorites[4], as the environmental temperature in which meteorites were held before the entry into the Earth's atmosphere is suggested to be very low[5].

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[See also p. 476 of this issue of *Nature*.]

FRESH NATRO CARBONATITE LAVA FROM OLDOINYO L'ENGAI

By DR. C. G. B. Du BOIS, J. FURST, DR. N. J. GUEST and D. J. JENNINGS

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OLDOINYO L'ENGAI has the distinction of being the only active volcano in East Africa. It forms a striking steep-sided cone rising to 9,443 ft. at the south end of Lake Natron in northern Tanganyika in the Rift Valley.

Immediately south of L'Engai lies the carbonatite volcano Kerimasi. The petrography of both, together with that of rocks collected during geological mapping in the surrounding area, including the volcanoes of Mosonik and Shombole north of L'Engai, indicate that all occur in a petrographic province characterized by richness in sodium and calcium. Among the rock types described are phonolites, nephelinites, melitite-basalt, rocks of the ijolite–melteigite–urtite series and biotite–pyroxenite. The presence of fenites and tveitåsites at L'Engai suggested that it may have a carbonatite at its heart[1].

Around the summit of the cone is a white mantle of 'soda' which increased in extent immediately after an eruption in 1954, when the upper reaches of the volcano and the interior of the latest crater resembled a snow-scape[2]. At that time a cone of dark lava grew on the newer crater floor but has now almost disappeared partly due to weathering and partly owing to subsequent fairly regular eruptions involving conelet formation.

The 1954 eruption at L'Engai was studied by Guest[2]. A specimen of soda crust collected shortly after an eruption was found to contain about 50 per cent of material insoluble in water of which 70 per cent was identified as calcium carbonate by J. R. Harpum, adding support to the idea that the volcano may overlie a carbonatite. In two samples of soda crust the Geological Survey Laboratories in Tanganyika determined the two major soluble salts as sodium carbonate 23·6 per cent and 40·2 per cent, and sodium carbonate 3·4 per cent and 8·5 per cent, respectively.

In August 1961, L'Engai was climbed by a party of six, three of whom descended several hundred feet, mostly on soda-covered ash, on to the floor of the inner crater. Guest observed that the latter had changed considerably since 1954. Several new cones were present, all eroded to a greater or lesser degree, and small flows on the crater floor showed many of the classical structures characteristic of lavas. Some activity was in progress, ash and boulders being ejected from at least one vent while fumaroles and solfatara were vigorously in action elsewhere. Rock samples collected varied from fresh, hot dark lava types to paler-coloured not so fresh types. To avoid further exposure to the atmosphere the samples were immediately sealed in stout polythene containers for transporting to the laboratory.

The fresh rock is soft, dark grey, holocrystalline medium- to fine-grained and granular in texture. On exposure to the air the surface becomes white within two days. Left for a few weeks it crumbles to a damp white powder which X-ray analysis shows consists of a mixture of calcite and trona. Thin sections were prepared in the usual manner except that paraffin was used throughout in place of water because some 40 per cent of the rock consists of water-soluble carbonates. The thin sections were examined by Du Bois.

The chief mineral present appears to be a new carbonate similar in some respects to the artificial double carbonate of calcium and sodium described by Winchell[3], but showing some important differences. It is colourless, highly birefringent, length-slow, uniaxial negative and occurs as euhedral laths 0·25–1 mm long having a length/breadth ratio of between 6 : 1 and 3 : 1. Some basal sections show complex multiple twinning reminiscent of leucite. Cloudy rounded crystals of comparable size yield a similar X-ray pattern and may represent a previous generation of the same mineral which has undergone partial alteration and magmatic abrasion. Black dust and minute grains, possibly of pyrite, are present interstitially and also as inclusions. Purple fluorite is present as tiny crystals in some sections, and X-ray study of acid-insoluble residues of some lava specimens shows up to 1·4 per cent calcium fluoride although no fluorine was detected in the particular samples analysed. Flow textures are developed locally, the lathy crystals having sub-parallel arrangement. Some voids occur between boundaries of adjacent crystals and account for the differences in the true and apparent specific gravity of the rock.

Chemical analyses of two samples of fresh lava are given in Table 1.

Table 1

	*L*1	*L*4
SiO_2	1·18	1·12
R_2O_3	1·64	1 70
MgO	1·48	2·35
CaO	19·09	17·52
BaO	1·05	1·02
SrO	0·89	0 85
Na_2O	29·00	30·00
K_2O	6·90	7·50
H_2O	1·81	1·91
CO_2	31·98	30·73
Cl	2·07	3·03
SO_3	2·79	2·88
S	0 08	0·13
	99 91	100·74
Less O for S and Cl	0·57	0·75
Total	99·34	99·99

Analyst: J. Furst

Specific gravity of *L*1, 2·38 (apparent); 2·51 (true); 2·55 (calculated).

*L*1: Natro-carbonatite collected from uppermost flow on the south-westerly side of a cluster of cones on the east side of crater floor.

*L*4: Natro-carbonatite collected very hot from lava flow beneath *L*1.

These can be compared with results obtained by von Knorring and Du Bois[4], who described a carbonatitic lava from Uganda containing about 13 per cent silica. It is of particular interest that both samples contain less than 2 per cent silica and 78–80 per cent total calcium oxide, sodium oxide and carbon dioxide. Both samples have high barium and strontium content, which is characteristic of carbonatite and certain related rocks[5–7]. The water content of 1·8–1·9 per cent is very considerably less than that reported elsewhere[8], and part or all of this may in fact be hygroscopic water drawn from the atmosphere during crushing of the samples for analysis.

In order to calculate the composition of the lava from the chemical analysis using only known minerals, the need arises to include 6–8 per cent of lime. Assuming for the moment that modal lime is present in the fresh lava an explanation for its existence could be provided by postulating the partial breakdown of a complex calcium–sodium carbonate to yield lime and some carbon dioxide. Winchell[3] mentions that $Na_2Ca_2(CO_3)_3$ fuses at 812° C in an atmosphere of carbon dioxide; but if an attempt is made to synthesize this compound by mixing together stoichiometric proportions of anhydrous sodium and calcium carbonates, and heating to that temperature in air, the result is a loss of some carbon dioxide and a white residue which shows strong CaO lines when an X-ray powder photograph is taken. Photographs of fresh L'Engai lava, however, show no such lines, neither are there any present attributable to calcium hydroxide. Other tests, both physical and chemical, also fail to reveal the presence of calcium oxide, hydroxide or bicarbonate in the fresh rock. The inference is that the lava was formed under PT conditions which very probably included an atmosphere of carbon dioxide which, in turn, permitted the formation of a new carbonate mineral. This is believed to be a complex carbonate of calcium and sodium with perhaps some potassium. Because of its rapid alteration to calcite and trona by atmospheric water and carbon dioxide its existence has probably escaped notice in the past.

In view of the extraordinary nature of this new lava[7] it is proposed to call it 'natro-carbonatite'.

We thank Dr. William Pulfrey, Commissioner, Mines and Geological Department, Kenya, for advice, and Mr. P. Wentzel for assistance with transport in 1961.

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ACTIVATION ANALYSIS OF PAPER CHROMATOGRAMS FOR IODINE ($^{127}I \rightarrow {}^{128}I$)

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IN recent investigations of the iodoamino-acids extractable from human serum it became important to ascertain whether the ceric–arsenite method's indication of iodine (iodine-127) in the iodotyrosine spot really depended on iodine rather than on some unexpected artefact. Activation analysis of the chromatogram's spots was therefore performed, the paper being activated directly[1–3]. It soon emerged that such an analysis of paper required a stringent post-irradiation purification to eliminate from the sample the other more active radiochemical products also activated in the paper. The method finally evolved for this purpose is given here, as it may be of interest to other workers.

The chromatograms, prepared from extracts of human sera, as described elsewhere[5], were run in duplicate along with some blank strips. Preliminary staining of one chromatogram with ceric–arsenite provided a guide for cutting off the appropriate areas from the other strip; pieces up to 6 sq. in. could be used. From the blank strips, areas of similar size and distance from the origin were cut, one to be used as a standard after spotting with standard potassium iodide, and the other as a blank. All the papers were then sealed in polythene envelopes and placed together in aluminium screw cans, each of which contained two or three papers, usually a chromatogram spot and a standard. Blank strips were also included.

A series of such cans were inserted in the pile at a thermal neutron flux of 10^{12} n/cm²/sec, and irradiated for either 1 or 0·5 h. Our evidence given in this article now suggests 0·5 h as the preferable time. Iodine-128 (half-life = 25 min) was produced by an (n, γ) reaction on iodine-127.

In preliminary experiments[1], the Medical Research Council cyclotron had been used to produce a neutron beam by means of the beryllium-9 (d, n) beryllium-10 reaction, the fast neutrons produced being partially thermalized by a block of paraffin wax. However, although similar activation has been obtained, it was at a varying and insufficient activity due to the lower and less constant neutron flux (approximately 10^{10} n/cm²/sec).

Radiochemical separation (to separate radioactive iodine from other more abundant radioisotopes produced):

After preliminary trials had found that simpler methods, including that of Bowen[4], were inadequate for this purpose, the following method was evolved: (*a*) On removal,

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* Wellcome Trust Research Fellow.

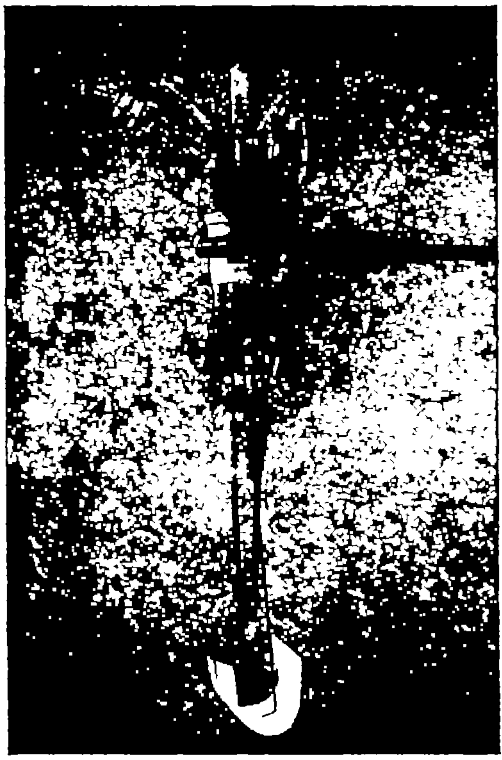

Fig. 1. Ignition head: 1, exhaust tube; 2a, electrode; 2b, electrode; 3a, to pressure gauge; 3b, to pressure gauge; 4, to oxygen cylinder; 5, fuse wire

each paper was spotted with 0·1 ml. of a concentrated 'carrier' solution containing 5-mg each of Cl⁻, Br⁻ and I⁻.

(*b*) A preliminary combustion of the paper was then carried out in a modified Shöniger flask in order to destroy the excess of reducing substances, unless the area of paper was less than 1 sq. in. The paper was fixed on a fuse wire and the ignition head (Fig. 1) was then placed in a 500-ml. flask, in the bottom of which had been placed a small beaker with 2 ml. saturated sodium hydroxide. By means of the exhaust tubing the flask was then evacuated (to < 100 mm mercury), and then filled with oxygen to 360 mm mercury, when ignition through the fuse from a 6-V battery could follow safely. After settling for 3 min, the flask was opened and the ignition head washed down with 10 ml. water and removed. After shaking, the flask was ready for use in the next step.

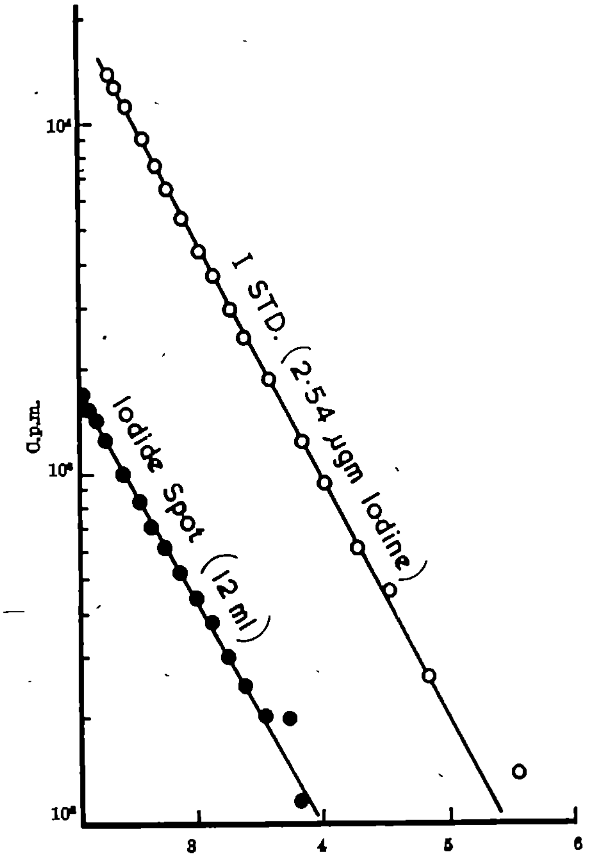

Fig. 2. Activation analysis of chromatogram paper after activation for 0·5 h: iodide spot and its standard from normal serum

Table 1. Recoveries and Times for Steps in Radiochemical Separation

Step	Iodine-131 Recoveries Alone per cent	Iodine-131 Recoveries Cumulative	No.	Bromine-82 losses	No.	Time (min)	
Preliminary combustion	87 (82–93)		8	99·87 (99·86–99·89)	4	8–10	42–49
Digestion	99 (99–100)	87 (82–93)	10			9–10	
Distillation	98 (95–100)	85 (80–91)	5			15–17	
Silver iodide precipitation	99 (98–100)	84 (79–90)	10			10–12	
Sodium iodide supernatant	98 (95–100)	84 (79–90)	2+				

Table 2. Sensitivities of Some Published Methods of Activation Analysis contrasted with that of the Cerio-Arsenite Method

	Author	Chemical separation method	Counting	Time from activation to counting	Neutron flux	Sensitivity (g iodine)
Activation analysis	Bowen (ref. 4) (whole blood)	Acid digestion ($H_3PO_4+HNO_3$)→ distillation and CCl_4 extraction AgI ppt for counting	G.M.	1 h for 6 samples	10^{12} n/cm²/sec	10^{-10}
	Kellershohn *et al.* (ref. 5) Whole blood, plasma, serum, extracts, PBI.	(a) Acid digestion ($CrO_3+H_2SO_4$)→ distillation, CCl_4 extraction AgI ppt for counting (b) Pre-activation separation with subsequent CCl_4 extraction	G.M. γ-spectrom. with scin. crystal	30 min Approx. 20 min	6 × 10^{12} n/cm²/sec ,,	10^{-10} 10^{-9}
	Stärk (ref. 6) Serum paper chromatogram	CCl_4 extraction and AgI ppt.	2π G.M. flow counter	50 min	4 × 10^{12} n/cm²/sec	10^{-9}
	Dimitriadou *et al.* (ref. 2) Serum paper chromatogram	(Method described) (See this text)	Well scin. counter	50 min	10^{12} n/cm²/sec	10^{-9}
Cerio-arsenite	Bowden *et al.* (ref. 7) Paper chromatograms		Densitometry			1–10 × 10^{-9}
	Dimitriadou *et al.*		Densitometry			6 × 10^{-9}

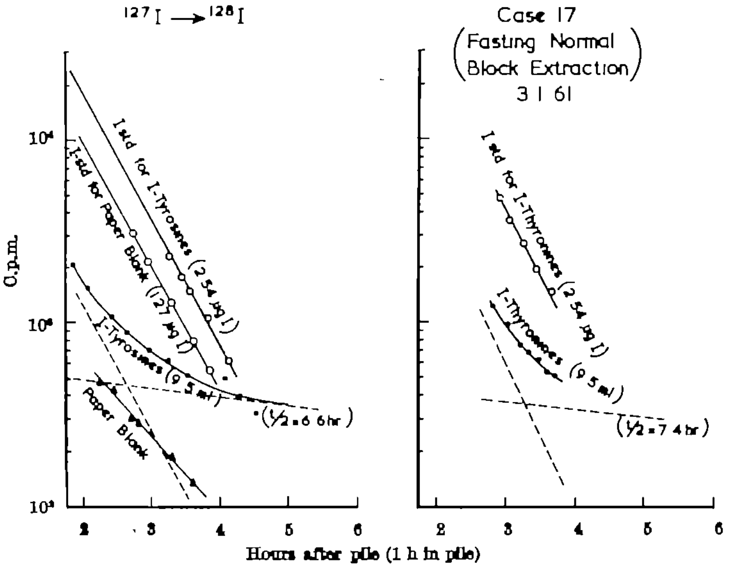

Fig. 3. Activation analysis of chromatogram paper after activation for 1 h: iodotyrosine and iodothyronine spots from extract (refs. 9a and b) of normal serum, with paper blank and standard

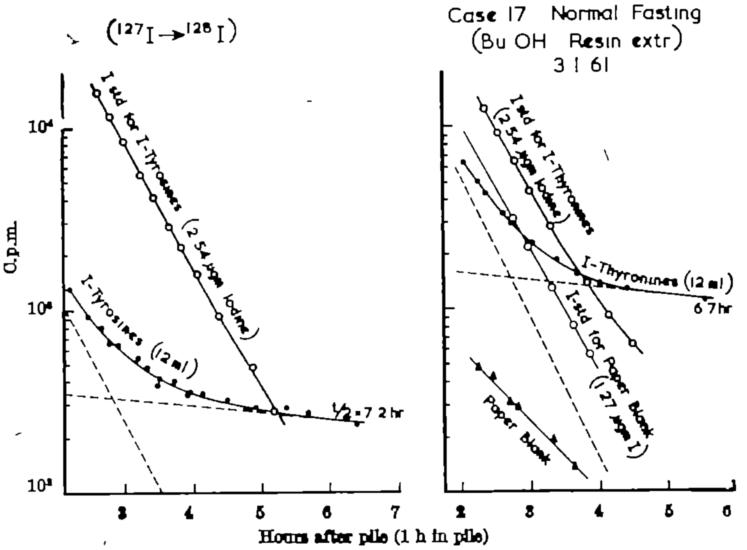

Fig. 4. Activation analysis of chromatogram paper after activation for 1 h: iodotyrosine and iodothyronine spots from resin extract (ref. 3) of normal serum, with paper blank and standard

(c) Acid digestion was then carried out by adding 6 or 12 ml. 60 per cent chromium trioxide and 25 or 50 ml. 70 per cent sulphuric acid and heating to 190° C, and repeating this after cooling to 160° C.

(d) Distillation followed in a Chaney-type still; after adding 10 or 20 ml. phosphorous acid, distillation was continued for 15 min at 140°–150° C, a few ml. of hydrogen peroxide being added during this phase to facilitate transfer. The iodine evolved was trapped in 1 ml. 2 N sodium hydroxide and 2 ml. of 10 per cent sodium sulphite.

(e) Final purification of the distillate was effected first by precipitation as silver iodide by adding 1 per cent silver nitrate at 60° C after acidification with sulphuric acid, then by centrifugation, washing and drying the precipitate with acetone, followed by the addition of 11 ml. 10 per cent sodium sulphide, mixing and centrifuging and counting 10 ml. aliquots of supernatant.

The radioactive iodine was assayed in a well-type scintillation counter; a pulse height analyser, if available, would have improved the sensitivity of the assay. Preliminary experiments had shown that the counting efficiency was approximately equivalent whether an end-window Geiger–Müller counter was used for β-counting or a well-type scintillation counter for γ-counting, the counting efficiencies being estimated as 13·5 and 8·5 per cent respectively. Each sample was assayed to at least a 3 per cent statistical accuracy at frequent intervals, and the decay curves plotted in order to check radiochemical purity (see Figs. 2–4). Several of these curves had to be resolved into two components, but not those obtained after only half an hour's activation. Final estimates of the amount of iodine-127 were made by direct comparison of the unknown with the standard irradiated with it.

Some sample results are shown in Figs. 2–4. Note the single decay line after activation for 0·5 h (Fig. 2), and also of all standards, and that the paper blank did not reveal any iodine. The sensitivity is similar with either 0·5 h or 1 h activation, and corresponds to 3×10^{-9} g iodine-127. (This amount, that is, $2{\cdot}7$–$3{\cdot}7 \times 10^{-9}$ g iodine-127, was found to give count-rates twice those of background at 50 min after irradiation. We calculated that 0·29–0·39 μc. iodine-128/μg iodine-127 had been produced.) Recovery checks of the purification procedure showed, using iodine-131 as tracer, more than 80 per cent recovery (Table 1), and elimination of 99·9 per cent of bromine-82; the losses of chlorine should be at least as great.

Figs. 2–4 show that this is a practicable activation analysis. Our early experience had shown that it needed an adequate neutron flux (about 10^{12} n/cm^2/sec), and that if the entire paper was to be irradiated a stringent subsequent purification would be needed. The method described can be used to treat up to 6 sq. in. of paper, does not need a concurrent recovery check and apparently gives adequate purification. Other authors (Table 2) have described methods for activation analysis of iodine-127 applicable to blood, extracts or paper chromatograms, using equivalent or greater neutron fluxes and irradiation times of 30 min, but they obtained lower recoveries after less stringent purification procedures and were limited to small pieces of paper. Two of these have been applied to serum[4,5], and the third[6] to chromatograms. Their reported sensitivities were equivalent to our own, ranging from 10^{-9} to 10^{-10} g iodine.

The method described, needing 1 h for radiochemical purification, should be applicable with higher sensitivity than we found, for our counting had not usually begun until 2–2½ h (Figs. 2–4) after the sample left the pile, because of delay in their delivery to the laboratory. Following 1 h activation, evidence of a long-lived contaminant emerged in our rather delayed counts at 2½–4 h from the pile (Figs. 3 and 4); this may have been sodium-24 (half-life = 15 h), or iodine-126 (half-life = 13 days). Iodine-126 seems an unlikely product as the threshold for the iodine-127 (n, 2n) iodine-126 reaction is approximately 10 MeV, and the percentage of such fast neutrons in *Bepo* is very small. Samples obtained after activation for 0·5 h (Fig. 2) were free from this contaminant, which earlier assay should also minimize.

Activation analysis, although practicable, need not supplant the useful ceric–arsenite method. We have found[6] that this latter method can detect iodine compounds on chromatograms over the range 0·006–0·8 μg iodine, and so its sensitivity, reaching to 6×10^{-9} g iodine, is comparable with that of activation analysis. However, this ceric–arsenite method may give false indication of iodine, as, for example, with the reducing agents of the thiourea group, which had been recently found to give artefacts on chromatograms[1]. Fortunately, activation is available as a useful check whenever the specificity of the ceric–arsenite method comes in doubt.

We thank Dr. G. E. Harrison of the Medical Research Council Radiobiological Research Unit, Harwell, who put his laboratory at our disposal for these experiments.

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ASSAY OF TRITIUM, CARBON-14 AND SULPHUR-35 IN WOOL BY LIQUID-SCINTILLATION COUNTING

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IN the past we have assayed sulphur-35 in wool, labelled biosynthetically, by end-window Geiger–Müller counting of the wool itself or of benzidine sulphate prepared from it[1]. Wool labelled with carbon-14 has been assayed either by the end-window technique or by combusting the sample and gas-counting the resulting carbon dioxide by the method of Bradley, Holloway and McFarlane[2].

Recently we have found that wool labelled with these isotopes or with tritium can be assayed directly by the liquid-scintillation method. Samples were clipped from three sheep which had received an intravenous injection of DL-[^{3}H]lysine, L-[^{14}C]phenylalanine and L-[^{35}S]cystine respectively. Each batch of wool was washed successively with ether, ethanol and water, and was dried overnight at 105° C. Samples were then weighed into screw-cap vials, and 18 ml. of toluene containing 2,5-diphenyloxazole (0·4 per cent w/v) and 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (0·01 per cent w/v) was added to each. The radioactivity was then assayed in a liquid-scintillation counter ('Tri-Carb 314*EX*', Packard Instrument Co., La Grange, Illinois), at 0° C under 'balance point'[3] conditions, with background counting rates of 30–35 c.p.m. depending on the actual settings.

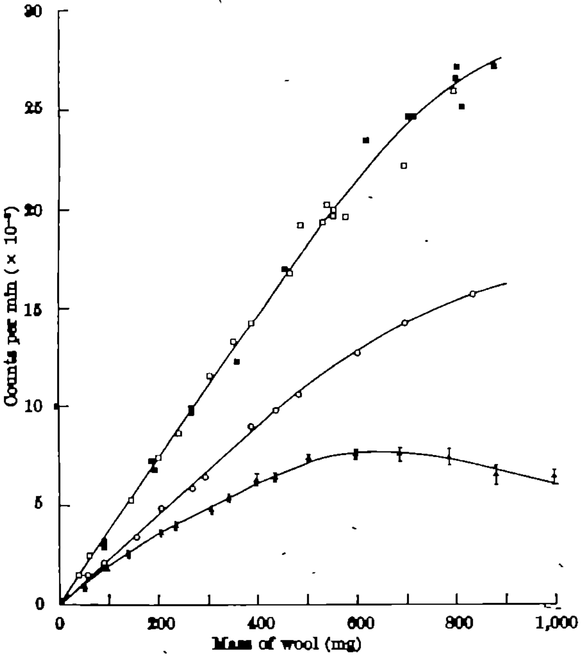

Fig. 1. Relationship between counting rate and mass of wool for three samples of wool labelled with single isotopes

○, ^{14}C-wool; ■, □ ^{35}S-wool samples counted 12 weeks apart; ▲, △ ^{3}H-wool samples counted 2 weeks apart; vertical lines are ± *S.D.*

The results are shown in Fig. 1. The counting rates were proportional to the mass of ^{14}C- or ^{35}S-labelled wool up to approximately 500 mg, but with larger amounts the counting rate per g fell progressively. In the case of the ^{3}H-labelled wool the relationship between counting rate and mass was almost linear up to about 200 mg. With larger amounts the counting rate per g fell much more rapidly than for ^{14}C- or ^{35}S-labelled wool until, over the range 500–800 mg, a plateau was reached over which the counting rate was practically constant. The departure from linearity in all cases is due to absorption of light photons with consequent loss of counts. Within the linear ranges for carbon-14 and sulphur-35, or within the plateau region for tritium, the results have a standard deviation of about ± 3 per cent. As indicated in Fig. 1 there was no change in the counting efficiency over a period of 12 weeks.

A linear relationship between counting rate and mass was also obtained with ^{14}C- and ^{35}S-labelled wool in a liquid-scintillation counter of the single-tube type (Ekco type *N*664) which was operated at room temperature with a background of 70 c.p.m. The efficiencies for these isotopes were comparable with those for the coincidence type of instrument, but the curves were linear only up to 200 mg of wool.

The counting efficiency was determined by comparing the results with those obtained by oxidizing samples (100 mg) of each lot of wool by the oxygen-flask technique, substantially as described by Kalberer and Rutschmann[4]. The combustion products from tritiated wool were shaken with 7 ml. methyl cellosolve. Those from wool labelled with carbon-14 or sulphur-35 were shaken with 12 ml. ethanolamine/methyl cellosolve (1 : 9, v/v). Measured amounts (5 and 10 ml. respectively) of the resulting solutions were then mixed with 9 ml. of the scintillating solution previously described, and the counting rates measured in the 'Tri-Carb' counter, again under balance-point conditions. The efficiency of counting tritium and carbon-14 under these conditions was established by assaying accurately weighed amounts of [^{3}H]hexadecane (2 μc./g) and of sodium [^{14}C]carbonate (0·9 μc./g aqueous solution), each of which was calibrated and supplied by the Radiochemical Centre, Amersham. The results in Table 1 show that carbon-14 and sulphur-35 were detected with approximately the same efficiency by the two methods, and that tritium was detected 6·6 times as efficiently by the oxidation technique as by the direct method. These results appear reasonable when the maximum ranges for the β-particles from tritium, carbon-14 and sulphur-35 (2·3, 285 and 317μ respectively[5] in material with a density of 1), are compared with the average radii of wool fibres (7–15μ) from fine- to strong-wool Merino sheep. (The apparent specific gravity of wool[6] in toluene is 1·306.)

Table 1. COMPARISON OF LIQUID-SCINTILLATION COUNTING OF WHOLE WOOL AND OF WOOL OXIDIZED BY THE OXYGEN FLASK TECHNIQUE

Isotope	Counts/min/mg wool ± *S.D.* Direct	Counts/min/mg wool ± *S.D.* Oxidized
^{3}H	2·01 ± 0·05	18·2 ± 0·4 (7·42% *)
^{14}C	21·5 ± 0·7	23·5 ± 0·5 (48·4% †)
^{35}S	42·9 ± 1·1	41·7 ± 1·8

* Efficiency measured by assay of standard [^{3}H]hexadecane.
† Efficiency measured by assay of standard sodium [^{14}C]carbonate.

Using Libby's equation[7] for the absorption of the β-particles from carbon-14 and sulphur-35 it may be calculated that a 50 per cent change in the average diameter of the wool fibres would produce no more than a 4 per cent change in the counting rate/g. Thus, reliable relative values for the specific activity of wool samples labelled with these isotopes can be obtained, even in experiments resulting in marked changes in fibre diameter.

Wool fibres labelled with tritium apparently behave as 'infinitely' thick sources. If the tritium is assumed to be distributed uniformly throughout the fibre, the counting rate will be proportional to the volume of the outer annulus from which the β-emission is detectable. This volume equals $\pi l(r^2-(r-R)^2)$, or more simply $\pi l(2Rr-R^2)$, where l is the length of the fibre, r is the radius and R is the thickness of the annulus. The extra radioactivity detected at the ends is negligible for fibres more than 500μ long (about 2 days' growth). Therefore the proportion of the total radioactivity that is counted in wool of uniform specific activity will equal the volume of the annulus multiplied by a constant (k) to allow for self-absorption in the annulus, and divided by the total volume of the fibre:

$$\text{Proportion of tritium counted} = \frac{k\pi l(2Rr-R^2)}{\pi r^2 l} = \frac{k(2Rr-R^2)}{r^2} \qquad (1)$$

Since the average range of the β-particles is small by comparison with the radii of the wool fibres, equation (1) may be further simplified:

$$\text{Proportion of tritium counted} \doteqdot \frac{K}{r} \qquad (2)$$

where K is a constant. Thus the counting rate per g of wool will be approximately proportional to the inverse of the average radius of the fibres. More care will obviously be needed in using the direct method for wool labelled with tritium.

The direct counting of wool is much quicker and easier than the methods used previously. It is accurate and, because of the high efficiencies and relatively large sample sizes, is very sensitive. For example, to produce a net counting rate of 30 c.p.m. (that is, equal to the background rate) from 0·5 g of wool a specific activity of 6×10^{-5} μc./g would be required for carbon-14 or sulphur-35, and 3×10^{-3} μc./g for tritium.

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DISTANCES OF ATOMS FROM THE 'BEST' PLANES OF CHOLESTERYL IODIDE

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THE configuration of the cholesteryl iodide molecule may be quantitatively described by referring its atoms to the series of planes ($A1$, $A2$, $A3$, etc.) illustrated in Fig. 1. The structure of cholesteryl iodide (form B, $P2_1$, a_0 = 12·57 Å, b_0 = 9·04 Å, c_0 = 21·89 Å, β = 149°) was determined by Carlisle and Crowfoot[1]. The fractional monoclinic atomic co-ordinates of Carlisle and Crowfoot (x, y, z) were converted to cartesian atomic co-ordinates in angstroms (p, q, r) as follows: $p = a_0 \sin\beta$, $q = b_0$, and $r = zc_0 + xa_0 \cos\beta$. The p, q, r co-ordinates were afterwards used for calculating the equations ($ax + by + cz = d$) of the best planes of the cholesteryl iodide molecule, the coefficients of which are given in Table 1. The root mean square distances of the atoms from one or several of the best planes have also been calculated and are listed in Table 2. The perpendicular distance of each atom from one or more of the best planes is recorded in Table 3, in which atoms lying between the origin of the unit cell and the plane have a negative value, whereas those lying on the opposite side of the plane from the origin have a positive value.

The best plane through all the carbon atoms of the cholesteryl iodide molecule has the equation $0·08x + 0·99y - 0·03z = 2·22$. The root mean square distance of carbon atoms (C_1–C_{27}) from this plane is 0·58 Å. The root mean square distance of carbons C_1–C_{17} from the best plane ($0·23x + 0·97y - 0·01z = 2·71$) through the steroid nucleus is 0·28 Å. The root mean square distances of the atoms comprising each of the planes which represent

Table 1. COEFFICIENTS a*, b*, c*, d† IN THE EQUATIONS ($ax+by+cz = d$) OF THE BEST PLANES OF CHOLESTERYL IODIDE

	a	b	c	d
Steroid nucleus ($C1$–$C17$)	0·23	0·97	−0·01	2·71
Entire molecule ($C1$–$C27$)	0·08	0·99	−0·03	2·22
A	0·40	0·91	0·06	2·95
$A1$	0·26	0·64	0·72	2·66
$A2$	0·35	0·89	−0·29	2·07
$A3$	0·44	0·80	0·43	3·91
B	0·14	0·99	−0·05	2·29
$B1$	0·04	0·99	−0·17	1·63
$B2$	0·13	0·98	−0·17	1·83
$B3$	0·22	0·89	0·41	4·70
C	0·21	0·98	0·00	2·67
$C1$	0·16	0·87	0·47	4·80
$C2$	0·18	0·92	−0·34	0·80
$C3$	0·24	0·82	0·52	6·40
D	−0·17	−0·84	0·52	2·17
$D1$	0·06	0·96	0·28	4·43
$D2$	0·22	0·94	−0·26	0·89
$A3B1$	0·26	0·88	0·38	3·77
$B3C1$	0·19	0·87	0·45	4·80
$C3D1$	0·16	0·90	0·40	5·67

* Direction cosines of normal to plane.
† Perpendicular distance from origin to plane.

Table 2. ROOT MEAN SQUARE DISTANCE ($\sqrt{\bar{\lambda}}$) OF THE ATOMS OF CHOLESTERYL IODIDE FROM THE BEST PLANES

	$\sqrt{\bar{\lambda}}$ (Å)
Steroid nucleus	0·28
Entire molecule	0·58
A	0·29
$A1$	0·00
$A2$	0·11
$A3$	0·00
B	0·17
$B1$	0·00
$B2$	0·10
$B3$	0·00
C	0·26
$C1$	0·00
$C2$	0·07
$C3$	0·00
D	0·14
$D1$	0·00
$D2$	0·18
$A3B1$	0·24
$B3C1$	0·04
$C3D1$	0·13

Table 3. PERPENDICULAR DISTANCE (Å) OF INDIVIDUAL ATOMS FROM ONE OR MORE OF THE MEAN PLANES OF CHOLESTERYL IODIDE

	A	*B*	*B*1	*B*2	*B*3	*C*	*C*1	*C*2	*C*3	*D*	*D*1	*D*2	Steroid nucleus	Entire molecule
C_1	+0·31												+0·43	+0·87
C_2	−0·44												−0·22	+0·29
C_3	−0·37												+0·48	+0·80
C_4	−0·20												−0·36	−0·25
C_5	+0·12	−0·08	0·00										−0·17	−0·11
C_6		+0·10	0·00	+0·10									+0·14	+0·02
C_7		+0·08		−0·10	0·00								+0·19	+0·03
C_8		−0·26			0·00	−0·18	0·00	+0·07					−0·22	−0·21
C_9		+0·27		+0·10	0·00	−0·22	0·00						+0·15	+0·38
C_{10}	−0·14	−0·11	0·00	−0·10									−0·29	−0·03
C_{11}						−0·33	0·00	−0·07					+0·07	−0·04
C_{12}						+0·34		+0·08	0·00				+0·22	+0·58
C_{13}						−0·26			0·00	+0·06	0·00	−0·13	−0·35	+0·20
C_{14}						+0·20		−0·07	0·00	−0·79	0·00		+0·15	+0·11
C_{15}										−0·07	0·00	+0·14	+0·01	−0·22
C_{16}										+0·02		−0·21	−0·04	−0·21
C_{17}										+0·17		+0·20	+0·33	+0·40
C_{18}						−1·66			−1·23	+1·15	−1·32		−1·77	−1·52
C_{19}	−1·74	−1·61											−1·85	−1·62
C_{20}										+1·30		−0·78	−0·38	−0·22
C_{21}													+0·07	+0·43
C_{22}													+0·53	+0·55
C_{23}													−0·31	−0·33
C_{24}													+0·70	+0·58
C_{25}													−0·29	−0·41
C_{26}													−0·63	−0·43
C_{27}													+0·99	+0·78
I	−0·42												−0·21	

the major subdivisions of the steroid nucleus, all of rings *A*, *B*, *C* and *D* are respectively 0·29 Å, 0·17 Å, 0·26 Å and 0·14 Å. If the ring system is further subdivided, *A*1, *A*2, *A*3, etc., we obtain a series of best plane equations (Table 1) which may be used to describe the intra-ring chair configuration of atoms. When used as references for the calculation of root mean square distances (Table 2), these equations also illustrate the degrees of planarity of the various portions of the intra-nuclear chair configuration. Thus it can be seen that the 'seat' portion of the chairs or partial chairs concerned, namely, *A*2, *B*2, *C*2 and *D*2, exhibit nearly the same degree of planarity with root mean square distances of 0·11 Å, 0·10 Å, 0·07 Å and 0·18 Å.

The angles between the various best planes of the intra-ring components have been calculated and are shown in Table 3. On consideration of these angles, the following steric features of the cholesteryl iodide molecule are evident:

(1) Chair *A* is unsymmetrical with angles of 117° (*A*1 ∧ *A*2) and 137° (*A*2 ∧ *A*3).

(2) Chair *B* is unsymmetrical with angles of 175° (*B*1 ∧ *B*2) and 146° (*B*2 ∧ *B*3), the approach of angle *B*1 ∧ *B*2 to 180° reflecting the presence of the double bond between C_5 and C_6.

(3) Chair *C* is more or less symmetrical with angles of 132° (*C*1 ∧ *C*2) and 129° (*C*2 ∧ *C*3).

(4) The join *B*3*C*1 is essentially planar, as is shown by both the 176° angle *B*3 ∧ *C*1 and the 0·04 Å root mean square distance of atoms C_7, C_8, C_9 and C_{11} from the *B*3*C*1 best plane ($0{\cdot}19x + 0{\cdot}87y + 0{\cdot}45z = 4{\cdot}80$).

(5) The *A*3*B*1 and *C*3*D*1 joins have angles of 134° and 161° respectively.

The root mean square distances of the cholesteryl iodide molecule may be compared with those of 4-bromoœstrone[3] and 4-bromoœstradiol[4]. A surprising similarity exists among the distances of atoms from the steroid nucleus, these being 0·28 Å, 0·26 Å and 0·24 Å respectively. Other similarities are seen where the distances are respectively: (*a*) 0·26 Å, 0·27 Å and 0·26 Å in ring *C*; (*b*) 0·07 Å, 0·00 Å, and 0·04 Å in ring *C*2; and (*c*) 0·14 Å, 0·18 Å, and 0·22 Å in ring *D*. In cholesteryl iodide the root mean square distance of atoms from ring *A* is 0·29 Å, whereas in the two œstrogens the average root mean square distance is 0·025 Å. This dissimiliarity is due to the dissimilarity of the bonding structure of the *A*-ring in the three compounds; in cholesteryl iodide it is saturated, while in the two œstrogens it is aromatic. Root mean square distance dissimilarity due to dissimilar bond distribution is also exemplified by ring *B*, which in cholesteryl iodide

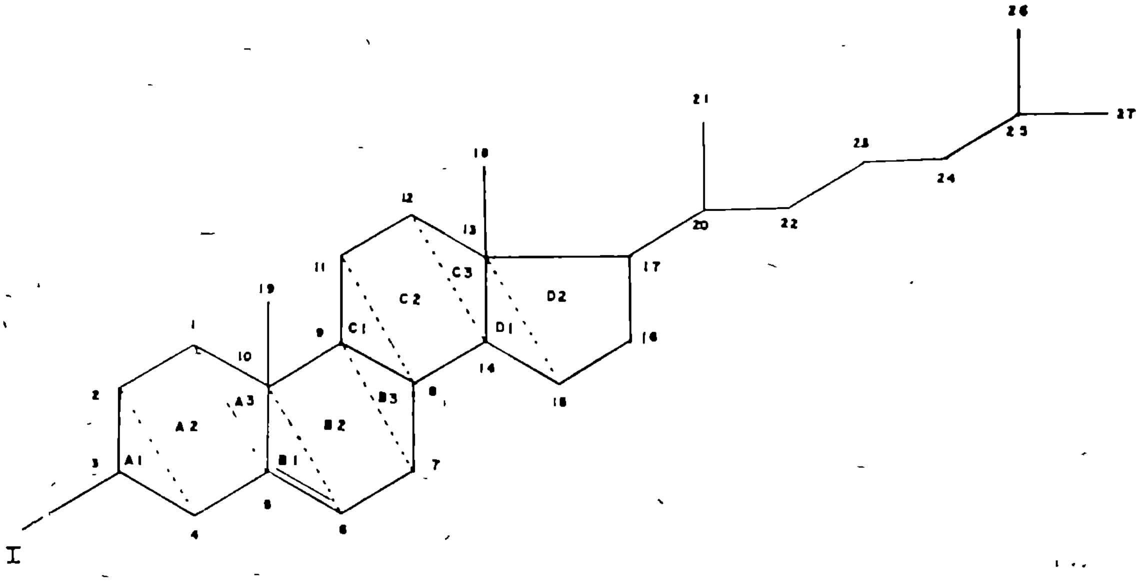

Fig. 1. Cholesteryl iodide

has a distance of 0·17 Å and in 4-bromooestrone and 4-bromooestradiol has distances of 0·29 Å and 0·23 Å respectively. A root mean square dissimilarity, which is effected by changes in side group bonding, is illustrated by ring *D*2, in which the distances are 0·18 Å, 0·01 Å and 0·12 Å respectively.

In view of the evidence presented here, it is clear that the root mean square distances of atoms from the best planes of equivalent portions of the steroid nucleus are approximately equal, except when disrupted by variations in the bonding scheme.

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CYTOLOGICAL DEMONSTRATION OF THE CLONAL NATURE OF SPLEEN COLONIES DERIVED FROM TRANSPLANTED MOUSE MARROW CELLS

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IN normal mouse hæmatopoietic tissue, there is a class of cells which, on being transplanted into heavily irradiated mice, can proliferate and form macroscopic colonies. In the spleen, the colonies formed in this manner are discrete and easy to count[1,2]. Microscopically, each colony appears as a cluster of hæmatopoietic cells, many of which are dividing; and often, within a given colony, the cells which are observed indicate that differentiation is occurring along three lines, into cells of the erythrocytic, granulocytic and megakaryocytic series, respectively[1].

In vitro techniques, which permit investigations of clonal populations, have greatly advanced the knowledge of the genetic and physiological properties of cells[3]. It was therefore desirable to prove whether or not spleen colonies each develop from single cells and hence are clones; for if they are, it would be possible to study clonal populations of hæmatopoietic cells *in vivo*. Evidence from previous experiments supporting the view that the colonies are clones is as follows: (1) the curve relating the number of nucleated marrow cells that are transplanted to the number of colonies that develop in the spleen is linear, and shows no initial threshold[1,2]; (2) the radiation survival curve of cells that can form colonies closely resembles the survival curves obtained for single cells in cell culture[4] or tumour transplants[5]. These observations provide only indirect evidence that the colonies are clones. It seemed desirable, therefore, to attempt to obtain direct evidence for the unicellular origin of the colonies.

At first glance the easiest way to accomplish this aim would be to make use of marrow cells that have a known chromosome marker, such as that found in *T*6/+ mice[6], and to transplant into irradiated mice a mixture of marrow cells with a marker and marrow cells with no marker. If the colonies that developed in the spleen were always composed of cells with markers or cells without markers it would, of course, suggest the single-cell origin of colonies; but it would not prove the single-cell origin of colonies, because the experiment would not rule out the possibility that the dispersion of donor cells was not complete and that the injected material contained undispersed aggregates of two or more cells which were the units that gave rise to colonies. The procedure used for ruling out this possibility was based on the observation of Barnes *et al.*[7] that the re-population of the hæmatopoietic organs of heavily irradiated mice could be accomplished by a small number of cells, and that the clones originating from these few cells were sometimes characterized by unique abnormal karyotypes. This suggested that if heavily irradiated marrow were utilized for the formation of spleen colonies, some of the cells containing chromosome aberrations, generated at random by radiation, might retain the ability to proliferate and give rise to progeny carrying a recognizable, persistent chromosome abnormality. If a spleen colony develops from one such cell, it would be expected that all the cells of the colony would carry the same abnormality. If, however, a spleen colony derives from an undispersed aggregate of two or more cells, one would not expect all the cells of the colony to have the same abnormal karyotype, since the probability of inducing by radiation the same abnormality in two or more cells of an aggregate is vanishingly small.

The details of the spleen colony technique and the radiation procedures involved have been described elsewhere[1,2]. The particular procedure in the present experiment was to expose recipient Swiss mice of either sex to an initial X-ray dose of 250 rads. Each mouse then received an injection of 10^7 nucleated bone-marrow cells, obtained from the femora of female donors of the same strain. After an interval of 1 h, the host mice, bearing the transplanted cells, were irradiated using a cobalt-60 γ-ray source with two doses of 325 rads each, separated by an interval of 4 h. In this way, the transplanted cells received a total dose of 650 rads *in vivo*, while the host received the larger dose of 900 rads necessary to suppress the proliferation of its own colony-forming cells. As predicted from previous experiments[8], this radiation schedule yielded spleens containing small numbers of discrete colonies.

Eleven days later, all recipient mice were injected intraperitoneally with 'Colcemid' (desacetylmethyl colchicine, Ciba) and killed approximately 1 h afterwards. Their spleens were removed and individual discrete colonies were carefully dissected out. The colonies were then prepared for chromosomal analysis according to a modification of the method of Fox and Zeiss[9].

Each colony was placed in 2 ml. of phosphate-buffered saline which was then diluted in 4 equal steps, with distilled water, to 5 times its original volume. An interval of 10–15 min was allowed to elapse after each dilution step. The colonies were fixed for 1 h in a solution of 3 parts absolute alcohol and 1 part glacial acetic acid at 4° C. They were then transferred into a solution of 40 per cent acetic acid (4° C) where they were kept for a period of 1–2 h. Each colony was afterwards transferred into 0·5 ml. of 60 per cent acetic acid (4° C) in a small plastic cup where it was teased apart by two fine needles and suspended by gentle pipetting. Small drops of this suspension were placed on the surface of a microscopic slide which had been frozen in the manner described by Fox and Zeiss[9]. The slide was immediately heated and dried over a gentle Bunsen flame. Staining was performed under a coverslip with 2 per cent aceto-orcein.

Slides from each colony were systematically scanned under 100 times magnification and every metaphase cell which appeared sufficiently well spread without its cytoplasm being ruptured was carefully searched under oil-immersion, at 1,000 times magnification, for the presence of an abnormal karyotype. If the first 25 meta-

Table 1

Colony with abnormal karyotype	No. of metaphases scored	Abnormal chromosomes present	Frequency of abnormal chromosomes in cells having given number of chromosomes: 35 or less	36	37	38	39	40	Total No. of cells with given chromosome condition	Per cent of cells with at least one marker characteristic of colony
A	100	Minute plus long	6	0	4	8	63	0	81	99
		Minute only	0	2	1	1	9	2	15	
		Long only	0	0	1	2	0	0	3	
		None	0	0	0	0	0	1	1	
		Total cells	6	2	6	11	72	3		
B	100	Dicentric	0	2	4	5	14	70	95	95
		None	0	0	0	2	2	1	5	
		Total cells	0	2	4	7	16	71		
C	100	Minute plus dicentric	0	0	4	6	7	74	91	97
		Minute only	0	1	0	1	0	4	6	
		Dicentric only	0	0	0	0	0	0	0	
		None	0	0	0	1	0	2	3	
		Total cells	0	1	4	8	7	80		
D	75	Minute	0	2	6	6	10	49	73	97
		None	0	0	0	0	0	2	2	
		Total cells	0	2	6	6	10	51		

phase cells of any given colony so examined failed to reveal a readily identifiable chromosome aberration, that colony was scored as containing no obvious marker. Since female donor marrow was used, the presence, in the cells of the colonies examined, of the third unpaired small chromosome characteristic of the male karyotype[10] was avoided; this facilitated identifying any odd minute chromosome encountered as a radiation-induced marker.

A total of 42 colonies obtained from 36 animals were examined in the foregoing manner. Of these colonies, 4 contained cells with an obviously abnormal karyotype. Table 1 summarizes the types of chromosome markers encountered and the frequencies of these markers within the cells of each colony. For example, mitotic figures from colony *A* showed three characteristic abnormalities: (1) a modal chromosome number of 39; (2) an abnormally small chromosome; (3) an abnormally long sub-telocentric chromosome (Fig. 1, top). Eighty-one of 100 metaphase cells examined manifested all three abnormalities; and 99 per cent of the cells contained at least one of these distinguishing characteristics. The occasional inability to score one or the other of the two markers is probably the result of the following technical difficulties: (1) cytoplasmic rupture and chromosome loss; or (2) excessive overcrowding and overlapping of chromosomes in less-than-optimally spread metaphase cells. In the latter circumstance, whenever it was impossible to delineate with certainty one or other of the marker chromosomes, the cell was scored as lacking that particular marker. Of the 3 cells from colony *A* which were recorded as containing 40 chromosomes, two were very difficult to count because of overcrowding of the chromosomes, and the chromosome number recorded for them may represent a miscount. Similar considerations are applicable to the interpretation of the data compiled for the other 3 marked colonies. A representative complement from colony *C* is shown in Fig. 1 (bottom). It is characterized by an abnormally small chromosome and a long chromosome which is either a dicentric or has a prominent secondary constriction.

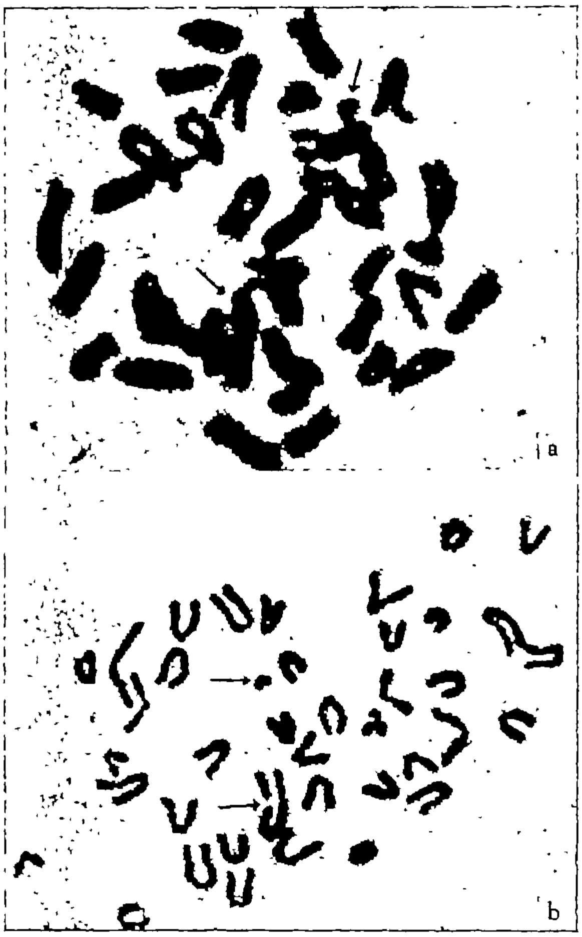

Fig. 1. Representative chromosome complements from two of the colonies listed in Table 1. The abnormal chromosomes are indicated by arrows. (× 1,430)
a, Colony *A*, the chromosome count is 39 instead of the normal 40; *b* colony *C*

Experiments were performed to test for the presence of pre-existing abnormal karyotypes among the colony-forming cells of normal donor marrow. Groups of Swiss mice were exposed to total-body doses of 900 rads; and, following irradiation, each mouse received 5×10^4 nucleated bone-marrow cells from female donors of the same strain. No further irradiation was given. Of 55 colonies examined from these controls, all contained, exclusively, mitoses with the normal female diploid complement of 40 chromosomes. This finding rendered unlikely the existence, within the donor marrow, of uniquely and uniformly marked colony-forming cell aggregates. The 4 abnormal karyotypes were thus the result of chromosome damage by ionizing radiation.

The results given in Table 1 show that when recognizable marker chromosomes are present in the cells of a colony, an overwhelming majority of the cells contain the same markers. Since normal mouse marrow contains no uniquely and uniformly marked colony-forming cell aggregates, and since chromosome breakage by radiation is a random process, rendering highly improbable the generation of an identical abnormality in each of the cells of a hypothetical colony-forming aggregate, it can be concluded that all the cells in these marked colonies were derived from a single cell in which a chromosome aberration was induced by radiation. Thus, in every colony where direct cytological evidence is available this evidence indicates that the colony is a clone. Because the karyotype of the mouse does not permit the identification of individual autosomes, only gross changes in the chromosomes may be identified readily. In the experiments reported here, this was possible for only approximately 10 per cent of the colonies. Nevertheless, if the direct cytological evidence is considered together with the indirect support provided by the dilution and radiation-survival data, the general view that spleen colonies are clones is a most reasonable conclusion. The spleen colony procedure may, therefore, be regarded as an *in vivo* single-cell technique, analogous to the well-known *in vitro* single-cell experimental systems[9]. The advantages inherent in the clonal approach to the study of cell biology are thus available for future investigations on hæmatopoiesis.

We thank Dr. A. A. Axelrad for his advice and Miss R. Wyncoll and Messrs. P. Csordas and J. Hicks for technical assistance. This investigation was supported by the Defence Research Board (grant 9350-14), the National Cancer Institute of Canada, and the Medical Research Council, Canada (grant *MA* 1282). One of us (A. J. B.) is a Fellow of the Medical Research Council, Canada.

Note added in proof. In the four additional marked colonies encountered after the submission of this article, the markers have been unique and present in all the cells.

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ELASTASE AND THROMBOGENESIS

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DURING the past two decades numerous theories have been developed concerning the various lesions associated with arterial disease. To account for the topographically discrete nature of the mural thrombi implicated by one such hypothesis[1], the suggestion has been made that some fundamental change in the vessel wall must precede fibrin deposition[2]. Although absorption on surfaces such as glass can activate certain of the factors necessary for plasma coagulation[3] *in vitro*, the physical state of the surface of the vessel wall would not appear to determine the site of thrombogenesis since arterial grafts and traumatized regions[4] do not necessarily promote clot formation. The reason for the localization of thrombus formation remains a mystery, and observations which increase the chances of explaining the phenomenon may be of prime importance as starting points for future research and for attempts at therapy.

Since the physical properties of the intimal surface alone cannot explain the vagaries of thrombus production, one must consider the possibility of other changes in the vascular tissue, causing local alterations in the equilibrium between fibrogenesis and fibrinolysis; an equilibrium which under normal conditions would appear to determine the fluidity of the circulating plasma[5]. Activation of the former process or inhibition of the latter could produce the desired effect. We discuss in the present article certain observations which point to the possible existence of localized centres of clotting activation.

The clotting mechanism consists of a complex interplay of factors derived from plasma, platelets and tissue, and activation of the clotting process with the resultant formation of mural thrombi could be due to the localization of one or more of the essential components on the surface of the vascular tissue.

The pancreatic enzyme elastase is not normally considered as a component of the clotting system, but it has been demonstrated that the presence of low concentrations of elastase appreciably shortens the clotting time *in vitro*[6]. The point of intervention of the enzyme has not been proved although Bagdy has shown that it occurs much earlier than the transformation of fibrinogen to fibrin[7]. The results illustrated in Fig. 1 indicate that elastase may in fact act on the platelets, assisting in the release of various platelet factors. Carefully collected platelet-rich plasma responds to the addition of increasing concentrations of elastase over a range of 0–25 μg/ml. to give increasingly shorter recalcification times[8], but this response disappears completely if the platelets are previously degraded by mechanical manipulation.

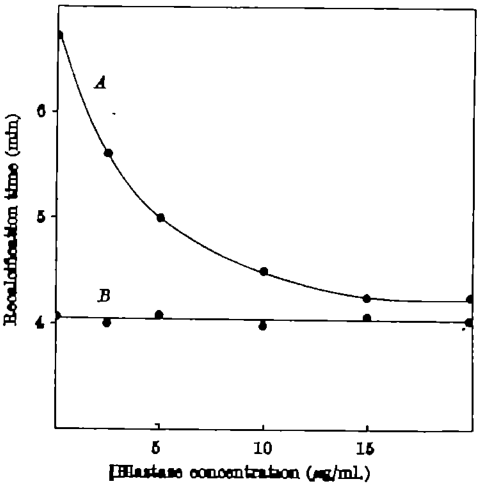

Fig. 1. A typical effect of free elastase on the recalcification time of: *A*, platelet-rich plasma; and *B*, plasma with mechanically degraded platelets. The mixture contained 0·1 ml. 0·2 M sodium chloride, 0·1 0·033 M calcium chloride, 0·1 ml. citrated plasma from a 21-year-old normal male and the appropriate amount of elastase (E_1I^{14}) dissolved in 0·1 m. 0·9 per cent sodium chloride and was incubated at 37° with gentle inversion in a polythene-stoppered tube, following the addition of the plasma

One difficulty which arises in any attempt to associate elastase with systemic activity is the fact that, not only has the enzyme never been identified in plasma but also its acinar origin[9] would appear to preclude a systemic function. Rationalization of these facts with the observations that elastase fed to animals has marked systemic activity[10] may indicate that readsorption through the intestinal mucosa may be possible. The failure to identify elastase in plasma may mean that it is never present at levels high enough for estimation and this may imply that elastase passes rapidly into an immobilized state, possibly by adsorption on to the vascular tissue. Evidence in favour of this hypothesis is already available.

Saxl[11] and Yu and Blumenthal[12] have reported the presence in aortic tissue of a substance variously described as a lipoprotein or mucopolysaccharide but having properties of both and possibly identifiable as a lipomucoprotein. This material, described by Saxl as present throughout the aorta of young subjects, but progressively disappearing from the lumen outwards with age, prevents the dissolution of aortic tissue (frozen sections) under the action of elastase alone. When isolated from aortic tissue[12] it has the power of inhibiting elastolysis *in vitro* by attachment to the substrate rather than to the enzyme. Whether such a complex is still capable of adsorbing elastase is still without proof, but it has been shown[13] that elastin modified by acetylation[14] is capable of adsorbing twice as much enzyme while its susceptibility to elastolysis is reduced by 80 per cent. It may also be significant that the only reported preparation of elastase from mammalian tissue outside the pancreas is that by Gore and Larkey[15] in a crude polysaccharide fraction from aorta. The possibility may exist therefore that elastase becomes adsorbed on to vascular tissue, and evidence is available in support of the concept that in this condition it is capable of intervening in the clotting reaction.

Pancreatic elastase[16] differs from the enzyme derived from bacterial sources in that all attempts to separate elastolytic activity (with solid elastin as substrate) from generalized proteolysis (with soluble proteins such as α-elastin, casein, insulin, etc., as substrates) have been without success[17]. In this enzyme therefore it would appear that both types of activity are essential functions of one molecular species.

The differing rates at which elastase attacks elastin and other proteins[18] may be due to either of two reasons: the physical state of the two types of substrate may affect the rate even if the active centres are identical, or alternatively the enzyme may have two active centres. Recently, evidence has been presented in support of this latter concept[19]. Human plasma inhibits the elastolytic system competitively, whereas its effect on the generalized proteolytic system is non-competitive. From a topographical point of view, this may indicate that whereas the inhibitor attaches itself to the centre through which the enzyme and substrate react in the case of elastolysis, this point of attachment is not the site of enzyme substrate complex formation in the case of generalized proteolysis. The enzyme may therefore be assumed to have two dissimilar active centres.

Because of this property it is theoretically possible for elastase to react simultaneously with two substrates, and if, as suggested here, one of these is solid elastin the proteolytic system will assume the same form as the original elastolytic system but in reverse, the enzyme being solid and the substrate in solution.

Experiments have been carried out to determine whether elastase adsorbed in this fashion on a solid substrate still has the power to take part in the clotting reaction as estimated by the determination of recalcification times[6]. To simplify the system, purified elastin[20] was used rather than whole elastic tissue and this was suitably modified by acetylation[14] to increase the degree of elastase adsorption while suppressing elastolysis. Since the amount of elastase taken up by acetylated elastin (59 per cent of the amount present in the surrounding fluid) is dependent on this latter concentration, it proved impossible to remove excess elastase by washing before the addition of the components of the recalcification system since both the wash water and the plasma and calcium chloride solutions added afterwards would elute elastase from the acetylated elastin. Systems were therefore prepared in the presence and absence of acetylated elastin and measurements of recalcification times made at a variety of enzyme concentrations.

Direct assessment of the effects of acetylated elastin over the range of enzyme concentrations referred to here was rendered difficult on account of a marked prolongation of clotting time, which was apparent in systems in which the modified elastin was added alone to the other components of the recalcification system. This phenomenon was not investigated further, but was presumably brought about by the adsorption of certain unspecified components of the clotting system on the surface of the solid. However, use could be made of a variation in response observed in about 50 per cent of the sera studied. In these cases the decrease in recalcification time over the range of elastase concentrations from 0–15 μg/ml. is followed at higher concentrations of enzyme by an increase and subsequent decrease, giving a region of minimum elastase effect at about 25 μg/ml. Movement of this trough to higher concentrations of elastase in systems containing acetylated elastin would indicate that the effective concentration of enzyme was altered by adsorption on to the solid. The results did not, in fact, show this movement. The curves (Fig. 2) obtained for a group of normal sera were compared and, allowing for the difference due to the basal effect of the solid, could be shown to be statistically identical. It may be assumed therefore that elastase has exactly the same activating effect on the clotting reaction when adsorbed on elastin as when free.

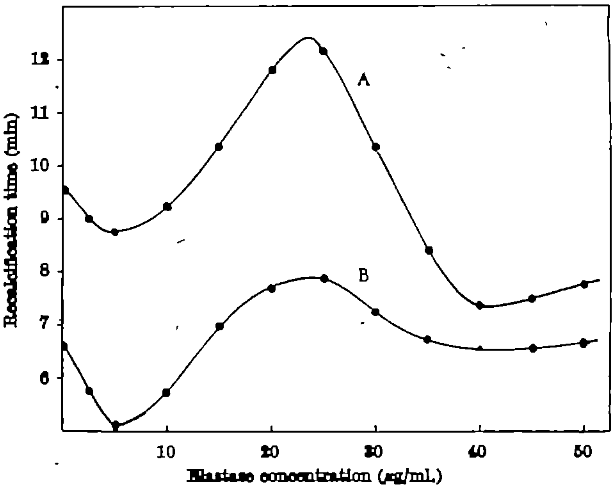

Fig. 2. The effect of elastase in: *A*, the free state; and *B*, adsorbed on acetylated elastin on the recalcification time of a typical plasma from a 19-year-old normal female. Conditions as in Fig. 1

In view of these observations, the suggestion may be made, as a working hypothesis, that elastase, adsorbed on to the lipopolysaccharide rich regions of aortic elastica, revealed following damage to the endothelial lining, may result in the presentation to the surrounding plasma of a proteolytic enzyme system capable of bringing about localized clotting and hence of initiating thrombus formation in these regions.

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β-HYDROXYASPARTATE PATHWAY: A NEW ROUTE FOR BIOSYNTHESES FROM GLYOXYLATE

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WHEN micro-organisms grow on glycollate as sole source of carbon, reactions ancillary to the tricarboxylic acid cycle must occur to replenish, from the C_2-growth substrate, the intermediates of the cycle which have been removed in the course of cellular syntheses. In micro-organisms such as *E. coli* or *Pseudomonas* sp., the necessary net formation of tricarboxylic acid cycle intermediates is effected via the 'glycerate pathway'[1]. In this pathway, glyoxylate (derived from the oxidation of glycollate) undergoes a condensation reaction in which 2 mol. units of glyoxylate yield 1 mol. unit each of tartronic semi-aldehyde and carbon dioxide[2-4]: the enzyme catalysing this process has been named glyoxylic acid carboligase[3]. The tartronic semi-aldehyde thus formed is afterwards reduced[4-6] to glyceric acid with the aid of tartronic semi-aldehyde reductase. In the presence of adenosine triphosphate and *Pseudomonas* extracts, glyceric acid has been shown[5] to yield pyruvate, this transformation being effected by the well-established Embden–Meyerhof sequence. The net effect of these reactions is to transform 2 mol. units of glycollate to 1 mol. unit of carbon dioxide and 1 of pyruvate which can either be carboxylated to oxaloacetate[3] or, after oxidation to acetyl coenzyme *A*, condense with a third mol. unit of glyoxylate[8] to yield malate[8,9]: in either case, the *de novo* formation of intermediates of the tricarboxylic acid cycle from glycollate has been effected.

Although the reactions of the glycerate pathway have been demonstrated in a wide variety of micro-organisms (for review, see ref. 10), a major problem was posed by the observation that *Micrococcus denitrificans*, which grows readily on glycollate as sole carbon source with either oxygen or nitrate as electron acceptor, is devoid of the key enzymes of this pathway: no glyoxylic acid carboligase and only traces of tartronic semi-aldehyde reductase were detected in extracts of the glycollate-grown organism. The necessary nett formation of tricarboxylic acid cycle intermediates must therefore be achieved by a different route.

An indication of the nature of this route has been obtained from studies with glycollate-grown *M. denitrificans* and with cell-free extracts prepared therefrom. The results obtained are consistent with the view that the growth of *M. denitrificans* on glycollate as sole source of carbon involves a hitherto unknown metabolic route in which *erythro* β-hydroxyaspartate is an intermediate (Scheme 1). The main points of evidence in support of this scheme may be summarized as follows:

(1) When *M. denitrificans*, growing on glycollate, were incubated with [1-^{14}C]glycollate for brief periods, isotope appeared in glycine, malate and aspartate at the earliest times. In contrast to the results obtained with glycollate-grown *Pseudomonas*[5] and *E. coli*[9], no isotope was detected in glycerate or phosphoglycerate.

(2) Extracts of glycollate-grown *M. denitrificans*, prepared either by ultrasonic treatment or by passage through a French press, oxidized glycollate to glyoxylate: this oxidation was greatly stimulated by the addition of phenazine methosulphate.

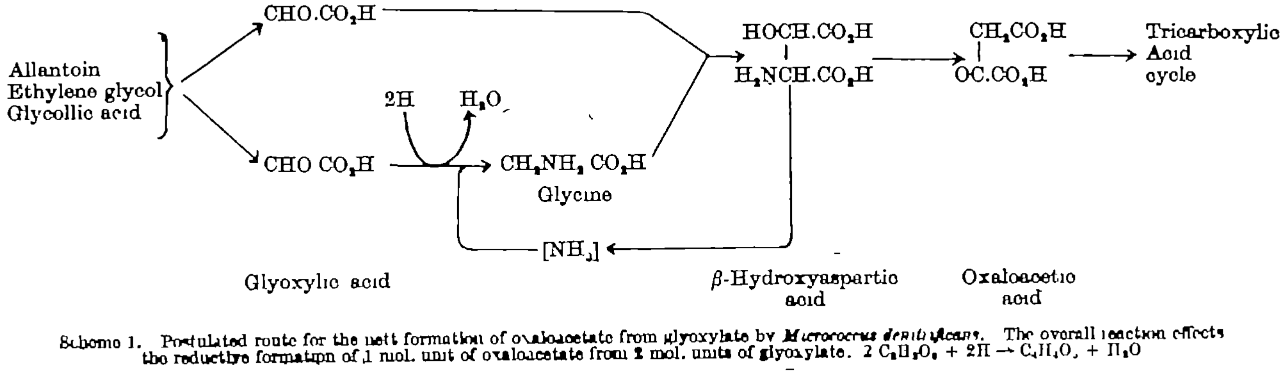
(3) Although *M. denitrificans* grew readily on glycollate, growth on glyoxylate was poor and on glycine, negligible. However, the addition of glycine to a medium, otherwise containing glyoxylate as sole carbon source, stimulated growth.

(4) Sonic extracts of the glycollate-grown organism catalysed the formation of oxaloacetate as measured by the increase in absorbancy at 262 mμ, when both glyoxylate and glycine were added: no such increase in absorbancy was detected if glyoxylate or glycine was added alone. The probable condensation product of glyoxylate and glycine, β-hydroxyaspartate, yielded oxaloacetate in this system at a rate greater than that observed with equimolar concentrations of glyoxylate and glycine.

(5) Sonic extracts, which were rich in a malate dehydrogenase capable of utilizing reduced nicotinamide–adenine dinucleotide phosphate ($NADPH_2$) as electron donor, catalysed the oxidation of $NADPH_2$ when glyoxylate, but not when glycine, was added. However, the rate of $NADPH_2$ oxidation in the presence of glyoxylate was greatly stimulated by the further addition of glycine. Synthetic DL-β-hydroxyaspartate[11] reacted in this system at a rate greater than was found with equimolar concentrations of glyoxylate and glycine: after resolution of the synthetic substrate, it was established[12] that only the *erythro* isomer reacted.

(6) In the presence of $NADPH_2$ or a $NADPH_2$-generating system, the crude extracts catalysed the formation of labelled malate from either [1-^{14}C]glyoxylate and unlabelled glycine or from [1-^{14}C]glycine and unlabelled glyoxylate. The yield of labelled malate was decreased considerably if $NADPH_2$ or the unlabelled substrates were omitted from the incubation mixtures. In these experiments, the formation of labelled β-hydroxyaspartate was also detected.

(7) When extracts were incubated with DL-*erythro* β-hydroxyaspartate and semi-carbazide, at *p*H 6·5, the absorbancy at 252 mμ increased linearly at a rate proportional to the quantities of extract and of substrate added: this indicated the enzymatic formation of the $\rangle C{=}N-$



Scheme 1. Postulated route for the nett formation of oxaloacetate from glyoxylate by *Micrococcus denitrificans*. The overall reaction effects the reductive formation of 1 mol. unit of oxaloacetate from 2 mol. units of glyoxylate. 2 $C_2H_2O_3$ + 2H → $C_4H_4O_5$ + H_2O

linkage of a semi-carbazone. The product of the reaction, converted to the 2,4-dinitrophenylhydrazone and analysed by paper chromatography, was found to be identical with the derivative formed from authentic oxaloacetate[11]. Labelled β-hydroxyaspartate, eluted from chromatograms (see paragraph 6), yielded radioactive oxaloacetic 2,4-dinitrophenylhydrazone in this system.

(8) When extracts of glycollate-grown cells were incubated anaerobically with oxaloacetate, adenosine triphosphate, and cobalt sulphate (under assay conditions similar to those described by Utter and Kurahashi[13]), the rapid evolution of carbon dioxide indicated the presence of phosphopyruvate carboxylase (*E.C.* 4.1.1.32). When [1-^{14}C]glyoxylate and unlabelled glycine were used as enzymatic precursors of oxaloacetate, the carbon dioxide evolved was highly radioactive whereas pyruvate, isolated as the 2,4-dinitrophenylhydrazone, contained only traces of isotope. With unlabelled glyoxylate and [1-^{14}C]glycine, on the other hand, the pyruvate isolated was highly radioactive and the evolved carbon dioxide contained relatively little isotope. Since *erythro* DL-β-hydroxyaspartate also reacted rapidly as enzymatic precursor of pyruvate and carbon dioxide in this system, these findings suggest[12] that β-hydroxyaspartate, formed from glyoxylate and glycine, is transformed to oxaloacetate via a dehydratase similar in action to other dehydratases known to act on α-amino-β-hydroxyacids (*E.C.* 4.2.1.13–4.2.1.16).

(9) The enzyme responsible for the enzymatic formation of oxaloacetate from *erythro* β-hydroxyaspartate, tentatively named 'β-hydroxyaspartate dehydratase'[12] was also present in high activity in extracts prepared from *M. denitrificans* grown on glyoxylate, glyoxylate plus glycine, or substrates known to be catabolized to glyoxylate (such as ethylene glycol or allantoin), as carbon sources. Extracts from cells which had been grown on acetate or succinate were virtually devoid of this enzyme.

(10) Succinate-grown *M. denitrificans*, placed into growth medium containing glycollate as sole carbon source, rapidly formed β-hydroxyaspartate dehydratase during the lag period prior to the onset of growth. When succinate-grown cells continued to grow in a medium containing 5 mM succinate and 25 mM glycollate as carbon source the initial period of logarithmic growth was followed by a stationary phase, which was again followed by renewed growth. During this typical diauxic lag, the low levels of β-hydroxyaspartate dehydratase increased approximately ten-fold and reached a level which was maintained during the subsequent growth on glycollate[14].

The biosynthetic pathway outlined in Scheme I is similar in effect to the glyoxylate cycle[15,16] in which the net formation of C_4-dicarboxylic acids is achieved by the direct condensation of two C_2-units: however, the formation of the C_4-compound (β-hydroxyaspartate) appears to be catalysed by an aldolase-type condensing enzyme which does not necessitate prior activation of one of the C_2-reactants to the coenzyme *A* derivative. The β-hydroxyaspartate pathway differs from other routes of biosynthesis from glyoxylate, such as the glycerate pathway[1] established in other micro-organisms, or the glycollate pathway[17] described in plants, in avoiding the loss of carbon dioxide concomitant with the initial formation from the C_2-substrate of a compound containing more than two carbons.

Full details of this work, which was supported in part by the U.S. Air Force under grant No. *AF–EOAR* 62–77, monitored by the European Office, Office of Aerospace Research, will be published elsewhere.

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MANIPULATION OF SALT SOLUTION—FRESH WATER DRINKING REGIMEN AND EATING BEHAVIOUR IN RATS

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IN an earlier investigation[1] the effects of marked and moderate food restriction on consumption of freely chosen 1 per cent salt solution, and the possible effects of excessive salt intake on survival, body-weight, reproduction, and the weights of certain organs of the rat were reported in detail. During a three week period of maintenance of reduced weight, free choice (salt solution and water) and control (water only) groups both required the same amounts of food to maintain their weights. Yet the animals in the choice situation increased their consumption of the salt solution to 25 per cent of their body-weight, while the fresh water intake of both groups remained stable. In keeping with Hebb's[2] contention that hunger be considered neither as a particular condition of the body, nor a set of sensations from the stomach, but as an organized neural activity, capable of arousal like any other conceptual process, it was suggested that the intake of water alone failed to provide the animals with an associative link between the consummatory response made (that is, drinking) and appetite, whereas salt did. Put another way, the suggestion was made that fresh water did not serve, as did salt, to arouse a pattern of learned behaviour associated with appetite and/or hunger. The findings were interpreted as lending support to the role of exteroceptive stimuli in activities frequently viewed as responses to internal needs, or as primary biological drives.

The present article is concerned with the eating behaviour, after food deprivation, of rats drinking only water, or water and a 1 per cent salt solution. The experiment seeks to determine whether freely elected alterations in fluid regimen affect the animal's consumption of food. It reflects an interest in the investigation of the role of change in, or manipulation of, external stimuli as a motivator of behaviour.

Adult male albino rats (Sherman strain) were used for these studies. The animals were individually housed on shavings in roomy cages. The rats were raised on a standard ration of 'Rockland *D*-Free' pellets and, at a median age of 135 days (range, 74–162 days), were transferred to a purified diet[3] containing (per cent) 30 lactalbumin, 54 dextrose, 10 lard, 2 cellulose, 0·5 calcium carbonate, and adequate amounts of all known vitamins. To this diet was added 3·5 per cent of *U.S. Pharmacopeia* salt mixture XIII, which allowed a dietary sodium

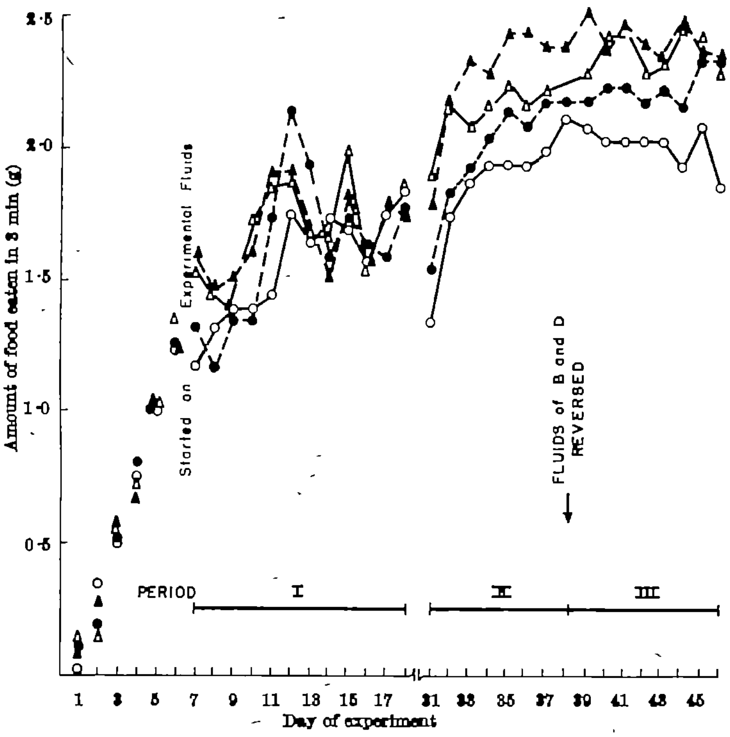

Fig. 1. Amount of food eaten in 3 min by rats drinking water or 1 per cent salt solution and water during each period of the experiment. Each point represents an average determination for 8 animals. The results for the first 5 days of the experiment represent the average daily 3-min amount of food consumed by the groups while they were all drinking water, that is, before the start of the experiment proper. These points have been included only to lend continuity to the graph by showing the gradual increase, with experience, in the quantity of food consumed in a fixed period of time. Group *A* drank water throughout the experiment; *B* was transferred from water to salt solution and water; *C* drank salt solution and water throughout; *D* was switched from salt solution and water to water alone. ○, Group *A*; △, group *B*; ●, group *C*; ▲, group *D*

chloride level of approximately 0·15 per cent. This level had been found to be adequate for growth, survival, and reproduction in our rat colony, and it was our intention to avoid limiting factors in the diet *per se* which might interfere with such functions.

The rats were placed in the experimental room one week before testing so that they might become adjusted to the daily noises around them. Two identical bottles were placed on each cage; both contained water for the water only condition, and one water, and the other a 1 per cent salt solution, for the choice situation. As is well known, rats, when offered this choice, consistently prefer the 1 per cent salt solution. The possibility that specific position preferences among the animals might influence the choice of fluid was controlled by alternating the positions of the bottles on the cages. The feeding tests, which were conducted in a small room built into the larger one, were carried out between 5 a.m. and 8 a.m. in order to minimize possibly distracting noise. The amount of food eaten in 3 min, after 24 h of food deprivation, was measured. The animal was not given any food beyond that eaten in 3 min during the initial series of these determinations. Later, the 3-min measurements were continued, but each rat was given a total of 10 g of food, which it could finish in its cage. The rats were taken for testing alternately from experimental and control groups.

In detail, an eating test was conducted as follows: A glass cup containing food was weighed to 0·05 g and placed in a stainless steel box, 7 × 10 × 6·5 in. The box was evenly illuminated by a fluorescent light six feet above. The rat was carried into the experimental room and placed in the box with its head toward the food, and the stop watch was started. A wire mesh cover was placed very quietly on the box and the experimenter stepped behind a shield to observe and record the behaviour of the animal. At the end of 3 min the watch was stopped (the click stopped the rat from eating), the food cup was taken from the rat, its paws and mouth were dusted into the cup, any spilled diet was replaced, and the cup was reweighed. The rat was returned to its cage together with its food cup during the period when it was being given a fixed amount to eat.

At a median age of 197 days, food was withdrawn, and the animals were weighed. On each of the next five days (days 1–5 of the experiment) they were given 3-min eating tests, but no additional food. On day 5 the animals were distributed into four groups of 8 rats each, matched on the basis of the total amount of food eaten in the five 3-min tests, the amount eaten during the last trial, their body-weight before food deprivation, the percentage of body-weight lost during the trials, and the amount of urination and/or defæcation in the test box. The average weights of the groups at this time were as follows: group *A*, 450 g (range, 399–513 g); *B*, 444 g (348–498 g); *C*, 443 g (359–513 g); *D*, 458 g (403–510 g). Groups *A* and *B* continued to drink water and groups *C* and *D* now were given a choice of drinking water or a 1 per cent salt solution. On day 6, the groups were given another 3-min eating test without additional food. The continuation of the experiment will be discussed in terms of three periods.

Period I. On days 7–18, all animals were given 10 g of food, and the amount eaten in 3 min in the test box was determined. During this period the 3-min food consumptions of the different groups showed no clear or consistent patterns of similarity or difference (Fig. 1). It was thought that more time (that is, a longer habituation period) should be allotted the animals on their basic fluid intakes before any reversal of fluids was carried out, in order to permit some organization of the consummatory response to take place. Accordingly, the rats were given 10 g of food daily, on days 19–30, to be eaten in their cages, and no determinations were made.

Period II. On days 31–38, 3-min eating tests were again carried out, and the rats were permitted to finish their 10 g rations in their cages. After the ingestion of 10 g on day 38, the fluid intake of group *B* was switched from water to a choice of 1 per cent salt solution and water. At the same time group *D* was switched from salt solution and water, to water only. The fluids offered the animals in group *A* (water) and *C* (salt solution and water) remained unchanged.

Period III. On days 39–46, 3-min feeding tests were carried out and the rats were permitted to finish their 10 g rations in their cages.

The average daily amount of food consumed in 3 min by the rats in each group is plotted in Fig. 1. Though the appearance of the curves during periods II and III suggested that differences existed among the groups, a simple analysis of variance of the average 3-min food consumption of the groups, carried out for each of the periods, indicated that these differences were not statistically significant.

The cross-over design of the experiment permitted each animal in groups *B* and *D* to serve as its own control when comparing the amount of food eaten under the different drinking programmes. In periods II and III, 8 rats (group *B*) received water and then water and a 1 per cent salt solution; the 8 animals in group *D* received water and salt solution and then water. In order to minimize residual effects from preceding drinking regimens,

Table 1. AVERAGE QUANTITY OF FOOD (G) CONSUMED IN 3 MIN ON LAST FOUR DAYS OF PERIOD II AND PERIOD III, BY EACH RAT IN GROUPS *B* AND *D* WHEN DRINKING WATER AND WHEN DRINKING SALT SOLUTION AND WATER

The differences (*d*) in food consumption under the two drinking conditions also are included

Rat No.	1% salt solution and water	Water	*d*
Group *B*	Period III	Period II	
35	2·23	2·30	−0·07
30	2·10	2·07	0·03
11	2·82	2·50	0·32
12	2·42	2·12	0·30
18	3·10	2·80	0·30
20	1·60	1·48	0·12
4	2·40	2·15	0·25
56	2·48	2·18	0·30
Group *D*	Period II	Period III	
10	2·87	3·07	−0·20
23	1·50	1·53	−0·03
17	3·20	3·32	−0·12
16	2·45	2·37	0·08
7	2·13	1·90	0·23
42	2·07	2·20	−0·13
29	2·45	2·53	−0·08
8	2·68	2·38	0·30
			$\bar{d} = 0·10$

The cross-over analysis of 3-min food consumptions is based on these results. The standard deviation of $\bar{d}$ (defined below) was calculated by the formula:

$$\sigma d = \sqrt{\frac{\Sigma(d - \bar{d})^2 - \dfrac{(\Sigma \text{period I} - \Sigma \text{period II})^2}{30}}{28}}$$

where d = the difference, for each rat, between the average amount of food eaten in a series of days while drinking water and while drinking salt solution and water; $\bar{d}$ = the mean of the above differences; Σperiod I = the sum of the average consumptions of food of all animals for period I, regardless of their fluid intakes; Σperiod II = the same as above for period II.

This formulation of the standard deviation contains a correction factor which takes into account the control, by the cross-over design, of changes in food consumption due to time, habituation phenomena, and the like.

only the food consumptions on the last 4 days of periods II and III were used for determining the average amounts eaten (Table 1). The average 3-min intake of food (during periods II and III) of each rat (in groups *B* and *D*), while drinking water, was subtracted from its average intake when in the choice situation and the mean and standard deviation of these differences were determined (see foot of Table 1 for procedure followed). The average difference ($\bar{d}$ in Table 1) in the amount of food eaten under the two conditions of fluid intake amounted to 0·10 ± 0·041 g, with the average 3-min consumption of food being significantly higher ($t = 2·41$; $P < 0·05$) when the rats drank salt solution and water than when they drank only water.

The difference between the average daily 3-min food consumption on the last 4 days of period II and of period III was then tabulated for each rat in each of the four groups. A single classification analysis of variance of these data, preceded by a corrected Bartlett's test for homogeneity of variance which failed to reach significance ($\chi^2 = 6·91$; d.f. = 3; $P > 0·05$), demonstrated a significantly higher intergroup than intragroup variance ($F = 3·47$; d.f. = 3·28; $P < 0·05$). To determine which group differences, if any, were responsible for the significant *F* ratio, the intergroup difference significant at the 0·05 level was calculated by the Tukey test[4] and found to be 0·22 g. A significant difference existed between groups *A* and *B*; group *B* increased its 3-min food consumption by 0·19 g when changed to the choice situation, whereas the food consumption of group *A*, which remained on water, decreased by 0·04 g in period III. The significant difference in food consumption noted earlier between groups *B* and *D* did not appear under the more stringent conditions of the analysis of variance, where the combined variance of all groups, rather than the variances of just groups *B* and *D*, were involved in significance testing. Rather than carry out several tests among the different groups, thus increasing the risk of securing significant differences by chance, we chose to use the more penalizing analysis of variance. In either case, the point was established that, following a change in fluid intake, animals ate significantly more food in 3 min when drinking salt solution and water than when drinking only water.

The curves in Fig. 1 resemble those published by others[5-7] who have studied the effects of deprivation on eating behaviour. Ghent[5] plotted the number of seconds spent eating during the first minute of availability of food or water, after deprivation of either, against day of testing. She found, as we also observed, that the animals did not eat immediately when first offered food, but, with continued experience in the testing situation, gradually ate increasingly more during the fixed period of time. This increase in amount eaten was due to more time spent in eating during the fixed time interval, rather than to an increase in the speed with which the animals ate.

The effects of changes in drinking regimen on food consumption were greatest among the animals transferred from water to the choice situation (group *B*). Though this switch was followed by a clear increase in food consumption, the removal of salt solution involved in the transfer in group *D* did not result in a decrease in eating. Conceivably, these findings could be viewed as contradictory. To do so, however, would presuppose a logic in behaviour which, in fact, remains to be demonstrated; moreover, such a view would ignore the obvious possibility of changes in behaviour due to learning. It is not unreasonable to assume that the presence of a particular set of sensory stimuli may lead to a type of behaviour which is sufficiently rewarding so that it is maintained even when these stimuli are removed.

The freely chosen intake of salt solution then, following a change in drinking fluids, was associated with a significant increase in food consumption.

Change or manipulation of external stimuli as a motivator of behaviour has been examined before. Campbell and Sheffield[8] found that rats deprived of food for 24 h showed a marked increase in cage activity when changes in the stimulating conditions in their environment occurred. They suggested that food deprivation reduces the animal's threshold to environmental stimulation, that is, sensitizes it to stimulus change. Similar results were obtained with water deprivation[9]. Teghtsoonian and Campbell[10] observed that food-deprived animals, maintained in a situation where environmental stimulation was markedly restricted, showed a decided reduction in the level of maximum activity during deprivation and in the rate at which this maximum was reached. Another group of food-deprived animals, living in a bustling laboratory environment, showed marked increases in cage activity, ranging to 400 per cent of the pre-deprivation level. These investigations, and others (for example, ref. 11), emphasize the role of exteroceptive stimuli as instigators of behaviour, where sensory stimulation becomes a reinforcer of behaviour in its own right, independently of the part it plays in drive or need reduction[12].

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PHOTOCHEMISTRY OF A NICOTINAMIDE METABOLITE, N'-METHYL-4-PYRIDONE-3-CARBOXAMIDE

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IN the course of paper chromatographic investigations of nicotinamide metabolites in human plasma[1,2], it was noted that one of these, later identified[3] as N'-methyl-4-pyridone-3-carboxamide (4-PY), occasionally exhibited a blue fluorescence in ultra-violet light at 360 mμ. Further examination showed that the phenomenon was due to the production of a fluorescent compound or compounds on the paper under the influence of ultra-violet light. Its intermittent occurrence was readily attributable to the varying amounts of ultra-violet radiation to which different chromatograms were exposed for purposes of visual inspection and contact photography. Although the fluorescence was most easily seen on excitation with 360 mμ light, this wave-length had very little photochemical effect. On the other hand, exposure to light from a 254 mμ lamp (the spectral emission of which is shown in Fig. 1) caused a sample of 4-PY spotted on Whatman No. 1 paper to appear to 'melt away' in a few seconds. As the absorption diminished a faint purple glow developed, which in 360 mμ light changed to the characteristic strong whitish-blue fluorescence. The intensity of the fluorescence increased with duration of exposure to radiation until maximum conversion had occurred.

Proof of the formation of at least one new compound was obtained by reversing the chromatogram after irradiation, and running it in the opposite direction (Fig. 2). The unchanged 4-PY ran with its usual velocity, whereas the photoproduct remained at the new origin. A number of different solvent systems, including distilled water, failed to move, and hence to elute, this material from the origin. It was therefore concluded that, under the influence of irradiation at 254 mμ, 4-PY forms a stable fluorescent compound or compounds with cellulose.

Experiments were then carried out to determine whether similar effects could be produced by irradiation of 4-PY in solution. A solution of 4-PY in absolute ethanol at a concentration of 10 μg/ml. was irradiated in a quartz microcuvette situated as close as possible to the 254 mμ hand-lamp. At intervals the cuvette was removed and the absorption spectrum of the solution determined with a Beckman *DU* spectrophotometer. Fig. 3 shows the changes produced. It will be seen that the untreated compound in ethanol exhibits a major peak at 258 mμ and a shoulder at 285 mμ. As irradiation proceeds the 258 mμ maximum diminishes and eventually disappears. The 285 mμ inflection shows little change in intensity but undergoes a bathochromic shift, giving rise to a peak at 305 mμ. In aqueous solution a slight reduction in the intensity of the 258 mμ maximum is the only change observed.

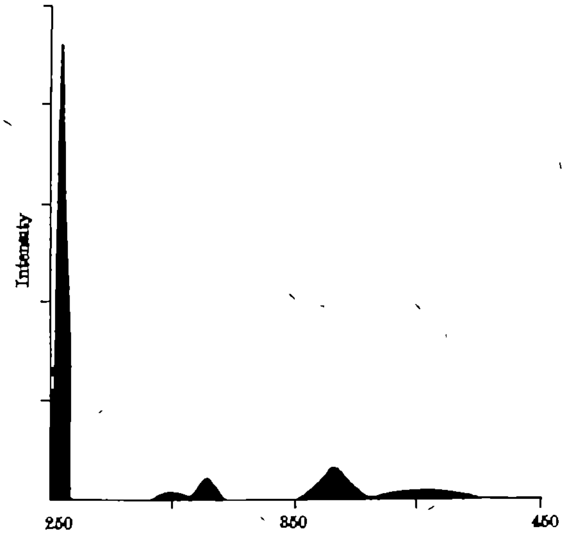

Fig. 1. Spectral emission of Mineralight *SL* 2537 hand-lamp in the ultra-violet region

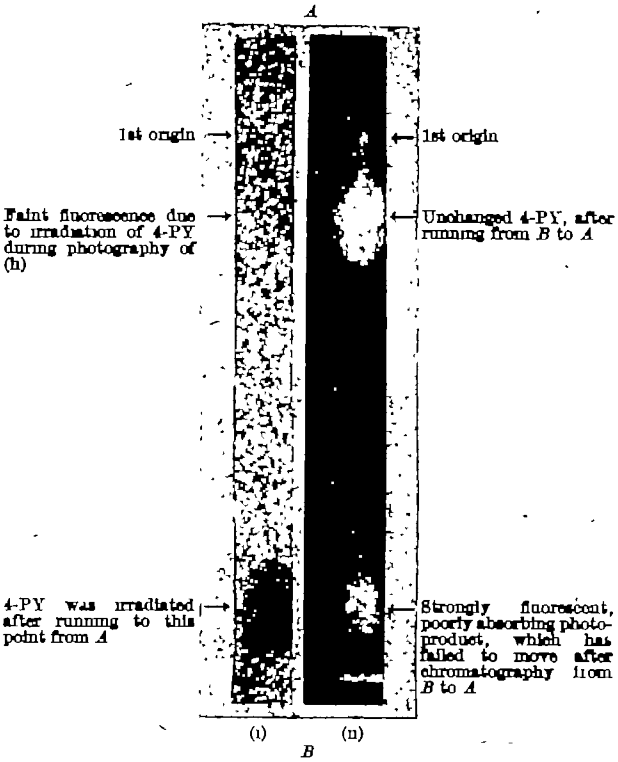
A
1st origin
1st origin
Faint fluorescence due to irradiation of 4-PY during photography of (ii)
Unchanged 4-PY, after running from B to A
4-PY was irradiated after running to this point from A
Strongly fluorescent, poorly absorbing photo-product, which has failed to move after chromatography from B to A
(i)
(ii)
B

Fig. 2. Chromatogram of 4-PY after running from *A* to *B*, irradiating, and then running from *B* to *A*. (i), Photograph of fluorescing spots, taken at 360 mμ, with filter (ref. 1); (ii), photograph of absorbing spots, taken at 254 mμ

To exclude the possibility that the changes produced on paper were due to heat from the lamp, a sample of 4-PY spotted on Whatman No. 1 paper was heated in an oven at 100° C for periods of up to 30 min. No fluorescence developed. That the changes in ethanol were also not due to heat was shown by the absence of spectral change in a control solution, screened by aluminium foil, which was placed next to the test solution throughout the irradiation period.

When viewed with the 360 mμ lamp, a bluish-green fluorescence is visible in solutions of 4-PY which have previously been exposed to radiation from the 254 mμ lamp. The changes in the intensity of fluorescence with duration of irradiation are shown in Fig. 4. After irradiation for 30 min the solution was taken to dryness and chromatographed on paper in butanol–ammonia. At least two spots showing dark blue fluorescence were seen.

The fluorescence spectra before and after irradiation were determined using an Aminco-Bowman spectrophotofluorometer (Fig. 5). It will be seen that the fluorescence intensity of the irradiated solution is actually less than that of the unirradiated solution. The fact that the former is visible to the naked eye, whereas the latter is

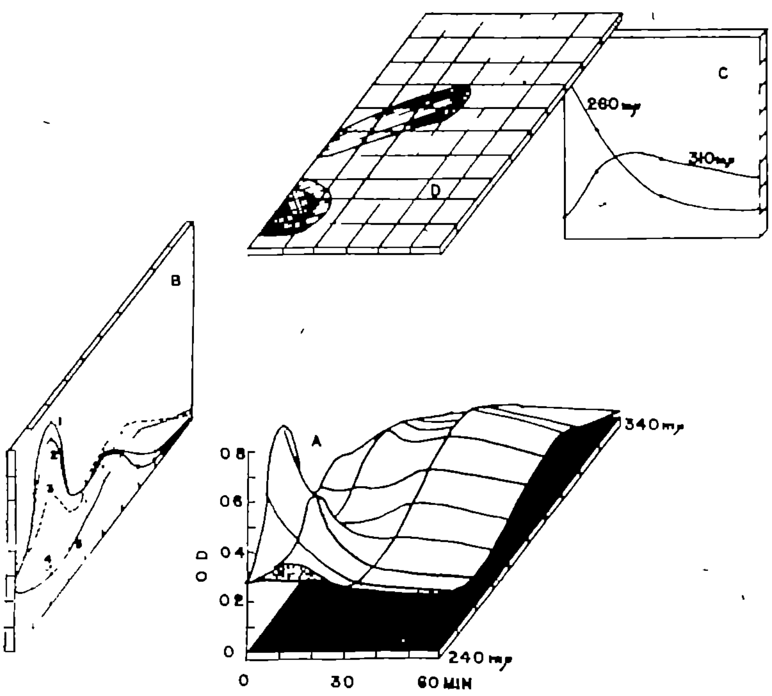

Fig. 3. Absorption stereospectrum of 4-PY in ethanol during ultra-violet irradiation. *A*, Stereospectrum. *B*, (1) spectrum before irradiation; (2) spectrum after 1 min irradiation; (3) spectrum after 8 min irradiation; (4) spectrum after 26 min irradiation; (5) spectrum after 56 min irradiation. *C*, Changes in absorption at 260 mμ and 310 mμ with time. *D*, Areas shaded by parallel lines represent optical density (O D.) greater than 0 3, cross-hatched area O.D. greater than 0 4, and black area O.D. greater than 0 6

not, may be attributed partly to the shift in the fluorescence towards the visible region after irradiation. In addition, it was found that the activation maximum of the unirradiated solution is 300 mμ. compared with 320 mμ for the irradiated solution. (These values are approximate, due to the presence of a scatter peak in the region concerned.) Inspection with the 360-mμ source would therefore excite the fluorescence of the irradiated solution more efficiently For the determination of the emission and activation spectra it is essential to use 'fluorometric grade' absolute ethanol (Hartman-Leddon Co., Philadelphia, Pa.) to eliminate the spurious fluorescence exhibited by reagent grade absolute ethanol in the 400 mμ region.

Although it is known that a number of compounds on paper chromatograms undergo decomposition on prolonged exposure to ultra-violet light[4], the present example is unusual in that it possesses extreme sensitivity to such radiation, and gives rise to a strongly fluorescent photoproduct. This property permits the ready identification of 4-PY among other ultra-violet-absorbing spots. The photoproduct itself displays the distinctive property of being completely immovable in all solvent systems tried, including distilled water. This phenomenon suggests an irreversible association with a constituent of the chromatographic paper, such as hydroxyl or aldehydic groups of cellulose.

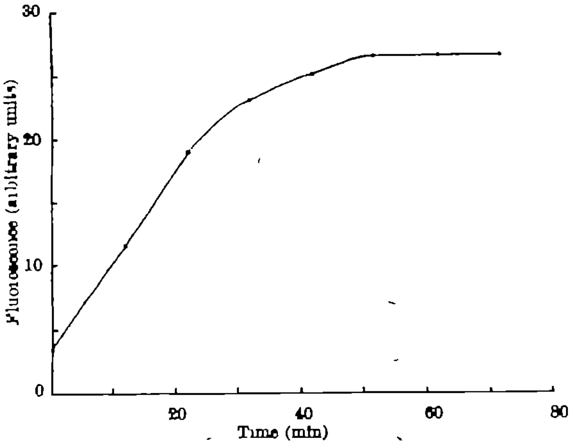

Fig 4 Development of fluorescence with time in irradiated solution of 4-PY in ethanol

The only other comparable situation which we have been able to trace occurs in the field of amino-acid chromatography.

In 1948 Phillips[5] showed that the α-amino-acids and peptides resulting from enzymatic degradation of insulin fluoresced on paper chromatograms. The intensity of fluorescence was greatest when high drying temperatures were used, but some fluorescence developed even when the papers were dried at 37° C. The following year Patton *et al.*[6] showed that this fluorescence remained behind on the paper after washing with water. They demonstrated that the paper was essential for the development of fluorescence, which failed to appear when amino-acids were heated on glass plates. These workers suggested that the fluorescent change is analogous to the browning reaction. Similar results were reported in 1950 by Woiwood[7], who pointed out that most amino-acids fluoresce feebly in the pure crystalline state, although tryptophan, histidine and citrulline fluoresce strongly. When heated with cellulose some twenty amino-acids fluoresced, as did a number of simple peptides and amines. The amino-group alone appeared to be necessary for the reaction which, it was suggested, occurred with aldehydic groups derived from modified or short-chain celluloses present in the paper. We have already indicated that heat does not induce fluorescence in 4-PY on paper.

The effects of ultra-violet radiation on 4-PY in ethanol resemble those obtained on paper chromatograms in that a diminution in light absorption is accompanied by the development of fluorescence. The nature of the fluorophore(s) is unknown. Slomp *et al.*[8] and Taylor *et al.*[9] have proposed a dimeric structure for the product of ultra-violet irradiation of *N*′-methyl-2-pyridone. However, apart from structural differences between this compound and that used in the present investigation, their experi-

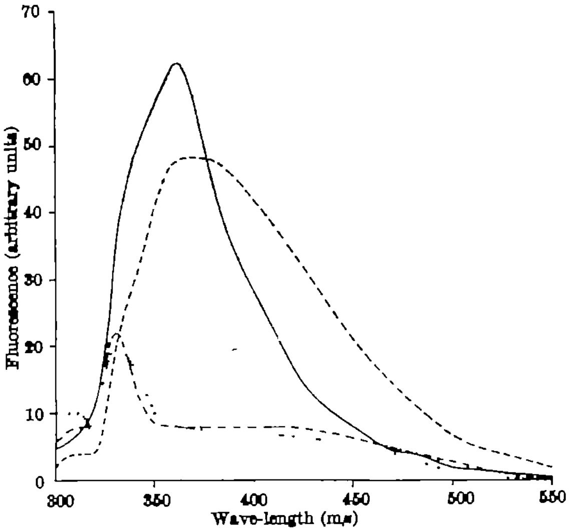

Fig. 5. Fluorescence spectrum of 4-PY in ethanol (10 μg/ml.) before and after 30 min irradiation. Activation monochromator set at 300 mμ ———, 4-PY before irradiation; ----, 4-PY after irradiation; · · ·, absolute ethanol (fluorometric grade) before irradiation, ·– –, absolute ethanol (fluorometric grade) after irradiation

ments were carried out in aqueous solution in which, as we have shown, the spectrum of 4-PY is unchanged. The spectral shift observed in ethanolic solutions of 4-PY suggests reduction of the pyridone with preservation of the quinonoid structure.

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INTERPRETATION OF INHIBITION KINETICS OF COENZYME-SUBSTRATE REACTIONS, AND THE ROLE OF ZINC IN ALCOHOL DEHYDROGENASES

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SEVERAL types of inhibition are distinguished experimentally by the effects of a fixed inhibitor concentration on the slopes and intercepts of plots of initial rate data according to Lineweaver and Burk[1], or by the point of intersection when plots in presence and absence of inhibitor are extrapolated. They receive theoretical interpretation by the inclusion of compounds *EI* and *ESI*, with dissociation constants K_{EI} and K_{ESI} respectively, in the reaction scheme proposed by Michaelis and Menten[2] for a single substrate reaction. The general relation between initial rate in the steady state, v_0, the enzyme concentration e, the substrate concentration s, and the inhibitor concentration i, takes the form:

$$1/v_0 = (1 + i/K_{ESI})/V + (1 + i/K_{EI})K_m/Vs$$

or:

$$e/v_0 = \varphi_0(1 + i/K_{ESI}) + \varphi_1(1 + i/K_{EI})/s$$

The parameters V and K_m, or φ_0 and φ_1, and K_{EI} and K_{ESI} can be determined experimentally from plots of e/v_0 against $1/s$. Increased slope with unchanged intercept, or intersection on the abscissa, indicates competitive inhibition ($K_{ESI} = \infty$). Increased intercept with unchanged slope, that is, parallel plots, has been designated uncompetitive inhibition ($K_{EI} = \infty$). Equal proportionate increase of slope and intercept, or intersection on the ordinate, denotes non-competitive inhibition ($K_{EI} = K_{ESI}$). 'Mixed' types are competitive–non-competitive, with greater proportionate increase of slope than intercept and intersection above the ordinate ($K_{EI} < K_{ESI}$), and non-competitive–uncompetitive, with greater proportionate increase of intercept than slope and intersection below the ordinate ($K_{EI} > K_{ESI}$).

The behaviour of inhibitors in coenzyme-substrate reactions has also been described in these terms, both with reference to the coenzyme, from initial rate measurements with constant substrate concentration, and with reference to the substrate, by experiments with constant coenzyme concentration. The underlying assumption appears to be that if the concentration of either the substrate or the coenzyme is maintained at a level much greater than its Michaelis constant, the reaction will be kinetically equivalent to a single substrate reaction. This is not necessarily so[3], and it cannot be assumed[4] that inhibition equations deduced for a single substrate reaction are applicable to coenzyme-substrate reactions.

The main purpose of this article is to show that on the basis of a compulsory order mechanism, by which the coenzyme is bound to the enzyme first, it is to be expected that an inhibitor which competes with the coenzyme only will in general exhibit different 'types' of behaviour with reference to the substrates for the forward and reverse reactions. Experimental observations of this kind with the zinc-binding inhibitor *ortho*phenanthroline and liver alcohol dehydrogenase have been considered[5] inconsistent with a compulsory order mechanism, and have invoked conclusions regarding the binding of the substrates which appear to be unjustified[5-7].

Such a mechanism is represented by the following reactions:

$$\begin{aligned}
&E + I \rightleftharpoons E.I\\
&E + C \rightleftharpoons E.C\\
&E.C + S \rightleftharpoons E.C.S \rightleftharpoons E.C'.S' \rightleftharpoons E.C' + S' \qquad (1)\\
&E.C' \rightleftharpoons E + C'
\end{aligned}$$

The initial rate equation in the steady state, whether or not the ternary complexes $E.C.S$ and $E.C'.S'$ are included, takes the form[8,9]:

$$e/v_0 = \varphi_0 + \varphi_1\,(1 + i/K_{EI})/c + \varphi_2/s + \varphi_{12}\,(1 + i/K_{EI})/cs$$

With the reciprocal of the coenzyme concentration, $1/c$, as variable:

$$e/v_0 = (\varphi_0 + \varphi_2/s) + (\varphi_1 + \varphi_{12}/s)(1 + i/K_{EI})/c \qquad (2)$$

and with any constant values of substrate and inhibitor concentration, s and i, the effect of the inhibitor will be to increase the slope by the factor $(1 + i/K_{EI})$, from which K_{EI} may be calculated, while the intercept will be unchanged, that is, competitive behaviour, as expected.

With $1/s$ as variable:

$$e/v_0 = [\varphi_0 + \varphi_1(1 + i/K_{EI})/c] + [\varphi_2 + \varphi_{12}(1 + i/K_{EI})/c]/s \qquad (3)$$

The effect of the inhibitor will be to increase both slope and intercept, but not necessarily in the same proportion. The relative increases will be determined by the relative values of φ_0, φ_1, φ_2 and φ_{12}, initial rate parameters which may be estimated experimentally in absence of inhibitor. Re-arranging equation (3):

$$e/v_0 = \varphi_0\left[1 + \frac{\varphi_1}{\varphi_0}\left(\frac{1 + i/K_{EI}}{c}\right)\right] + \varphi_2\left[1 + \frac{\varphi_{12}}{\varphi_2}\left(\frac{1 + i/K_{EI}}{c}\right)\right]\frac{1}{s} \qquad (4)$$

or in alternative symbols[9,10]:

$$1/v_0 = \frac{1}{V}\left[1 + K_{m1}\left(\frac{1 + i/K_{EI}}{c}\right)\right] + \frac{K_{m2}}{V}\left[1 + K_{EC}\left(\frac{1 + i/K_{EI}}{c}\right)\right]\frac{1}{s}$$

V is the maximum rate; $\varphi_1/\varphi_0 = K_{m1}$, the Michaelis constant for the coenzyme; $\varphi_2/\varphi_0 = K_{m2}$, the Michaelis constant for the substrate; and $\varphi_{12}/\varphi_2 = K_{EC}$, the dissociation constant of the enzyme–coenzyme compound.

The 'type' of behaviour of the inhibitor towards the substrate will therefore depend on the relative values of K_{m1} and K_{EC}. Only if these two quantities are equal will the slope and intercept be increased by the same proportion, indicating 'non-competitive inhibition'. If $K_{m1} < K_{EC}$, the inhibition will be 'competitive–non-competitive', or if the inequality is large, almost 'purely competitive'. If $K_{m1} > K_{EC}$, the inhibition will be 'uncompetitive' or 'non-competitive–uncompetitive'.

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A Michaelis constant may be greater or smaller than the true dissociation constant[3]. For a number of dehydrogenases that use the coenzyme nicotinamide–adenine dinucleotide (NAD+), including lactate dehydrogenase[11], yeast[12] and liver[13,14] alcohol dehydrogenases and malic dehydrogenase[15], for which initial rate data at pH 6–8 are available, it is found[16] that for the reduced coenzyme, NADH, $K_{m1} > K_{EC}$ (that is, $\varphi_1/\varphi_0 > \varphi_{12}/\varphi_2$), while in the reverse reaction for NAD+, $K'_{m1} < K_{EC}'$. These complementary relations for the oxidized and reduced coenzymes are to be expected theoretically[8] for a compulsory order mechanism. (For the special case that ternary complexes $E.C.S$ and $E.C'.S'$ are not kinetically significant (that is, the Theorell–Chance[17] mechanism) it can be shown[16] that $K_{m1}/K_{EC} = K_{EC'}/K'_{m1}$.) It follows from equation (4) that an inhibitor that competes with the coenzyme should in general show different 'types' of behaviour towards the substrate in the forward and reverse reaction. Moreover, since the relative values of K_{m1} and K_{EC} may vary with pH, so also may the 'type' of inhibition.

Hoch, Williams and Vallee[6] interpreted experimental data for the inhibition of yeast alcohol dehydrogenase by *ortho*phenanthroline on the basis of mechanism (1), except that the ternary complexes were omitted. They concluded that the inhibitor showed non-competitive behaviour towards both ethanol and acetaldehyde, which they considered, mistakenly as has been shown, to be required by this mechanism[5]. Their data with the highest inhibitor concentration indicate that the inhibition is in fact competitive–non-competitive towards ethanol and non-competitive–uncompetitive towards acetaldehyde, in accordance with the preceding discussion.

With liver alcohol dehydrogenase, more marked differences between the apparent behaviour of *ortho*phenanthroline towards the two substrates have been detected and variously interpreted. It is of some interest, therefore, to show that as with the yeast enzyme the experimental data are in accordance with mechanism (1), and the fact that for this enzyme, in neutral solution, the values of K_{m1}/K_{EC} for NADH, and K_{EC}'/K'_{m1} for NAD+, are large. This can be done by substitution in equations (2) and (4) of experimental values for the initial rate parameters reported by me[13]. Equation (4) then becomes, for the reaction of ethanol and NAD+:

$$e/v_0 = 0{\cdot}37\left[1 + \frac{3{\cdot}0(1 + i/K_{EI})}{[\mathrm{NAD^+}]}\right] + 110\left[1 + \frac{65(1 + i/K_{EI})}{[\mathrm{NAD^+}]}\right]\frac{1}{[\mathrm{C_2H_5OH}]}$$

and for the reaction of acetaldehyde and NADH:

$$e/v_0 = 0{\cdot}0075\left[1 + \frac{13(1 + i/K_{EI})}{[\mathrm{NADH}]}\right] + 3{\cdot}3\left[1 + \frac{0{\cdot}42(1 + i/K_{EI})}{[\mathrm{NADH}]}\right]\frac{1}{[\mathrm{CH_3CHO}]}$$

The Lineweaver–Burk plots in Figs. 1*c* and *d* were calculated from these equations with $i = 120$ μM and $K_{EI} = 2{\cdot}5$ μM, and the fixed concentrations of coenzymes indicated. Figs. 1*a* and *b* were obtained by similar substitutions in equation (2). It should be added that larger values for the fixed concentrations would merely require a greater inhibitor concentration to produce comparable effects, and would not alter their nature; and also that if the initial rate parameters reported by Theorell and McKee[18] are used, the same types of plot are obtained.

It is evident that the 'partially competitive' type of inhibition towards ethanol and the 'non-competitive–uncompetitive' behaviour towards acetaldehyde are predicted by mechanism (1), and provide no grounds for earlier conclusions that the two substrates are bound at different sites on the enzyme, and that the ethanol-binding site interacts with, or is adjacent to, that responsible for coenzyme and *ortho*phenanthroline binding[7]; or that the substrates must have an effect on the binding of *ortho*phenanthroline to liver alcohol dehydrogenase, and that the mechanism may be different to that for the yeast enzyme[6].

All workers agree that *ortho*phenanthroline is strictly competitive towards the coenzymes, but there are discrepancies between the values obtained for K_{EI}. Plane and Theorell[6] did not interpret their data in terms of a compulsory order mechanism, however, but considered that binary complexes of enzyme and substrate are formed. With this assumption, they calculated values for K_{EI} of 3·0, 0·013 and 11 μM from data corresponding to Figs. 1*a*, *b* and *c* respectively. To account for these apparent discrepancies, they postulated ternary complexes of enzyme, inhibitor and substrate, and estimated their dissociation constants. There are no such discrepancies, however, if their data are interpreted in terms of mechanism (1). By means of equations (2) and (4), values close to 2·5 μM for K_{EI} are obtained from all three plots; the close agreement between the slopes of the competitive plots against the two coenzymes is apparent on inspection. The theoretical plots in Fig. 1 are in fact calculated for the same concentrations of inhibitor, coenzymes and substrates as those used by Plane and Theorell, and resemble their experimental plots closely. The value of 2·5 μM is also in reasonable agreement with 4 μM calculated in a similar manner by Vallee, Williams and Hoch[5] from competition with NADH under slightly different conditions. These workers obtained a much higher value from competition with NAD+, but this may arise from the high ethanol concentration

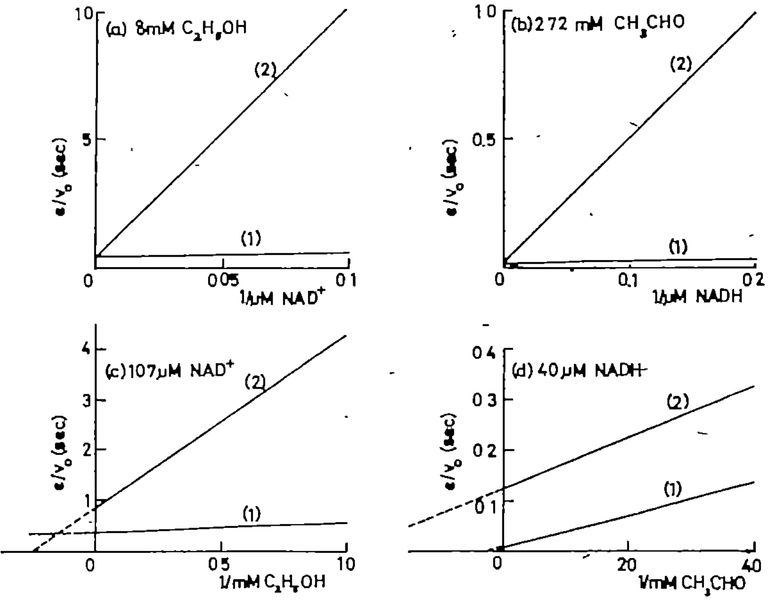

Fig. 1. Effects of an inhibitor which competes with the coenzyme on the initial rate of the liver alcohol dehydrogenase reaction at pH 7·1 and 23·5°, on the assumption of a compulsory order mechanism. The reciprocals of specific initial rates e/v_0 are plotted against the reciprocals of the coenzyme (*a*, *b*) and substrate (*c*, *d*) concentrations. The plots (1) for the uninhibited reaction were calculated from experimental values for the initial rate parameters (ref. 13), and the plots (2) showing the theoretical effects of 120 μM inhibitor, with $K_{EI} = 2{\cdot}5$ μM, by equations (2) and (4) in the text

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used, which causes substrate inhibition and renders equation (2) inapplicable. It appears that the kinetic data for the inhibition of liver alcohol dehydrogenase are consistent with mechanism (1) and a value of about 2·5 μM for K_{EI} at pH 7 and 23·5°. A discrepancy remains, however, between this value and that obtained by spectrophotometric measurements[19].

Vallee *et al.*[5,9] conclude from the absence of strictly competitive behaviour of *ortho*phenanthroline towards the substrates that the latter cannot be bound to zinc in ternary complexes of yeast and liver alcohol dehydrogenases. If the substrate were so bound it might be expected that competition between *ortho*phenanthroline and substrate for $E.C$ in mechanism (1) would occur with formation of $E.C.I$. This would introduce the inhibition factor $(1 + i/K_{ECI})$ in the term φ_1/s in the initial rate equation. It may also imply the formation of the product complex $E.C.'I$, which would give $\varphi_0(1 + i/K_{EC'I})$ as the maximum rate, and the compound EI_2 also. In any event, there is no evidence of such effects in the experimental data.

Theorell *et al.* assume a random order mechanism for liver alcohol dehydrogenase[6,18,20], with kinetically significant binary enzyme-substrate complexes. If the substrates were bound to zinc, competition from *ortho*phenanthroline would again be expected, in this case for the free enzyme also. Theorell and McKee[20] explain the absence of strict competition by assuming that of the three available bonds of octahedral zinc, the inhibitor can only utilize the same two co-ordination sites as the coenzyme, and cannot compete for the third substrate site. The evidence for ternary complexes of enzyme, *ortho*phenanthroline and substrate adduced by Plane and Theorell[6] is taken to support this explanation. The 'partially competitive' behaviour towards ethanol, but not acetaldehyde, is attributed to a stabilizing effect of ethanol on the binding of NAD+.

The compulsory order mechanism (1) seems to provide a simpler explanation of the experimental data which, contrary to the conclusions of Vallee *et al.*[5,9], does not preclude the binding of substrate to zinc in the ternary complex, as suggested by Theorell and McKee[20]. If the metal were the centre of an octahedral ternary complex, with three bonds to the protein, and two to the bidentate coenzyme, the absence of competition between the bidentate *ortho*phenanthroline and substrate for the remaining co-ordination site of the enzyme–coenzyme complex would be understandable. Thus if binary enzyme-substrate complexes are not assumed, no further assumptions are needed to explain the behaviour of the *ortho*phenanthroline towards the substrates. Indeed, the conformity of the inhibition data to the initial rate equation derived from a compulsory-order mechanism and the absence of competitive behaviour towards both substrates, might be construed as evidence against the view that such complexes are kinetically significant. Unlike the coenzymes, *ortho*phenanthroline would not be expected to have binding sites on the enzyme other than those to zinc, as Theorell and McKee[20] remark; it seems improbable, therefore, that of the three co-ordination sites of the free enzyme, only the two utilized by the coenzyme are available to the *ortho*phenanthroline, as they suggest.

It may be concluded that inhibition kinetics with *ortho*phenanthroline provide valid evidence that the coenzymes are bound to zinc in the enzyme–coenzyme complexes of yeast and liver alcohol dehydrogenases, and are consistent with, but do not prove, the binding of substrates to zinc in the reactive ternary complex in a compulsory order mechanism. It may also be concluded that in kinetic studies of inhibition of coenzyme-substrate reactions, it is desirable that measurements be made with simultaneous variation of both coenzyme and substrate concentrations, so that the effects of the inhibitor on all the terms in the initial rate equation may be evaluated and used to assess the mechanism.

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FORMATION AND DISTRIBUTION OF AMYLOSE AND AMYLOPECTIN IN THE STARCH GRANULE*

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STARCH granules are biological objects and as such closely linked to the genotype and the physiology of the plant which produces them. Although most starch granules contain linear and branched molecules of the α-1,4-glucosidic type, with α-1.6 linkages at the branching points, there is much heterogeneity in these two fractions. There is also an enormous variation in their ratio and in their packing to a paracrystalline pattern. It is well known that there is a different shape of starch granule for every plant species.

This variability in properties is surprising when we consider that the basic chemistry is the same for all starches and that all plastids have the fundamental ability to make starch in their ground substance[1]. It suggests the presence of a complex of interfering factors which makes the production of starch in plants an extremely variable process.

We will limit the discussion to the plastids of higher plants, and we ask: How are starch granules formed, what enzymes play a part in their synthesis, how can modifying factors influence the deposition of a mixture of linear and branched molecules and their relative amounts? If one could give detailed answers to all these questions, the fine structure of the different types of starch granules would be clear. Unfortunately, this is not yet possible and much has still to be done.

(1) *The plastid and starch formation.* Chlorophyll-containing plastids (chloroplasts) have been most extensively studied because they form the photosynthetic apparatus and are available in quantity. We will deal

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only with the amyloplast, which forms the storage-type of starch granule. Only in exceptional cases is the amyloplast coloured (as in *Pellionia*). When examined with the electron microscope[2] it appears to be a vesicle surrounded by a double membrane, with few, if any, membranous structures inside. Nevertheless, amyloplasts from various plants show typical structural characteristics which must have a genetical origin and which are associated with the chemical composition of the plastid. The influence of genetical factors has been clearly demonstrated in the shape and composition of starch granules from various corn varieties. (I have summarized these aspects elsewhere[3].)

At the time of starch formation, potassium permanganate causes the production of a fine homogeneous precipitate in the plastid. This precipitate disappears when the first starch deposit is formed and may therefore represent an accumulation of precursor substance with reducing properties. With a new supply of carbohydrate similar particles, also visible after potassium permanganate treatment, appear; electron pictures suggest that again they represent precursor material which is added to the starch granule. The particles range in size from 250–300 Å, and the smallness of the droplets they represent explains an earlier observation that starch may be deposited in extremely thin layers[4]. Growth of starch granules by apposition has been fully demonstrated, but so far we did not know how it actually occurs. Lengthening of molecules at the periphery of the granule was thought to be the mechanism by which growth takes place. However, this theory does not take into account the inability of retrograded molecules to act as primers. The electron picture may give a better representation of the real situation.

The possibility that starch crystallizes out from a coacervate was suggested in 1946 by MacMasters *et al.*[5]. If it is true that tiny coacervate droplets carry fresh material towards the periphery of the starch granule, then a new approach to our understanding of the composition of the granule is possible. To appreciate this we have now to consider the enzyme systems involved in starch synthesis.

(2) *Starch synthesizing enzymes.* Phosphorylase has been thought to be responsible for starch synthesis (in collaboration with the *Q*-enzyme, which has branching action), since it was found in those parts of plants where starch accumulates and because of its location inside the plastids. Doubts about its function have been expressed before (see my summary elsewhere), but more recently it was even rejected as a starch synthesizing enzyme *in vivo*. The reasons for this rejection can be read in an article by Whelan[6]. A new enzyme proposed is one that uses uridine diphosphoglucose (UDPG) as a glucose donor[7]. Other possible systems, suggested by Whelan and by Erlander, have been discussed earlier[3,6].

The implication is that an enzyme may be present, but that it has no physiological function. Such instances can actually be found. In the plant epidermis, for example, plastids contain phosphorylase, although under natural conditions they never form starch[8]. It is possible that the function was lost during the process of evolution; such loss in the epidermis would not discredit the enzyme as a starch synthesizer in other tissues. In addition, it becomes more and more clear that organisms are versatile and can adapt their metabolic pathways to varying conditions. We know as yet very little about the co-ordination between enzyme systems which may be involved in the production of starch. It is reasonable to assume that they all play their part at times.

The enzyme discovered by Leloir *et al.*[7] needs preformed starch to make more starch. Since the enzyme could not be extracted from the starch granules, it is probably located inside the granules and provides for some addition to existing molecules *in situ*. Such growth by intussusception will be limited in extent. This mechanism does not explain how the starch was formed in the first instance, or how there can be linear and branched molecules. As Porter[10] rightly remarks, *de novo* synthesis of starch may yet depend on the presence of phosphorylase.

What more does the Leloir enzyme do? It makes oligosaccharides (up to 6 units) from maltose and maltotriose in the presence of UDPG, but it was found that these oligosaccharides cannot act as intermediates in starch synthesis. They could, of course, act as primers for phosphorylase.

Turner *et al.*[11,12] have shown that UDPG is an excellent source of glucose-1-phosphate (G-1-P), the substrate for phosphorylase, from which this enzyme may make starch even without primers being present[13]. This G-1-P is produced from sucrose, the main sugar of transport in plants. In potato plants which had assimilated $^{14}CO_2$, sucrose was found to be transported down the stem and to spread evenly throughout the tuber, where it appeared in the cytoplasm of the cells. From there radioactivity entered the plastids, the membranes of which must have some regulatory function. Finally, the starch granules become radioactive, in the first instance in those parts of the tuber where phosphorylase activity was highest[4].

Our conclusion must be that phosphorylase is the predominant starch synthesizing enzyme and that it can be excellently co-ordinated with a UDPG or related system. Further discussion in this article will be based on this conclusion.

At this junction I wish to stress again that studies of tissue homogenates cannot give a proper insight into the processes inside the plastid. As Krech[9] has pointed out, in such homogenates various substances come together which would never meet in the intact cell. This is one reason why the ratio inorganic/organic phosphate, as observed in tissues, is not so important as was thought before. We have to study the plastids themselves in undamaged, healthy cells, in order to discover their mechanism.

(3) *The general distribution pattern of amylose and amylopectin.* One of the most difficult problems facing starch chemistry is to explain why there are linear molecules at all, when we know that a mixture of phosphorylase and branching enzyme (often indicated respectively as *P*- and *Q*-enzyme) always gives only a branched product in a solution containing G-1-P and primer molecules. Both enzymes have been found in normal corn endosperm (containing starch with about 25 per cent amylose), waxy corn (1 per cent amylose) and wrinkled pea (65 per cent amylose and more). Assuming a different *P*/*Q*-ratio for these plants does not help in elucidating the differences between their starches.

Both *P*- and *Q*-enzymes must be present with their substrates in the plastid, because that is the place where the starch granule is formed. Phosphorylase has been repeatedly detected histochemically inside the plastid, but we have to confirm the localization of *Q*-enzyme and the other substances involved. Since G-1-P penetrates only very slowly into the cells[9] and a UDPG-enzyme system was found in association with the starch[7], it is likely that sucrose is admitted to the plastid and converted while inside. The mechanism by which this could happen has to be investigated. We also do not know how the enzymes (and other proteins) become included.

Now the stage is set for the production of starch. In principle we may expect the same reactions to happen as are demonstrated in the test tube, and the question therefore arises: what are the factors that can modify this standard pattern? In other words, How is the hereditary chemical composition of the plastid reflected in the structure of the starch granule it produces?

One of the effects appears to be that for most starches crystallization from a coacervate takes place before all the linear molecules have become branched. We may assume that the amylopectin is formed rapidly, but that the *Q*-enzyme will reach entangled linear molecules with increasing difficulty. This would explain the presence of an intermediary fraction and slight branching in longer molecules[14,21] when crystallization takes place at this stage in the tiny coacervate droplets. In waxy starch *Q*-enzyme appears to be able to branch completely the original linear molecules before crystallization starts.

Since each coacervate droplet produces a mixture of linear and branched molecules in ordinary starch, and an immense number are required to build up a new shell, we do not expect *a priori* a difference in location between the two fractions. They must show an even distribution throughout the granule in the form of mixed crystals, which is in agreement with the results of microscopic investigations[3].

Only 1/5 to 1/3 of the total chain-length in the starch granule shows orderly arrangement in crystalline patterns[14]. Amorphous regions are therefore found both within and in between the shells, those in between forming the so-called amorphous layers[3]. Permutoid reactions will occur in the more accessible amorphous regions in the first instance, and this is the reason why amylase action spreads tangentially from the corrosion canals in cereal starches, and why treatment with dilute acid (lintnerization) causes separation of layers in practically all starches. During these processes amylopectin is degraded by preference[15–18], and this result indicates that both amylose and amylopectin are also present in the amorphous regions. We conclude with Hess[19] that the only difference between the shells and the amorphous layers is in their water content, and this must be related to a periodic supply of carbohydrate. When there are no layers present, as in cereal starches which were produced under conditions of constant illumination, there is an uninterrupted addition of coacervate droplets. The fundamental process remains the same and therefore we do not expect chemical differences between granules with or without layers (when studied in the same species of plant).

It will scarcely be necessary to stress again[20] that there is no layer with special chemical properties surrounding the starch granule.

The assumption that the concentration of amylopectin increases towards the outside of the granule was based on an incorrect interpretation of the swelling phenomena (for a discussion see my earlier work[21]). Recently, Samotus and Schwimmer[22] demonstrated an increase in esterified phosphate during the development of the potato starch granule. Combining this result with growth by apposition only and the assumption that esterified phosphate is located at the branching points[23] could lead to the conclusion that there is an increase in the degree of branching in a centrifugal direction (in the potato starch granule).

The potato starch granule shows unique behaviour in many respects. I have given arguments which could support an increase of amylopectin content within one layer of the potato starch granule[21] (separation of layers after leaching out the longest molecules). It is, admittedly, very difficult to demonstrate such fine differences in structure with certainty. On no account, however, should one use specific observations, made on potato starch granules, to draw general conclusions about the cereal starches[21].

Finally, there are the waxy types to be considered. These starch granules consist mainly of amylopectin, so that obviously there is no chemical difference between the layers. Many of them contain a low percentage of amylose, which is located in the centre of the starch granule[3]. These cores can easily be isolated and consist of a globular particle, often surrounded by one or two layers. They were well known in waxy corn starch, but I found them also in grenadilla and waxy sorghum starch. Since a 'blue core' cannot be detected in the earliest starch deposit, one wonders whether they are caused by an after-production of linear molecules (which may become slightly branched as they grow longer); this would be a possible conclusion on the basis of recent chemical results[24]. A similar explanation might be given to the increase in linear component observed in all cereal starches[3] and wrinkled pea starch[24] during their development. I have always maintained that, while growth is by apposition, some intussusception could easily occur, especially since parts of the plastid matrix may become included as the starch granule grows[3]. The peculiarities mentioned deserve intensive study.

(4) *Factors which can influence starch deposition in the plastid.* We assume that: (1) the chemical composition of the plastid is genetically fixed; (2) phosphorylase is the initial starch synthesizing enzyme; (3) amylose is formed first and then transformed into amylopectin by *Q*-enzyme; (4) starch crystallizes out in microcoacervate droplets before branching is complete (ordinary starches) or after it has been completed (waxy varieties).

Some of the factors that may have an influence on this process are listed here; they may differ between species and between organs of one plant.

(*a*) The quantity of water available for hydration is known to influence the type of crystallization: less water available causes a tendency towards the *A*-spectrum[3].

(*b*) Higher temperature also brings about stronger molecule association; both *in vivo*[25] and *in vitro*[3] the X-ray spectrum is shifted towards the *A*-type. Higher temperature can have the effect of producing amylose chains of shorter length[26].

(*c*) Fatty acids and phospholipids cause stronger retrogradation, especially of the linear fraction.

(*d*) Inorganic phosphate limits the chain-length of amylose by suppressing its formation[27]. Esterified phosphate increases swelling power (as in the potato starch granule).

(*e*) Many salts retard retrogradation[28], and some influence the crystallization pattern[29]. In this connexion it may be significant that potato starch has the highest ash content of all[30], with potassium as the main cation.

(*f*) Glucose may inhibit amylose formation[27].

(*g*) Sucrose competes successfully for hydration water[3]. This may be an important factor in all seeds where high sucrose concentration is associated with decreased starch production. Such seeds shrink when they are dried; their starch has low swelling power and high amylose content. Examples are: wrinkled pea, sweet corn, and corn with the gene *sh*-2.

(*h*) Proteins can form complexes with the starch molecules[3]. Interaction with enzymes like amylases may shorten the chain-length of the linear fraction[24].

(*i*) The type and quantity of primer has a great influence; the concentration of G-1-P may be equally significant[3].

I consider the factors last mentioned under (*i*) to be of prime importance, because they initiate starch formation. There may be factors other than the few enumerated, but it will be clear from the examples cited that starch formation in the plastid is a very complex process. At present it is impossible to correlate the factors with known starch properties, but there is a wide field open for research in this direction. All we can say now is that variations in starch granule structure are probably associated with the complexity of the plastid.

Substances like saponins and chlorogenic acid, which are known to have an inhibitory action on starch formation, occur in the vacuoles and not in the plastids; they are of little interest in this connexion.

In coacervate droplets linear molecules will be continuously formed and gradually subjected to the branching action of *Q*-enzyme. During their formation the molecules are subjected to the influence of substances in the plastid. Crystallization brings an abrupt end to this process, and could leave a minority of molecules unbranched, some slightly branched[21], and others unfinished on their way to amylopectin. A good approach for further research would be the study of plastid models in which various factors are allowed to act under controlled conditions, to test the validity of the foregoing concept.

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FUNGAL DISEASE IN MALAYAN TOADS: AN ACUTE LETHAL INFLAMMATORY REACTION

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Bufo melanostictus collected around Kuala Lumpur for laboratory class dissection work showed a high incidence of tumour-like structures on the skin (Fig. 1). Out of 471 toads examined to date, 218 were infected externally. No difference in the percentage of infected animals was found between males and females, and random samples collected at different periods over a year showed no seasonal variation. The commonest sites of infection were the ventral and dorsal surfaces and the hind limbs, the incidence being higher on the ventral surface. Of the 218 infected toads 76 were infected internally and the organs commonly infected were the liver and kidney, but the spleen, ovary, heart (ventricle) and the lung were occasionally involved. No toads were found to be infected internally unless at least one external lesion was also present.

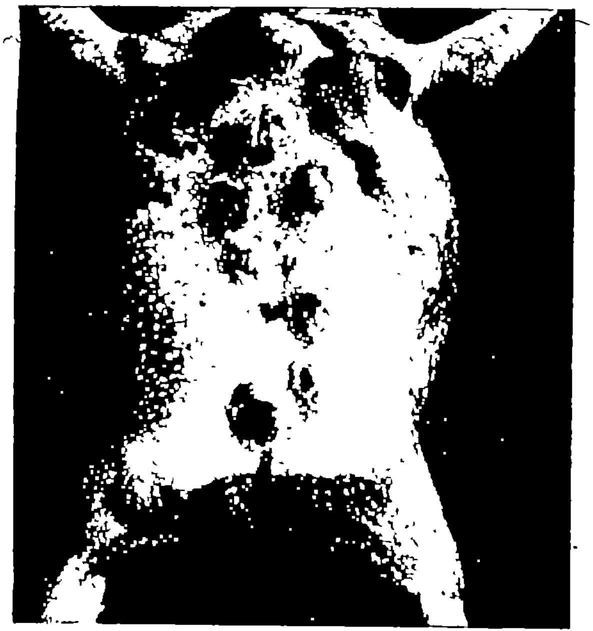

Fig. 1. Ventral view of a toad showing external lesions. Note the large lesion at the top of the sternum

Gross appearance of lesions. The external lesions appeared as pale red or whitish tumour-like structures which varied in size from small nodules to structures of 10–20 mm in diameter. The smaller nodules were hard, whitish and fibrous, while the larger and older lesions tended to be soft and reddish and were frequently ulcerated. The internal lesions were usually whitish and varied in size from small nodules to large structures, sometimes 2–3 times the size of the kidneys.

Histopathology of the lesions. The lesions from the skin and internal organs were almost identical in histology, that is, between the cellular elements were found fungal hyphæ consisting of dark-staining, separate hyphæ frequently composed of globular segments (Fig. 2). Fruiting bodies were never present in the tissues. The individual segments of the hyphæ were usually widely separated by large masses of inflammatory tissue, a condition which contrasts with that reported by Elkan[1] in *Bufo bufo*, where large masses of mycelium had induced inflammatory reaction in the epidermis and the renal tissues.

The inflammatory tissue surrounding the fungal hyphæ consisted mainly of monocytes interspersed with fibrocytes. Foreign-body giant cells with 5–100 or more nuclei were seen in various regions of the sections, usually surrounding part of a fungal hypha. The number and size of giant cells varied according to the site of infection and in the skin lesions they frequently contained 25–50 nuclei. In the liver 100 nuclei could be counted in some of the giant cells but in the kidney, heart and lung lesions they usually had only 5–10 nuclei.

Lesions on the skin consisted of inflammatory tissue which completely replaced the connective and muscular layers in the dermis. The blood vessels in this region were dilated to 10–20 times the size of normal blood vessels in the dermis (Fig. 3). The epidermal layer above the infected region was invariably thickened except in advanced lesions where ulceration had occurred and no epidermal layer was present. A few mitotic cells could be seen among the inflammatory tissue, but these

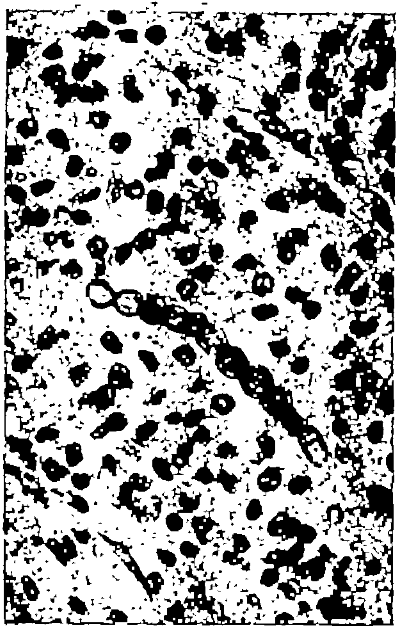

Fig. 2. Photomicrograph of fungal mycelium surrounded by fibrocytes and monocytes

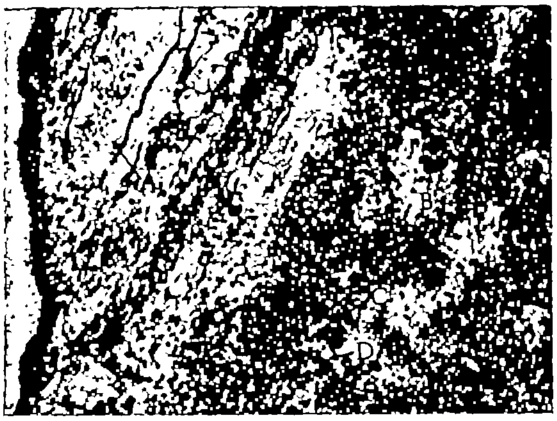

Fig. 3. Photomicrograph of a surface lesion showing dilated blood vessels (*A*), and inflammatory tissue with giant cells, *B*, *C* and *D*

were especially common in and below the hyperplastic epidermis. In one experiment (see further on) the most pronounced effect of strapping the fungus on the back of the toad was the enlargement of poison glands and accumulation of monocytes along their ducts. Study of small young lesions showed that the primary sites of infection are the poison glands. The poison glands have openings to the exterior, and presumably the fungus enters the poison gland through the duct.

The internal lesions on the liver and kidney were histologically similar to the external lesions. There was an accumulation of monocytes and fibrocytes. Giant cells were common in the liver tissue, although only a few were found in the kidney tissue. The blood vessels in the lesions were enlarged. In liver and kidneys with advanced lesions, inflammatory tissue completely replaced the liver lobules and kidney tubules. In the periphery of the lesions the tubules were intact and bits of hyphæ associated with the inflammatory tissue occurred in between the tubules. Only three cases of infection were found in the heart. All these were heavily infected and generally very little cardiac tissue was left intact.

Transplantation of inflammatory tissue. Inflammatory tissue was transplanted subcutaneously with a trocar and cannula after mincing with a fine pair of scissors. The transplanted tissue grew to 20–30 mm in about 10 days, killing all the toads within three weeks. On dissection the tissue was found to be heavily vascularized, and infective centres occurred on the liver and kidney in a majority of toads. Presumably the fungus was carried from the primary site of inoculation to these secondary sites of infection.

The severe reaction produced in such a short time indicates that the fungus is highly toxic and lethal to the toads.

Inoculation of crude extract. Crude extracts of inflammatory tissue were prepared by mincing the tissue in sterile water in glass homogenizers. The cellular elements were removed by centrifuging at 1,200*g* and the extract inoculated subcutaneously and intraperitoneally. Toads injected developed the disease at the sites of inoculation and died within two months. Histological examination of sections of the infected tissue again showed the presence of fungal hyphæ. This indicates that some hyphæ were present in the extract.

Isolation of fungus. Freshly caught toads bearing severe external lesions were anæsthetized, surface washed with mercuric chloride and dissected under sterile conditions. Small nodules on the liver were removed and macerated in sterile saline solution. The macerate was suspended in sterile distilled water and a dilution series made in malt agar, containing 100 units penicillin and 50 μg streptomycin mixture/ml. After 4 days incubation at 25° C, hyphæ growing from segments of liver tissue were removed and sub-cultured on to nutrient agar containing no antibiotics to test for contamination by bacteria. Pure cultures of the fungus were transferred to liquid Czapek-Dox solution supplemented with autoclaved, bacterially filtered toad-liver extract.

Inoculation of fungus. After three weeks' growth in liquid culture the mycelium was removed and macerated in sterile distilled water. This macerated fungal material was inoculated subcutaneously and intraperitoneally into healthy toads with a trocar and cannula. The animals were kept in large aquaria and after 10 days were anæsthetized and dissected.

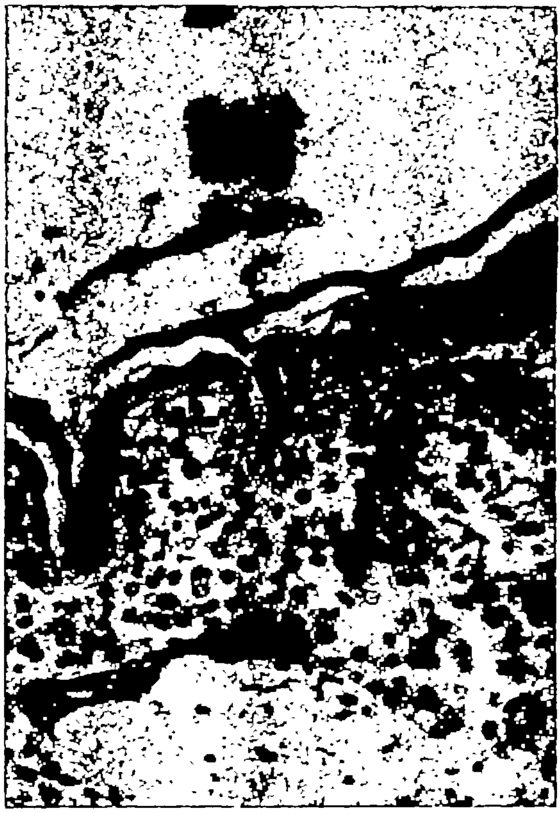

Fig. 4. Photomicrograph of the abdominal wall showing emergence of fungal hyphæ

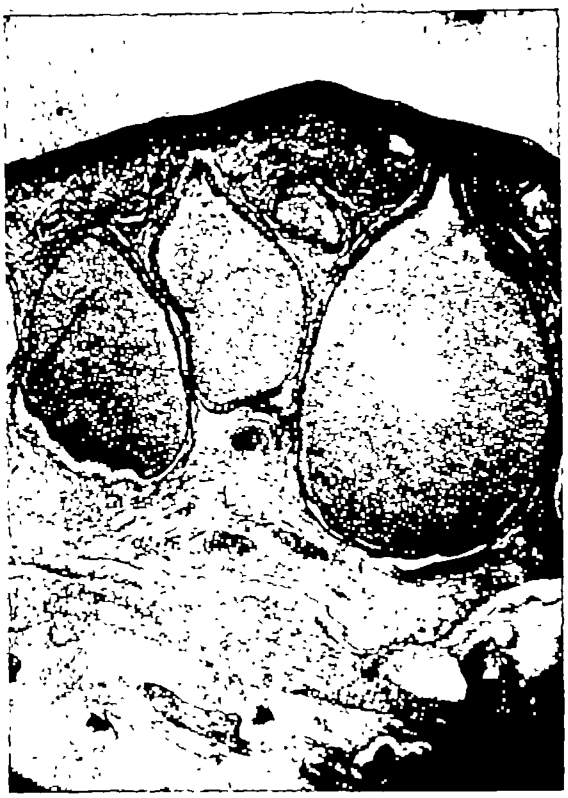

Fig. 5. Photomicrograph of the epidermal and sub-epidermal layer showing gross enlargement of poison glands and hyperplasia of epidermal layer

Series I: Subcutaneous inoculation. Macroscopically, the only observable symptom was the development of small nodular structures in the sub-epidermal connective tissues. In section these were seen to consist entirely of inflammatory tissue containing intercellular fungal hyphæ.

Series II: Intra-abdominal inoculation. The most obvious macroscopic symptom was the marked inflammatory reaction at the site of abdominal puncture. At this point the abdominal wall became greatly thickened and nodulated. The inflammatory tissue was seen to be composed of monocytes and fibrocytes arranged in whorls, that is, each piece of hypha became the locus of cellular accumulation. The muscular layers of the outer abdominal wall consisted of normal fibrillar layers between which were developed masses of small nucleated cells similar to those making up the inflammatory tissue. In many instances the muscular layers had been completely destroyed by the inflammatory tissue. Where this had occurred hyphæ had grown through the abdominal wall and penetrated the sub-epidermal layers resulting in the development of hyphæ on the outer surface of the animal (Fig. 4), thus facilitating dissemination of the fungus. In some sections the fungal hyphæ had migrated towards the epidermal layer and entered the poison glands from the inside, together with an accumulation of phagocytic monocytes. Associated with inflammation of the abdominal wall, organs within the abdominal cavity invariably developed small white nodules similar to those found in naturally infected toads. Sections of the liver, kidney and lungs showed marked inflammation at the site of infection and the development of migratory hyphæ and inflammatory tissue between the tubules at the periphery of the lesions.

Attempts to establish infection via the epidermis failed due to the difficulty of maintaining a skin/fungus contact over a period of several days' incubation. In cases where contact had been established for 5 days the most obvious histological feature was the increase in thickness of the dermal layers due to the enlargement of the poison glands beneath the epidermis and hyperplasia of the epidermal cells (Fig. 5).

We thank Dr. E. Elkan of the Group 9 Pathological Laboratory, Watford, England, for his advice throughout the investigation and to Prof. J. R. Hendrickson and Prof. M. E. D. Poore for help in the preparation of the manuscript.

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'WASTING DISEASE' FOLLOWING THYMECTOMY IN THE HAMSTER

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SINCE thymectomy has been shown to impair the immune response in rats[1], mice[2], chickens[3] and rabbits[4], this procedure was undertaken in the golden hamster (*Mesocricetus auratus*), in an attempt to develop a group of animals which might be tolerant to transplanted human leukæmia and lymphoma. Male and female hamsters were thymectomized 1–4 weeks following birth, and in addition, some animals were partially thymectomized and sham operated. It was soon apparent that total thymectomy in male (but not in female) hamsters 1–4 weeks of age resulted in the development of a disease which had many of the gross and clinical features of runt disease[5]. Histologically, there was a marked generalized reduction in lymphoid tissue.

Eighty-four nursing hamsters 1–4 weeks of age were totally thymectomized. The animals were anæsthetized with intraperitoneal pentobarbital sodium. A midline incision was made through the skin and superficial fascia from the mid-cervical area to the level of the sixth rib and then extended down to the deep fascia. A longitudinal incision was then made through the middle of the sternum and extended to the level of the fourth rib exposing the bilobed thymus. The gland was freed from the surrounding structures by blunt dissection. All bleeding was easily controlled with a cotton-tipped applicator stick. After removal of the thymus, interrupted sutures were placed in the pectoralis muscles, and the skin was closed by continuous sutures. There was a 5 per cent mortality due to respiratory arrest, overdosage of anæsthesia and cannibalism.

The total removal of the thymus gland was followed by the rapid development (4–14 days) of a severe, progressive, fatal disease, characterized by weight loss, wasting, lethargy, dorsal kyphosis, periorbital and facial œdema, rough, unkempt fur and posterior cervical alopecia, unsteady gait and diarrhœa or constipation (Figs. 1 and 2). Anæmia, slight reticulocytosis, thrombocytopænia, mild leukopænia and an absolute reduction in circulating lymphocytes were also found. Only males were affected: thus of 44 males thymectomized, 28 developed the wasting syndrome; however, of 36 females thymectomized, none showed any indications of the disorder.

Post-mortem examination of the affected animals revealed no gross evidence of sepsis and cultures of the surgical site and heart blood were negative. There was a striking depletion of body fat, no evidence of residual

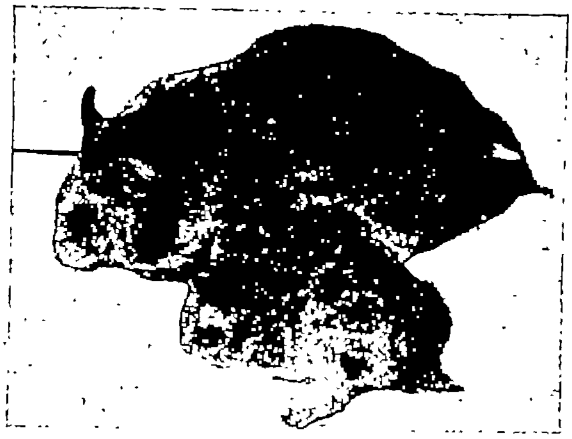

Fig. 1. Sham-thymectomized and thymectomized hamsters, 4 weeks after birth. The smaller thymectomized animal shows loss of weight wasting, unkempt fur, dorsal kyphosis and periorbital œdema

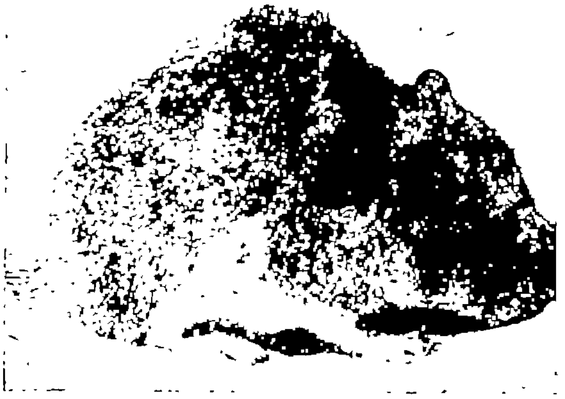

Fig. 2. Close-up of thymectomized hamster of Fig. 1 showing in more detail the features of 'wasting disease' and having the general appearance of runt disease (homologous or secondary disease)

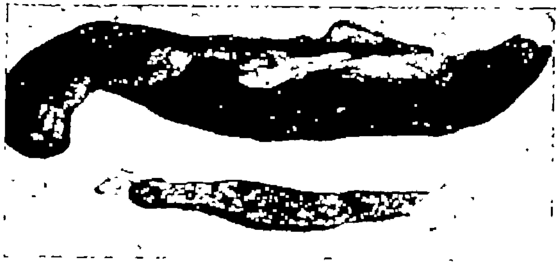

Fig. 3. Marked reduction in spleen size in a thymectomized hamster compared with its litter mate, sham-thymectomized, control

thymic tissue, a marked reduction in spleen size (Fig. 3) and an almost complete absence of Malpighian corpuscles in the spleen (Fig. 4); Peyer's patches were similarly reduced. These gross and histological changes were not observed in sham-thymectomized, partially thymectomized or control hamsters (Fig. 5).

It is obvious that thymectomy induced a state of what might be called wasting, cachexia, or runting. Was the condition observed 'runt disease' or did it merely simulate it more or less closely? Runt disease (homologous or secondary disease) is almost universally conceded to be a graft versus host reaction produced by the administration of immunologically competent cells to an immunologically neutral recipient. The various immunological reactions of the genetically different grafted tissue against the recipient's cells and tissues are thought to induce this experimental disorder[4–7]. Runt disease produced by the injection of parental strain spleen cells into normal adult F_1 hybrid mice is characterized by hæmolytic anæmia with a positive antiglobulin test, leukopænia, thrombocytopænia, splenomegaly, loss of weight, lethargy, hunched appearance, rough unkempt fur with partial alopecia in the posterior cervical area, facial and periorbital œdema and hypothermia[8].

There is a striking similarity between the various clinical features of the 'wasting disease' produced in thymectomized hamsters and the changes produced in mice injected with parental strain spleen cells. The hæmatological features, that is, anæmia, mild reticulocytosis, leukopænia and thrombocytopænia, are perhaps similar although a positive Coombs' antiglobulin test has not been demonstrated. Pathologically, the 'wasting disease' of the hamster differs from runt disease of the mouse in that the former does not have splenomegaly, but on the contrary is characterized by a generalized depletion of lymphoid tissue, with a reduction of Malpighian corpuscles, Peyer's patches and peripheral blood lymphocytes. The histology of runt disease is variable,

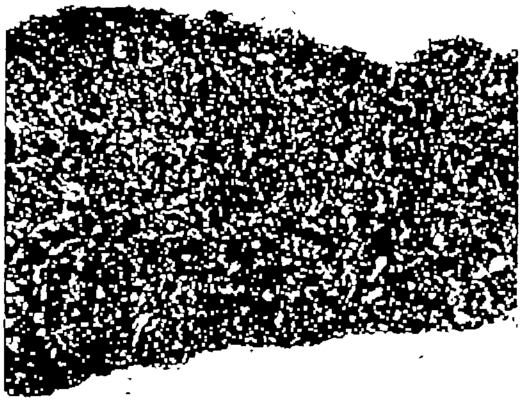

Fig. 4. Section of small spleen in Fig. 3 (thymectomized hamster) showing absence of Malpighian corpuscles (× 350)

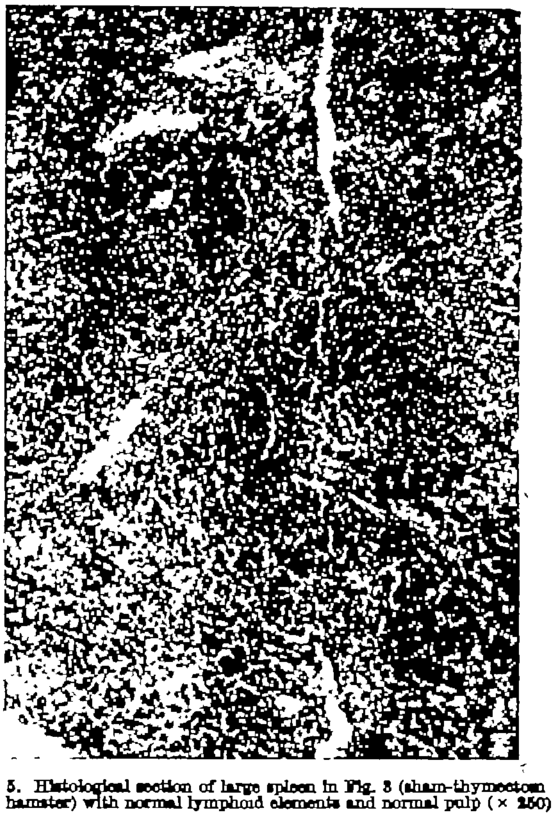

Fig. 5. Histological section of large spleen in Fig. 3 (sham-thymectomized hamster) with normal lymphoid elements and normal pulp (× 250)

although some observers allude to a great reduction in lymphoid elements in the spleen and lymph nodes[8].

In view of the many striking similarities between runt disease and the 'wasting disease' which was induced in new-born hamsters by thymectomy, one may speculate that they are either similar 'end-products' of different mechanisms or that the fundamental mechanism may be the same in both instances. A graft-versus-host phenomenon can be ruled out in the thymectomized animals. There is a possibility that the depleted lymphoid tissue might lead to an unbalanced state of antigen excess to antibody production, which could in some manner lead to runting. Or perhaps the thymus is involved in runt disease, a subject not discussed in the literature of that disorder until very recently. Thus, Billingham *et al.*[9] recently noted that injection of thymic tissue failed to induce runt disease in F_1 hybrid rats and thymic atrophy occurred in runted rats. We have noted thymic atrophy in runted male F_1 hybrid LAF_1 mice injected with parental strain spleen cells.

It is noteworthy that depletion of lymphoid tissue has been considered to be an important factor in the production of runt disease[4]. Mueller *et al.*[3] made chickens lymphoid deficient by 19-nortestosterone, and noted signs of a disease that was similar to that seen in our thymectomized hamsters. It should be noted that splenectomy of the hamster has not resulted in any disease.

A condition which has been called 'thymic cachexia' has been observed by other investigators in other species[10–12] and the various features as described are comparable with those noted in the hamster. Similarities between post-thymectomy wasting and runt disease have not been previously mentioned. The failure of females to develop the wasting syndrome following thymectomy is of considerable interest and requires further study. This and other observations are at present under way to define more clearly the role of the thymus gland in the gross reduction of lymphoid tissue that follows total thymectomy of the new-born hamster, and its relation to the wasting disorder.

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ANTIGENIC RELATIONSHIP BETWEEN HOST AND PARASITE

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IT has been suggested that the immune response of the host may exert selective pressure on 'fitness' for survival of many parasitic species. In those situations where the effect of the immune response is contemporary with the development of the parasite within the host, it is expected that selection for survival may only favour variants of the parasite which display reduced antigenic disparity with the host[1]. It was also suggested that for the evolution of the host/parasite relationship a reduction of disparity would only be necessary with those antigenic characters of the parasite, which stimulate immune responses which adversely affect survival of the parasitic species. It seems likely that many other antigenic characters stimulate a variety of immunological responses in the host; but if these in no way influence 'fitness' of the parasitic species then a progressive change in the causal antigens would not be a requirement for evolution of the host/parasite relationship. Such characters which do not stimulate the mechanism of resistance of the host may undergo mutation in a random fashion, and this genetic change may be expressed without progressive drift for reduction of antigenic disparity with the natural host.

The experiment described in this article was designed to seek evidence of reduction of antigenic disparity associated with selection for 'fitness' characters[1]. It is based on a comparison of the serological responses evoked by larval and adult antigens of the parasite *Haemonchus contortus* in the natural host (sheep) with those evoked in the rabbit. It is deduced, as an *a priori* consequence of hypothesis, that the natural host will fail to recognize 'fitness' antigens, or will show relatively little immunological responsiveness to them while reacting with normal vigour to those antigens of the parasite which do not influence 'fitness' of the organism. Conversely, it is expected that with the rabbit, which is not a natural host for this parasite, there will be serological responses to a greater range of antigens because there has not been the same reduction in disparity due to selection of 'fitness' antigens during an evolutionary period. By the use of the Ouchterlony gel-diffusion technique, evidence was obtained which supports the thesis that certain antigens of the parasite show reduced disparity with the natural host (sheep), and that these relevant antigens are shared by the larval and adult stages of development of the parasite.

For the experiments, antigens were prepared by homogenizing the infective larvae and adult worms of *Haemonchus contortus* in physiological saline. The larval and adult-worm homogenates were then fractionated by differential centrifugation. The crude particulate material of the homogenates was sedimented by centrifugation at 4,000 r.p.m. for 1 h at 2°–4° C in an angle-head centrifuge. The supernatant fluids were then centrifuged at 144,000*g* for 1 h in a Spinco model *L* ultra-centrifuge. The clear gelatinous pellet sedimented by ultra-centrifugation was redissolved in physiological saline. The crude particulate material, and the sediment and clear supernatant obtained after ultra-centrifugation, were used for the production of antisera in sheep and rabbits. The soluble antigenic fractions, that is, the redissolved gelatinous pellets and supernatant fluids of both larval and adult-worm preparations, were used in the Ouchterlony-plate technique to characterize the antibodies in the rabbit and sheep sera and to assess their specificity.

Eight rabbits and eight sheep, born and reared worm-free, received the larval and adult-worm antigens by four intraperitoneal injections of the fractions spaced at 3-weekly intervals. Blood for serum samples was collected from the marginal ear vein of the rabbits and from the jugular vein of the sheep ten days after the fourth injection of the antigen.

Gel-diffusion plates were prepared from 1 per cent Oxoid 'Ionagar No. 2' in physiological saline with merthiolate added as preservative to a concentration of 1 in 10,000 parts. Agar plates were poured to a depth of 7 mm and five symmetrically placed wells, 8 mm in diameter, were cut with a cork borer and centred 16 mm from a central well also 8 mm in diameter. Each central well was charged with one of the soluble larval or adult-worm antigenic fractions and the peripheral wells with the appropriate undiluted antisera. The plates were incubated at 30° C and bands of antigen/antibody precipitate were usually fully developed in five to six days.

The results of the gel-diffusion studies have been consistent. In comparison with the antisera produced in rabbits, larval and adult-worm antisera produced in the natural host (sheep) showed a relatively high degree of specificity of reaction with antigens from the corresponding developmental stage of the parasite.

With sheep antisera, multiple antigen/antibody bands were formed during the course of double diffusion of the larval antisera with the larval fraction used for immunization. Furthermore, tests with each of these larval antisera and the other soluble larval fraction produced cross-reactions. This finding was expected because of the crude nature of the antigenic fractions. However, none of the four larval antisera reacted with the crude antigenic fractions similarly prepared from adult worms. The four sheep-antisera to adult-worm fractions showed a similar degree of immunological specificity but in the reverse direction. These sera precipitated with the adult-worm fraction used for immunization and cross-reactions with the other adult-worm fractions were also observed. Thus the specificity of antisera prepared in the sheep was demonstrated by the failure of the larval antisera to react with the adult-worm antigens and vice versa.

The larval and adult-worm antisera produced in rabbits precipitated with the injected antigenic fractions, and cross-reactions were observed with larval or adult-worm fractions other than the particular fraction used for immunization. In addition to the cross-reaction with fractions from the corresponding developmental stage of the parasite, the rabbit antisera also reacted with the other category of antigenic fractions, that is, the rabbit larval antisera reacted with the adult-worm fractions and the adult-worm antisera reacted with larval antigens. These cross-reactions were in sharp contrast with the results obtained with sheep antisera.

The relative unresponsiveness of the natural host (sheep) to those antigens which are common to the larval and adult stage of development of the parasite suggests that such antigens constitute 'fitness characters'. It is relevant in this context that infecting parasites, when superimposed on an existing infection, may not develop beyond the larval stage. Roberts and Keith[2] have extended this finding by showing that, after the differential removal of adult worms by treatment of a *Haemonchus placei* infection of cattle with phenothiazine, the remaining larvæ that had been arrested in their growth may then complete their development. If, as Roberts and Keith have suggested, this phenomenon of arrested growth in larvæ is based on an immunological response of the host to the infection, then the results of the experiments just described indicate that the control exercised by the host on larval development could be stimulated not only by antigens in larvæ but also by certain antigens in adults which are common to both stages of development.

It is interesting to note that Michel[3] has recently suggested that resistance to *Dictyocaulus viviparus* in cattle is also a reaction to antigens produced by both early parasitic stages of the organism and by mature adults. The observations described in the present study are consistent with these views.

Further work on the relationship of antigens from host and parasite is being undertaken with the aid of the more refined technique of immune electrophoresis.

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GROSS STRUCTURE OF HÆMOGLOBIN A_3 AND THE NATURE OF ITS BINDING TO CHROMIUM-51

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HÆMOGLOBIN from the red cells of normal persons when separated by electrophoresis at alkaline *p*H is not homogeneous. Rather, it consists of three components: the major fraction, hæmoglobin A_1, constituting about 90 per cent; the slow minor component, hæmoglobin A_2, constituting 2–3 per cent; finally, the fast minor component, hæmoglobin A_3, ranging in quantity from 3 to 10 per cent of the total hæmoglobin. Synthesis of hæmoglobin A_1 and A_2 is under direct genetic control. The nature of hæmoglobin A_3 is less clear, and it has been postulated that it is a senescent form or breakdown product of hæmoglobin A_1 (ref. 1).

One of the most striking characteristics of hæmoglobin A_3 concerns its properties with respect to tagging with chromium-51. Metrakis, in 1957[2], and afterwards Malcolm and Ranney[3] and Ebaugh *et al.*[4] have noted that when hæmoglobin labelled with chromium-51, obtained from normal red cells, is electrophoresed at alkaline *p*H, the hæmoglobin A_3 fraction contains disproportionately large amounts of radioactivity. Using starch block electrophoresis at *p*H 8·6, we also have noted that although hæmoglobin A_3 constitutes only 5–10 per cent of the total hæmoglobin, 32–60 per cent of the total chromium-51 radioactivity is associated with the hæmoglobin A_3 fraction.

Investigations previously reported from our laboratory indicated that chromium-51 binding to hæmoglobin was largely a function of the β-polypeptide chains[5]. This conclusion was based first on the finding that hæmoglobin H (β_4^A) had approximately twice the specific activity per unit of hæmoglobin as did hæmoglobin A_1 ($\alpha_2^A\beta_2^A$). This observation has been confirmed and extended by Rigas[6]. Secondly, when hæmoglobin A_1 labelled with chromium-51 was mixed with non-radioactive hæmoglobin C ($\alpha_2^A\beta_2^C$) and the mixture then subjected to acid dissociation and recombination, only a 2–3 per cent increase in the radioactivity associated with the hæmoglobin C fraction was observed. This would indicate β-chain binding in the order of 95+ per cent. Schneiderman has repeated similar recombination studies and confirmed these findings[7].

Ebaugh *et al.*[4] studied polypeptide chain binding of chromium-51 by means of the Wilson and Smith technique of gradient elution with acid urea from a weak cationic resin column[8]. Under the condition used by Ebaugh, chromium-51 activity was found to be largely associated with the β-chain fractions. However, Chernoff, using a similar technique, observed what was interpreted as α chain binding[9].

Since hæmoglobin A_3 has such unusual chromium-51 binding properties, it was decided to investigate the pattern of chromium-51 activity and gross polypeptide chain composition of hæmoglobin A_2. Hæmoglobin A_1 and hæmoglobin A_2 were obtained by multiple starch block electrophoretic separations in veronal buffer *p*H 8·6. Approximately 200 mg of hæmoglobin A_2 and 500 mg of hæmoglobin A_1 were collected. The hæmoglobin solutions were dialysed free of buffer. Tagging was then accomplished by the addition of approximately 25μ of chromium-51 (as $Na_2{}^{51}CrO_4$) per gramme of hæmoglobin, and incubation of the mixture at room temperature for 4 h. The radioactive hæmoglobin solutions were then dialysed against 11·7 per cent formic acid before adsorption on 25 g of washed 'Amberlite *CG*-50' type II resin. The resin with adsorbed hæmoglobin was then added to the top of a column containing 75 g of wet resin. 1 l. of 2 M urea, *p*H 1·9, was passed through the column before starting the continuous molarity gradient from 2 to 8 M with acid urea. Three hundred, 12–15 ml. fractions of eluate were collected. The optical density at 280 M μ of the even-numbered fractions was read in the Beckman *DU* spectrophotometer. The corrected counts/min/ml. eluate of the odd-numbered fractions were determined in a scintillation counter with a 3-in. well-type sodium iodide crystal.

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The results of the separation of radioactive hæmoglobin A_1 are depicted in Fig. 1. The first protein peak separated by this method consists largely of α-chains, while the second peak contains predominantly β-chains[8]. More than 95 per cent of the total radioactivity that was associated with protein-containing fractions was found in the β-chain region, confirming Ebaugh's results.

In addition, in agreement with Ebaugh, we observed a large initial loss of radioactivity long before the first protein peak. Chernoff apparently did not encounter such an initial loss, perhaps because of his modifications of the chromatographic technique[9], in which 3 M urea is used initially and a discontinuous acid urea gradient is used.

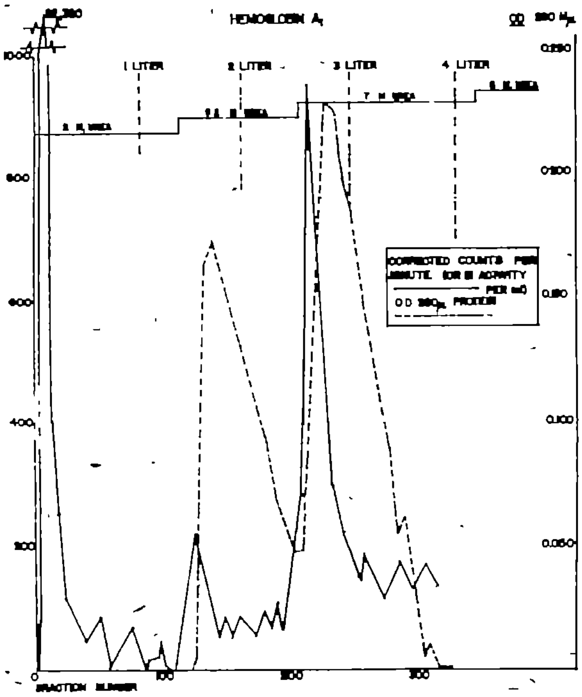

Fig. 1

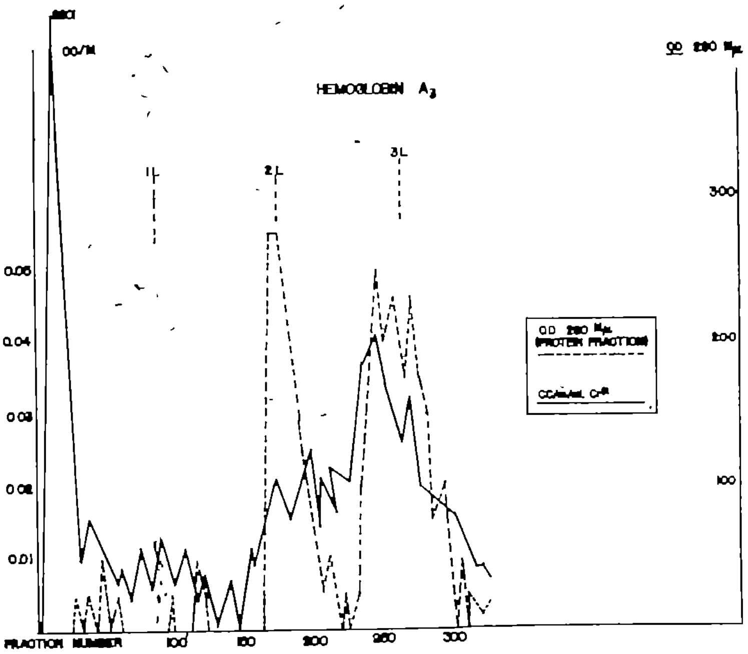

Fig. 2

When hæmoglobin A_2 labelled with chromium-51 was separated in the same way, the patterns for both protein and chromium-51 activity were similar to those obtained with hæmoglobin A_1 (Fig. 2). Because of the lesser amount of hæmoglobin A_2 used, both protein and chromium-51 curves were of less magnitude. However, the ratio of optical density to corrected counts per minute for the β-chain fraction of hæmoglobin A_1 and hæmoglobin A_2 were similar.

The observation of a similar pattern of the protein peaks obtained with hæmoglobin A_2 and A_1 indicates that there is no gross molecular alteration of hæmoglobin A_2, and that α- and β-chains are present in roughly equal proportions in hæmoglobin A_2.

1 per cent solutions of hæmoglobin A_1 and A_2 were placed in separate bags and dialysed for 24 h in a single container against an aqueous solution containing 100 μc. of $Na_2{}^{51}CrO_4$. After tagging had been thus accomplished, the hæmoglobins were dialysed against distilled water for 12 h to eliminate all unbound isotope. The specific radioactivity for hæmoglobin A_1 and A_2 was found to be the same. This suggests that, under these experimental conditions, there is no significant difference in binding capacity of hæmoglobins A_1 and A_2.

These studies suggest that the increased activity of chromium-51 observed in the hæmoglobin A_2 fraction after electrophoresis at alkaline *p*H is not due to some peculiar polypeptide configuration of hæmoglobin A_2. Rather, it is likely that since the chromium-51–hæmoglobin A_1 complex is more electronegatively charged than hæmoglobin A_1 alone, the complex has a more rapid anodal migration. Hence, after electrophoresis, the hæmoglobin molecules tagged with chromium-51 may become concentrated in the hæmoglobin A_2 fraction, producing the very high specific activity which has been repeatedly observed.

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LETTERS TO THE EDITOR

ASTROPHYSICS

A New Method of directly measuring the Galactic Magnetic Field

WITH the announcement of the existence of polarization in the Crab Nebula at a wave-length of 21 cm[1], a new method of determining the galactic magnetic field becomes available—the measurement of the Faraday rotation of the plane of polarization due to the presence of anomalous dispersion in the neighbourhood of the 21-cm line of neutral hydrogen. This may be calculated as follows:

The index of refraction due to hydrogen atoms at frequency ν_s is given by:

$$n = 1 + \frac{e^2 N(\nu_0) f}{4\pi m \nu} \; \frac{\nu_s - \nu}{(\nu_0 - \nu)^2 + \left(\frac{\Gamma}{4\pi}\right)^2} \cdot \frac{1}{4}$$

where f is the Ladenburg f for the transition, $3 \cdot 8 \times 10^{-12}$. The factor 1/4 appears because only the atoms in the lower state cause a phase shift. Summing over all hydrogen atoms:

$$n - 1 = \frac{e^2 f}{16\pi m \nu} \int_{-\infty}^{\infty} \frac{(\nu_s - \nu) N(\nu_s)}{(\nu_s - \nu)^2 + \left(\frac{\Gamma}{4\pi}\right)^2} \, d\nu_s$$

When $\Gamma \rightarrow 0$ this integral becomes a Cauchy principal value.

$$n - 1 = \frac{e^2 f}{16\pi m \nu} \text{ c.p.v.} \int_{-\infty}^{\infty} \frac{N(\nu_s)}{\nu_0 - \nu} \, d\nu_s$$

A ray is thus delayed beyond a ray *in vacuo* by:

$$\Delta t = \int \frac{n - 1}{c} \, ds = \frac{ef}{16\pi m\nu c} \text{ c.p.v.} \int_{-\infty}^{\infty} \frac{\int N(\nu_s) ds}{\nu_s - \nu} \, d\nu_s$$

which corresponds to a phase lag of:

$$\Delta\varphi = \omega \Delta t = \frac{e^2 f}{8mc} \text{ c.p.v.} \int_{-\infty}^{\infty} \frac{\int N(\nu_s) ds}{\nu_s - \nu} \, d\nu_0$$

The optical depth $\tau(\nu_s)$ is given by:

$$\tau(\nu_0) = \frac{\nu}{c} \beta \int \frac{N(\nu_s)}{T_s} \, ds$$

where $\beta = 5 \cdot 45 \times 10^{-14}$ cm^3 sec^{-1} deg. K, and T_s is the spin temperature.

If we assume T_s constant:

$$\Delta\varphi = \frac{e^2 f}{8m\beta\nu} T_s \text{ c.p.v.} \int_{-\infty}^{\infty} \frac{\tau(\nu_s)}{\nu_s - \nu} \, d\nu_0$$

The Faraday rotation ψ is the change in phase between the right and left circularly polarized components. If they are separated in resonant frequency by:

$$\Delta\nu = 2 \cdot 8 \times 10^6 B \text{ c.p.s.}$$

then

$$\psi = \frac{e^2 f}{8m\beta\nu} \mathrm{T}_s \frac{d}{d\nu} \left(\text{c.p.v.} \int_{-\infty}^{\infty} \frac{\tau(\nu_s) d\nu_s}{\nu_0 - \nu} \right) \Delta\nu$$

numerically:

$$\psi = 6 \cdot 22 \, T_s \frac{d}{d\nu} \left(\text{c.p.v.} \int_{-\infty}^{\infty} \frac{\tau(\nu_s) d\nu_s}{\nu_s - \nu} \right) \Delta\nu$$

The absorption profile observed with a single paraboloid has been taken from Clark, Radhakrishnan and Wilson[2] to derive the frequency-dependent part of this expression. The resultant for a field of 10^{-6} gauss and $T_s = 100°$ K is plotted in Fig. 1. Some smoothing has inadvertently been introduced in the process of numerical integration. Since ψ is essentially dependent on the second derivative of optical depth with respect to frequency, the estimated rotation is somewhat sensitive to the band-width and saturation corrections to the measured absorption profile.

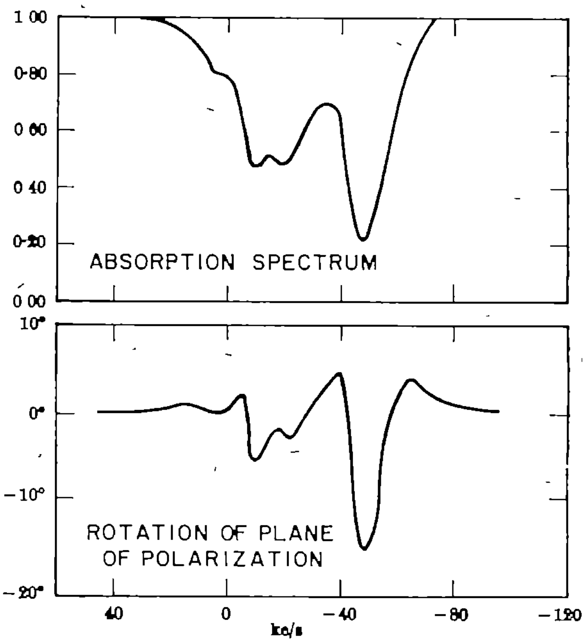

Fig. 1. Absorption profile and Faraday rotation in the Crab Nebula

The measurement of this rotation is in some ways not as difficult as an absolute measurement of the angle and amount of the polarization since instrumental polarization effects need not be considered in finding the frequency dependence of the polarization. Thus the question again reverts to having a sufficiently good signal to noise ratio to detect the polarization. For small magnetic fields the method is comparable in sensitivity with the more conventional measurement of Zeeman splitting in right and left circular polarizations.

This work was supported by the U.S. Office of Naval Research under Contract *Nonr* 220(19).

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RADIOPHYSICS

Radio Observations of Jupiter during 1962

IT has been suggested by Barrow[1] that a rigorous comparison of solar, geomagnetic and Jupiter activity in radio emission cannot be made until continuous observation of Jupiter is possible throughout an apparition. The results summarized in this communication will, it is hoped, help to fill some gaps in the available figures for the 1962 apparition.

Standard communications receivers, suitably modified, were used in conjunction with single folded dipole aerials

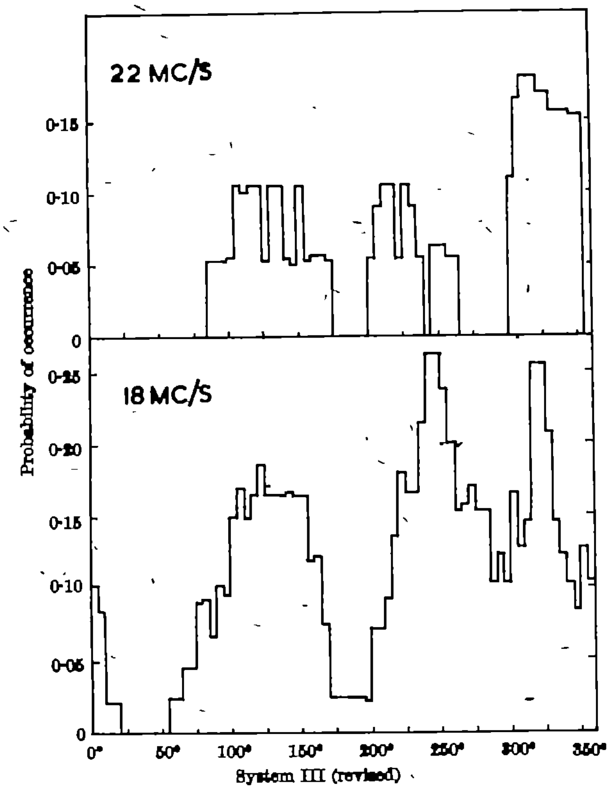

Fig. 1. Occurrence probabilities of radio noise from Jupiter at 18 and 22 Mc/s, July–October 1962

to record signals at frequencies close to 18 and 22 Mc/s, at a site (*A*) near Grahamstown, South Africa. Another standard communications receiver, with an eight-dipole broadside array, was operated at a frequency close to 18 Mc/s to record the noise at a site (*B*) near East London. Observations were made at site *A* each night from July 16–17 until October 15–16, that is, on 92 nights. Jupiter was observable by the equipment from about 2200 h to 0300 h U.T. at the beginning of the period of observation and from 1800 h to 2300 h at the end of it. Observations at site *B* were more irregular but results for 26 nights are available. Recordings were continually monitored aurally at site *B*, but only occasionally at site *A*. Identification of Jupiter noise was made by comparison of records from the two sites, where available, and otherwise by the characteristics of the recorded trace.

Signals identified as coming from Jupiter were observed on 38 nights at site *A* and 14 nights at site *B*. Interference from thunderstorms, apparatus failure, etc., prevented useful recordings on 32 nights at site *A* and from August 29 onwards at site *B*.

The histograms in Fig. 1 show the occurrence probability for each 5° interval of central-meridian longitude on Jupiter, in terms of the revised system III co-ordinates[1]. The occurrence probability is defined as the ratio of the number of events occurring while the central meridian lay in the 5° interval concerned to the total number of times during which that interval was under observation in interference-free conditions. Three sources, at longitudes of about 120°, 240° and 320°, are clearly shown, in agreement with the observations previously reported by other workers[1,2]. The maximum signal strength was of the order of 10^{-20} W/m^2 c/s.

We wish to point out that any investigation of the relationship between solar phenomena and Jupiter's activity must be complicated by the fact that Jupiter's orbital period of 11·9 years almost coincides with the length of the sunspot cycle. It is worthy of note that during the period of minimum activity found by Carr *et al.*[2] the south pole of Jupiter was inclined towards the Earth. The minimum would therefore be consistent with the theory that the radio noise may be produced by particles trapped in Jupiter's magnetic field interacting with the Jovian atmosphere in a possible magnetic anomaly in the vicinity of the north pole.

It is planned to extend observations to a wider frequency band during the 1963 apparition.

We wish to thank the South African Council for Scientific and Industrial Research for a grant for apparatus, the Council of Rhodes University for a grant in aid of transport expenses and the Grahamstown City Council for permission to set up the receiving station on municipal land. We also thank Mr. S. F. H. J. Archer, who suggested the problem and collaborated with us in the early stages.

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High-resolution Observations of the Galactic Radiation at 4·7 Mc/s

As part of a programme of radio astronomical measurements at wave frequencies less than 10 Mc/s, observations of the distribution of sky brightness at 4·7 Mc/s were made in Tasmania during the winter of 1962.

The antenna system was a filled-in array 4,000 ft. east–west by 1,000 ft. north–south. It was connected to receivers in which the signals were added in six independent phase arrangements in such a way that the intensity of the cosmic noise at six declinations could be recorded simultaneously. The antenna beam-width was 3° × 11°. The band-width of the receivers was 4 kc/s and minimum reading recorders were used to reduce interference from atmospherics and transmitting stations.

During the nights of May, June, July and August, the ionospheric critical penetration frequency f_0F_2 was normally less than 3 Mc/s for 12–14 h each night. On most nights it was less than 2 Mc/s for lesser periods, and on some less than 1 Mc/s. As a result it was possible to obtain many observations which covered a range of southern declinations of 50°.

Fig. 1 shows examples of records for five declinations. Four of these were recorded on the same night. A notable feature of those for declinations −27° to −47° is the pronounced absorption trough between 1600 *R.A.* and 1900 *R.A.* A peak in the intensity is visible through this trough at declination −27°, that is, in the direction of the galactic centre. This absorption is consistent with a layer of ionized hydrogen in the plane of the galaxy, several hundred parsecs in thickness[1–3].

Two external galaxies *NGC* 5128 and *NGC* 1316 were recorded. The record of Fig. 1 shows *NGC* 5128. These sources had previously been detected with an interferometer at 4·8 Mc/s[4]. In addition, fine detail was observed in the absorption trough as indicated in Fig. 1. This appeared to be due to bright objects of angular size less than the beam-width of the antenna. It was very weak and requires higher angular resolution for further investigation.

The main conclusion to be drawn from this preliminary report is that high-resolution radio astronomy is possible at frequencies less than 5 Mc/s using ground-based receivers in suitable locations. The sky appears to contain as much interesting detail as at higher frequencies. Because of the strong absorption, objects seen near the

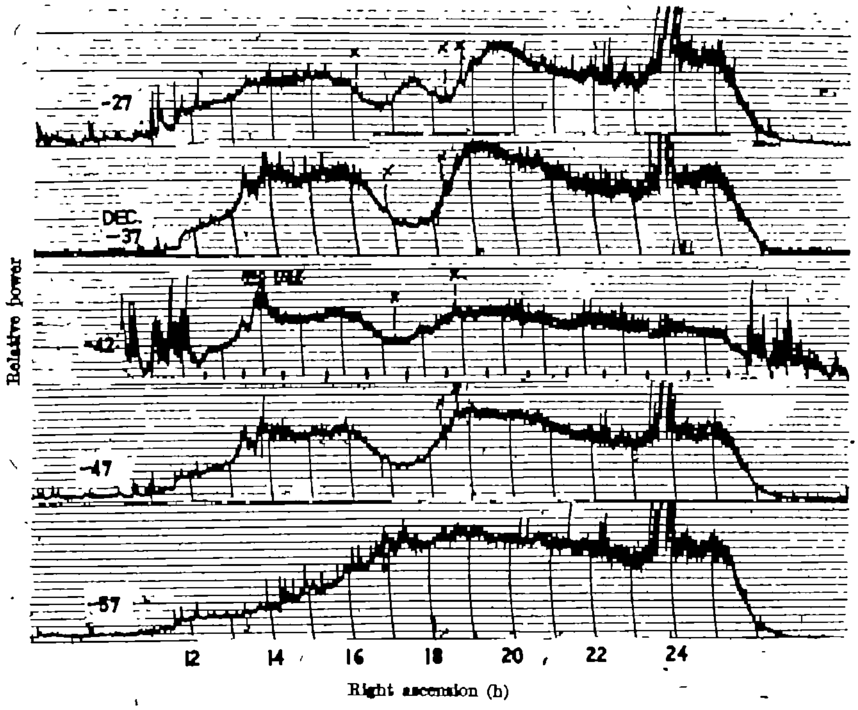

Fig. 1. Records of cosmic radio noise at 4·7 Mc/s. Antenna beam width, 3° × 11°. Discrete detail between 1600 *R.A.* and 1900 *R.A.* is indicated thus *I*

plane of the Milky Way are likely to be located within a few kiloparsecs of the Sun and there would seem to be a possibility of establishing a distance scale for such objects by observations in the 1–10 Mc/s band. Since the optical depth of the interstellar medium varies as the square of the wave frequency, progressive reduction in the frequency rapidly reduces the radial limits of observation. At the same time, the study of the absorption provides a sensitive way of investigating the interstellar medium.

This work is supported financially by the Australian Radio Research Board.

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GEOLOGY

Thermo-chemical Remanent Magnetization of Rocks

When titanomagnetites, $x\mathrm{Fe_2TiO_4}\cdot(1-x)\mathrm{Fe_3O_4}$, are heated in the atmosphere at a certain high temperature, they are oxidized, becoming solid solutions of titanomagnetites and titanomaghaemites, $y\mathrm{FeTiO_3}\cdot(1-y)\mathrm{Fe_2O_3}$ having a spinal crystal structure[1-4].

The oxidation process is proved[1-4] to be represented simply by $\mathrm{Fe^{2+}}\rightarrow\mathrm{Fe^{3+}}$, a ratio (Fe + Ti)/Fe, being kept invariant per unit molecule. This results in a certain amount of vacancies in the crystal lattice, accompanied by a decrease in lattice constant (a) of the cubic crystal and an increase in Curie temperature (Tc).

For example, a titanomagnetite of 40 mol per cent $\mathrm{Fe_2TiO_4}\cdot$60 mol per cent $\mathrm{Fe_3O_4}$ ($\mathrm{FeO} : \mathrm{Fe_2O_3} : \mathrm{TiO_2}$ = 58 : 25 : 17 in mol per cent), having a = 8·440 Å and Tc = 365° C, becomes a solid solution of titanomagnetite and titanomaghaemite, with a composition of $\mathrm{FeO} : \mathrm{Fe_2O_3} : \mathrm{TiO_2}$ = 38 : 44 : 18. a = 8·407 Å and Tc = 560° C, after the original specimen has been heated about 2 h at 470° C in the atmosphere.

In other words, the original titanomagnetite is paramagnetic at 470° C at the initial instant of the oxidation procedure, whereas the oxidized specimen is ferrimagnetic at the same temperature.

If the oxidation procedure is performed in a magnetic field, H, the oxidized titanomaghaemite–titanomagnetite acquires a fairly stable remanent magnetization the direction of which is the same as that of H and the intensity of which depends on the intensity of H.

The remanent magnetization mentioned here is different from the chemical remanent magnetization in that this new process occurs only at high temperatures whereas the ordinary chemical remanent magnetization involving a change from a non-magnetic to a ferri- (or ferro-) magnetic state occurs at atmospheric temperature[5-7].

Verhoogen[8] also has suggested a possibility of this kind of remanent magnetization based on his studies on oxidation of iron–titanium oxides in igneous rocks. The new type of remanent magnetization may be called thermo-chemical remanent magnetization (TCRM).

Fig. 1 illustrates a process of development of thermo-chemical remanent magnetization (Jr) with time of oxidation for an initial constituent of x = 40 per cent titanomagnetite in H = 41·0 oe at temperature T = 470° C. Development with time of intensity of magnetization, $J = Jr + \chi H$, at 470° C is also shown in Fig. 1.

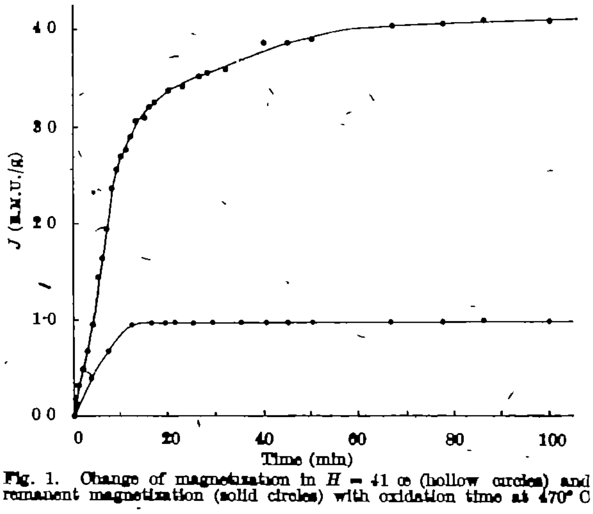

Fig. 1. Change of magnetization in H = 41 oe (hollow circles) and remanent magnetization (solid circles) with oxidation time at 470° C

The intensity of thermo-chemical remanent magnetization, $Jr(T)$, thus acquired at T = 470° C increases, due to an increase in the spontaneous magnetization, when the specimen is cooled to atmospheric temperature in non-magnetic space.

Fig. 2 illustrates the a.c.-demagnetization curve of the thermo-chemical remanent magnetization together with the a.c.-demagnetization curves of isothermal remanent magnetization and thermo-remanent magnetization of the same oxidized specimen. It is shown in Fig. 1 that the thermo-chemical remanent magnetization is as stable as the thermo-remanent magnetization and is much more stable than the isothermal remanent magnetization

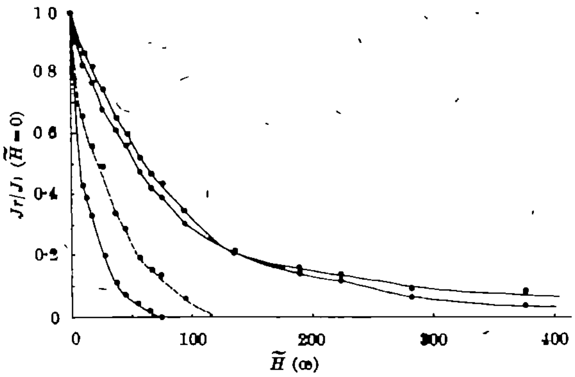

Fig. 2. Demagnetization by alternating magnetic field of various types of remanent magnetization of titanomaghemite (size *B*). —○—, TCRM (H = 41 oe); —○—, TRM (H = 20·5 oe); – – – ○ – – –, IRM produced at 470° C by 41 oe, —●—, IRM produced at 30° C by 100 oe

against the a.c.-demagnetization. The stability of the thermo-chemical remanent magnetization is, therefore, quite similar to that of the ordinary chemical remanent magnetization.

The results of preliminary studies on the thermo-chemical remanent magnetization during a thermo-chemical change from titanomagnetites to titanomaghemites are summarized in Table 1.

Table 1. Original sample, 40 per cent Fe_2TiO_4·60 per cent Fe_3O_4. Heat treatment, 2 h at 470° C in air

A-group specimens (0·5μ < mean diameter of grains ≤ 1·0μ. Initial coercive force H_c = 90 oe)

H (oe)	TCRM (measured at 30° C) (E.M.U./g)	TCRM (measured at 470° C) (E.M.U./g)	IRM (produced at 470° C, measured at 30° C) (E.M.U./g)	IRM (produced at 30° C, measured at 30° C) (E.M.U./g)
0·0	0·0	0·0	0·0	0·0
20·5	1·30	0·542	—	—
41·0	2·06	0·843	0·560	0·140

B-group specimens (1μ < mean diameter of grains < 10μ. Initial coercive force, H_c = 32 oe)

H (oe)	TCRM (measured at 30° C) (E.M.U./g)	TCRM (measured at 470° C) (E.M.U./g)	IRM (produced at 470° C, measured at 30° C) (E.M.U./g)	IRM (produced at 30° C, measured at 30° C) (E.M.U./g)
0·0	0·00	0·00	0·00	0·00
20·5	1·18	0·73	—	0·143
41·0	1·77	0·97	1·19	0·445

Although the intensity of isothermal remanent magnetization is very sensitive to the grain size of the ferrimagnetics, that of the thermo-chemical remanent magnetization is not sensitive. As already reported[1], the degree of oxidation of titanomagnetites can be primarily controlled by the heating temperature, so long as the heating time ranges from an hour to days. The development of thermo-chemical remanent magnetization dealt with here may be interpreted as due to an increase of the total volume of the oxidized parts within the specimen while individual oxidized parts can attain almost finally oxidized states rather quickly, say within 10 min. This interpretation seems to be supported by a fact that a ratio $Jr/J = Jr/(Jr + xH)$ is kept nearly constant, being about 0·3, throughout the entire time of the oxidation process.

Some remanent magnetization of oxidized natural rocks can be attributed to the thermo-chemical remanent magnetization described here. Results of detailed studies will be published elsewhere.

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[See also p. 444 of this issue of *Nature*.]

Replacement in the Octahedral and Interlayer Positions in Micas

IN a paper read at the eleventh National Clay Conference, Ottawa, 1962[1], one of us (I. Th. R.) presented results of a radioactive tracer technique and infra-red spectroscopy applied to dioctahedral and trioctahedral micas. The mineral powders had been pre-treated with deuterium oxide and tritium-bearing water. The results indicated that dioctahedral hydrous micas contained water molecules in interlayer positions substituting some of the K^+ ions of the ideal end member. The necessary charge balance in the crystal model was achieved by substituting some of the apical O's by OH (or by filling in H^+ in vacant positions of the octahedral layer).

The communication by White *et al.*[2] strongly indicates that lithium may enter vacant positions of dioctahedral micas, expelling potassium from the interlayer position. In continuation of the work reported we have treated muscovite from Bjortjenn, Aust-Agder. and phlogopite from Skåtøy, south-eastern Norway (Table 1), with tritium-bearing water and tritium-water containing 1·2 m lithium chloride and 1·1 m rubidium chloride.

1 g of mineral powder (D_e < 5μ) was heated for 6 days to 125° C in quartz tubes together with the reagents mentioned. Afterwards the samples were washed with distilled water to remove all solved alkali, and afterwards the samples were heated at a rate of 100° C/h in a tube furnace. The water phase given off was collected, and the

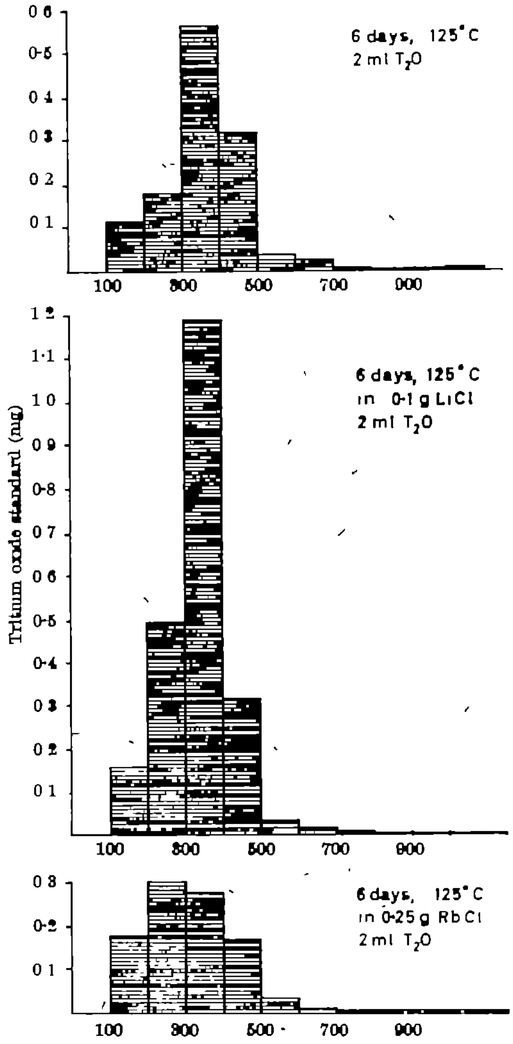

Fig. 1. Determination of tritium content in water fractions given off by gradual heating of muscovite after six days at 125° C in tritium-bearing water

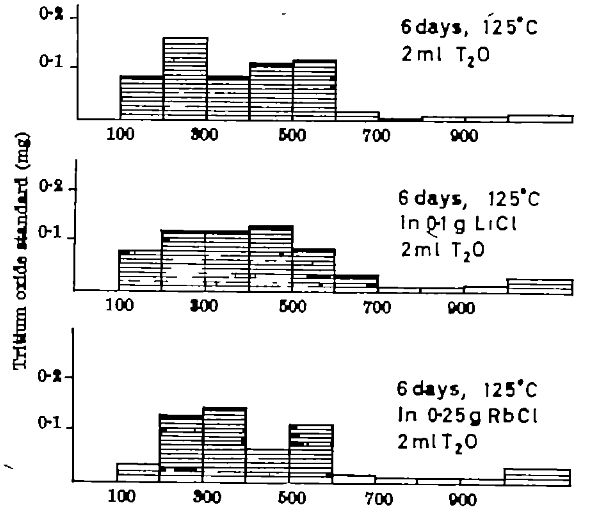

Fig. 2. Determination of tritium content in water fractions given off by gradual heating of phlogopite after six days at 125° C in tritium-bearing water

Table 1

Muscovite (%)		Phlogopite (%)	
SiO_2	44·30	SiO_2	40·32
Al_2O_3	35·95	TiO_2	3·54
Fe_2O_3	3·26	Al_2O_3	12·63
MnO	0·09	Fe_2O_3	2·30
K_2O	10·20	FeO	5·08
Na_2O	0·55	MgO	22·02
H_2O (< 110° C)	0·03	Na_2O	0·36
H_2O (> 110° C)	5·47	K_2O	9·32
	Σ 99·86	H_2O (+)	4·00
		F	0·30
			Σ 99·82

In addition (%)
V 1/10
Ni and Cu 1/100
Mn and Co 1/1,000
Ca not found (< 1/10)
} spectrographically

Table 2. ALKALIS SOLVED AFTER SIX DAYS AT 125° C AS PERCENTAGE OF ALKALI CONTENT OF THE MINERAL POWDERS

		H_2O	1·2 m LiCl	1·1 m RbCl
Muscovite	Na	7·2	16·5	12·7
	K	0·8	5·6	2·9
Phlogopite	Na	13·8	11·5	11·5
	K	1·7	2·7	1·9

amount of tritium oxide therein determined for each 100° C in the interval from 100° to 1,200° C. The results for tritium are given in Figs. 1 and 2. The amount of alkali solved from the mineral by the different treatments is given in Table 2.

The results support the concept that water molecules are present in the interlayer position of hydro-muscovites. Further, it is shown that by treatment with lithium chloride considerably more alkali is liberated from the di-octahedral micas than by the other treatments. Further, the maximum amount of tritium enters the lattice by this treatment. The replacement $Li^+ + H_2O$ against K^+ supports the opinion by White *et al.* that lithium enters into the octahedral position in the dioctahedral micas expelling a potassium from the interlayer sheet. The hole left gives place to a water molecule.

By the trioctahedral phlogopite no such replacement in the octahedral layer is possible. Consequently no significant difference is seen by the various treatments.

By treatment with lithium chloride we seem to obtain mixed crystals between $KAl_2(Si_3Al)O_{10}(OH)_2$ and $H_2O(Al_2Li)(Si_3Al)O_{10}(OH)_2$ compared to our concept of hydro-muscovite as mixed crystal between $KAl_2(Si_3Al)O_{10}$-$(OH)_2$ and $H_2O(Al_2H)(Si_3Al)O_{10}(OH)_2$ or $H_2OAl_2(Si_3Al)O_9$-$(OH)_3$.

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PHYSICS

Electron Microscopic Examination of Highly Radioactive Fall-Out Particles

MICROSCOPIC examination of highly radioactive fall-out particles, produced by the series of large-scale nuclear test explosions carried out during September and October 1961, revealed that although they were quite or nearly spherical, their surface states were different from particle to particle and some of them were much creased or had small spherical particles attached to their surface[1]. Electron microscopic examination followed by electron photomicrography has been carried out to examine their surface state in detail. A vacuum-evaporated silica film backed with evaporated carbon film was used for holding a sample particle. A small drop of benzene was put on the silica film which was supported on a 150 sheet mesh, then a sample particle which had been picked up by the tip of a fine needle was transferred into the drop seen under a microscope. The sample particle was left on the film after the drop evaporated out. Electron photomicrographs of four particles, *A*, *B*, *C* and *D*, were taken. In Table 1 are given their diameters, radioactivities and fractionation factors for $^{141}Ce + ^{144}Ce$ (ref. 2). Fig. 1 (1,2,3) gives electron photomicrographs of particles *A*, *B* and *C*, respectively. Small particles adhered to the surface are thought to be fine fall-out particles which are micron size or smaller[3]. Fig. 1 (5) is a close-up of a part of particle *C*. The diameter of this small particle is about 1·4μ.

Table 1

	Mean diam. (μ)	Estimated β-activity at the beginning of November 1961 when the particle fell on the ground surface (mμc.)	Fractionation factor for $^{141}Ce + ^{144}Ce$
Particle *A*	16·3	26·6	0·21
Particle *B*	18·9	50·2	0·36
Particle *C*	17·2	57·5	0·21
Particle *D*	17·5	29·9	0·29

During electron microscopic examination, it was experienced that the particle surface became smooth and small particles adhered to the surface disappeared by the evaporation caused by too intense electron beam. Fig. 1 (4) shows the smooth surface, and the broken silica film thus occurred during examination of particle *C*. The same situation observed on particle *D* is shown in Fig. 6, in which a more smooth surface formed by a more intense electron beam is seen.

As already reported[4–6], the uptake of radioactive substance through the leaf surface was confirmed to take place at the point where a highly radioactive fall-out particle was located. This fact suggests that a highly radioactive fall-out particle has easily soluble components.

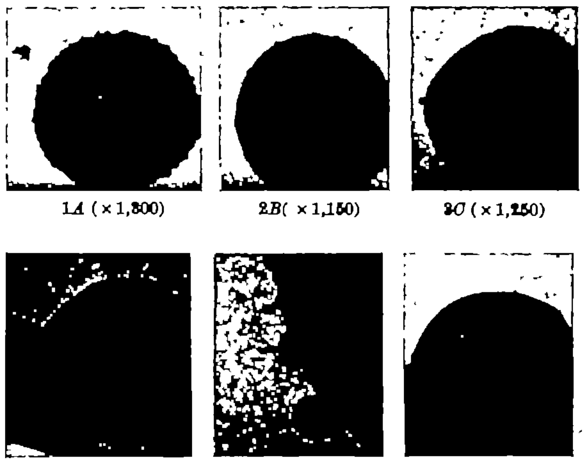
1*A* (×1,300) 2*B* (×1,150) 3*C* (×1,250)
4*C* (×4,600) 5*C* (×1,250) 6*D* (×1,200)

Fig. 1. Radioactive fall-out particles

On the other hand, it has been confirmed by the aid of an autoradiographic technique that some fall-out particles are impossible to be dissolved even in hot aqua regia.

The surface of a highly radioactive fall-out particle is assumed to be as follows. The main part of the particle was produced by condensation followed by solidification of explosion products at relatively high temperature immediately after explosion. On the surface of the particle thus produced, volatile elements such as ^{131}I and ^{106}Ru condensed to form a film at relatively low temperature and at the same time submicron size particles happened to be attached through collision.

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Activation Analysis of Particulate Air Contaminants

Typical air samples contain particulate air contaminants in concentration of 100–2,000 μg/m^3. Standard methods for analysing the composition of these contaminants are tedious and often insensitive[1]. The application of activation analysis for the inorganic elements appears to be attractive as a fast, sensitive and highly specific method. Preliminary irradiations of samples collected on filter paper have been performed and have shown that the method is feasible. Identification of several radioactive species has been accomplished, and quantitative estimates have been made. One problem has been that the filter paper as well as the contaminants activate, necessitating the irradiation of a control sample to correct for the effect.

The airborne particulate material was collected using Whatman No. 41 filter paper. Collection efficiency was approximately 99 per cent for particles greater than 0·1μ in diameter. The filter paper containing the sample, together with a filter paper control, were then irradiated in the University of Florida training reactor. β-activities were measured with a 2π proportional counter and γ-activities were measured with a single-channel pulse height analyser equipped with a well-type sodium iodide (Tl) crystal. In addition, one sample was studied with a multichannel analyser used in conjunction with a sodium iodide (Tl) crystal.

A description of the activation samples and irradiation histories is given in Table 1. As indicated there, several of the samples were collected in environments containing a very high particulate concentration.

Typical decay curves of the β-activity of several samples are shown in Fig. 1. Also shown there is the activity of the filter paper control which was irradiated at the same time as one of the samples. After subtracting the activity of the filter paper control, the decay curves were analysed for the half-life of the various components. Results of these analyses are shown in Table 2 together with tentative identification of the isotopic species responsible for the activities. The presence of barium in sample Nos. 2 and 6 is attributed to the fact that these samples were collected during an operation which involved barytes concrete dust.

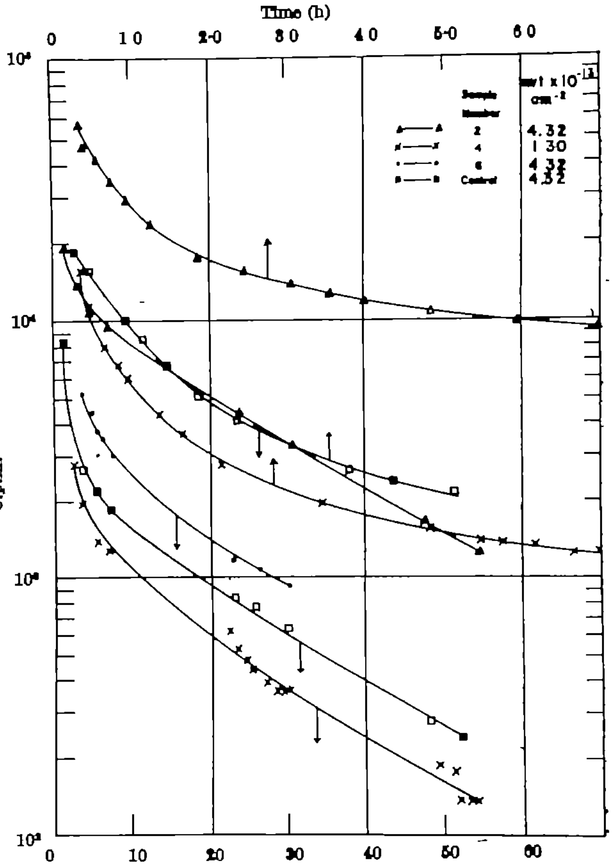

Fig. 1

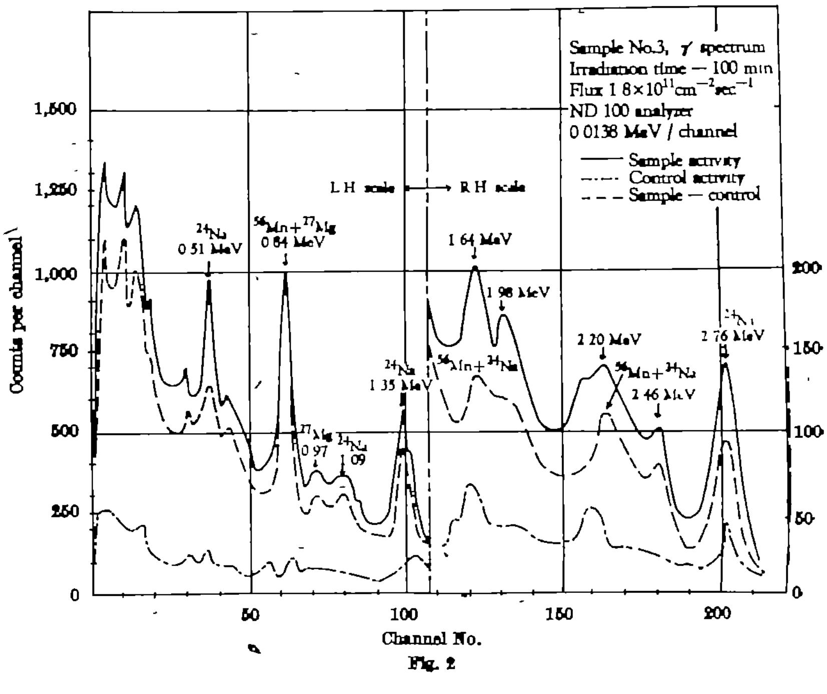

Fig. 2

Table 1. Air Contaminant Activation History

Sample No.	Air vol. (m³)	Particulate conc. (μg/m³)	Activation sample weight (mg)	Activation date	Irradiation ϕth (10^{11}/cm²/sec)	t (min)
1*a*	76·4	135	0·780	14/10/60	1·8 × 10^{-3}	30
1*b*	76·4	135	0·780	28/10/60	0·18	10
1*c*	76·4	135	0 780	16/11/60	0·18	10
1*d*	76·4	135	0·780	24/2/61	1·8	15
2*a*	59·1	2,000	14·2	17/11/61	0·72	10
2*b*	59 1	2,000	14·2	14/11/61	0·72	10
3	57·8	1,880	9·55	19/12/61	1·8	100
4	6·3	*	*	8/1/62	0·72	3
5	9·8	3,020	14·8	17/1/62	0·72	10
6	10 1	370	1·85	25/1/62	0 72	10

* No observation.

Table 2. Half-life Analysis of Samples

Sample No.	Half-life of β activity*	Half-life of γ activity*	Isotope
1*b*, 1*c*, 1*d*	14·1 h		Sodium-24
	2·9 h		Manganese-56
	81·0 min		Barium-139
2	15·0 h	15·8 h	Sodium-24
	72·0 min	81·0 min	Barium-139
	19·5 min	25·0 min	Iodine-128 (?)
	3·3 min	2·8 min	Aluminium-28
4	13·6 h		Sodium-24
	44·0 min		Chlorine-38
	8·0 min		Magnesium-27
	3·3 min		Aluminium-28
5	14·3 h		Sodium-24
	42·0 min		Chlorine-38
	8·0 min		Magnesium-27
	3 5 min		Aluminium-28
	40 0 sec		Oxygen-19
6	15·0 h		Sodium-24
	72 0 min		Barium-139
	12·0 min		Magnesium-27
	3 3 min		Aluminium-28

* Typical estimated *S.D.* of half-life: 9 per cent

In order to investigate the γ-spectrum from the activated contaminants, sample No. 3 was irradiated for 100 min together with a filter paper control. Following the irradiation the γ-spectra of the sample and the control were measured with a 256-channel analyser. The results of this measurement are shown in Fig. 2. The principal activities in the corrected spectrum appear to be due to sodium-24 and manganese-56.

An obvious need in improving the sensitivity of the method is to use filter paper having the lowest possible content of constituents which activate and interfere with the sample determination. Accordingly, samples of Whatman No. 41 (0·00009 g of ash per piece), Whatman No. 42 (0·00006 g of ash per piece) and 'Millipore' were irradiated. The results indicated that Whatman No. 41 and 'Millipore' contained approximately equal constituents, while Whatman No. 42 contained only about 10 per cent equivalent constituents (in terms of the activation cross-section, $V\Sigma_{act}$). On this basis Whatman No. 42 would have been a better choice than Whatman No. 41 filter paper. The 'Millipore' paper has the advantage that it can be dried to a constant weight, thus permitting a quantitative correction for the activities due to the filter paper.

If one assumes the contaminant in sample No. 5 is concrete dust (1·80 weight per cent magnesium), then the observed 8-min activity may be attributed to 266 μg of magnesium. This value agrees very well with the value of 271 μg calculated from the observed activity after corrections are made for sample decay and counter efficiency. Similarly, for sample No. 2 one obtains 254 μg of magnesium (14·1 mg total × 1·80 weight per cent magnesium) as compared with 245 μg calculated from the measured activity.

We plan to improve the precision of the method as follows: (1) use radiochemical separation to aid in identification of specific elements; (2) use standards and standard carriers to ensure reproducible quantitative data.

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Bath-tub Vortex

With reference to Prof. A. H. Shapiro's recent communication[1] on the above subject, I venture to call attention to the fact that in 1908 the Austrian physicist, O. Turmlitz, described careful and effective experiments which demonstrated the effect of the rotation of the Earth on the outflow of water through a central aperture, in a paper entitled "Ein neuer physikalischer Beweis der Achsendrehung der Erde"[2].

I dealt at some length with the whole question of rotation at outflow in a lecture on "Whirlpools and Vortices"[3] given in 1938.

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CERAMICS

High-pressure Densification of Glass and the Effects of Shear

When certain oxide glasses are temporarily compressed at high pressures, a large 'permanent' increase of density may occur when the pressure is released. For example, Bridgman and Simon[1] reported that the density of vitreous silica was increased 7 per cent after room temperature compression at 200 kilobars; below an apparent threshold pressure of about 100 kilobars, however, no densification was possible at room temperature. Roy and Cohen[2] afterwards reported that a density increase of 7 per cent was achieved at only 55 kilobars, and that from 20 to 160 kilobars densification of silica glass at room temperature was a linear function of pressure. Despite the unexplained large disparity between these results, it was proposed[2] that silica glass be used as a pressure calibrant for the range 10–200 kilobars. More recently, Christiansen, Kistler and Gogarty[3] furnished yet another different set of results. These and some results of the present work at room temperature are shown in Fig. 1. The results of Roy and Cohen which were presented as changes of refractive index have been converted to changes of density from a calibration obtained by us. The results of curves *A*, *B*, and *C* were all obtained on the opposed-anvil apparatus. Whereas Roy and Cohen used powdered glass, the samples of the other workers were in the form of thin glass disks. The results reported here were obtained on solid cylinders about 2 mm in diameter and 2 mm in height with a pyrophyllite–alumina cell in a 'belt' apparatus[4].

Bridgman[5] had questioned whether densification of glass was possible under pure hydrostatic condition without the co-operation of shear. In one experiment on soda glass in which densification of several per cent was first observed, this large increase was no longer possible after the cell design was altered to decrease shearing forces during compression. Christiansen *et al.*[3] have suggested that a larger degree of shear was possibly present in their experiment resulting in the disagreement with Bridgman's results. The very much higher densification reported by Roy and Cohen[2] is understandable in view of the powdered samples used. A greater degree of shear is expected when irregular particles in contact with one another undergo sliding motion on compression. A further cause may be due to localized heating arising from friction, since densification increases with increasing temperature at constant pressure.

In the present work, large differences are found in the densification of silica glass dependent on the degree of shear present. Thus, with an alumina container, the densification at 60 kilobars and 400° C for 2 min is 14 per

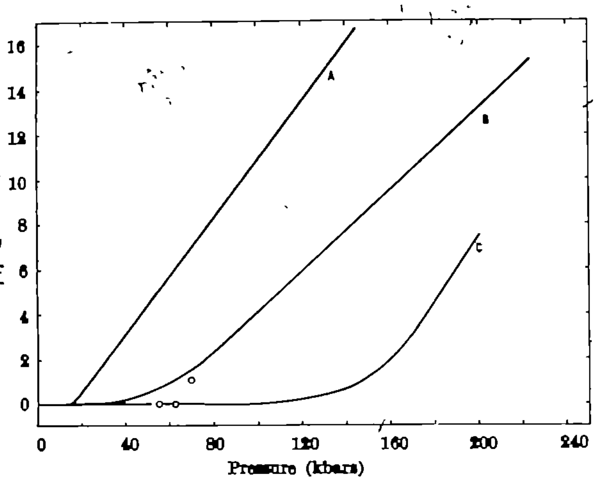

Fig. 1. Increase of density with applied pressure at room temperature for silica glass. *A*, Roy and Cohen (ref. 2); *B*, Christiansen *et al.* (ref. 3); *C*, Bridgman and Simon (ref. 1); O, present work, alumina cell

cent. When the container is silver chloride, it is only 4 per cent. Cohen and Roy[6] reported that, at 40 kilobars, densification of approximately 4 and 10 per cent was achieved at 300° and 500° C, respectively. When a solid piece of silica glass was embedded in silver chloride, we found the corresponding results to be 0 and 1·5 per cent.

Roy and Cohen[2] suggested that "for each pressure and temperature there is a pseudo-equilibrium volume for silica glass" and that this volume is attained in less than 15 sec in their experiment. Since densification increases with shear, the effect of time on the comparatively less dense glass must be considered. Christiansen *et al.*[3] found that at about 100 kilobars the observed densification of about 4 per cent is unchanged from 1 min to more than 2 h. The corresponding value of Roy and Cohen is 11 per cent. At 400° C and 60 kilobars, we have found that no further densification was observed from 4 min to 1 h in a silver chloride cell. The apparent final value of 7 per cent is still far less than that of 14 per cent attained in the alumina cell in 2 min. It appears, then, the final volume attainable is dependent not only on pressure, temperature, and time, but on the degree of shear as well.

The use of glass as a pressure calibrant is further complicated by the high annealing rates at atmospheric pressure. Some typical results are shown in Fig. 2. If densification had occurred at elevated temperatures and pressures, and the pressure is decreased prior to cooling, the significance of the density of the cooled glass must be dependent on the cooling rate.

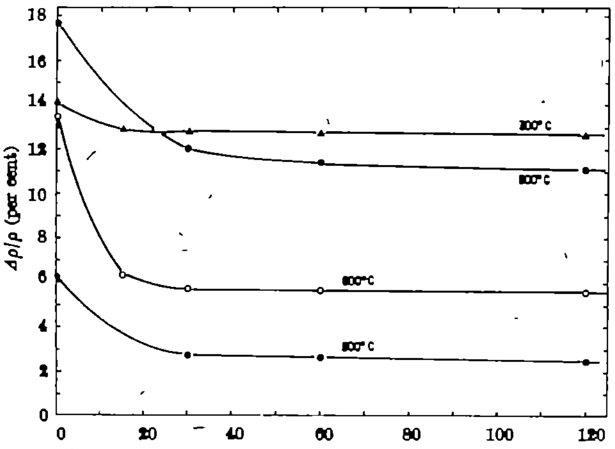

Fig. 2. Decrease of density of densified silica glass as a function of time by annealing at various temperatures and atmospheric pressure

Details of both the densification and annealing studies will be published elsewhere. We thank Dr. W. F. Claussen for the high-pressure facilities made available.

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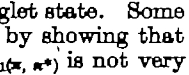
RADIATION CHEMISTRY

Origin of the Phosphorescence Radiation in Aromatic Hydrocarbons

WHEREAS the triplet nature of the (π, π^*) phosphorescent state of aromatic hydrocarbons has been definitely proved by the electron spin resonance investigations[1], the origin of the phosphorescence radiation is far from being understood.

Pure triplet–singlet transitions are forbidden according to the electric dipole selection rules. Phosphorescence in fluorescein is found experimentally[2] to be an electric dipole radiation. Perturbation theory is thus applied to mix the emitting triplet state (T_1) with some singlet character, or the ground state (S_0) with some triplet character (because of energy separation arguments the latter mixing was unjustifiably neglected). Whereas different workers use the spin orbit interaction for the perturbation operator, they differ on the type of the mixing singlet state. Some workers[3,4] can explain the long lifetime by showing that the mixing between $S_{(\pi,\pi^*)}$ states and $T_{1(\pi,\pi^*)}$ is not very strong because the one and two centre integrals of the spin orbit matrix elements vanish[3] if the molecule has a plane of symmetry in the molecular plane and across the bonds. Other workers[5] use the (σ, π^*) singlet states and can also account for the observed lifetime. None of these calculations is accurate or strong enough to support or reject one or the other mechanism. Thus, theoretically, this is still an unsolved problem.

Experiments were designed to gain more insight about the radiation source of the phosphorescence process. Two different independent types of experiments were carried out. The first experiment was meant to examine the effect of bending the aromatic ring on the $T_1 \rightarrow S_0$ transition probability. Because of the nature of the (π, π^*) states and the form of the spin orbit Hamiltonian for these molecules, such mixing should be very sensitive to the degree of molecular planarity. Bending the aromatic ring would bring in contributions from the one and two centre integrals, which do not contribute[3] in the planar molecules. This should greatly decrease the natural lifetime of the phosphorescence if these were the mixing states. Theoretical analysis on the (σ, π^*) states has not yet been made. The phosphorescence characteristics of [2·2] para*cyclo*phane[6], with the structure



is studied. X-ray examination of this molecule showed[7] that each carbon atom in the ring goes through ~12° of non-planarity. The observed lifetime of its blue phosphorescence was found to be 3·3 sec, compared with 7 sec in benzene. The difference might very well be due to slightly more radiationless processes in the para*cyclo*phane molecule. It therefore seems that bending does not have any effect on the phosphorescence lifetime.

The polarization of the phosphorescence spectrum of these molecules is very valuable in determining the symmetry properties of the perturbing state. The method of photoselection[8] was used where the molecules studied are embedded in a plastic medium[9], and the polarization measurements were carried out at room and liquid nitrogen temperatures. The molecules chosen were naphthalene (D_{2h}), phenanthrene (C_{2v}), chrysene (C_{2h}), and coronene (D_{6h}). The results on the polarization of these molecules strongly indicate that the whole spectrum is polarized similarly and perpendicular to the $S_{(\pi,\ \pi^*)} \leftrightarrow S_0$ transitions; that is, the emission shows a great amount of out-of-plane polarization (the results on phenanthrene are shown in Fig. 1). The great variations in the electronic structure of the molecules examined in this work suggest that such results are general for aromatic hydrocarbons. Therefore, it can be concluded that in aromatic hydrocarbons the (π, π*) singlet states are not very important in mixing with the emitting (π, π*) triplet state. The important singlet (or triplet) state could be a (σ, π*), (π, σ*), or Rydberg (or ionization) state to which the transition from the ground (or the emitting triplet) state is polarized perpendicular to the molecular plane. It is further concluded that the polarization results are independent of the symmetry of the aromatic molecule.

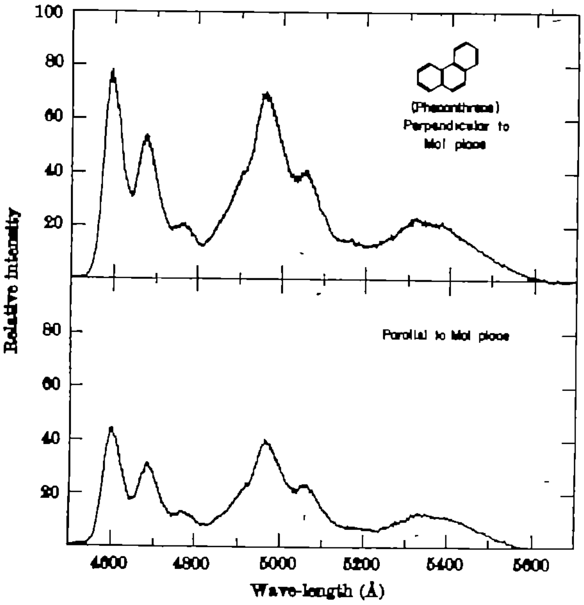


Fig. 1. Results on the polarization of the phosphorescence spectrum of phenanthrene using the method of photoselection. Excitation in the two perpendicularly polarized π, π* singlet states gave similar results and indicated that the phosphorescence is polarized perpendicular to the molecular plane

When corrected for the radiationless processes, it was found[10] that many of these hydrocarbons possess the same natural phosphorescence lifetime. It was also found[11] that many of the benzene derivatives have a phosphorescence spectrum with the same polarization characteristics as benzene. These results, together with those found in this work, indicate that the phosphorescence mechanism is insensitive to the symmetry, the planarity or to the details of the fine structure of these molecules. This is a rather strange result for a phenomenon which originates, according to our present understanding, from molecular perturbation effects. It might mean that the effect is mainly due to a one-centre interaction on the carbon atom or else the perturbing state is a high-energy one with mixed nature and so far separated from the molecule that it is insensitive to the finer details of its structure (for example, high-energy Rydberg or σ, π* states).

I thank Prof. D. J. Cram and his co-workers for providing me with the paracyclophane sample, Dr. W. Melhuish for providing the plastic samples, and the U.S. Atomic Energy Commission for its support of the research under contract No. AT(11-1)-34, Project No. 91.

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BIOPHYSICS

Thermochemiluminescence of Chlorophyll–Carotene Mixtures

ARNOLD[1] has measured the emission of light from chloroplasts on illumination and subsequent heating in air. Litvin *et al.*[2] established that oxidation of photoreduced chlorophyll is accompanied by chemiluminescence. Goedheer and Vegt[3] have oxidized chlorophyll in methanol solution with ferric ion and observed chemiluminescence on reducing with ferrous ion.

We have observed chemiluminescence on heating in air mixtures of chlorophyll *a* and all-*trans*-β-carotene which had been deposited from benzene solution on stainless steel disks.

Chlorophyll *a* was prepared according to a modification of the method of Jacobs[4], and the all-*trans*-β-carotene (obtained from Nutritional Biochemicals and from Mann Biochemicals) was examined spectroscopically to determ-

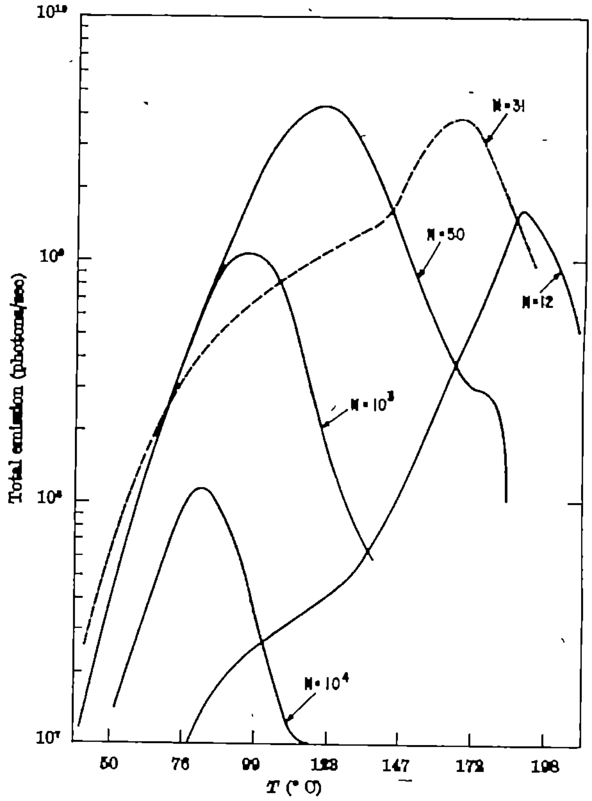

Fig. 1. Concentration dependence of the thermochemiluminescence of chlorophyll–β-carotene mixtures. β-Carotene: 1 mg/disk (~ 0·04 mg/cm²). *N*, Molar ratio of β-carotene/chlorophyll. Disks prepared and heated as described in text

ine its purity. No difference was observed between the behaviour of the carotene from the two sources. The deposited mixture was checked spectroscopically and found to be of uniform composition. Heating was done at 13° C/min, and detection was with a cooled R.C.A. No. 7102 photomultiplier isolated by a Corning No. 4600 filter and calibrated against a radiation thermocouple. Absolute calibration is conservatively estimated to be accurate to ± 50 per cent.

Table 1. TRANSMISSION BY CORNING FILTERS OF LIGHT EMITTED FROM CHLOROPHYLL a–β-CAROTENE MIXTURES

Filter No.	Transmission
2404	0·90
2418	0·93
2030	0·80
5850	0·25
5031	0·125
2550	0·03
2540	0

Fig. 1 shows typical data for the thermochemiluminescence of samples of 1 mg β-carotene with varying amounts of chlorophyll. (We have adopted this terminology as descriptive of the fact that the light is emitted during a chemical reaction which was catalysed by heating in the manner conventionally used in thermoluminescence work.) Self-absorption of the emitted light is not a problem at these low chlorophyll concentrations. Height of the lower temperature peak increases approximately linearly with increasing chlorophyll concentration until a carotene/chlorophyll molar ratio of 50 (0·037 mg carotene + 0·0031 mg chlorophyll/cm²) is reached. Beyond that the lower temperature peak is quenched very sharply with increasing chlorophyll content in the film, while the higher temperature peak comes up rapidly. Samples prepared in very dim light and samples illuminated for 5 min with a 500-W projection lamp prior to heating gave substantially the same results. We have measured the spectral distribution of the emitted light with a series of Corning graded filters. The results are given in Table 1. They are consistent with two peaks in emitted intensity, one centred at 6950 Å and the other, with relative intensity 0·6 as great, at 7450 Å. The results have been corrected for the response of the S–1 photosurface plus No. 4600 filter combination.

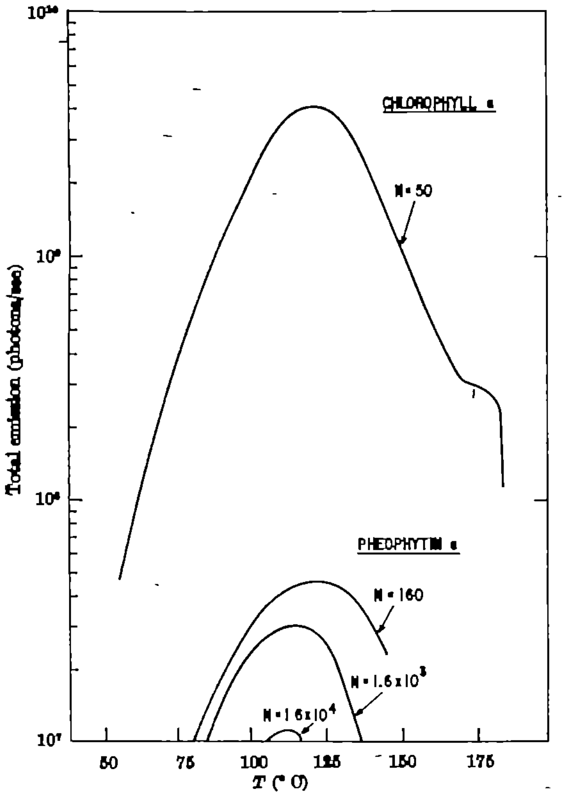

Fig. 2. Thermochemiluminescence of pheophytin–β-carotene mixtures. Concentrations and notations as in Fig. 1

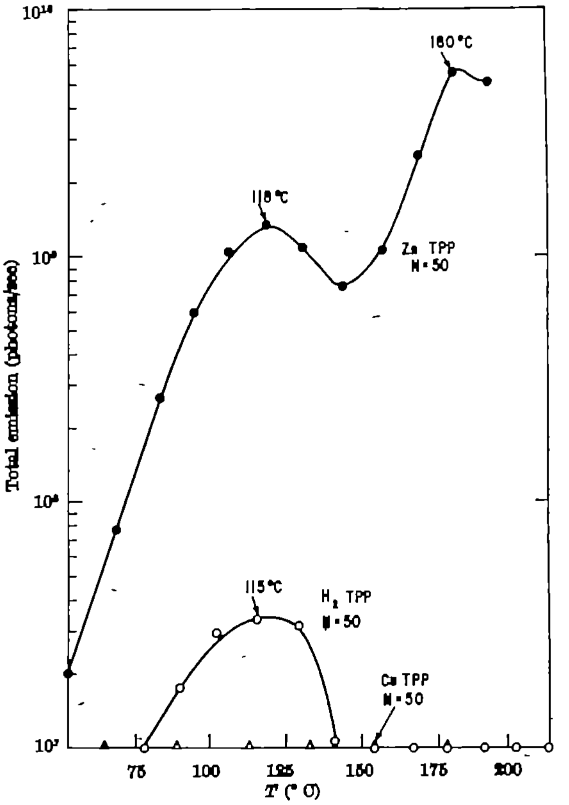

Fig. 3. Thermochemiluminescence of tetraphenyl porphin–β-carotene mixtures. ZnTPP, Zinc tetraphenyl porphin; CuTPP, copper tetraphenyl porphin; H_2TPP, free base tetraphenyl porphin. Concentrations and notations as in Fig. 1

We have measured the thermochemiluminescence of mixtures of carotene with an allomerized chlorophyll a prepared according to Gilman's method[5]. There appears to be very little difference between the results for chlorophyll a and allomerized chlorophyll a.

Mixtures of β-carotene and pheophytin a were examined with the results shown in Fig. 2. The striking decrease in emission may be noted by comparison with the curve reproduced from Fig. 1 for a carotene/chlorophyll ratio of 50. The decrease in luminescence accompanying removal of the magnesium from the chlorophyll is related to the interaction between the β-carotene and the porphyrin and is not a trivial effect due to loss of oscillator strength in the pheophytin red luminescence. This was established by measurement of the fluorescence yields of chlorophyll and pheophytin a and of allomerized chlorophyll a in benzene solution when excited by 400 and 430 mμ light. They were observed to differ by less than a factor of two. (We thank Dr. Charles Hurwitz of the Medical Research Laboratory, Veterans Administration Hospital, Albany, New York, for the opportunity to use his spectrofluorometer to make these measurements.)

Linschitz[6] has measured the chemiluminescence of tetralin–tetraphenylporphine (TPP) mixtures. He found that ZnTPP catalysed the chemiluminescent decomposition of tetralin hydroperoxide, and was itself gradually consumed in the process. The metal-free TPP did not interact to decompose the tetralin hydroperoxide.

We have measured the thermochemiluminescence of mixtures of ZnTPP, CuTPP, and H_2TPP (we thank Prof. H. Linschitz, Brandeis University, for the gift of samples of these materials) with β-carotene by dissolving the porphyrins in benzene and following the procedures described here. The results are shown in Fig. 3. The complete absence of chemiluminescence for the carotene–CuTPP mixture is not surprising in view of the fact that CuTPP is non-fluorescent. The fluorescence yields of ZnTPP and H_2TPP in benzene at 1 × 10^{-6} M, however, do not appear to differ by more than a factor of two, and, as in the case of the chlorophyll–pheophytin system, the decrease in luminescence upon removing the complexed metal from the porphyrin ring appears to stem from the change in porphyrin–carotene interactions.

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Photochemistry of Thymine

In spite of intensive work, much concerning the primary physical and chemical processes in the effects of radiation in DNA and its constituents remains obscure. Stimulated by the reported ultra-violet dimerization of thymine[1] and the peroxidation induced by ionizing radiations[2], we have re-investigated the photochemistry of thymine.

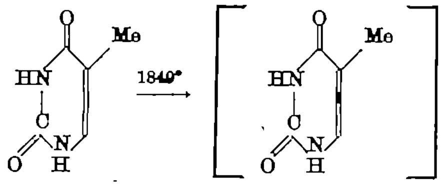
Thymine in aqueous solution has generally been supposed to be resistant to ultra-violet radiation, for example, Wierzchowski and Shugar[3], quoting a quantum yield for destruction of thymine of 4 × 10^{-4}. Using a 'Mineralight *R*-51' low-pressure mercury lamp in conjunction with a 1·5-mm Corning 7–54 filter to give pure 2537 Å radiation, we have re-determined this quantum yield at 4·3 × 10^{-4}. Oxygen seems to have no effect at the wave-length and no hydrogen peroxide was found. On removing the filter the rate of thymine has increased 80-fold while the intensity of 2537 Å radiation only increased by a factor of 3·8. However, it is known that this type of lamp, especially with the newer quartz envelopes, can emit an appreciable amount of radiation at 1849 Å (manufacturer's estimate ~5 per cent of total energy).

Accordingly, we suggest that the rapid photolysis of thymine is due to 1849 Å radiation and we estimate for this a quantum yield ~ 0·2.

We have further investigated the nature of the primary chemical reaction. Using a high-intensity flash discharge (measurements made at Illinois Institute of Technology on the apparatus of Dr. L. Grossweiner, whose co-operation and assistance are gratefully acknowledged) (8 kV, 22 μF) known to form small amounts of hydrogen peroxide in water (and hence emitting radiation in far ultra-violet) it was found that repeated flashing caused no loss of thymine in oxygen-free solution, whereas in air-equilibrated solution it caused a 25 per cent loss at 2·5 × 10^{-5} M and 14 per cent loss at 1 × 10^{-4} M. It is concluded that oxygen is necessary for the primary photochemical process at 1849 Å.

The nature of the photoproducts has been investigated. Reaction with I^- reagent at *p*H 4 (ref. 4) shows the presence of hydrogen peroxide (confirmed by reaction with titanium reagent[5]) and a hydroperoxide. Preliminary indications from the rate of reaction are that the hydroperoxide is 4-hydroperoxy-5-hydroxythymine, which is the opposite order of addition to that formed, presumably by free radical attack, on X-irradiation[6]. Further product identification is in progress.

Kinetic investigations show both the hydroperoxide and hydrogen peroxide to be major initial photolysis products and it is suggested that this behaviour can be understood in terms of the following reactions, in which the critical step is the electron transfer to oxygen from O_4 position of the excited state of thymine[2], followed by further reaction of thymine radical with oxygen[3].



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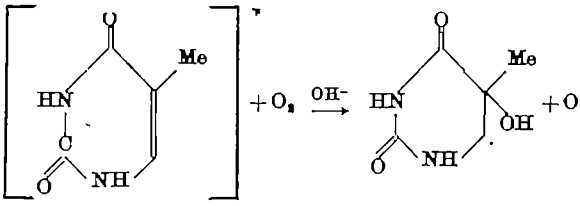

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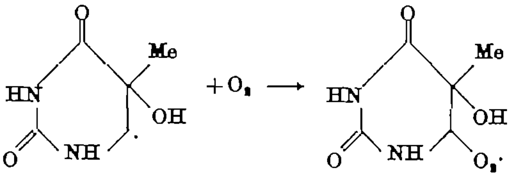

(3)

The O_2^- ion can give rise to hydrogen peroxide and the peroxy radical to the hydroperoxide.

It should be emphasized, however, that the nature of the absorption process at 1849 Å and of the excited state remain to be determined.

This investigation was supported in part by U.S. Public Health Service research grant *AM* 06420–01 from the National Institute of Arthritis and Metabolic Diseases.

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CHEMISTRY

Analysis of Partial Glyceride Mixtures

It has been emphasized by Hartman[1] that the computation of diglycerides and triglycerides in technical monoglyceride mixtures merely from the free hydroxyl content and amount of monoglyceride (both 1- and 2-forms) is liable to serious error. He suggested that a direct estimation of mono-, di-, and tri-glycerides was required particularly in view of the anomalies which appear to arise in attempting to assign a random distribution to the results.

Some years ago[2] a full analysis was carried out at this Station of the products arising from the alcoholysis at 240° C of linseed oil with glycerol (essentially an equilibrium mixture of glycerides) in the following manner:

(*a*) *Glycerol.* This was obtained from the hydroxyl values of the glyceride mixture before and after washing away the glycerol with water. Good agreement was found with the direct periodic acid oxidation method[3]. The hydroxyl value before washing was also taken as a measure of the glycerol/linseed oil proportions, thus correcting for any losses of glycerol that might have occurred during heating.

(*b*) 1- *and* 2-*monoglycerides*. Analysis for 1-monoglyceride was carried out on the washed sample (glycerol free) using a standard periodic acid oxidation procedure[4], the 2-monoglyceride content being obtained by a similar technique after isomerization of the sample with perchloric acid[5,6].

(*c*) 1,2-, 1,3- *and tri-glycerides*. The original glyceride mixture (including glycerol) was treated with methyl iodide and excess silver oxide to convert the hydroxyl groups to the corresponding methyl ethers. As a result of this methylation procedure the system no longer underwent isomerization and the tri-methoxy-propane (from the glycerol) and the 1,2- and 1,3-dimethoxy glycerides (from the 1- and 2-monoglycerides) could be removed by distillation without affecting the distribution of fatty acid radicals.

The residue after distillation consisted of 1- and 2-methoxy diglycerides and triglycerides from which, after saponification and acidification, 1- and 2-methoxy glycerol and free glycerol were obtained in aqueous solution. The free glycerol was then determined by a modification of the periodate technique[7] (liberation of formic acid) and the 1-methoxy glycerol by difference from the total consumption of periodate[8] and that consumed by the glycerol as determined here. The results could then be recalculated in terms of 1,2-diglyceride and triglyceride in the original reaction product. Thus, only the 1,3-diglyceride content need be calculated by difference.

The results of this analysis are shown in Table 1.

Table 1. ANALYSIS OF PARTIAL GLYCERIDES OBTAINED FROM 1 MOLE LINSEED OIL : 1·85 MOLE GLYCEROL AFTER HEATING FOR 2 H AT 240° C (WITH 0·1 PER CENT TRISODIUM PHOSPHATE CATALYST)

Component	Experimental analysis (% by weight)	Theoretical distributions (% by weight) (1) Assuming different 'reactivities' for 1- and 2-hydroxyl groups	(2) Assuming randomization of fatty acid radicals
Glycerol	6·5	6·5	6·5
1-Monoglycerides	36·5	39·5	28·3
2-Monoglycerides	4·4	5·1	14·2
1,2-Diglycerides	15·6	14·1	27·0
1,3-Diglycerides	32·7*	26·9	13·5
Triglycerides	4·3	7·9	10·5

* Calculated by difference.

Possible theoretical distributions are also included for comparison, calculations of the proportions of the various glycerides being made on a weight basis by:

(1) Assuming that the hydroxyl groups of glycerol have different 'reactivities' related to the ratio of 2-monoglyceride to 1-monoglyceride determined experimentally, namely, 0·13. This figure was the average found for several systems of varying linseed oil/glycerol proportions and also the ratio of 2- to 1- monostearin found on heating either pure 1- or pure 2-monostearin until equilibrium was established[2].

(2) Assuming equal 'reactivity' of all hydroxyl groups, that is, complete randomization of fatty acid radicals occurs.

The results in Table 1 show that the relative proportions of 1,2- and 1,3-diglycerides found are in good agreement with the theoretical distribution derived by assuming different reactivities for 1- and 2-hydroxyl groups. In fact almost an inverse ratio to that found would be required if the distribution were on a random basis.

Confusion in the literature has surely arisen from the use of the term 'random distribution' to describe experimental results when only the total monoglycerides present have been determined—the total diglycerides being obtained by difference, for example, in an early paper by Feuge and Bailey[9]. Clearly a true random distribution of acyl radicals cannot occur unless the ratio of 1- to 2-monoglycerides in the final equilibrium mixture is 2 : 1 by weight (experimental figures are normally closer to 8 : 1) and the ratio of 1,2- to 1,3-diglyceride is also 2 : 1 by weight.

I am not aware of any published figures for the individual diglyceride isomers of mixed glycerides and I have therefore directed attention to the work of the Paint Research Station in this field.

In more recent work at this Station the use of thin layer chromatography for the analysis of partial glyceride mixtures has been examined[10].

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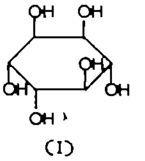
Occurrence of Configurational Inversion during Tritiation by the Wilzbach Method

LABELLING of organic molecules with tritium by the Wilzbach method[1] — exposure to tritium gas for a prolonged period—has become widely used, particularly for biochemical investigations. A considerable amount of decomposition always accompanies the formation of labelled material which, therefore, has to be very carefully purified. It is important to know whether replacement of hydrogen atoms on asymmetric carbon atoms may occur with inversion of configuration: if it does, diastereomers of the original compound will be formed, which may not be readily separated by recrystallization or other methods of purification. Absence of diastereomers has been reported in one case, that of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose[2], whereas 30 per cent racemization occurred on labelling of (–)-mandelic acid[3].

We have chosen (–)-inositol (I) for an investigation of the stereochemistry of Wilzbach labelling, because in this compound replacement of any hydrogen atom attached to carbon by tritium with inversion will result in the formation of a different isomer: myo- (II), allo- (III), or muco-inositol (IV)[4]. Inversion on any carbon can therefore readily be detected.

(–)-Inositol was exposed to tritium gas for eight weeks; equal amounts of each of myo-, allo-, and muco-inositol were then added, and the isomers were separated and purified by chromatography and chemical reactions. Each of the isomers was found to contain tritium, the ratio of specific activities being: (–)-inositol 1·0; myo-0·09, allo- 0·06, muco-inositol 0·03. Some inversion of every carbon atom has therefore occurred during the introduction of tritium.



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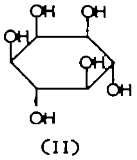

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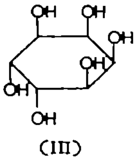

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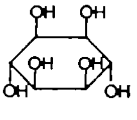

(IV)

Extensive intermolecular hydrogen bonding in a crystalline inositol would be expected to favour retention of configuration during tritiation. A non-crystalline derivative, hexa-*O*-methyl-(−)-inositol, was therefore submitted to Wilzbach tritiation, and it was found that inversion occurred to a far greater extent than in (−)-inositol, the ratios of specific activities being: (−)-inositol 1·0, myo-0·65, allo- 0·47, muco-inositol 0·33. In fact, since labelled (−)-inositol is produced by tritiation with retention at any one of the six carbon atoms, whereas each other inositol is formed by tritiation with inversion at either of two out of six carbon atoms, inversion is more extensive, on the average, than retention.

This example should serve as a warning that diastereomers may be encountered, after Wilzbach tritiation, in any compound containing asymmetric carbon atoms.

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Hypercritical Aerosol

THE inhomogeneity of fluids at close proximity to their critical point has long been known[1,2], well examined[3,4] and theoretically explained[5,6]. The 'critical opalescence' is in fact a two-phase system in the state of fine division, one of the phases being a gas; thus this state may be looked on as an aerosol. This point of view was expressed sixty years ago[7]. As an aerosol, the near-critical state is a very peculiar one: the specific gravity of the dispersed phase is but slightly different from that of the dispersing medium; the viscosity of the latter is considerably above the usual values for gases.

The purpose of the investigation reported here was to prepare and investigate the 'hypercritical aerosol' in a two-component system. Solutions of comparatively non-volatile substances, such as camphor and boric acid in liquid carbon dioxide, were examined in a cylindrical pressure cell 2 cm in diameter and 2 cm long, closed at both ends by appropriate transparent windows. By raising the temperature of the cell very slowly (so that the whole cell interior was at the same temperature), the transition in the gaseous state occurs homogeneously (Cagniard-Latour phenomenon), but, differently from the single component systems or the two-component ones with a common critical point, a cloud of particles persists above the critical point of the carbon dioxide. The cloud disappears slowly by sedimentation. The mean particle diameter for camphor, estimated from the sedimentation time—on the assumption that the gas is pure carbon dioxide at the measured temperature (32° C) and pressure (90 kg/cm^2) and the dispersed phase is pure camphor—is slightly less than 3μ. The persistence of an 'aerosol' of that size in a volume of only few cm^3 for several minutes is rather remarkable, but easily explained by the differences of the present experimental conditions from those to be found in the more familiar aerosols.

If the temperature of the cell is not rigorously uniform with a temperature gradient of a few tenths of a degree C, the bottom being warmer and below the critical temperature, a cloud above the liquid phase can be maintained indefinitely. In this event, boiling of the liquid occurs and the splashed droplets replenish the sedimentating cloud permanently. This kind of cloudiness, while being an aerosol near the critical point, is not a hypercritical aerosol, because of the presence of the liquid. This is a near-critical aerosol, which, in a homogeneous system, had been already prepared, not as described previously, by the use of a temperature gradient, but by exposing the liquid carbon dioxide, slightly below its critical point, to mechanical vibrations[8].

At the start of the experiments, 'Plexiglas' windows 2 cm thick were used. Carbon dioxide under 70 or more kg/cm^2 pressure is soluble in 'Plexiglas' and after releasing the pressure, gaseous bubbles, accompanied by the deformation of the surface, appear near the inner surface of the window. This effect is reversible, and does not weaken the window noticeably. But at 40° C and 100 kg/cm^2 pressure, camphor in the presence of liquid carbon dioxide enters instantly into solid solution in 'Plexiglas'. The resulting solid solution is pale yellow, opaque and with a mechanical strength much below that of 'Plexiglas'. That was in one case responsible for the failure of the window—somewhat hazardous at the working pressure.

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Constituents of Dental Calculus from Sheep

WHILE the crystalline composition of dental calculus in man is well elucidated, very little is known about such calculus in animals. We have had the opportunity of comparing the results of X-ray diffraction examinations of samples of dental calculus from sheep from Dundee, Scotland, and from Greenland. Powder diagrams were taken with copper *K*α-radiation in cameras of the Guinier type.

The two Danish samples, both from the same sheep, and one of the three Scottish samples exhibited a lustrous pigmentation. The material responsible for the pigmentation did not, however, show up in any of the diagrams. The results of X-ray analysis of the samples are shown in Table 1.

Table 1

	Danish samples		Scottish samples		
	1	2	1	2	3
	Pigmented	Pigmented			Pigmented
Major constituents	Brushite	Whitlockite	Apatite	Whitlockite and apatite	
Minor constituents	Whitlockite	Weddellite	—	Weddellite	

Diffraction lines due to brushite ($CaHPO_4,2H_2O$) were sharp, whereas those due to apatite and whitlockite (β-$Ca_3(PO_4)_2$) were quite diffuse indicating poorly crystallized compounds. Additional sharp lines with *d*-values 6·21 Å and 2·78 Å respectively could not be ascribed to any of the phosphates found in human dental calculus, but agree well with the two strongest lines of weddellite ($CaC_2O_4,2H_2O$) and in one case three other weak lines fitting the weddellite pattern could be detected. If the formation of this compound is likely in the saliva or the cud of ruminants we cannot judge. It has never been found in human dental calculus, but is well known as a constituent of urinary calculi. Three lines in human dental calculus patterns tentatively attributed to whewellite (CaC_2O_4,H_2O) by Tovborg Jensen and Danø[1] were almost certainly due to the presence of octocalcium phosphate, later identified as a common minor constituent of human dental calculus[2]. The latter compound was not found in any of the samples, although the presence of brushite in one specimen indicates a high supersaturation of sheep saliva with respect to all calculus forming calcium phosphates, brushite being the most soluble of these phosphates at *p*H above about 6 (ref. 3). Considering the high value (about 8·2) given in the literature as an average for the total saliva of sheep[4] the formation of brushite is unexpected. It seems likely that the pH of the saliva from all glands is not identical, but unfortunately the localities of the two samples from the same jaw are unknown.

Whitlockite has not previously been found in animal dental calculus. The principal constituent of dental calculus from the horse is calcite[5,6], while samples from dog, ferret and hamster contain poorly crystalline apatite and sometimes small amounts of calcite[6]. In human dental calculus the formation of whitlockite is related to the presence of magnesium in the precipitating fluid, the magnesian whitlockite showing a characteristic shift of the diffraction lines compared with β-tricalcium phosphate. The diffuseness of the whitlockite lines in the patterns from sheep calculus makes it difficult to discern a similar shift. However, ignition (1,000° C for 1 h) of Scottish samples 2 and 3 gave magnesian whitlockite containing about 10 atomic per cent magnesium, indicating a significant magnesium content of the original material.

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Selective Poisoning of Catalytic Activity

A RECENT investigation[1] has shown that the exchange reaction between alkylbenzenes and deuterium may provide a useful method of investigating changes in the catalytic activity of evaporated metal films subjected to thermal sintering. One of the most suitable alkylbenzenes for this purpose is p-xylene because it contains only two different kinds of hydrogen atoms—the six in the methyl groups and the four on the benzene ring. Nickel films laid down on a glass substrate at 0° C will catalyse the exchange of the first six hydrogen atoms (reaction I) about five times faster than the exchange of the remaining four (reaction II) at 0° C. Pre-sintering such nickel films for 30 min at 200° C before use reduces their activity for reaction I by a factor of about 600 but has an even more drastic effect of about 10^3 on the rate of reaction II. The earlier work did not establish whether these marked changes of catalytic activity on sintering were due solely to the influence of temperature on the films or whether contamination, and consequent poisoning of the films, had occurred by gas evolved from the reaction vessel. Because of this uncertainty, we have now carried out a series of experiments on the influence of carbon monoxide as a poison for reactions I and II.

Films, prepared as previously described[1], were subjected to the following treatment at 0° C: (1) admission of 10 mm of deuterium for 5 min; (2) evacuation for 40 sec; (3) admission of a known pressure of carbon monoxide with a 10-fold excess of deuterium for 15 min; (4) evacuation for 40 sec and then used as catalysts for the exchange of 1 mm of p-xylene and about 23 mm of deuterium. This complicated procedure was designed to ensure that the small quantities of carbon monoxide admitted would be adsorbed by displacement of deuterium only on the sites giving the greatest heat of adsorption and not merely randomly on the surface. The main features of the results for the rates of exchange at 0° C are summarized in Table 1. The figures in the fourth column were obtained from surface area data for similarly prepared nickel films[2] and an assumption of 7 Å² for the mean area of each nickel atom; they represent the maximum coverage of the surface if all carbon monoxide admitted was adsorbed and retained. The rate k_0 is the initial rate of disappearance of p-xylene-d_0 and corresponds to reaction I; k_6, the initial percentage rate of conversion of p-xylene-d_6 to products with more deuterium atoms, is a measure of the rate of reaction II. The rates quoted for experiments *G*, *H* and *J* were obtained by extrapolation of Arrhenius plots for rates at higher temperatures and each plot yielded an activation energy of about 20 kcal/mole for reaction I.

Table 1

Exp.	Weight of film (mg)	Molecules CO admitted (× 10^{17})	Approximate ratio of molecules of CO admitted to surface Ni atoms	k_0 (%/min)	k_6 (%/min)
A	10·5	0	0	50	8
B	12·3	1·2	0·04	60	13
C	16·7	1·6	0·04	45	3
D	10·8	2·0	0·07	18	0·05
E	10·9	2·8	0·10	21	0·02
F	11·1	5·1	0·18	1·8	$< 2 \times 10^{-3}$
G	11·8	8·1	0·27	0·03	$< 3 \times 10^{-4}$
H	8·8	16	0·65	0·02	$< 2 \times 10^{-4}$
J	8·5	160	7	$\sim 3 \times 10^{-4}$	—

Two runs were carried out on films laid down with the glass walls of the reaction vessel maintained at 200° C (after baking at 450° C) and the results were identical with those obtained previously[1] for films laid down at 0° C and presintered for 30 min at 200° C. Detailed rates for these experiments are not reported but were similar (about twice as fast) to those for experiment *G*.

It is convenient to discuss the results on the assumption that all carbon monoxide admitted in experiments *B*–*H* is adsorbed. A comparison of *B* and *C* with *A* suggests that the sites first covered by carbon monoxide are not important for the catalysis of either reaction. Presumably, the heat of adsorption of the hydrocarbon is too large on these sites, amounting to some 4 per cent of the surface, to permit easily reversible adsorption to occur. Experiments *C* and *D* show that adsorption of carbon monoxide on the next 3–6 per cent of the surface cuts down the rate of reaction II by more than a factor of 100 but only reduces the rate of reaction I by a factor of three. This is clear evidence that certain active centres on the nickel films are required to catalyse the exchange of the hydrogen atoms on the benzene ring of p-xylene and that these centres are selectively poisoned by carbon monoxide. A drastic reduction in the rate of reaction I is only obtained when more than 25 per cent of the surface is covered, indicating that some 20 per cent of the sites on the surface are efficient centres for this reaction.

We believe that the nickel films laid down with the glass maintained at 200° C will be substantially free of contamination. Such films, or films pre-sintered for 30 min at 200° C, contain practically no active centres for reaction II and very few for reaction I. It seems evident that thermal treatment, like poisoning by carbon monoxide, completely eliminates the centres for reaction II and also destroys most of the sites on which reaction I occurs as well. A possible explanation is that metastable metal atoms on the films are necessary to form the π-bonded adsorbed hydrocarbon intermediates[1,3] which are believed to be involved in the mechanism of the exchange reactions of the alkylbenzenes.

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Rheopexy in Dilute Polymer Solutions at Low Shear Gradients

ONE of us has pointed out that theoretical consideration of the hydrodynamic interaction of a very long flexible chain in laminar flow suggests that under certain conditions an increase in the viscosity of a polymer solution may occur with increase in the shear gradient[1]. Such rheopexy should be favoured with a solution of a linear polymer of very high molecular weight in an extremely viscous solvent, otherwise the theory predicts only a decrease in solution viscosity with increasing gradient such as has been reported experimentally in numerous instances. Generally, examples of rheopexy reported for polymer solutions occur at high gradients and reasonably have been attributed to the onset of turbulent flow, but this would not provide a likely explanation of an increase in viscosity reported by Selby and Hunstad which begins at only about 100 sec^{-1}. The latter results are consistent with the theory mentioned here inasmuch as the system studied was a polymethylmethacrylate of high molecular weight ($\sim 10^6$) dissolved in a mineral oil with a viscosity of several hundred poise but are not directly apposite because of the large concentration of polymer (3 per cent)[2]. A direct test of the theory has been undertaken by Wolff, who reported viscosity data extrapolated to infinite dilution for solutions of polystyrene (M up to 10^7) in a chlorinated diphenyl ($\eta \sim 1$ poise), with the conclusion that no increase in viscosity occurred[3]. The present communication reports further preliminary experiments designed to test the theory.

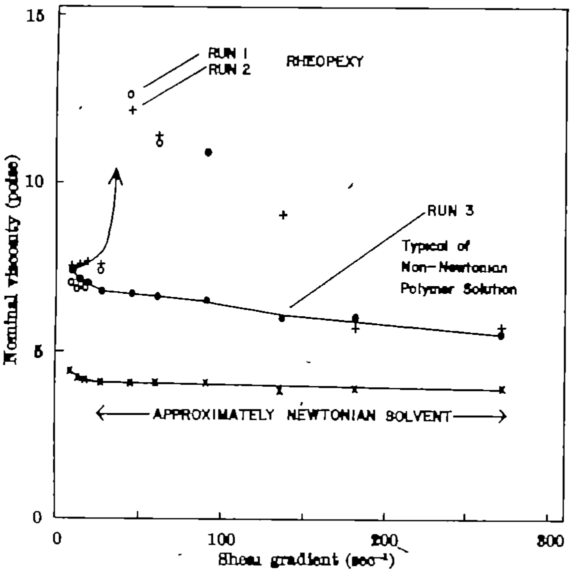

Fig. 1. Dependence of viscosity on shear gradient ×, Chlorinated diphenyl; ○, +, ●, solution of polymethylmethacrylate in chlorinated diphenyl

Polymethylmethacrylates, polymerized by conventional radical polymerization, of $M_w \sim 4\cdot2$ and $1\cdot0 \times 10^6$ were dissolved in a chlorinated diphenyl containing 0·2 per cent 4-tert-butylcatechol. Solutions were sheared at an ambient temperature of about 25° C in a plate and cone consistometer[4]. The results in Fig. 1 obtained with 2×10^{-3} g/ml. of the higher molecular weight polymer show an increase in nominal viscosity (the absolute calibration of the machine has not yet been checked) at low shear gradients, which is reproducible as a gross effect provided the solution is subjected to only limited shearing (cf. runs 1 and 2). After the more extensive shearing during run 2 plus 1 h at the highest gradient of 272 sec^{-1} the solution showed only a decrease in viscosity in two further runs (only run 3 is shown). However, when this same solution was cooled to near 0° C and its viscosity thereby increased to several hundred poise, marked rheopexy was again detected and could again be eliminated by prolonged shearing. Qualitatively similar results were obtained with the original solution in Cannon–Fenske capillary viscometers. By contrast, a more concentrated solution of the lower molecular weight polymer (5×10^{-3} g/ml.) showed no rheopexy at ambient temperature but only a decrease in viscosity with increasing gradient of the type generally reported for polymer solutions.

These experiments show that rheopexy is definitely observed in these solutions and at such low gradients that turbulent flow seems unlikely and, further, indicate that the occurrence of rheopexy is sensitive to viscosity and the length of the polymer molecules. Moreover, this latter sensitivity may be adduced to explain why rheopexy is eliminated by shearing with the acceptable postulate that the long polymer molecules are broken by the high shear forces applied. This general picture is consistent with theory but more critical and quantitative tests are necessary. The most important is to test whether, as required by theory, rheopexy can be observed in more dilute solutions. So far we have observed an effect near 0° C with concentrations of as little as $0\cdot13 \times 10^{-3}$ g/ml., but we are still trying to improve control of important experimental factors such as temperature in order to permit extrapolation of results to infinite dilution.

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Colorimetric Determination of 3,6-Anhydrogalactose with the Indolyl-3-acetic Acid Reagent

THE sugar 3,6-anhydrogalactose is a major constituent of the agarose fraction of agar, of kappa carrageenin, and of some other polysaccharides from marine algae[1]. This sugar can be determined spectrophotometrically as 5-hydroxymethyl-2-furaldehyde[2] or by an adaptation of the resorcinol test for ketoses[3]. The rate of degradation of 3,6-anhydrogalactose to 5-hydroxymethyl-2-furaldehyde suggests that any method used for the colorimetric determination of fructose should be applicable to 3,6-anhydrogalactose. Steinitz[4] compared six methods for the determination of fructose in body fluids, and favoured the method of Heyrovsky[5], who used β-indolylacetic acid as the colour reagent. The present communication describes the use of this reagent for the determination of 3,6-anhydrogalactose.

A standard solution of 3,6-anhydrogalactose was prepared from 3,6-anhydro-L-galactose diethylmercaptal[6]. Heyrovsky's procedure[5] was followed except that the tube was placed in the thermoregulated bath immediately after the hydrochloric acid was added. The colour-development curves showed that at λ 530 mμ maximum absorbance occurred in 5 h at 23·5°; in 1 h at 37° and in 7 min at 80° C. Because of the rapidity of the reaction at the higher temperatures, careful attention to the details of the procedure were necessary. Beer's law was followed with amounts up to 0·25 μM of 3,6-anhydrogalactose. The absorbance of the mercaptal was 7 per cent greater than with the free sugar, presumably because of the mercaptan formed in presence of the acid. A similar increase in absorbance occurred when ethyl mercaptan was present with fructose.

The reaction was applied to the determination of 3,6-anhydrogalactose in agar[3], and compared with the results obtained with the resorcinol reagent[8]. The results are shown in Table 1. Apparently the agar was not completely hydrolysed at the two lower temperatures. At temperatures above 37° C blank values were high and the colour was not stable, as Heyrovsky[7] also found when the reagent was applied to fructose. At 80° C the absorbance continued to increase for 2 h after the tubes had been removed from the bath.

Table 1. EFFECT OF TEMPERATURE ON THE YIELD OF 3,6-ANHYDROGALACTOSE FROM AGAR

Reagent	Temperature	Time	% in agar
Resorcinol	80°	10 min	44
Indolyl-3-acetic acid	23 5°	22 h	34
	37°	2 h	38*
	80°	7 min	43*

* Calculated from maximum absorbance values.

It was shown that the indolyl-3-acetic acid reagent can be used at 23·5° and 37° C to determine free 3,6-anhydrogalactose. It shows no advantage, however, over the resorcinol test[6,8], for the quantitative determination of 3,6-anhydrogalactose in a polysaccharide.

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A Lignin Carbohydrate Bond as revealed by Infra-red Spectroscopy

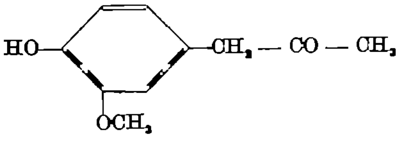
IN the course of spectroscopic investigation on the basic chemistry of lignins in wood and in unbleached kraft and sulphite pulps, we have recently obtained new evidence which suggests the existence of the long-sought covalent bond between lignin and carbohydrate in these materials. It appears to be an acetal or hemi-acetal bond, and, on cleavage, liberates a ketone group in the lignin. The work of others[1] indicates that the point of attachment may be mainly on the hemicellulose portion of the holocellulose.

We are examining the wood and pulp lignins by the method of high-resolution differential infra-red spectroscopy[2]. This is a quantitative application of the potassium bromide pellet technique, where the reference beam passes through an analytically determined amount of holocellulose (obtained by the *Tappi* method[3]) of the wood or pulp under examination in the sample beam of the spectrometer (Unicam *SP*–100). The quantity of holocellulose is calculated to cancel out the holocellulose spectrum in the sample, and only the spectrum of the lignin remains. The difference spectra are of generally good quality, and only two difficulties have been encountered. The first is that the *Tappi* holocellulose isolation fails to preserve all the acetyl groups, and the band due to the acetyl carbonyl is not completely cancelled. The second is that the holocellulose absorbs so strongly in the 1,000 cm^{-1} region that in that region the available light energy is reduced to the point that the difference spectrum is unreliable. In future work we intend to overcome these problems by using holocellulose isolated by milder methods, and by running the spectrum in the 1,000 cm^{-1} region on lower concentrations of sample, with ordinate scale expansion, if necessary.

We have now examined the differential spectra of eleven softwood and two hardwood species of wood, two kraft pulps (13·6 and 17·2 per cent lignin, respectively) and three sulphite pulps (11·8, 19·6 and 24·0 per cent lignin, respectively). We have also determined the spectra of thirty-five isolated lignins, obtained from different sources and by various techniques. These lignins included spruce milled-wood lignin, spruce and aspen dioxane lignins, spruce and poplar kraft alkali lignins, spruce and birch periodate lignins, spruce and birch lignosulphonic acids, and Klason lignins of thirteen wood species.

All the isolated lignins had an absorption band at 1,710 cm^{-1} ($\pm$ 15 cm^{-1}) of greater or lesser intensity, depending on the method of isolation, and this band was absent from all the differential spectra of wood and pulp lignins.

The results of earlier infra-red studies of isolated lignins do not agree on the assignment of the absorption band at 1,710 cm^{-1}. In an investigation of aspen native lignin, Smith[4] has presented evidence that a band near 1,710 cm^{-1} (actually 1,694 cm^{-1} in his measurements) is due to a carboxyl group of the type found in *p*-hydroxybenzoic acids. The band was not affected when the lignin was treated with sodium borohydride, but disappeared after alkali treatment. Marton[5] has studied milled-wood lignin (Björkman) from Norwegian spruce and also concluded that the band near 5·8μ (1,724 cm^{-1}) was due to carboxyl groups, since it remained after borohydride reduction of the lignin. However, Hergert[6] examined a series of native, milled-wood, and dioxane lignins, and found that the band was removed by borohydride, except when the lignin had been pretreated with alkali. From this evidence, and from an examination of model compounds, Hergert concluded that the 1,710 cm^{-1} absorption was due to a non-conjugated keto group of the type found in guaiacyl acetone (I), and he explained its behaviour on the basis of the enolizable nature of the ketone, its easy conversion to an enolate ion, and the air-oxidation of the unstable enolate to a carboxylic acid which could no longer be reduced by borohydride. According to Hergert's estimate the grouping represented in (I) occurs once in every twenty-five C_6–C_3 units of lignin.



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The literature contains several other isolated pieces of work, some supporting the carboxyl interpretation, and some the ketone, but the weight of evidence suggests that the 1,710 cm^{-1} absorption band in lignin arises largely from an enolizable aryl-β-keto group with, possibly, a small contribution from carboxyl[7].

Support for the β-keto structure is given by the work of Adler, who recently stated from chemical and ultra-violet spectroscopic evidence that half the total carbonyl in milled-wood lignin from Norwegian spruce is in the beta position, and that there is one such carbonyl for every ten methoxyl groups in the lignin[8].

The ketone interpretation is also supported by our differential spectra of the lignin in kraft pulps. If the lignin-carbohydrate bond were an ester bond, and separation of lignin from carbohydrate required its hydrolysis, then the differential spectra of the unbleached pulps should show absorption in the neighbourhood of 1,730 cm^{-1}. In fact they do not. Hence it is unlikely that the new group in the lignin is a carboxyl group.

The evidence from whole wood and sulphite pulp is less clear. These differential spectra do show weak absorptions at 1,730–40, but they are probably artefacts due to poor compensation by the holocellulose for the acetyl groups in the hemicelluloses of the wood and the pulp, as explained here.

All published spectra of isolated lignins appear to contain the 1,710 cm⁻¹ absorption band ('appear to' because not all have been printed with adequate clarity for comparison). The band is also present in Freudenberg's dehydrogenation polymers, and Freudenberg and co-workers[9] have pointed out that the strength of the band is influenced by the method of preparation of the polymer.

These observations are consistent with the conclusion that an acetal or hemi-acetal bond exists between the carbonyl groups of lignin and the hydroxyl groups of some portion of the holocellulose. This was first suggested as a hypothesis by Holmberg in 1925[10], and is supported by the results of a number of chemical investigations[11]. The work reported here is the first evidence obtained by purely physical means in such a way that the wood and pulp lignins were observed without having been modified by chemical reactions.

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BIOCHEMISTRY

Chemical Characterization of Kinins of Human Plasma

By the method of ammonium sulphate fractionation, human plasma can be separated into a part *A*, which is insoluble at 33 per cent saturation, and a part *B*, precipitated between 33 and 45 per cent saturation. When these precipitates are dissolved in water, both the solutions spontaneously develop the ability to cause contraction of the isolated rat uterus, apparently due to the liberation of plasma kinins. From the second preparation (precipitate *B*) the well-characterized kinins bradykinin and kallidin have already been isolated[1,2]. We have now undertaken detailed investigations of the neutral '33 per cent precipitate', *A*, with results which may be summarized as follows.

(1) The ammonium sulphate precipitate (*A*) contains a substance, apparently a proteolytic enzyme, which has been isolated (by a method to be described), and which liberates a kinin from 'preactive' human plasma[3]. This kinin can be distinguished from both bradykinin and kallidin and has been provisionally called kinin *E*.

(2) When the ammonium sulphate precipitate (*A*) is allowed to activate spontaneously in neutral solution, two kinins can be obtained which are different from either kinin *E*, bradykinin or kallidin. These have been referred to as kinins *F* and *S*.

These substances have been classed as plasma kinins on the grounds that they can bring about contractions of the isolated rat uterus, cause pain when applied to the exposed blister base, are unstable in plasma and are digested by pancreatic enzymes. All five compounds can be separated either by chromatography or by electrophoresis on paper. For each method the paper has been washed with 2 mM 'Versene' solution and then with water, in order to reduce adsorption of the peptides. Despite this precaution, however, substances *E* and *F* are often 'lost' during the separation, though whether by adsorption on the support or through destruction by the solvent is not yet clear.

Table 1

Solvent	*F*	*E*	*S*	Kall.	Brad.
BuOH/AcOH/H_2O	12	19	23	23	26
BuOH/Pyr./H_2O	Decomposed (?)	16	18	6	10

-The distance moved (cm) after 18 h chromatography of five plasma kinins on Whatman No. 1 paper washed for 24 h in 2 mM 'Versene' followed by water for 48 h. The strips were cut into 1-cm segments and eluted with Ringer's solution. The eluate was then tested for its ability to contract the isolated rat uterus. Crystalline synthetic bradykinin and kallidin were used.

Paper electrophoresis is particularly useful for the separation of bradykinin and kallidin from substance *S*, which migrates the fastest of the three (thus, at a field strength of 100 V/cm in 2 N acetic acid, *S* moved 24 cm; kallidin, 16 cm; bradykinin, 7 cm in 30 min). Substances *E* and *F* can also be resolved by this method; for example, in *p*H 7·4 pyridine/acetic acid buffer, at 100 V/cm, *E* moved 2 cm; kallidin, 4 cm; and *F*, 6 cm in 10 min, but after 30 min substances *E* and *F* could not be detected. In all cases migration was cathodic.

The chromatographic behaviour of all five kinins is summarized in Table 1, from which it appears that substance *S* and kallidin can be resolved in butanol/pyridine/water (1 : 1 : 1), but not in butanol/acetic acid/water (4 : 1 : 5). However, the latter solvent is the better for the identification of compounds *F* and *E*, since it brings about their inactivation at a slower rate.

From these results it appears that human plasma has a potential for the endogenous formation of several kinins. Moreover, since the isolated enzyme of precipitate *A* produces kinin *E* when added to human plasma, it cannot be plasma kallikrein, which forms bradykinin under these conditions[2]. Furthermore, substances *E* and *F* are formed at a rapid rate, whereas substance *S* reaches its maximum concentration only after about 3 h. Thus it is unlikely that substance *S* appears in reactions that produce 'kinin' rapidly; but it may be an important product of systems, such as those of acid or acetone-treated plasma[4-7], which react relatively slowly.

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Blood- and Serum-levels of Water-soluble Vitamins in Man and Animals

VITAMIN-LEVELS in human blood and serum have recently been reported[1]. Except for cyanocobalamin (vitamin B_{12})[2], the titre of vitamins of the B-group in animal bloods and sera has not been documented. Comparative values would be an aid in determining the influence of these vitamins as animal growth factors, and also for evaluating their influence when these fluids are included in microbial and tissue culture growth media. Here we report the vitamin titre of freshly drawn blood and serum of cattle, sheep, chicken, rabbit and horse, the biological fluids of which are commonly used for experimental work. Eight pooled samples from each species were obtained from Cappel Laboratories of West Chester, Penn. These fluids were assayed for: (*a*) thiamine with *Ochromonas malhamensis*[3]; (*b*) pantothenic acid with *Lactobacillus plantarum*[4]; (*c*) nicotinic acid and amide with *Tetrahymena pyriformis*[5]; (*d*) B_6 group (pyridoxine, pyridoxal, pyridoxamine) with *T. pyriformis*[6]; (*e*) unconjugated pteridines with *Crithidia fasciculata*[6]; (*f*) folic acid with *L. casei*[7]; vitamin B_{12} titres were determined with *L. leichmannii*, *Euglena gracilis*, *O. malhamensis* and *Escherichia coli* 113–3[8] for comparison. Table 1 gives the averages of the vitamin titres of blood and serum received at different times during the year to ensure adequate sampling. The standard diets of these animals contained no vitamin supplementation during any of the testing periods involved in this work.

The concentration of folic acid did not vary to any great extent in these six species. The same can be observed for thiamine, with the exception of the rabbit which was the representative rodent. The rabbit carried up to ten times as much of this vitamin than other species. A similar picture was seen in the case of vitamin B_{12} where the rabbit showed 5–100 times more B_{12} than man and the other species tested.

Table 1. COMPARISON OF THE VITAMIN TITRES OF WHOLE BLOOD AND SERUM OF ANIMALS AND MAN

(mμg/ml.)

	Man	Cattle	Sheep	Horse	Rabbit	Chicken
Thiamine						
Blood	30	52	45	25	182	14
Serum	27	22	11	15	132	5
Folic acid						
Blood	43	30	18	35	39	18
Serum	15	15	10	20	24	8
B_6 group						
Blood	52	250	60	70	34	50
Serum	35	210	127	50	57	56
Pteridine						
Blood	48	510	470	800	410	76
Serum	27	120	57	120	445	300
Pantothenic acid						
Blood	1,150	395	420	700	440	715
Serum	925	2,340	2,270	680	450	665
Nicotinic acid						
Blood	7,250	1,100	2,200	1,700	2,200	1,700
Serum	65	250	240	190	210	220
Vitamin B_{12}						
Blood	0·30	0·20	2·6	3·0	19	1·8
Serum	0·40	0·22	3·5	3·5	18	0·9

The values for the B_6 group were substantially elevated in the two ruminants tested, cattle and sheep. The unconjugated pteridines, which were included with the vitamins, were about 20 times higher in the four mammalian species than in man. On the other hand, nicotinic acid was 3–5 times higher in human blood than in any of the other animals tested. The most striking result is the inversion of the relation of the nicotinic acid titre in the serum. Here the sera of the five animal species contain 3–5 times more nicotinic acid than human serum. The nicotinic acid ratio between blood and serum is 100 : 1 in man, but 4 : 1 to 10 : 1 in the animals. Another feature is the high content of pantothenic acid in ruminant serum.

A different picture in the blood : serum ratio appears to characterize the chicken. The bird contains substantially more unconjugated pteridine in the serum than in the blood, in contrast to mammals. It contains only half as much vitamin B_{12} in the serum as in the blood, while in man and the other mammals the B_{12} ratio for blood to serum is about unity.

These unexpected discrepancies may be due to a variety of factors, especially diet. Since man was the only carnivore included in this investigation, these multiple differences existing between man and animals may be based on the diet. Moreover, possible differences existing between man and other carnivores may be due to the large quantity of dairy products and fats in the human diet, which probably accounts for the peculiarity of the human nicotinic acid picture. Secondly, the variation in the vitamin picture may be due to the position of these species in evolutionary development. The chicken and perhaps all birds can be expected to display differences in a parameter regulated by diffusion because of the different nature of their blood corpuscles. It is rather surprising in how many facets their vitamin picture is congruent with that seen in mammals.

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Improved Recoveries of Protein from a Starch–'Pevikon C-870' Gel

A METHOD for the elution of proteins from starch gel has been described[1] involving the setting of starch gel segments into a 'Pevikon C-870' (PVK) block and then, by performing electrophoresis, proteins are recovered from PVK. This technique affords a good recovery of proteins contaminated by a negligible amount of soluble starch; but it is time-consuming if many gel segments have to be handled.

The experiments reported here constitute an alternative procedure: hydrolysed starch (Connaught Laboratories) is thoroughly mixed with PVK (Stockholms Superfosfat Fabriks Aktiebolag) in buffer solution and the suspension is heated until, after being almost solidified, it becomes less viscous near boiling point. The starch–PVK gel appears white and the mechanical strength is slightly inferior to that of starch gel.

Up to 40–70 mg of human serum proteins were electrophoretically separated either with the gel slab (17·0 × 30·0 × 0·65 cm) in vertical position and with the buffers indicated by Smithies[2] for 15 h using a potential gradient of 7 V/cm, or with the gel (30·5 × 21·0 × 1·0 cm) in horizontal position according to Poulik[3] for 9 h with a potential gradient of 8 V/cm. Electrophoresis was performed at room temperature, a running-water cooling system being used only for the gel in horizontal position.

In order to obtain maximum recovery of proteins from the gel, high resolving power and satisfactory mechanical strength of the gel, several experiments were carried out and the concentration selected was: starch (Lot 150) 10·5 g; PVK 126 g; buffer solution 100 ml. The starch–PVK (g): buffer solution (g) ratio is 1·36: if it was increased (> 1·42) the preparation of gel was very difficult and protein mobilities decreased progressively. On the other hand, by lowering the ratio (< 1·30) the gel tended to melt on handling.

Electrophoretic and autoradiographic patterns of human serum as obtained by using either starch or starch–PVK gel are compared in Fig. 1. The starch–PVK gel retains the high resolving power typical of the starch gel although the localization of some proteins is changed.

Protein recoveries were tested as follows: human serum proteins were labelled[4] with iodine-131 and electrophoretically separated. The top half of the gel was stained[5] and, after washing, autoradiography was carried out. Selected segments (2·0 × 1·0 × 0·4–0·5 cm) of the bottom half of the gel were put into glass tubes, 0·15 M sodium chloride solution being added up to a volume of 3 ml. The tubes were frozen at −40° C three times for 8 h each time, and afterwards were counted for total radioactivity. Elution of

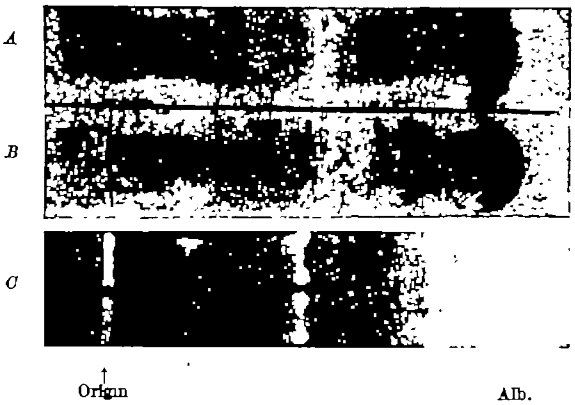

Fig. 1. Stained starch–PVK gel (*A*) and starch gel (*B*) electrophoretic patterns of human serum. Starch–PVK gel autoradiographs (*C*) of human ^{131}I-serum after being separated by electrophoresis (vertical)

proteins was accomplished by squashing the gel in a metal-glass syringe, a filter paper disk preventing the gel mass escaping. After centrifugation (1,500*g*, 30 min) the clear supernatant was brought up to 3 ml. and counted in order to determine the percentage of recovered radioactivity. This procedure was preliminarily tested and only 2–4 per cent of original radioactivity was lost in handling.

Table 1 reports percentages of eluted radioactivity and of iodine-131 trichloroacetic acid (TCA, final concentration 10 per cent) soluble radioactivity estimated on the eluates. Results were easily reproducible.

Table 1. Percentages* of recovered radioactivity from starch (10·5 g per cent) gel and from starch–PVK (10·5–126·0 g per cent) gel after electrophoretic separation of human serum ^{131}I-proteins by using Poulik's modification of Smithies's method. Percentages of ^{131}I-TCA soluble radioactivity were estimated on the eluates

	Albumin	Post-albumins	Trans-ferrin	Hapto-globin	γ-Globulin
Starch-gel	90	51	51	30	27
^{131}I-TCA soluble radioactivity	2·8	4·8	6·6	5·8	8·2
Starch–PVK gel	95	75	76	67	66
^{131}I-TCA soluble radioactivity	2·3	3·7	5·4	4·1	6·7
Increase in recovery	5	24	25	37	39

* Each value is an average of at least two electrophoretic runs.

The slightly higher recovery of albumin from the starch–PVK gel was also confirmed by photometric measurement of the eluates containing bromophenol blue bound to the albumin. The relatively high values of ^{131}I-TCA soluble radioactivity are mainly due to the freezing of proteins, as the values of the original ^{131}I-sera were about 1 per cent. The eluted radioactive fractions, after being concentrated by pressure dialysis, were again electrophoretically separated with some carrier serum and the radioactive peaks were located in their original positions by counting small pieces of stained gel.

Samples of starch gel and of starch–PVK gel were cut with a freezing microtome, and slices of about 20μ were examined by microscopy. With the concentration of starch–PVK gel used here, the different-sized and irregular PVK granules appeared closely interposed in the starch chain net (Fig. 2).

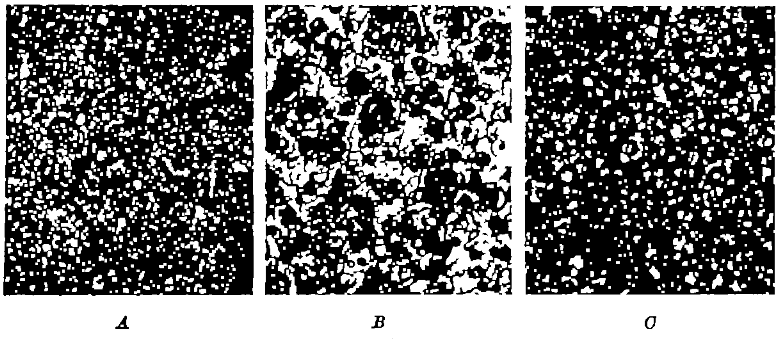

Fig. 2. Photographs of: *A*, starch-gel section; *B*, starch (10·5 g)–PVK (21·0 g) gel section; *C*, starch (10·5 g)–PVK (126·0 g) gel section (× c. 36)

It is known that proteins are easily eluted from PVK[6,7] and that increase in protein recoveries depends on the PVK concentration (Table 2); consequently, the higher protein recoveries from starch-PVK gel are due to the PVK itself as well as to the gel broken down during freezing.

Table 2. Percentages of recovered radioactivity after electrophoretic separation of human ^{131}I-serum by starch gel and starch–PVK gels

Starch (g)–PVK (g) per 100 ml. buffer solution		Haptoglobin	Transferrin	γ-Globulin
10·5	—	29	51	27
10·5	15·7	31	62	36
10·5	31·5	42	70	45
10·5	42·0	39	74	47
10·5	52·5	54	70	50
10·5	68·3	61	73	62
10·5	84·0	62	74	61
10·5	99·8	66	73	63
10·5	115·5	67	77	—
10·5	126·0	67	75	66
10·5	141·8	68	77	67

The amount of soluble starch, as investigated by addition of iodine to the eluates, was slightly increased in the starch–PVK samples.

It is concluded that starch–PVK gel can be used for separating and recovering proteins when presence of soluble starch does not interfere with their estimation.

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Reduction of Glyceric Acid to Glyceraldehyde by Lithium Aluminium Hydride

CARBOHYDRATE acids generally can be converted into the corresponding aldoses by amalgam reduction of a lactone. In cases where lactonization does not really occur or where lactones are unstable, few alternative procedures are available for bringing about this conversion.

We now find that a possible route exists for this purpose by conversion of a suitably protected (for example, isopropylidene) aldonic ester into the *N*-methyl-*N*-phenylamide[1], followed by its reduction with an ethereal solution of lithium aluminium hydride[2]. The reactions are conducted at room temperature, predominantly in non-aqueous media.

Using methyl isopropylidene-DL-glycerate, the reduction was accomplished in the following way. A Grignard reagent was formed from equimolar quantities of methyl magnesium iodide and *N*-methylaniline and to this the isopropylidene ester (0·01 mole) was added under nitrogen at 0°. After 30 min at room temperature the mixture was washed successively with dilute sulphuric acid and with water at −10°. Evaporation of the solvent from the dried separated organic layer gave *N*-phenyl-*N*-methyl isopropylideneglyceramide (b.p. 87°–89°, 0·008 mm, n_D^{18} 1·5230; found, N, 5·8 per cent; $C_{13}H_{17}NO_3$ requires N, 5·95 per cent). The substituted amide was treated with lithium aluminium hydride (0·05 mole) in anhydrous ether for 18 h at room temperature. After addition of ethyl acetate, the solution was washed with dilute sulphuric acid (15 ml. 5 N). The aqueous layer was neutralized by passage through a column of 'Amberlite *IR* 120 (H$^+$)'

and then by addition of $BaCO_3$. Evaporation of the solvent gave DL-glyceraldehyde (30–40 per cent yield), characterized chromatographically and by preparation of the 2,4-dinitrophenylhydrazone (m.p. 157° alone or mixed with an authentic sample, the infraspectrum of which was identical).

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Urinary Amino-acids on Phenylalanine–Tyrosine-supplemented Diets

It has been previously demonstrated[1] that feeding rats diets high in DL-phenylalanine and L-tyrosine caused the animals to excrete phenylpyruvic acid in their urine. Along with the excretion of phenylpyruvic acid, psychological tests on the animals revealed a disturbance in the temporal discriminate learning pattern. These experimental changes have been compared with those accompanying phenylketonuria.

The foregoing results have been corroborated[2] and have also been extended[3] by feeding excessive quantities of the L-isomer of phenylalanine. However, the influence of these diets on the renal threshold of other amino-acids has not been studied. It is possible that this could be important in assessing any toxic effects of such diets. The following experiments were therefore designed to evaluate this effect.

Eight experimental and four control rats (3 weeks old) were placed on commercial rat chow (Rockland). The diet of the experimental group was supplemented with 3·75 per cent DL-phenylalanine and 3·75 per cent L-tyrosine. All animals were housed individually in metabolic cages, and 24-h urines were collected over a six-week period. Gains in weight in all animals were satisfactory.

Although it has been reported[4] that diets containing 5 per cent DL-phenylalanine or 5 per cent L-tyrosine have a growth-depressing effect when fed in low protein diets to weanling rats, significant depression of growth was not observed in this study at the 3·75 per cent level. Presumably the alteration in the balance of the free amino-acids in plasma was not sufficient to exert deleterious effects.

Aliquots of urine were analysed weekly for phenylpyruvic acid[5], and at the end of the sixth week pooled aliquots of the control and test urines were prepared[6] for ion-exchange chromatography by removing ammonia *in vacuo* and adjusting the *p*H to 2·2. At the completion of this experiment, it was repeated with a second group of animals.

In agreement with previous findings, phenylpyruvic acid (PPA) excretion was markedly elevated on the experimental diet. The excretion of PPA was observed within 12 h after placing the animals on the diet; however, no attempts were made to assess the behavioural consequences of such a regimen.

The individual urinary free amino-acid excretion of the control and 'phenylketonuric' rats is shown in Table 1. Increases in threonine, serine, glutamine, cystine, cystathionine, lysine and 1-methyl histidine were noted while decreases were observed in aspartic and glutamic acid, glycine, alanine. Phenylalanine and tyrosine concentrations were not measured because of the possibility of contamination from the diet. The total excretion in mg/24 h was 15·09 for controls and 15·66 for the experimental animals (phenylalanine and tyrosine not included). The results of the duplicate experiment were consistent with the findings already reported.

Table 1. Free Amino-acid Excretion

Amino-acids	Control (mg/24 h)	Exp. (mg/24 h)
Taurine	8·20	8·60
Aspartic acid	0·31	0·16
Threonine	0·20	0·68
Serine	0·22	0·38
Glutamine	0·32	0·98
Glutamic acid	0·72	0·50
Glycine	2·60	0·99
Alanine	0·96	0·55
Valine	0·10	0·10
Half-cystine	0·16	0·66
Cystathionine	0·10	0·20
Methionine	0·16	0·32
Isoleucine	0·19	0·07
Leucine	0·09	0·09
Tyrosine	0·19	—
Phenylalanine	0·18	—
β-Alanine	0·09	0·06
Lysine	0·31	0·66
3-Methyl histidine	0·10	0·20
1-Methyl histidine	0·14	0·23
Proline	0·12	0·18

An explanation of the differences between the control and experimental groups depends on a better knowledge of the factors influencing the excretory pattern in the rat. Although no particular correlation between the structure of the amino-acid and its excretion pattern was evident from the data, it was apparent that the supplemented diets did exert an influence on the excretion of the amino-acids. Similar experiments[7] in which galactosaemia was produced in the rat did not result in any explanation of the aminoaciduria in this condition.

Although the excretion patterns in the rat and in man are considerably different, attempts were made to correlate, at least qualitatively, the amino-acid pattern with that previously reported in humans[8]. Glycine and alanine excretion, which was decreased in the experimental animals, was also decreased in the phenylketonuric subject. On the other hand, many of the amino-acids which were excreted in excessive amounts by the animals were excreted in small amounts by the human. However, it is not possible to determine the significance of this phenomenon until additional subjects have been evaluated.

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Formation of Tyrosine-α-ketoglutarate Transaminase Activity *in vitro*

In the course of experiments designed to clarify the role of hydrocortisone and tyrosine in the stimulation of tyrosine-α-ketoglutarate transaminase activity, we observed small apparent increases of enzymatic activity in an *in vitro* system. This system is essentially that of Lingrel and Webster[1], with some modifications. No attempt was made in these experiments to distinguish between synthesis of apoenzyme and release of active enzyme, in contrast to the studies of Pitot and Cho[2] on the release of tryptophan pyrrolase activity.

The results are shown in Table 1; they are expressed as the difference in the number of μmoles of *p*-hydroxyphenylpyruvate produced in a 20-min incubation period per ml. of 600*g* supernatant between the beginning and the end of a 10-min pre-incubation period. The complete system is as follows: 6 ml. of a 600*g* supernatant from a rat liver homogenate prepared according to the method of Zamecnik and Keller[3]; 2·3 ml. of 0·1 M *tris*, *p*H 7·8; 1·2 ml. of 0·1 M *tris*, *p*H 7·8, containing 0·1 M potassium chloride; 10 μmoles each of ATP and DPN, 90 μmoles of α-ketoglutaric acid[4], 10 μmoles of crystalline manganese chloride, 1 mmole of glycine, all added as solid; 0·1 ml. of 0·1 M magnesium chloride; and water to 10 ml. Hydrocortisone acetate, 1 mg, and tyrosine, 6·5 mg, were also added in solid form. The system was shaken in a gyratory water-bath shaker at 37° C for 10 min; 1·0 ml. aliquots were removed for incubation at the beginning and at the end of the pre-incubation period. These aliquots were added to a system containing 8·0 ml. of 0·2 M phosphate buffer, *p*H 7·2, 0·5 ml. of a 300 μg/ml. solution of pyridoxal phosphate, 0·5 ml. of 0·3 M α-ketoglutarate, and 1·0 ml. of 0·06 M sodium diethyldithiocarbamate. This system was incubated at 37° C in the water-bath shaker; the reaction was started by the addition of 5·0 ml. of 0·012 M tyrosine made up in 0·2 M phosphate buffer, *p*H 7·2, and stopped at the end of 20 min by the addition of 0·5 ml. of 100 per cent trichloroacetic acid. The TCA precipitates were centrifuged and 2·0 ml. of the clear supernatants were taken for assay of *p*-hydroxyphenylpyruvic acid by the Briggs method[5], as modified by Canellakis and Cohen[6], except that 2·0 ml. of water and 4·0 ml. of the 'mixed reagent' were added to the 2·0 ml. of TCA supernatant.

All the experiments recorded here were performed with preparations derived from the livers of adrenalectomized male Wistar rats (Charles-River Breeding Laboratories, Brookline, Mass.). As can be seen from Table 1, the addition of hydrocortisone and tyrosine had no effect on the apparent increase. In this connexion, it should be noted here that Goldstein *et al.* have been able to stimulate both tyrosine-α-ketoglutarate transaminase activity and tryptophan pyrrolase activity in isolated perfused rat liver by means of hydrocortisone; this stimulation was prevented by puromycin[7]. There also appears to be a partial requirement for DPN in our *in vitro* system. In none of the experiments was there a significant decrease in the 10-min value as compared with the zero-time value. The increases were proportional to the amount of 600*g* supernatant in the pre-incubation system, and there was no increase when the supernatant was incubated with tyrosine and α-ketoglutarate alone, thus ruling out the possibility that the increases reflected simply endogenous formation of *p*-hydroxyphenylpyruvic acid. It also appears that the rate of increase observed here is roughly the same as that observed for the *in vivo* stimulation, at least in the early stages.

Table 1. APPARENT INCREASE OF TYROSINE-α-KETOGLUTARATE TRANSAMINASE ACTIVITY *in vitro*

System	No. of experiments	Δμmoles *p*-hydroxyphenylpyruvate/10 min pre-inc.
Complete	12	0·325 ± 0·118*
Complete + hydrocortisone and tyrosine	7	0 297 ± 0·119
Complete − DPN	2	0·164 ± 0·108
Control†	4	0 046 ± 0 101

* Average maximum error (see text for explanation).
† 6 ml. supernatant + 3·5 ml. *tris* buffer + 1·5 ml. water.

One of the major problems encountered in conducting these experiments was the difficulty in obtaining completely reproducible results; about one experiment in every four or five failed to show the expected result. Largely as a result of this, DPN was the only component of the system the necessity for which was explicitly tested; however, there appears to be a dependence on ATP also[8]. GTP (10 μmoles) and pyridoxal phosphate (120 μg) in some of the later experiments were added, although some experiments were successful without these additions.

The error indicated in Table 1 is not the standard error of the mean; rather, it is an estimate of the magnitude of the total error arising from the individual pipetting steps of incubation and assay. The magnitude of this error is about 2 per cent of the total activity for each time value. Since the zero-time activity was not a zero value, the overall error is about 4 per cent of the total activity. The increases are in the neighbourhood of 10 per cent of the total activity; thus the error is a rather large fraction of the increases. This error is about 1·5 times the standard error of the mean; strictly speaking, therefore, this represents an upper limit to the magnitude of the error.

These experiments do not rule out the possibility that the observed increases are due to an activator, analogous to the iron-porphyrin compounds in the case of tryptophan pyrrolase[9,10]. However, to our knowledge, there has been no evidence presented which indicates the existence of an activator. The available evidence, albeit of an indirect nature, points rather to protein synthesis more or less directly[7,11,12].

The small size of the increases and the difficulties with reproducibility suggest the existence of some unknown limiting factor or factors. RNA and a mixture of pentose nucleotides have been shown to have an effect *in vivo* which appears to be more specific than simply a stress mechanism[13,14]; perhaps it is possible that something like 'messenger RNA' breaks down rapidly in this system. Further work will be undertaken to clarify these various points.

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Microsomal L-Gulonolactone Dehydrogenase

IT has been demonstrated previously by several workers[1–4] that rat liver microsomes readily convert L-gulonolactone into L-ascorbic acid in the presence of oxygen. Recently, it has been found that the microsomes from the livers of goat and the rat can also convert L-gulonolactone into L-ascorbic acid under anaerobic conditions in the presence of a suitable electron acceptor. Various dyes were tested for their ability to act as electron acceptor with this enzyme; these included methylene blue, phenazine methosulphate, neotetrazolium chloride, 2,6-dichlorophenol indophenol and cytochrome *c*. Of these only phenazine methosulphate and 2,6-dichlorophenol indophenol were reduced by the enzyme in the presence of a substrate; the ability of 2,6-dichlorophenol indophenol as electron acceptor was about one-fifth compared with that of phenazine methosulphate (Table 1).

Table 1. SYNTHESIS OF L-ASCORBIC ACID UNDER ANAEROBIC CONDITIONS IN THE PRESENCE OF A SUITABLE ELECTRON ACCEPTOR

Electron acceptor added	Concentration used	Ascorbic acid formed (µmole) by: Rat liver microsomes D-Glucuronolactone	Rat liver microsomes L-Gulonolactone	Goat liver microsomes D-Glucuronolactone	Goat liver microsomes L-Gulonolactone
Methylene blue	2×10^{-4} M	0	0	0	0
Phenazine methosulphate	0·5 mg	0·31	0·60	0·44	0·82
Neotetrazolium chloride	1 mg	0	0	0	0
2 : 6-Dichlorophenol indophenol	2×10^{-4} M	0·05	0·12	0·08	0·17
Cytochrome *c*	1 mg	0	0	0	0

The test system contains: 20 mM sodium phosphate buffer, *p*H 7·4, 10 mM D-glucuronolactone, 0·25 ml. of microsomal dispersion (250 mg equivalent of wet liver), 50 mM KCN, phenazine methosulphate, 0·5 mg (kept in the side-arm of the Thunberg tube) in a total volume of 2·5 ml.; incubated for 20 min at 37° under anaerobic conditions. Ascorbic acid was estimated by Roe and Kuether's method (ref. 6).

When L-gulonolactone (5 mM) was used as substrate, the concentration of cyanide was 1 mM.

D-Glucuronolactone has also been found to be converted into L-ascorbic acid (as estimated by Roe and Kuether's[6] method after the removal of cyanide by acidification with hydrochloric acid and evaporating off the hydrocyanic acid) by goat liver microsomes under anaerobic conditions in the presence of phenazine methosulphate and 0·05 M potassium cyanide.

In a recent communication Bublitz[7] reported that 2,6-dichlorophenol indophenol dye was reduced by rat liver microsomes in the presence of phenazine methosulphate and L-gulonolactone under aerobic conditions and he termed this enzyme as L-gulonolactone dehydrogenase. According to him this L-gulonolactone dehydrogenase could only be solubilized with rattlesnake venom but not by bile salts and any other agent. Contrary to the observations of Bublitz[7], it has been found in this laboratory that L-gulonolactone dehydrogenase from rat or goat liver microsomes could be effectively solubilized with sodium deoxycholate according to the method of Chatterjee *et al.*[4] and the enzyme could be further concentrated by the addition of ammonium sulphate to a concentration of 30 per cent saturation.

The maximum formation of L-ascorbic acid takes place after an incubation period of 15 min and the optimum concentration of phenazine methosulphate has been found to be 0·5 mg; $\alpha\alpha'$-dipyridyl, ethylenediamine tetraacetic acid and 8-OH quinoline (10^{-3} M each) have no effect on the enzymatic activity. L-Gulonolactone dehydrogenase component has been found to be more stable than the oxidase. It has been found to retain its full activity even at *p*H 5·4. The oxidase activity is almost completely lost by keeping the soluble enzyme preparation at 45° in a water bath for 5 min, but this heat-treated enzyme preparation has been found to possess both L-gulonolactone dehydrogenase and D-glucurono reductase activities.

L-Gulonolactone dehydrogenase has been obtained free of any L-gulono oxidase activity by electrophoretic separation of the soluble enzyme at 0° on paper (Whatman 3 *MM*) in 0·1 M sodium phosphate buffer, *p*H 7·4, at a potential gradient of 6 V/cm for 3 h, the length of the run being 5·5 cm. The protein band (5·0–5·5 cm from the line of application) was eluted with 0·05 M sodium phosphate buffer, *p*H 7·4, containing 0·1 per cent sodium deoxycholate in a period of 30 min at 5°. Though there is not much increase in the specific activity the foregoing preparation has been found to convert both D-glucuronolactone and L-gulonolactone into L-ascorbic acid in the presence of phenazine methosulphate but not in the presence of air. A dehydrogenase preparation free of any oxidase activity has also been obtained by negative adsorption of the soluble enzyme on diethyl amino ethyl cellulose (DEAE-cellulose) column equilibrated with 0·01 M sodium phosphate buffer, *p*H 7·4. This has already been reported by Bublitz[7]. The enzyme preparation thus obtained can convert both D-glucuronolactone and L-gulonolactone into L-ascorbic acid in the presence of phenazine methosulphate but not in air, D-glucuronolactone requiring in addition cyanide in the system. This enzymic activity has been found to be inhibited by *p*-chloromercuribenzoate, the inhibition being reversed by GSH, showing the requirement of essential sulphydryl groups for its activity.

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Fungitoxicity of 2,4-Dichloro-3,5-dinitrobenzoates

MANY aromatic compounds containing halogen substituents activated by the presence of electronegative groups, particularly nitro groups, in *ortho* and *para* positions are fungitoxic. For example, 2,4-dinitrofluorobenzene and its 5-halogenated derivatives protect cotton fabric from attack by *Myrothecium verrucaria*[1], 1,5-dichloro-2,4-dinitrobenzene is a soil fungicide[2] and 1-chloro-2,4-dinitronaphthalene controls a number of plant pathogenic fungi[3]. The influence of the introduction of a carboxy or carboalkoxy substituent into compounds of this type has not received much attention although the fungicidal activity of certain chlorodinitrobenzoates is reported in recent patent literature[4].

Table 1. 2,4-DICHLORO-3,5-DINITROBENZOATES

Ester	m.p. (° C)	Formula	Analysis Found (%) C	Analysis Found (%) H	Analysis Required (%) C	Analysis Required (%) H
Methyl	84	$C_8H_4Cl_2N_2O_6$	32·9	1·1	32·5	1·4
Ethyl	101	$C_9H_6Cl_2N_2O_6$	35·2	1·6	35·0	1·9
Isopropyl	117	$C_{10}H_8Cl_2N_2O_6$	37 3	2·5	37·15	2 5

We have examined simple aliphatic esters of 2,4-dichloro-3,5-dinitrobenzoic acid[5] (Table 1) as protectant foliage sprays in the greenhouse for the control of *Alternaria solani* on tomatoes, *Erysiphe graminis* on oats and *Peronospora tabacina* on tobacco. A standard greenhouse test[6] was used for assessing control of *A. solani* and basically similar techniques were adopted with *E. graminis* and *P. tabacina*. The benzoates (prepared by Fischer esterification of the parent acid) were fungitoxic (Table 2). The methyl ester in particular gave good control of the three pathogens and in a more detailed examination against *A. solani* it had an ED_{95} value of 0·01. Tetramethylthiuram disulphide in the same experiment had an

Table 2. FUNGITOXICITY OF 2,4-DICHLORO-3,5-DINITROBENZOATES

Ester	Concentration (p.p.m.)	Disease (% of unsprayed control) *A. solani*	Disease (% of unsprayed control) *E. graminis*	Disease (% of unsprayed control) *P. tabacina*
Methyl	500	1	0	0
	100	1	0	10
Ethyl	500	0	0	60
	100	4	1	80
Isopropyl	500	25	0	20
	100	75	0	40
Free acid	500	100	90	100

ED_{50} of 0·08. The free acid was much less active, presumably because the presence of the polar carboxy group reduced the ability of the molecule to penetrate the fungal cells.

We detected no phytotoxicity with these compounds at 500 p.p.m., the highest concentration tested, nor did we observe symptoms of plant growth effects known to be associated with certain halogenated nitrobenzoic acids and their esters[7,8].

The reactivity of the halogen groups is almost certainly responsible for the fungitoxicity of the 2,4-dichloro-3,5-dinitrobenzoates. In this respect their mode of action probably resembles the pathways of fungitoxicity suggested for chloranil, dichlone[9–11] and 2,4-dinitrofluorobenzene[12], where labile halogen atoms are considered to react with essential amino or mercapto groups in the micro-organisms. This view is supported by the knowledge that the elimination of the chlorine atom of 2,4-dinitrochlorobenzene by amines[13] or mercaptans[14] occurs in mild conditions. Likewise, both the chlorine atoms of methyl 2,4-dichloro-3,5-dinitrobenzoate were readily replaced, for example, by methylamino and phenylthio groups to give methyl 2,4-di(methylamino)-3,5-dinitrobenzoate (m.p. 208°. Found: N, 19·7. $C_{10}H_{12}N_4O_6$ requires N, 19·7 per cent) and methyl 2,4-di(phenylthio)-3,5-dinitrobenzoate (m.p. 104°. Found: C, 54·4; H, 3·2. $C_{20}H_{14}N_2O_6S_2$ requires C, 54·3; H, 3·2 per cent) respectively. Neither reaction product was fungitoxic at 500 p.p.m.

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PHYSIOLOGY

Antihistaminic Action of Lysozyme

It was previously shown that heparin and mucin bind histamine[1,2]. For this reason we worked with an enzyme acting on mucopolysaccharides, and looked for some modifications of histamine action. Lysozyme inhibits the action of histamine on the isolated guinea pig's ileum. The threshold is about 10^{-8} (w/v). No inhibitor action was observed towards acetylcholine, serotonin and barium.

When injected intravenously into a guinea pig the lysozyme counteracts the bronchospasm produced by intravenously injected histamine. The method used is very much like that of Konzett and Rossler[3]. The threshold is about 10 µg per animal. No inhibitory action was observed towards the bronchospasm elicited by acetylcholine and serotonin (Fig. 1).

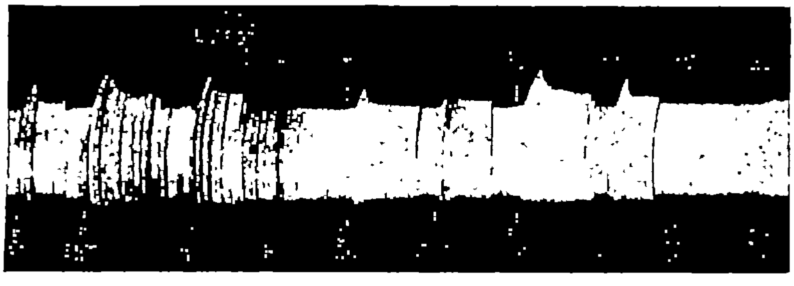

Fig. 1. Effect of lysozyme on the bronchospasm produced in a male guinea pig (500 g) by histamine dichloride (*H*), acetylcholine chloride (*AC*) and serotonin creatinine sulphate (5HT). Doses in µg. Time in min from lysozyme injection

Lysozyme prevented the shock produced in guinea pigs by the intravenous injection of histamine. In our experiments, 5 mg of lysozyme were injected intravenously and then histamine was given by the same route 10 min later. In such conditions, the LD_{50} becomes 300 times greater.

These investigations[4] suggest that lysozyme is a natural antihistaminic.

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Lubrication in Synovial Joints

A CRITICAL review of the work of Barnett, Davies, Charnley and MacConaill[1–4] indicates that there exists a tendency to explain the lubricating conditions in synovial joints by either the lubricating properties of synovial fluid, or by the lubricating properties of the articular surfaces. The proponents of these hypotheses applied the theories of hydrodynamic lubrication or boundary lubrication, respectively. Neither of these theories can explain, however, the lubrication in joints; both are much too crude for this purpose. The nearest approach to reality could be given, perhaps, by the recent elasto-hydrodynamic theories[5,6], but even in these, when applied to synovial joints, a discrepancy exists between the factual evidence and the theoretical results. In fact, a discrepancy between the experimental and the calculated values of rates of shear, or between the calculated and the experimental values of viscosity of synovial fluid, is of an order of 100,000. That the synovial fluid is indeed required for proper lubrication and functioning of joints may be judged from the effect of a fall in temperature on the increased resistance of joints to movement, and also on a decrease in the exertible external force[7]. Both phenomena would be due to an increase in the viscosity of synovial fluid and, to some degree, also due to the changes in the elasticity of the cartilage. A decrease of the viscosity of synovial fluid and a change of the rheological characteristics of this fluid from the thixotropic type into a Newtonian type was observed in cases of rheumatoid arthritis[8–11]; in such cases the joint temperature was higher than anticipated from relative clinical activity[8].

A normal synovial fluid exhibits non-Newtonian, thixotropic, properties. Its viscosity is low at high rates of shear, and it is high (reversibly) at low rates of shear. As the product of viscosity and of the rate of movement is nearly constant, synovial fluid is capable of carrying a constant load; the load-bearing properties of synovial fluid are independent of the rate of movement. The synovial fluid shows also some degree of elasticity[12]. On repeated shearing the consistency of fluid may increase[13,14]. The elastic properties of synovial fluid have two important consequences. First, the fluid cannot be squeezed out from the space between the articular surfaces: secondly, a sudden impact causes a development of transient and instantaneous dilatant properties which help to cushion the shock of impact on the articular surfaces. A simultaneous elastic deformation of the cartilage decreases the pressure by increasing the contact area and by permitting, most likely, an existence of a fluid film of nearly constant thickness. The latter is promoted also by the synovial fat pads, which, as suggested by Barnett *et al.*[2,15], reduce the thickness of film while not taking part in the actual load-bearing mechanism.

Due to the elastic deformation (in the plane normal to the plane of the articular surfaces) of cartilage, the length of the contact area between surfaces is much increased. This permits the film thickness to be independent of load. Perhaps the term 'contact' is not exactly a correct one, as a film of synovial fluid will be interposed between these surfaces. The degree of deformation in the cartilage will depend on the elasticity and the thickness of the cartilage, and on the load.

The elastic deformation of cartilage may exist also in the plane parallel to the plane of articular surfaces. The lateral-transverse movements of these surfaces decrease the actual velocity gradient existing in the film of synovial fluid.

The velocity gradient is decreased also due to the fact that only reciprocating movements may exist in synovial joints. The thixotropic break-down of synovial fluid will correspond to some equivalent equilibrium level of rate of shear, this level being much lower than the nominal rate of shear[16].

It can be stated that so long as the synovial fluid is normal (that is, it is thixotropic and elastic) and so long as the articular cartilage remains elastic, the articulate surfaces are never in an immediate contact and, consequently, the boundary lubrication does not take place. The elasto-hydrodynamic lubrication involving lateral-transverse movements of the cartilage is paramount.

If, however, the pathological changes in synovial fluid will deprive it of its content of hyaluronic acid and/or of the hyaluronic acid–protein complex, the resulting decrease in viscosity and a change in rheological type of synovial fluid will impair lubrication. A subsequent damage to the articular surfaces, due to high friction, will consist of lesions, fibrositis and adhesions. In the absence of a thixotropic and elastic (and wetting) fluid, only a boundary-type of lubrication may exist.

If some pathological changes occur, in the first place, in the articular cartilage, while the synovial fluid is still normal, a decrease in the degree of smoothness, elasticity and resiliency of the articular surfaces will affect the synovial fluid. A decrease in the normal (vertical) and lateral deformations of cartilage will lead to large increases in local pressures and to an increase in the velocity gradient across the gap between the opposing surfaces. Synovial fluid will be forced to perform under much higher rates of shear than under usual conditions. Both phenomena, that is, the decrease in contact area and the increase in the rate of shear, will lead to a degradation of hyaluronic acid–protein complex, in a manner analogous to mechanical degradation of polymers[17,18]. A degradation of synovial fluid (that is, a decrease in its molecular weight) will impair its lubricating properties, will prevent its action as a shock-absorber, and, finally, it will permit even this fluid to be squeezed out from the space between the surfaces.

A decrease in the lubricating action of synovial fluid will lead to tear and wear of cartilage[1], and to an increase of the intra-articular temperature[8], which, in their turn, will cause a further deterioration of joint structure.

The recent controversy between Charnley, on one hand, and Barnett, Davies and MacConaill, on the other, indicates the complexity of problems related to joint lubrication. The controversy, as indeed pointed out by the latter, is more of an argument about words than about things. However, some aspects could be appraised.

Charnley is right in saying that hydrodynamic lubrication is not suited to a reciprocal motion and that it is not achieved under large loads and small movements; but the lubrication theory mentioned is based on the classical hydrodynamic theory and not on the modern modifications of the same. Charnley states that as the coefficient of friction is constant for all speeds of movement, the lubrication must be of the boundary type. This does not necessarily follow; because, as the product of the rate of movement and of the viscosity of synovial fluid is nearly constant, friction will depend only on the load, even if the classical hydrodynamic theory of lubrication were applied. Barnett *et al.* state, on the other hand, that one should not deduce from the fact that the boundary lubrication implies affinity between the lubricant and the surfaces that this affinity implies boundary lubrication. Barnett, Davies and MacConaill are correct in stressing that the essential characteristic of joint lubrication is the existence of a fluid film. They neglect, however, the importance of the resiliency of articular cartilage, which point is well appreciated by Charnley. Neither Charnley nor Barnett *et al.* noted the existence or the significance of the lateral elastic deformations of articular surfaces.

It should be evident that both the synovial fluid and the articular cartilage play an important part in the joint lubrication. An application of the obsolete forms of hydrodynamic and boundary theories to the foregoing problem may be thus rather futile.

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Effect of Tetanus on the Parental Behaviour in the Rat

PREVIOUS experiments on the placental barrier to tetanus toxin in the rat revealed that mothers suffering from tetanus were able to deliver a healthy litter[1]. While some of these mothers cared for their new-born, others neglected them. The present investigation was therefore undertaken to determine to what extent tetanus might affect parental behaviour, and the effect of this behaviour on the offspring.

The œstrous cycle and the date of copulation were determined for 40 albino rats of the Wistar strain. At various intervals of time, 3–5 minimum lethal doses of tetanus toxin (supplied by courtesy of Lederle Laboratories, Pearl River, New York) (0·1 ml./100 g of body-weight) were injected into the gastrocnemius muscle or the femoral vein of each pregnant female. Injected animals were kept in breeding cages under standard conditions adapted for our animal quarters. The criteria

Table 1. THE EFFECT OF TETANUS ON PARENTAL BEHAVIOUR IN THE RAT

3–5 minimum lethal doses of tetanus toxin were injected intravenously or intramuscularly into each pregnant rat. Criteria of the parental behaviour were: nest building, cleaning the new-born, retrieval, and nursing of the young

Group No.	No. of animals	Time of injection	Maternal symptoms during delivery	Parental behaviour	Survival period of the mother	No. of surviving young relative to the total
1	14	79–144 h prior to parturition	Generalized tetanus	Poor to absent	Av. 149·3 h (130–164 h)	2/129
2	10	52–72 h prior to parturition	Advanced tetanus	Limited	Av. 145·5 h (139–158 h)	48/103
3	6	24–48 h prior to parturition	Beginning tetanus	Good	Av. 120 h (96–145 h)	49/55
4	8	0–144 h after parturition	None	Good	Av. 72·6 h (65–87 h)	70/83

of parental behaviour were determined by: nest-building, licking of vulva, cleaning of the new-born, eating of the afterbirth, retrieving, nursing and suckling[2].

In Table 1 groups of animals are tabulated according to the time of toxin injection and the results obtained. The greatest percentage of death among the new-born (98 per cent) occurred in Group 1. These animals exhibited symptoms of tetanus hypersensitivity and excitability during preparturitional nesting periods. As tetanus progressed to generalized spasticity of the body, the animals became too seriously handicapped to lick the vulva and to assist the foetuses during delivery. Parturition was uneventful, with the exception of four cases of prolonged periods of labour and two deaths which occurred during delivery as a result of tetanus. At birth, most of the offspring were alive and appeared healthy. However, due to maternal neglect, the majority died while still wrapped in the foetal membranes attached to the placenta. In some instances the mother cleaned the young but did not eat the placenta. These conditions prevented effective nursing of the young. In group 2, milder symptoms of tetanus were present at the time of parturition. The majority of these mothers were able to lick the vulva, clean the young, and eat the afterbirth. Symptoms of generalized tetanus, however, developed soon after delivery and interfered with the retrieving, nesting, and nursing activities. The young were found scattered in the cage, cold and starving. Only 47 per cent of the new-born survived the mother.

In groups 3 and 4 the percentage of new-born survivals increased to 89 and 84 per cent respectively. Initial symptoms of tetanus were present at the time of delivery in group 3 only, but in both these groups the symptoms of tetanus progressed to death. Here also, general tetanus prevented adequate maternal care.

New-born rats, taken from each group and transferred to a foster mother, survived. Although histological examination of the mammary glands indicated glandular activity in all four groups of the tetanus-injected animals, this does not imply that milk ejection was not affected in some cases. Milk ejection is known to be inhibited by emotional stress, pain, disturbances of the autonomic nervous system, and electrical stimulation or lesions of the hypothalamus[3]. The stress of tetanus experienced by the mother during parturition might have inhibited milk ejection in our experiments. As a note of interest, pregnant rats injected with tetanus toxin prior to parturition had a prolonged survival period as compared with postpartum injected rats.

Lehrman[2], in his detailed discussion of parental behaviour in birds and infra-human mammals, lists many external and internal conditions which influence the parent–young relationship. Mechanical prevention of the licking phenomenon, trauma to the nervous system, hormonal changes, and various external stimuli could perhaps reproduce most of the behavioural deviations observed in our experiment. Tetanus toxin acts on the central nervous system by suppressing synaptic inhibition[4]. It also effects higher nervous activity and disturbs the conditioned behaviour of the rat[5]. The convulsive state, locomotor and autonomic disturbances present in tetanus may contribute to the effect of tetanus on maternal behaviour and lactation in the rat.

Although the present investigation of parental behaviour in tetanus was of general nature, it adequately demonstrates that a neurotropic bacterial toxin can influence considerably the parental behaviour of the rat and thus effect the survival of the new-born.

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Sensory Deprivation and Augmentation in the Examination of Enzyme Adaptation

THE formation of new enzymes in bacteria was first described by Wortman[1] in 1882. Since then the subject has been extensively examined, and is now well recognized. For example, certain strains of *Esch. coli* do not possess the enzyme β-glucuronidase in detectable amounts. If these strains are grown in a medium containing glucuronides or related compounds they quickly synthesize the enzyme. This induction of enzymes has been shown to involve other enzymes as well as β-glucuronidase and, furthermore, is due to actual new enzyme formation and not merely to increased enzyme activity nor to the emergence of a mutant strain (although this latter mechanism can also operate). The whole subject has been reviewed by Pollock[2]. The opposite phenomenon of enzyme repression, in which non-optimal amounts of a substrate in the bacterial environment result in a decrease or disappearance of the enzyme, is also well known. This has been shown to be due to actual inhibition of enzyme synthesis[3].

Similar enzyme induction in mammals, in which it is usually known as enzyme adaptation, has also been extensively studied and there can be no doubt that it is a valid concept. However, the vast majority of experiments in mammalian enzyme adaptation have depended upon alterations in dietary intake of substances (including parenteral administration), hormone administration or endocrine gland ablation. The subsequent enzyme assays were generally made on whole organs such as liver, kidney, muscle, etc.[4]. The changes found are thus often difficult to evaluate in relation to the known functions of a particular cell which in these organs are multifarious. Difficulties in producing a constantly high intracellular level of a substrate may well militate against an attempt to test the phenomenon experimentally as has been implied by Burnet and Fenner[5]. Some other means of producing this than the foregoing methods must be found, and it is suggested that enforced changes in the rate of the normal activity of individual cells would be a more specific method. One drawback to such a procedure is that more than one enzyme system would be directly involved. Nevertheless, the experiment would probably be worth while. For example, the injection of large amounts of pilocarpine in mice has been shown to cause a decrease in activity of alkaline phosphatase[6] and succinate dehydrogenase[7] in the plantar sweat glands, also physical rotation of rabbits on special turntables causes an initial fall in succinate dehydrogenase and cytochrome oxidase activities in the neurones of Deiters's nucleus[6]. Repeated daily stimulation for 7 days, on the other hand, results in an increase in activity in these two enzymes.

The inference to be drawn from these experiments is that excessive activity of a cell or tissue will result in an initial loss of enzyme either by protein catabolism as has been suggested[8] or by loss into tissue fluids or secretions[7], for example, urine and sweat. The enzyme is afterwards resynthesized and increasing amounts appear as the hyperactivity continues. It may then be supposed that the increased enzyme activity is probably the result of increased amounts of intracellular substrate made available by the continued stimulation.

Decrease in enzyme activity in mammalian cells other than as a concomitant of pathological processes does not appear to occur so frequently. In relation to the present hypothesis, however, it would be expected to occur in cells undergoing disuse atrophy and, in fact, it has been shown that malic dehydrogenase activity in muscles decreases in simple disuse atrophy as well as in paralytic poliomyelitis[9]. Such enzyme changes may well be the first manifestations of disuse atrophy. Disuse atrophy, however, is usually the result of pathological processes which may themselves cause changes in the enzymes being investigated. Experimentally the least pathogenic of techniques producing disuse of an organ would seem to be some form of sensory deprivation without physical restraint, that is, alterations in the animals' environment not alteration of the animals themselves. The most practical form this could take would be neuronal deafferentiation, for example, visual or auditory deprivation. Subsequent examination of the geniculate bodies and visual cortex might disclose enzyme changes of disuse atrophy.

If, in fact, such changes were found significant light may be thrown on the pathogenesis of transneuronal degeneration. This is a phenomenon first described in the lateral geniculate body in 1913 by Minkowski[10], and despite several claims to the contrary[11] had not been conclusively shown to occur elsewhere in the nervous system until recently when it was found in the trigeminal ganglion[12,13] and in the auditory pathways[14]. In this condition section of the optic (trigeminal, auditory) nerve leads not only to degeneration of the primary neurones but also to the second and even third order neurones far removed from the site of operation (eye enucleation, alcohol injection of ganglion, ablation of the cochlea). The degeneration or atrophy in these higher order neurones is thus trans-synaptic and, although several explanations have been advanced, for example, reparative gliosis, none is wholly satisfactory. On the other hand, disuse atrophy with enzyme loss as envisaged here would provide a wholly adequate explanation subject to the proviso that for practical purposes these neurones form synapses with the corresponding lower order neurones and with no other part of the nervous system. In such a case deafferentiation leads to the non-receipt of stimuli by otherwise intact higher order neurones and results in disuse atrophy. The lateral geniculate body in man is stated to receive synapses only from the optic tract[15]. Claims to find transneuronal degeneration in other parts of the nervous system[11,15] have not been confirmed or successful. Owing to the richness of interconnexions between nerve roots, nerve tracts and sensory and motor neurones in the spinal cord together with the multifocal origin of synapses on any one nerve cell, it would appear almost impossible to deafferentiate any single neurone completely in this situation.

In monkeys transneuronal degeneration has been shown to occur as early as the 7th day following optic nerve section[16]. In these animals all the cells of the lateral geniculate body project to the cerebral cortex and there are no association neurones, whereas in the cat, in which it takes some 63 days for transneuronal degeneration to appear, the lateral geniculate body contains many small non-degenerating cells whose axons terminate on nearby neurones[16]. These neurones possess an average of 40 synaptic end-bulbs in contact with their surfaces[17], and these may originate from association neurones[15]. Thus differences in the rates of onset of transneuronal degeneration between species are probably not due to species-specific metabolic differences but to differences in anatomical richness of connexion.

The hypothesis presented here can be summarized briefly. It is that the amount of activity of a given enzyme in a cell (or organ) is dependent, *inter alia*, on the overall activity of that cell. It is, in a teleological sense, a biological manifestation of the economic principle of 'supply and demand'. This hypothesis of supply and demand can be extended back so that not merely enzymes are affected but also RNA, and possibly messenger RNA and ultimately DNA. This is to some extent supported by the work of Cook *et al.*[15], who showed that in neurones undergoing transneuronal degeneration there was a progressive loss of Nissl substance which contains RNA. They also found a decrease in the nuclear and nucleolar sizes. Furthermore, it has been shown that the rate of turnover of Nissl substance is accelerated by activity of the neurone[8,18].

Experimental procedures designed to test the hypothesis can be made using either sensory deprivation or augmentation, but these techniques will, of course, only show whether the principle is tenable in the nervous system. It should be possible to devise other techniques enforcing under- or over-activity in other systems such as the locomotor organs or digestive tract.

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Protein Digestibility and Ingestion

Bender[1] has directed attention to the importance of the correct definition and usage of terms adopted in discussions on protein nutrition, in particular to the distinction between 'apparent' and 'true' as adjectives qualifying absorption and retention. Thus the 'apparent digestibility' (AD) of a protein is the ratio of the difference of the ingested and fæcal nitrogen to the ingested nitrogen, expressed as a percentage, while the 'true digestibility' (TD) makes allowance for the nitrogen in the fæces of non-dietary origin (the so-called metabolic nitrogen, F_s), which is subtracted from the total fæcal nitrogen; true digestibility is therefore always greater than apparent digestibility.

It is reasonable to assume that the true digestibility of a particular protein, or foodstuff, is a constant figure, something peculiar to the protein itself, and independent of the level at which it is fed, or the quantity consumed. It may also be assumed, and commonly is, that F_e is constant and independent of dietary nitrogen intake (I). If these assumptions are made it is easy to show that the relation of apparent digestibility to dietary nitrogen intake is hyperbolic:

$$AD = TD - F_e\,(100/I) \qquad (1)$$

There have been several reports in the literature of apparent digestibility varying with intake of nitrogen, or with percentage of nitrogen in the diet, which usually amounts to much the same thing, true digestibility remaining constant[1–6]; this communication merely attempts to consider the matter quantitatively. A similar treatment was used by Greaves and Scott[7] in analysing data obtained with cats, though there it was assumed that F_e was related to the total weight of dry food consumed: in this case I in equation 1 becomes the percentage of nitrogen in the diet.

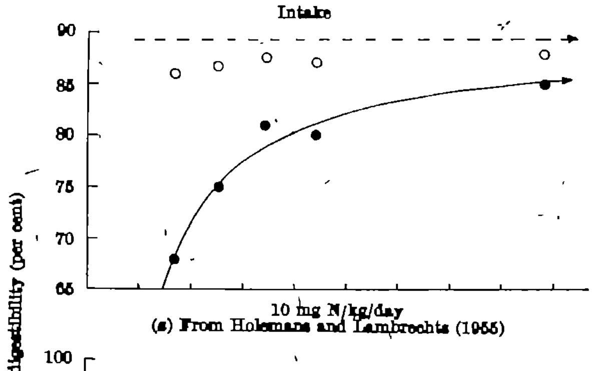
The results of Holemans and Lambrechts[8] and Phillips and Ladell[9], both obtained from human subjects, allow calculations to be made which permit curves of the type described by equation (1) to be fitted; the results of doing so are shown in Fig. 1. The equations of the curves are, respectively:

$$y = 89{\cdot}2 - 35{\cdot}3\,(100/x) \qquad (2a)$$

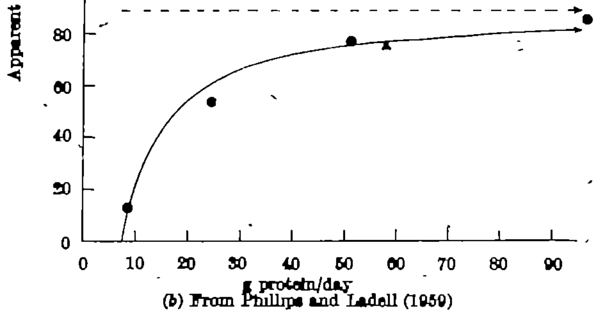
$$\text{and } y = 88{\cdot}7 - 6{\cdot}84\,(100/x) \qquad (2b)$$

and it is seen that they fit the experimental points closely. (The equations are obtained by calculating by the method of least squares a straight line regression of AD on the reciprocal of I. The negative slope of the line is then $(-)\,F_e$, and the intercept on the y-axis is TD.)

The units of the x-axis in the two parts of Fig. 1 are different, and are indicated in the caption. The curve in Fig. 1b was calculated from the observations represented by the black circles: the triangle represents an experiment in which a different source of protein was used but nevertheless it fits the line well.



(a) From Holemans and Lambrechts (1955)



(b) From Phillips and Ladell (1959)

Fig. 1. Variation of apparent digestibility of protein with dietary intake

By comparing equation 2 with 1, the true digestibility of the protein in the two experiments is seen to be 89·2 per cent and 88·7 per cent, and these values are indicated by horizontal dotted lines in Figs. 1a and b. Similarly, F_e in the first instance is seen to be 35·3 mg nitrogen/kg body-weight/day (Fig. 1a), and in the second 6·84 g protein/day (Fig. 1b). Phillips and Ladell[9] themselves estimate F_e by a different method to be 8·9 g protein/day; Holemans and Lambrechts[8] estimated F_e in their experiment to be 30 mg nitrogen/kg body-weight/day, and using this figure they calculated values for true digestibility at each level of intake: their results are plotted in Fig. 1a as open circles.

Pellett[6] suggested that the rise in apparent digestibility from 56 to 80 per cent which he observed when his pig was transferred from a 4·5 per cent protein diet on which it had subsisted for over 6 months, to a diet containing 7·5 per cent protein, was due to an increased production of digestive enzymes. While it has been conclusively shown that pancreatic function is depressed in cases of severe protein malnutrition[10,11] it is nevertheless true that a substantial rise in apparent digestibility would be expected to have occurred for the reasons advanced here. What lends greater support to Pellett's interpretation is his observation that whereas the rise in apparent digestibility mentioned here was immediate, when the pig was returned to the 4·5 per cent protein diet the apparent digestibility took several weeks to return to the original low level, falling finally to 30 per cent in the week before the animal's death. Furthermore, if one attempts to construct a line for Pellett's data in the manner of Fig. 1 it is immediately apparent that the change in the dietary level cannot of itself have been a sufficient cause of the change in apparent digestibility.

The fact that it is possible to fit so closely a theoretical curve of the type described by equation 1 to experimental data argues that the assumptions on which equation 1 is based, namely, the constancy and independence of intake of nitrogen of true digestibility and F_e, are reasonably valid. It is suggested that the process of fitting such a curve to experimental pairs of observations of apparent digestibility and dietary nitrogen intake may, in certain circumstances, be a useful means of estimating true digestibility or F_e, and relevant and important when conclusions are being drawn about the efficacy of digestion in experimental situations.

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PHARMACOLOGY

Cholinergic–Adrenergic Dual Action of γ-Aminobutyric Acid Methylester on a Preparation of Toad's Sinus–Atrium

THE effects of γ-aminobutyric acid methylester, $NH_2(CH_2)_3COOCH_3$ (I), its N-monomethyl (II), N-dimethyl (III) and N-trimethyl (IV) substituents were recently examined on the isolated heart of a toad by Takahashi[1]. He reported the positive inotropic effect induced by (I) and (II), no effect by (III) and the negative

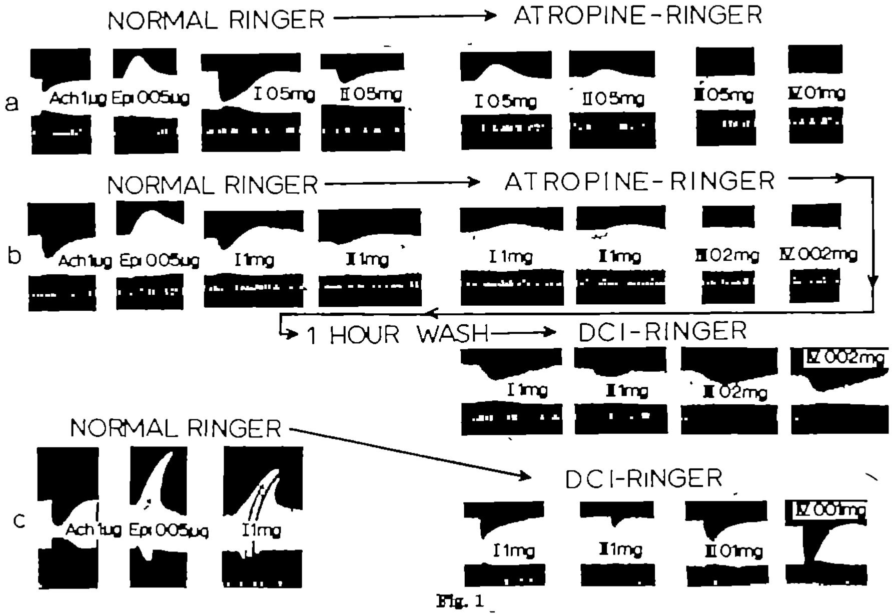

Fig. 1

inotropic effect by (IV). The induction of a quite opposite response by the difference of methyl moiety at the terminal nitrogen stimulated our interest to clarify the mode of action of these compounds. In this experiment a sinus–atrium preparation of a toad's heart was used, of which double cannulæ were so constructed that the Ringer solution perfused thoroughly the inner surface of sinus and atrium. The inner cannula was attached through rubber tubes to Mariotte bottles which contained normal Ringer, atropine-Ringer (0·01 mg/ml.) and dichlorisoprenaline (DCI)-Ringer (0·02 mg/ml.) solution. Perfusion pressure was 20 cm water, and 0·1 ml. of the drug solution was injected into the tube to the cannula. Experiments were performed during September–October, 1961, and during February–March, 1962.

The results obtained in the normal Ringer perfusion were somewhat different from those reported previously. When the normal Ringer solution was perfused to the sinus–atrium preparation of the non-treated animals, (I) and (II) in a dose of 0·5–1 mg usually induced the positive inotropic response on autumn animals (Fig. 1c), but the spring animals showed the negative inotropic response (Fig. 1a), occasionally followed by a positive inotropic one (Fig. 1b). In every preparation either the positive or the negative inotropic response was stronger in (I) than in (II). Meanwhile (III) and (IV) showed simply the negative inotropic response in every case in both seasons, the potency of which was around ten times larger in (IV) (0·01–0·02 mg) than in (III) (0·1–0·2 mg) for obtaining a 50 per cent decrease of contraction.

When the perfusing fluid was changed to atropine-Ringer solution, the negative inotropic effect previously observed by injection of these compounds, that is, (I) and (II) on spring animals, and (III) and (IV) in both seasons, disappeared, and the positive inotropic response occurred in the same dose of (I) and (II) (Fig. 1a and 1b). On the contrary, when the DCI-Ringer was perfused, the positive inotropic response commonly induced by (I) and (II) on autumn animals was completely blocked and a negative inotropic effect came out (Fig. 1b and 1c).

Concerning the cholinergic and adrenergic receptors Burn *et al.*[2] have developed the concept of cathecholamine release induced by acetylcholine of which action could be released by atropine on the rabbit atrium[3]. However, on the toad's sinus–atrium preparation with the atropine-Ringer perfusion, acetylcholine did not induce a positive inotropic effect even in the quite large range of dose used (0·01–250 µgm). Meanwhile, Japanese physiologists have carried out extensive investigations on the characteristic response of the trans-membrane potential of the toad's atrium tissue on sympathetic and parasympathetic stimulation and also on the administration of adrenaline and acetylcholine[4]. They confirmed the cholinergic excitation through the typical pattern of the trans-membrane potential when adrenergic reaction of adrenaline was ended and suggested one possible way to explain this phenomenon, such as acetylcholine release by administration of adrenaline[5]. However, neither the Burn's concept nor the Japanese physiologists' suggestion could explain the phenomenon by use of γ-aminobutyric acid methylester (I) and *N*-methyl γ-aminobutyric acid methylester (II) in the toad's heart. The experimental evidence here suggests that the adrenergic and cholinergic receptors of the toad's heart have a common part in response to the drug and there is probably neither a release of catecholamine induced by acetylcholine nor a release of acetylcholine induced by catecholamine. We believe that there is not enough differentiation of the adrenergic receptor from the cholinergic receptor in phylogenetically underdeveloped vertebrates.

In addition, these compounds have a simple negative inotropic action on Langendorff's preparation of the guinea pig and dog's heart lung preparation, especially prominent in (IV), which was prevented by atropine. Neither such dual activity nor the positive inotropic effect after atropine treatment was observed in the hearts of warm-blooded animals at all.

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Histamine-liberating Effect of Magnesium Deficiency in the Rat

Dietary magnesium deficiency in the rat gives rise, after a few days, to a characteristic peripheral vasodilation with erythema and œdema[1]. This change lasts for about a week, regresses and then re-appears later on. The signs of magnesium deficiency have been tentatively ascribed to a secondary vitamin B deficiency[2] and have also been compared to human neurodermatitis[3]. However, it was shown recently that magnesium deficiency induces a decrease in the number of subcutaneous mast cells[4], as well as an impairment in mast cell regeneration[5]. These observations are indicative of a change in histamine distribution that could be ascertained by measuring plasma and urinary histamine.

Male Sprague–Dawley rats (100–110 g body-weight) were fed a magnesium-deficient casein diet[6], and received demineralized water as drinking fluid; controls were

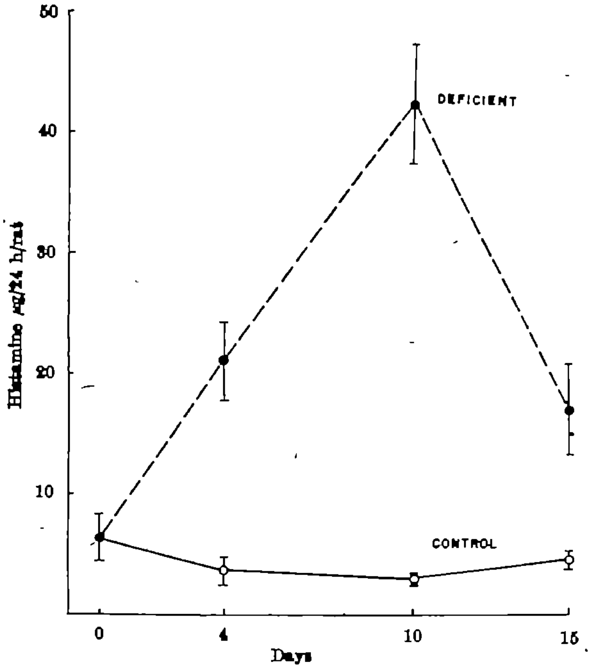

Fig. 1. Effect of a magnesium-deficient diet on urinary-free histamine secretion in male rats. Vertical lines indicate standard errors; each point represents the average of four pooled urine samples of three rats each

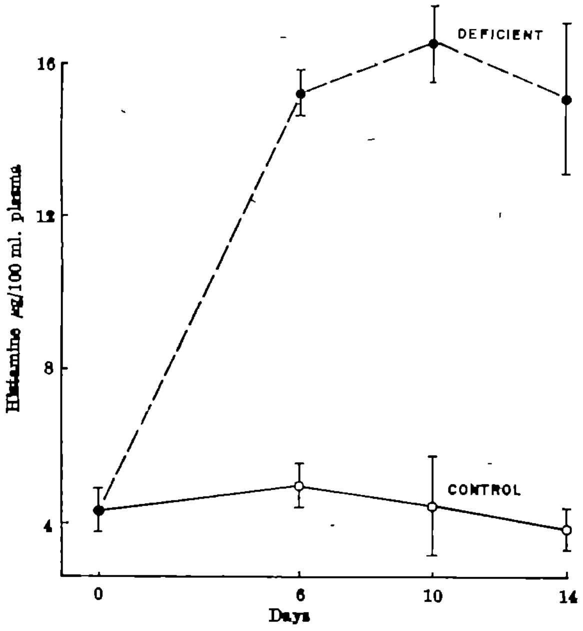

Fig. 2. Plasma histamine in magnesium-deficient rats. The higher values coincide with the period of maximal peripheral vasodilation. Each point is the average of five rats

given the same diet into which had been blended 0·6 g of magnesium sulphate per 100 g of diet. Metabolism cages were used for collection of urine; three rats were placed in each cage and urine samples were collected into bottles containing 0·3 ml. of concentrated hydrochloric acid[7]. The free histamine in the urine was determined by the guinea pig ileum assay; plasma histamine was measured by Lowry's method[8].

As shown in Figs. 1 and 2, both urinary and plasma histamine increase significantly in magnesium-deficient rats. This finding together with the fact that magnesium deficiency induces peripheral vasodilation[1], mast cell degranulation[4] and a decrease in tissue histamine[5] indicates that an endogenous histamine liberation occurs comparable with that observed after injection of histamine liberators such as compound 48/80 (refs. 9 and 10). There is yet no satisfactory explanation for this phenomenon. The release of histamine could be a direct effect of the deficiency or the result of accompanying changes in tissue concentrations of other ions such as calcium and potassium[11]. Another alternative could be that the lack of magnesium induces a decrease in the binding capacity of tissues for histamine, thereby causing a passive release of the amine. We are now testing these hypotheses in an investigation of the mechanism of histamine release.

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Tryptamine in the Blood and Urine of a Patient with a Carcinoid Tumour

PATIENTS with the carcinoid syndrome typically show high blood levels of 5-hydroxytryptamine and increased urinary excretion of 5-hydroxyindolyl-3-acetic acid. Variants of the tumour have, however, been described in which the major secretion is 5-hydroxytryptophan[1].

Recently, during studies of blood of a male patient with a carcinoid tumour with extensive metastases, an indole compound was detected which failed to give the fluorescence or colour reactions typical of 5-hydroxyindole compounds.

This substance was not detected in similar examinations of blood samples from normal individuals or from another carcinoid patient. The identity of this compound with tryptamine was indicated by the following evidence:

(*a*) R_F values identical with authentic tryptamine on paper chromatograms in three solvent systems: (1) 20 per cent potassium chloride (w/v); (2) butan-1-ol/acetic acid/water (12 : 3 : 5 by vol.); (3) propan-2-ol/aqueous ammonia solution (sp. gr. 0·88)/water (20 : 1 : 2 by vol.).

(*b*) Norharman formation, detected by fluorescence characteristics following condensation of tryptamine with formaldehyde and subsequent dehydrogenation of the tetrahydronorharman with hydrogen peroxide[2].

This patient was also found to be excreting large amounts of tryptamine in the urine as estimated by the method of Sjoerdsma, Oates, Zaltzman and Udenfriend[3]. In a 24-h urine sample the excretion was 84 mg, which is about 1,000 times the normal level of 0·036–0·12 mg/day (ref. 3). It is of interest that the urinary indolyl-3-acetic acid and its acid hydrolysable conjugates showed little or no rise above the normal concentrations, estimated by the method of Weissbach, King, Sjoerdsma and Udenfriend[4]. The urinary indolyl-3-acetic acid was found to be 12 mg/24 h (normal, 3·1–8·1 mg/24 h). After preliminary acid hydrolysis of the urine, the estimates increased to 15 mg/24 h (normal, 3·2–13·8 mg/24 h).

So far as we are aware, this is the first report of the finding of tryptamine in human blood either in normal human beings or patients with carcinoid syndrome.

This work was done with the help of Mr. Adam Smith, Western General Hospital, Edinburgh, whose patient this is.

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HÆMATOLOGY

Distribution of Haptoglobin among Thais

HAPTOGLOBIN type distribution has been determined on 682 Thais. All were normal, healthy male adults in the Royal Thai Army. Almost without exception their place of birth was confined to the provinces of north central and north eastern Thailand. This area of the kingdom was chosen for the survey as the one in which the population would be the most representative of the Thai people. It was felt that in this region there would be a minimum of disturbance of the Thai haptoglobin gene frequencies by recent (that is, past several centuries) admixture with non-Thai groups such as Burmese, Chinese, or Malayans. The blood samples were heparinized and centrifuged and the plasmas transferred to merthiolated vials immediately after collection and afterwards transported to our laboratory for electrophoretic analysis. Haptoglobin type identification was accomplished by the Smithies vertical starch-gel electrophoresis procedure[1] with minor modifications.

Table 1. HAPTOGLOBIN DISTRIBUTIONS IN ASIAN ETHNIC GROUPS

Ethnic groups	No. of subjects tested	Percentage distribution of haptoglobin types				Gene frequency	
		0–0	1–1	2–1	2–2	Hp^1	Hp^2
Thais (present work)	682	2·3	5·7	37·1	54·8	0·24	0·76
Chinese in Taiwan (ref. 2)	172	0	9·3	37·7	52·9	0·28	0·72
Chinese in Malaya and Australia (ref. 3)	167	1·2	10·8	34·1	53·9	0·28	0·72
Malays (ref. 3)	236	0·8	5·1	36·0*	58·1	0·24	0·76
Japanese (ref. 4)	349	1·4	6·0	35·0	57·6	0·24	0·75
Indians, Malaya (ref. 3)	219	1·8	1·8	14·6*	81·7	0·09	0·91
Indians, United States (ref. 5)	74	0	4·0	28·4	67·6	0·18	0·82

* Percentages shown for 2–1 haptoglobins include for Malays and Indians in Malaya, 0·4 and 0·9 per cent, respectively, of type 2–1 modified haptoglobins. No cases of 2–1 modified form were reported for the other ethnic groups in Table 1.

The group studied appears to be a reasonably homogeneous sample; the haptoglobin distribution obtained for the group as a whole is essentially the same as the separate distributions obtained by dividing the group into provincial sub-groups. By contrast, preliminary studies on smaller groups of individuals from the extreme northern portion of Thailand near Burma indicate a somewhat different distribution. A summary of the results of the study appears in Table 1 along with the haptoglobin distributions which have been reported for other Asian ethnic groups[2–5]. Based on the small numbers reported, the distributions of the Thai, Malayan, Japanese, and Chinese groups appear to be closely similar. The Thai, Malayan and Japanese groups, all with Hp^1 gene frequencies of approximately 0·24 according to present knowledge, resemble each other slightly more than they resemble the Chinese group, which has been reported[2,3] to have an Hp^1 gene frequency of approximately 0·28. (Current investigations in our laboratory on a larger series of Chinese inhabitants of Taiwan suggest that the frequency for their Hp^1 gene may prove to be slightly higher than the 0·28 value reported earlier[2].) The two small Indian groups examined in Malaya[3] and the United States[5] appear to exhibit a lower Hp^1 gene frequency than the other Asian ethnic groups. With respect to haptoglobin gene type distributions the Thai, Malayan, and Japanese groups fall between the Indian and Chinese groups; however, because both the latter groups are huge in number and diverse in nature, they may be expected to show variation in distribution of haptoglobin genotypes. A more detailed presentation of the provincial distribution of the Thai haptoglobin types and their accompanying hæmoglobin types will be presented elsewhere.

We thank the medical officers and technicians under Colonel Boon-Yang Vejjabool, Royal Thai Army Medical Corps, for collecting the blood specimens used in this work. The views expressed here are our own and are not to be construed as official or reflecting the views of the U.S. Navy Department or the Naval Service at large. This work is being supported in part by contract *Nonr*–1228 (14) between the Office of Naval Research and Northwestern University, and in part by a fund provided under Public Law 480, Section 104 (c).

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Catalase Activity in Young Red Cells

THE process of ageing of mature red blood cells is thought to be related to a progressive deterioration of one or more metabolic functions on which the morphological integrity of the cell appears to depend[1]. At present, there is insufficient evidence available to permit an identification of the critical processes within the cell that control and delineate its life span. Recent investigation of this problem has been focused at the enzyme-level, and it has been reported that young red cells have increased levels of catalase[2] as well as other enzymes[3,4] which fall progressively as the cells age.

Investigations on red cell ageing carried out in this laboratory have provided evidence that another enzyme, glutamic-oxaloacetic transaminase (GOT), was markedly elevated in young cells[5,6]. During subsequent clinical application of these findings to the evaluation of erythropoiesis in anæmia[7], it was considered desirable to compare the red cell levels of GOT and catalase to determine which of these enzymes might provide the most sensitive indication of the proliferation of young cells into the peripheral blood. It is the purpose of this report to present data which are not in accord with the presently accepted view[1,2] that catalase is significantly elevated in young red cells.

Enzyme analyses were carried out directly on diluted hæmolysates prepared from washed red cells or on hæmolysate fractions obtained after serial osmotic hæmolysis[8]. GOT was estimated by a slightly modified enzyme-linked spectrophotometric assay procedure[9]. Catalase activity was determined as outlined by Beutler *et al.*[2] without modification. The hæmoglobin content of the various solutions was estimated from the optical density at 540 mμ, based on previous calibration with a manometric oxygen capacity method. When used, radioactively tagged red cells were obtained from hæm-

Table 1. RADIOACTIVITY, CATALASE AND GOT ACTIVITY RATIOS IN HÆMOLYSATE FRACTIONS AFTER SERIAL OSMOTIC LYSIS

Fraction No.	Saline conc. (%)	Ratio (activity of fraction)/(activity of whole sample): Radio-activity (2)* A	GOT (9) B	Catalase (5) C	Mean % hæmolysis in fraction D
1	0·9	1·65	2·23	0·76	0·05
2	0·7	1·42	1·88	0·84	0·06
3	0·6	1·31	1·65	0·91	0·04
4	0·55	1·25	1·64	0·89	0·05
5	0·525	1·30	1·29	0·85	0·06
6	0·50	0·79	0·99	0·86	0·09
7	0·475	0·22	0·80	0·93	0·60
8	0·45	0·30	0·64	0·86	9·4
9	0·40	0·88	0·73	1·02	36
10	0·35	1·33	0·97	1·00	42
11	0·25	2·50	1·51	1·02	10·6
12	0·10	3·30	2·20	0·98	1·3
Whole sample		1·0	1·0	1·0	

* Numbers in parentheses refer to the number of experiments.

Table 2. GOT AND CATALASE ACTIVITY IN WASHED RED CELL SAMPLES FROM PATIENTS WITH NORMAL AND HIGH GOT-LEVELS

Subjects	No.	GOT (U/100 mg Hb)	Catalase (U/100 mg Hb*)
Group *A*	13	390 (260–480)	1,182 (710–1,440)
Group *B*	16	801 (540–1,170)	1,019 (690–1,320)

*Unit equals millimoles hydrogen peroxide destroyed.

atological normal subjects 6–10 days after the administration of 10 μc. of ferrous-59 citrate.

Successive exposure of washed red cells labelled with iron-59 to hypotonic saline solutions ranging from 0·9 down to 0·1 per cent has already been shown to produce a number of hæmolysate fractions which reflect a rough separation of cells of different ages in normal human subjects[10] and in rats[6]. Identical treatment of human red cells labelled with iron-59 provided similar results. A typical experiment (average of 2) in which the ratio of specific radioactivity of each sample to that of the whole blood is presented for each of the hæmolysate fractions (Table 1, col. *A*) indicates the adequate separation of young and old cells that this procedure can provide. Enzyme analyses were carried out for catalase and GOT activity in normal blood samples after exposure to the identical serial osmotic lysis. The elevated ratios of the GOT activity of the individual hæmolysate fractions to that of the whole unfractionated red cell sample (Table 1, col. *C*) reflect the increased enzyme activity due to the contribution of lysed young cells, substantiated by the increased radioactivity ratios in the same fractions (Table 1, col. *A*). This increase is clearly absent in the catalase data (Table 1, col. *C*). The presence of two populations of young cells, suggested by the high radioactivity ratios in the first 5 fractions, has already been observed[1,6,10]. Although there is no clear explanation for this finding, it may reflect an 'infant mortality'[1] that is in no way related to the general characteristics of the majority of young cells. The low catalase ratios apparent in these same fractions reflect the lability of catalase in extremely dilute solutions[9]. Separate experiments have shown that, under the conditions imposed by the serial osmotic fragility technique, there is a 15 per cent loss of enzyme activity in the extremely dilute hæmolysate fractions 1 to 5 (Table 1). No such loss of activity could be demonstrated in the more concentrated fractions.

Previous investigations have already indicated that red cell GOT-levels are markedly increased in peripheral blood samples containing increased numbers of young cells. In order to corroborate our findings that catalase activity is not significantly elevated under these conditions, both enzymes were studied in a series of washed red cell samples from 29 patients with normal (*A*) and elevated (*B*) red cell GOT-levels. The results (Table 2) clearly demonstrate the absence of any significant difference in the mean catalase-levels of the two groups despite the clear indication of the presence of young red cells provided by the two-fold increase in GOT activity.

There are a number of possible explanations for the discrepancy between these findings and those previously reported by Allison and Burn[2]. Although their findings are undoubtedly valid with respect to cholinesterase, and possibly glyoxalase, the extremely limited number of determinations of catalase activity provide some doubt as to the advisability of including this enzyme in their rather general conclusion. In addition, these investigators used either reticulocyte-rich samples or normal red cells after they had been transfused. Their findings may thus have no bearing on the normal physiological ageing process of young, mature, non-reticulated erythrocytes. The present data, on the other hand, are derived from a normal red cell population or from samples enriched with young cells without substantial reticulocytosis. The similarity in the findings with both types of samples indicates that catalase should not be included in the group of enzymes known to be increased in young, mature cells, decreasing with subsequent *in vivo* ageing.

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Ratio Adenosine Triphosphate/Adenosine Diphosphate in Platelet-rich Plasma in Hæmorrhagic Disorders (Von Willebrand and Glanzmann Disease)

ADENOSINE triphosphate (ATP) is found in large amounts in blood platelets[1] and disappears rapidly during clotting[2] while ADP increases; this nucleotide is able to aggregate platelets[3-6]. ATP and AMP act as inhibitors in this reaction[7,8].

Therefore, I tested the amount of ATP and ADP in platelet-rich plasma in seven normal patients, eight cases of Von Willebrand disease. five patients with Glanzmann thrombasthenia—one with (type I) and four without (type II) anomalies of glycolysis[4]—six patients with a congenital defect in a clotting factor: three AHG (VIII) deficiencies. one Christmas factor (IX), one proconvertin (VII) and one Stuart-Prower (X) deficiency. Lastly, I treated six normal plasmas with an antiserotonic drug inducing *in vitro* thrombasthenia[9].

Platelet-rich plasma was prepared as follows: blood was collected by venous puncture in silicone glassware at 4° C, centrifuged slowly (1,200 r.p.m. for 10 min) and the supernatant treated with perchloric acid (aqueous solution 6 per cent). The supernatant, after centrifugation, was neutralized and filtered. The methods used for ATP determinations were those of Biochemica test Bohringer *TC J* and for ADP the Biochemica test Böhringer *TC K*, except for the fact that I used 0·08 ml. of DPNH instead

Table 1. PLASMATIC ASSAYS (MEAN VALUES)

	No. of patients	ATP	ADP	ATP/ADP
Control	7	7·71*	6·23	1·24
Von Willebrand	8	*11·53*	6·23	1·85
Glanzmann disease:				
Type I	1	*1·7*	4·25	0·4
Type II	4	8·47	*4·16*	2·03
Various disorders of blood coagulation	6	8·80	5·57	1·57
Experimental thrombasthenia	6	8·23	6·51	1·26

* In mg for 10^{11} platelets.

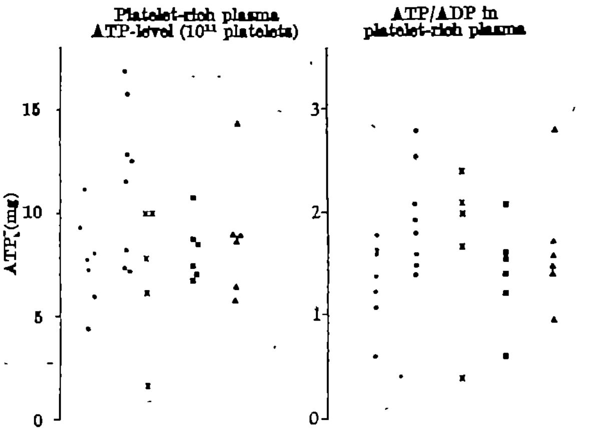

Fig. 1. ●, Control; ○, Von Willebrand; ×, Glanzmann disease; □, drug-induced thrombasthenia; △, blood coagulation disorders

of 0·1 ml; all the measurements were made in a Jobin Yvon Maroc II spectrophotometer at 366 Å. Results are expressed in mg for 10^{11} platelets (Fig. 1); the ratio ATP/ADP is on the right side in Fig. 1.

The mean values (normal and pathological) are given in Table 1. The difference of the ATP amount between normal subjects and patients with Von Willebrand disease is significant, and the coefficient of correlation is 2·36 (*S.E.* < 5 per cent). The amount of ADP is the same in both (Table 1). In Glanzmann disease with anomaly in glycolysis (type I), ATP is depressed; in the other one (type II), ADP is decreased; in any event the ratio ATP/ADP is abnormal. In experimental thrombasthenia and in patients with blood coagulation disorders, the level of ATP and ADP is normal except the increased amount of ATP in the platelet rich plasma of one case of Stuart (factor X) deficiency.

The significance of these results is still unknown, but it can be seen that the ratio ATP/ADP is abnormal (higher or lower than normal) in the cases with an anomaly in the cohesion of the platelets to the vessel wall[4,9,10]. The site of action of ADP on platelets, the opposite role of ATP, higher than normal in Von Willebrand disease, merits further consideration.

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PATHOLOGY

Characters of Mammary Tumours in *BALB/c* Female Mice Foster-nursed by *C3H* and *RIII* Mothers

ANALYSIS of mammary tumours occurring in *C3H*, $BALB/c_f$, and *RIII* mice has indicated the existence of strain differences in the characters of neoplasms[1–10] (Table 1).

Table 1. CHARACTERIZATION OF THREE HIGH-CANCER-STRAIN MICE BY MEANS OF THE DOMINANT TUMOUR CHARACTERS

Tumour characters	Strains *C3H*	 $BALB/c_f$	 *RIII*
Clinical duration	short	short	long
Growth-rate	high	high	low
Partial regressions	rare	rare	frequent
Type of growth	regular*	regular*	irregular
Responsiveness to pregnancy	absent(?)	rare	frequent
Histological origin	nodules†	nodules†	plaques
Morphological picture	pure	pure	varied

* Sigmoidal, linear, parabolic or exponential curves.
† Hyperplastic alveolar nodules.

The question therefore arises: What causes the difference found in tumour behaviour in various strains? The causes might be only genetic, but it is interesting to note that in *C3H* and $BALB/c_f$ strains, though their genetic origin is different, the behaviour of tumours is parallel. In order to explain the results found, three facts deserve special consideration, namely: (*a*) *C3H* and $BALB/c_f$ mice, which grow tumours with similar behaviour, carry the same milk agent (the $BALB/c_f$ strain is *BALB/c* foster-nursed by *C3H*), whereas another type of agent is responsible for tumours in *RIII* mice; (*b*) mammary tumours showing the same characters as *RIII* tumours have been observed in *RIII* × *C57b* and reciprocal hybrids, which bear the same milk agent as their *RIII* parent[11,12]; (*c*) the existence of differences in behaviour between the *RIII* milk agent and that of other strains of mice has also recently been noticed by other investigators in different laboratories[13–15]. This leads one to suppose that the mammary tumour agent, like a true genic particle, might be the carrier of a number of specific tumour characters. In order to test this hypothesis foster-nursing experiments are being performed.

Two new lines of inbred mice have been established by crossed foster-nursing, namely: *RIII* foster-nursed by $BALB/c_f$, and *BALB/c* foster-nursed by *RIII*. Preliminary results from the former line have been published elsewhere[16]. The results obtained were in agreement with the working hypothesis suggested here. In fact, the behaviour of mammary tumours occurring in *RIII* mice foster-nursed by $BALB/c_f$ closely approached that of $BALB/c_f$ tumours. The data so far available on the behaviour of tumours in the *BALB/c* line foster-nursed by *RIII* are given here.

A litter of five *BALB/c* mice removed from its mother at birth was transferred to an *RIII* mother for suckling. At weaning, four $BALB/c_{f(RIII)}$ mice were still living, two females and two males. Both the females were mated and the line was then maintained by brother to sister mating. Now it has reached the seventh inbred generation and shows a high incidence of mammary tumours. Data concerning mammary tumours in breeding females already dead are given here.

Of 52 breeding female mice, either normally or forcibly bred, 49 or 94·2 per cent developed mammary tumours at an average age of 233 days. The average number of tumours per mouse was two. All the animals with or without mammary tumours delivered litters. The average number of litters per mouse was 4·8 in mice normally bred and 7·2 in those forcibly bred.

The behaviour of mammary tumours in *BALB/c* mice foster-nursed by *RIII*, compared with that in *BALB/c* mice foster-nursed by *C3H*, is summarized in Table 2. On the right and left sides of Table 2 are recorded the characters of tumours grown in the *RIII* and *C3H* strains, which provided the respective milk agents to the $BALB/c_f$ lines.

Table 2. CHARACTERS OF MAMMARY TUMOURS IN *BALB/c* FEMALE MICE FOSTER-NURSED BY *C3H* AND *RIII* MOTHERS

	C3H (donor) →	$BALB/c_f$ (*C3H*)	$BALB/c_f$ (*RIII*)	← *RIII* (donor)
Clinical duration (days)	49	59	104	103
Growth-rate (cm per week)	0·238	0·231	0·125	0·135
Partial regressions (per cent)	27	34	60	59
Type of growth (regular: per cent)	64·3	61·5	25	30
Responsiveness to pregnancy (per cent)	0*	19	83	80·3
Histological origin (prevalent)	nodules	nodules	plaques	plaques
Morphological picture (prevalent)	pure	pure	varied	varied

* Due to the low fertility of these animals at the age of tumour appearance, this figure concerns only a few tumours. A larger group of *C3H* mice is now under investigation for this character.

In *BALB/c* mice foster-nursed by *RIII* the clinical duration of tumours was 104 days, on average, and the average rate of tumour growth was 0·125 cm per week. Partial regressions were observed in 60 per cent of tumours. The type of growth was regular in 25 per cent of tumours. Responsiveness to pregnancy was observed in 82 per cent of tumours. Preliminary histological studies have shown that tumours in these mice arise frequently in plaques and present varied pictures.

The results presented here show that the behaviour of mammary tumours occurring in *BALB/c* mice foster-nursed by *RIII* closely approaches that of *RIII* tumours. By contrast, a striking difference in behaviour has been found between tumours which grow in the same strain (*BALB/c*) foster-nursed by different mothers (*C3H* and *RIII*). These results indicate that the tumour characters may be transferred from one strain to another by means of foster-nursing.

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Asbestosis in Non-Experimental Animals in South Africa

Asbestosis has been produced in monkeys, rabbits and guinea pigs exposed to crocidolite, amosite and chrysotile asbestos dusts in experimental dust rooms[1]. No record of asbestosis occurring in non-experimental animals has been found, although Kiviluoto found anthophyllite asbestos fibres in the lungs of a cow from the vicinity of an asbestos mine[2]. He suggested that this finding supported his contention that the mining process was not directly responsible for the inhalation of asbestos dust in those cases of bilateral pleural calcification in which there was no history of an industrial exposure to asbestos dust.

Recently it has been possible to examine the lungs of different animals which have lived in the vicinity of an asbestos mine or associated process.

Donkeys. Two donkeys had worked on an amosite asbestos mine in the northern part of the Transvaal Province hauling ore from the mine to the reef picking plant. At autopsy the lungs and heart of one of these which had worked for ten years were removed and sent to this Unit for examination. Histological examination showed the presence of an interstitial fibrosis associated with asbestos bodies and asbestos needles. A small part was taken from one of the lungs of the other donkey—the length of service of which is not known—and although preservation of the tissue was not good, numerous asbestos needles were found in histological sections after micro-incineration at 600° C.

The finding of asbestosis in these donkeys is of interest. Although Heppleston[3] found reticulin fibrosis in the small foci of dust deposits in the lungs of pit ponies from the steam coal mines in South Wales, the examination of the lungs of mules which had worked in the haulage ways of one of the South African gold mines did not show silicotic lesions. A considerable amount of dust, however, had accumulated in the tracheo-bronchial lymph glands.

Baboon. A troop of baboons lives in the hills near one of the crocidolite asbestos mills in the Kuruman area of the north-west part of the Cape Province, and has been seen near the mill on a number of occasions. One of this troop was shot after killing some of the livestock on a neighbouring farm, and the lungs and heart were submitted for examination. Macroscopically the lungs felt firmer than those of a normal laboratory monkey, and on histological examination there were focal areas of interstitial fibrosis associated with asbestos bodies and fibres.

Rattus namaquensis. In furthering the investigation which this Unit is undertaking into the relationship of the inhalation of asbestos dust to the development of malignancy in general and mesothelioma of the pleura in particular, an examination of the rodent population of different areas is being carried out. Twenty-three field rats of this species were trapped around the mill already mentioned and sent to this Unit by a member of the Department of Health. The rats were killed, and the thoracic organs removed. Histological examination of sections from the right lung showed definite evidence of asbestosis in eighteen and probable asbestosis in three rats.

These rodents do not normally move more than 25 yards from their burrows[4]; but it was noticeable that there were far fewer trapped than was expected from the number of nests found. The mortality rate in this colony appears to be high, but this is probably not due to asbestosis.

I thank Messrs. C. G. Coetzee and A. J. Prinsloo of the Department of Health for trapping the smaller animals and for the care taken to preserve the specimens.

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Tissue Mast Cells

While examining the histology of tuberculoid and lepromatous skin lesions of leprosy, we noted that there were many cells containing acid-fast granules in the cytoplasm, around the granulomatous collections, in the connective tissue and in relation to small capillaries in both types of the disease. These granules were spherical, and stained somewhat paler red than the acid-fast *Myco. leprae* with the Fite-Faraco modification of Ziehl-Neelsen stain using Harris's hæmatoxylin as counter-stain. These cells were rounded, oval, stellate or spindle-shaped with oval, rounded or bean-shaped nucleus. The cell membrane was indistinct, and in some cells the granules appeared extracellular.

On staining the sections made from the same tissue blocks with Lison's toluidine blue (differentiated and dehydrated with alcohol, cleared in xylol and mounted in balsam), it was found that the above cells corresponded morphologically and in distribution to the mast cells which were prominently shown by the red coloration of the cytoplasmic granules due to their alcohol-fast γ-metachromasia with the dye.

Afterwards, we stained by toluidine blue and Fite-Faraco-hæmatoxylin respectively consecutive sections of various tissues containing fairly large numbers of mast cells, namely, post-kala-azar dermal leishmaniasis, lupus vulgaris, chronic dermatitis of non-specific ætiology, dermatitis herpetiformis, acne vulgaris, lichen planus, adenoma sebaceum, porokeratosis, psoriasis, metastatic carcinoma of the omentum, portal cirrhosis, and mesenteric spreads from guinea pigs. In these conditions most of the mast cells, the distribution of which was demonstrated by toluidine blue stain, showed acid-fast granules in the corresponding sections stained by Fite-Faraco-hæmatoxylin stain.

Morphology, evolution, cytochemistry, functions in health and disease, etc., of the mast cells have been reviewed by Asboe-Hansen[1] and Riley[2]. It is generally agreed that the mast cell granules contain an acidic mucopolysaccharide and substances closely related to hyaluronic acid and heparin. These cells are also believed to contain histamine. The present investigation shows that the granules contain a substance that shows an acid-fast character with the Fite-Faraco stain.

In the course of his work on the early lesions of leprosy, Khanolkar[3] was the first to record the presence of certain cells that showed acid-fast granules (fuchsinophil cells) in sections stained by the Fite-Faraco method. The morphology and distribution of these cells as described by him and illustrated with drawings in colour in his paper appear to be identical with the mast cells seen in our tissue sections. Khanolkar regarded these granules as partly digested *Myco. lepræ* and the cells as adventitial cells or macrophages.

Our observations indicate that these cells are tissue mast cells, the granules of which have now been shown to contain among other substances an acid-fast material and that such mast cells are seen in a wide variety of conditions unrelated to mycobacterial infection.

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Utilization of Cell Chromosome Number for diagnosing Cancer Cells in Effusion

THE karyological study of cancer cells has recently been a field of increased interest. While noting that most human cancer cells have abnormal idiograms, the various contributions have been concerned largely with the theoretical nature of cancer and have provided only a minimal amount of clinically useful information[1-5]. Jacob[6] has suggested that malignancy may be diagnosed by chromosome counts. It therefore appears worthwhile to emphasize that determining the approximate number of chromosomes of cells frequently will provide accurate diagnoses of cancer.

In the work reported here 49 effusions from pleural or peritoneal spaces were analysed for chromosomes. The absolute diagnosis in each instance was determined by the patient's hospital course. The cancerous effusions were primarily those with ovarian or uterine tumours, but also included are effusions from patients with cancer of the stomach, lung and breast.

The laboratory technique requires minimal experience. The effusion fluid is slowly centrifuged, and the cell button is resuspended in 0·5 per cent sodium citrate for 20 min, the cells then fixed in 50 per cent acetic acid. The cell suspension is stained with acetic orcein and a drop of fluid then squashed between cover-slip and slide. Using a phase microscope, the preparation is searched for cells and a minimum of 10 cells must be counted.

For the purposes of 'marker' chromosome examination and stem line determination, it is necessary to photograph the better chromosome figures and prepare idiograms. This laborious step is not required if the purpose is only to determine the presence of cancer. Most cancer cells are readily identified because of their abnormal chromosome patterns.

Cancer cell chromosome counts in this work have ranged from 24 to more than 250. The majority have been between 54 and 84. In these human cancer preparations, many different chromosome numbers were found and there was frequent absence of a clear-cut model number (stem line) of chromosomes. Idiogram analysis will often show unusually long acrocentric chromosomes which are apparently associated with cancer.

The fluids were evaluated at the same time with standard cytological techniques for cancer cells. The results are shown in Table 1. In 5 out of 16 examples of benign effusion, inadequate chromosome preparations were found and no diagnosis could be made. In only 2 of the 31 cancerous effusions was this true.

Table 1. DIAGNOSIS OF CANCER CELLS IN EFFUSIONS, USING CHROMOSOMAL AND CYTOLOGICAL TECHNIQUES

Source	Total cases studied	Chromosome study Correct diagnosis	Chromosome study Incorrect diagnosis	Cytological study Correct diagnosis	Cytological study Incorrect diagnosis	Preparations inadequate for chromosome study
Non-cancer effusions	16	11	0	16	0	5
Cancer effusions	33	26	5	31	2	2

Five out of 33 chromosomal preparations of malignant cells were incorrectly diagnosed, either because the tumour had nearly normal chromosome patterns or because poor karyological technique obscured the total number of chromosomes. Fluids from two patients were incorrectly diagnosed as showing 'no malignant cells' by the cytological technique but were correctly diagnosed as cancer by chromosome analysis.

Improved results could be obtained by short-term culture techniques and by more detailed analysis of the cell preparations. But such modifications change a simple technique into an expensive and laborious procedure which can offer no competition for the standard cytological techniques. While a significant number of malignant effusions were called benign, no false positive diagnoses were made.

In addition to adding a simple collaborative technique to the usual cytological procedures, chromosome study has the advantage of contributing knowledge to our understanding of cancer.

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IMMUNOLOGY

A Freezing-thawing Technique for Concentrating Antibodies in Serum

WHEN frozen serum is centrifuged, thawing due to air friction takes place and the heavier molecules are precipitated. By this means it is possible to concentrate the proteins and electrolytes present in serum.

Bovine serum containing *Brucella abortus* agglutinins was placed in 10-ml. amounts in 10-ml. conical centrifuge tubes and the tubes were frozen overnight at −10° C. The tubes were then centrifuged at 3,800 r.p.m. for 30 min at

Table 1

Fraction	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,560	1/5,120	1/10,240	1/20,480	1/40,960	Sodium content (m.equiv./l.)
						Serum dilutions						
1	++++	+										20
2	++++	++++	++++	+								20
3	++++	++++	++++	++++	++							47
4	++++	++++	++++	++++	++++	++++	++++	++++	++++	++		115
5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	272
Control	++++	++++	++++	++++	++++	++++	+++	+				136

room temperature. A water clear, supernatant fraction was then removed (Fraction 1) and the remaining portion again frozen overnight at the same temperature. The procedure was repeated on two occasions (Fractions 2 and 3) and a thick viscous layer remained. This fraction was then divided on the basis of colour into an upper light yellow fraction (Fraction 4) and a lower deep amber fraction (Fraction 5).

Fractions obtained from 2 × 10 *ml. quantities of serum:* Fraction 1, 7·2 ml. after 30 min at 3,800 r.p.m.; Fraction 2, 4·2 ml. after 30 min at 3,800 r.p.m.; Fraction 3, 2·0 ml. after 30 min at 3,800 r.p.m.; Fraction 4, 2·4 ml. = upper light yellow layer of sediment; Fraction 5, 1·8 ml. = lower deep amber layer.

The serum fractions were kept at −10° C until used. These and a frozen control serum were allowed to thaw at room temperature and were then well shaken and submitted to a *Brucella abortus* agglutination test. The control serum had been taken from the same batch of serum and had been kept frozen at −10° C for 5 days. The *Brucella* antigen was standard concentrated Weybridge antigen diluted 1 in 10 with normal saline. Twofold dilutions of serum were made starting at 1 in 40. Readings were made after 24-h incubation at 37° C in a hot-air incubator.

An analysis of the sodium content of each fraction and the control serum was carried out using a Beckman flame spectrophotometer. The results are contained in Table 1.

By this method there was approximately an eight-fold increase of agglutinins in the 5th fraction over the control serum. The sodium content was doubled. The length of time chosen for centrifugation was that required to ensure complete thawing of the sample. Rubber liners were used in the centrifuge buckets and these influenced the thawing time.

The rate of thawing and the speed of centrifugation are factors which are likely to influence the rate of precipitation of the molecules, and these factors require further investigation. This technique may have an application in the concentration of toxins.

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Type-specific Immunity against Intracerebral Pertussis Infection in Mice

It has been generally assumed that smooth strains of *Bordetella pertussis*, recently isolated from human cases of whooping cough, belong to a single antigenic type[1]. But Andersen[2] showed that these freshly isolated strains may be divided into serologically different types. This heterogeneity of the heat-labile agglutinogens, enabling type-differentiation of strains, has been confirmed by Eldering *et al.*[3], by Lacey[4], and by Preston and Te Punga[5]. All strains possess a common heat-labile agglutinogen which Andersen designated 1; and they may possess also one or more of a series of similarly heat-labile antigens which she numbered 2, 3, 4, 5. Andersen and Bentzon[6] attempted unsuccessfully to demonstrate a relationship between agglutinogen-type of vaccines and their protective potency in mice. But their experiments were handicapped in two ways: first, their strains were of low virulence for mice; and secondly, they were using active immunity, so that specific immunity to each sero-type would be partially masked by cross-immunity due to the common (1) antigen.

However, it seemed to us likely that one or more of these agglutinogens may be concerned with immunity, since there is usually a close correlation between the protective potency of pertussis vaccine and its ability to produce agglutinin in mice[7]. But, like Andersen, we found that strains freshly isolated from children were all of low virulence for mice. In fact, we had only two strains which were virulent for mice, by intra-cerebral injection: the Kendrick strain (*W*.18–323), which is widely used for intra-cerebral challenge, and possesses antigens 1,2,3,4; and a unique strain (353/*Z*), which came to us originally from Dr. Standfast, Lister Institute, and which we had made sufficiently virulent by serial passage in mice—this strain was unique in that it possessed only the common (1) antigen.

We were anxious to attempt cross-immunity experiments with sero-types frequently isolated from children, for example, 1,3; and 1,2,4; but we were unable to enhance the mouse-virulence of these by passage. However, we eventually succeeded in producing a mouse-virulent strain possessing antigens 1,3 by exposing a freshly isolated strain of this sero-type, in log-phase, to each of the following mutagenic agents successively: 5-bromo-uracil (1/250), ultra-violet light, and proflavine (1/35,000). In conjunction with the unique type-1 strain, this type-1,3 strain provided the possibility of determining the importance of the 3 antigen in immunity. Mice were passively immunized with type-specific (absorbed) antiserum containing either 1 or 3 antibody, and then challenged 2 h later with one or other sero-type as shown in Table 1.

Table 1. Passive Protection of Mice with Type-specific (Absorbed) Antiserum containing Either 1 or 3 Antibody

Sero-type of challenge strain	Antibody in serum	No. of deaths/No. challenged Exp. 1	2	3	4	5	Total	(%)
1	(normal serum)	·	12/15	13/15	10/14	8/14	43/58	(74)
	1	0/14	1/15	1/14	4/15	2/14	8/72	(11)
	3	14/14	5/14	8/15	8/15	8/14	43/72	(60)
1,3	(normal serum)	·	13/15	7/15	10/15	7/14	37/59	(63)
	1	2/14	15/15	3/15	3/14	3/14	26/72	(36)
	3	5/14	3/15	2/14	7/15	1/14	18/72	(25)

It is clear that 1 antibody gave significant protection against challenge by the type-1 strain, whereas 3 antibody did not. Conversely, both 1 antibody and 3 antibody protected significantly against the type-1,3 strain. (The mortality in mice which were pre-treated with normal serum was not significantly different from that in untreated mice; the challenge inoculum was deliberately chosen to give a mortality of 60–70 per cent so as to provide the greatest chance of detecting significant protection.)

While these immunity experiments were being performed, we succeeded in producing a mouse-virulent mutant from a type-1,2,4 strain. This was compared with the type-1,3 strain in a similar series of passive immunity experiments, the results of which are shown in Table 2.

Clearly, both 1 antibody and 3 antibody significantly reduced the mortality from challenge with the type-1,3 strain, whereas 2,4 antibodies did not. Conversely, both 1 antibody and 2,4 antibodies protected significantly against the type-1,2,4 strain, whereas 3 antibody did not.

It is evident from Tables 1 and 2 that, although there is cross-immunity between pertussis sero-types because of the common (1) antibody, there is also type-specific immunity determined by the type-specific (2,3,4) antibodies.

Table 2. Passive Protection of Mice with Type-specific (Absorbed) Antiserum containing Either 1 or 3 or 2,4 Antibodies

Sero-type of challenge strain	Antibody in serum	No. of deaths/No. challenged Exp. 1	2	3	4	5	Total	(%)
	(normal serum)	·	13/15	7/15	10/15	7/14	37/59	(63)
1,3	1	2/14	15/15	3/15	3/14	3/14	26/72	(36)
	2, 4	6/14	10/15	9/15	11/14	6/14	42/72	(58)
	3	5/14	3/15	2/14	7/15	1/14	18/72	(25)
	(normal serum)	·	·	9/14	10/15	8/14	27/43	(63)
1,2,4	1	·	·	1/12	0/15	2/14	3/41	(7)
	2, 4	·	·	6/14	4/14	6/14	16/42	(38)
	3	·	·	5/15	12/15	8/14	25/44	(57)

Considering the possible application of these findings to immunity in children, we direct attention to the fact that unabsorbed immune sera may contain much higher levels of type-specific antibody than of common (1) antibody[5,9]. A child immunized with type-1,2,4 strains may therefore develop very little immunity to type-1,3 infection. This may possibly account for the increase in the proportion of type-1,3 strains isolated in recent years[4,9]. Moreover, type-specific immunity may account for the different potencies of a single vaccine used in different localities[7], depending on the sero-type of the local epidemic strain.

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RADIOBIOLOGY

Irradiation of Fruit and Simultaneous Measurement of Respiration

Fruit has often been irradiated for the purpose of examining the extension of storage life through control of infective organisms[1,2]. Fruit tissues, however, also lend themselves for studies in radiobiology; especially when high dose-levels are desirable to accentuate the primary cellular responses. Doses several orders of magnitude higher than those normally found to be lethal to animal systems can be tolerated by the fruit tissues, albeit with consequent manifestations of an altered metabolism.

The respiratory rate of the whole fruit serves as a measure of the metabolic response induced by irradiation. Limitations in the physical facilities have often necessitated a delay in the respiration measurements to various times after the exposure[3]. This may not only result in a misleading estimate of radiation response but also impose a severe limitation in studies of radiobiology where the critical need is to assess the initial radiation effect[4].

A recently installed 32,000-c. cobalt-60 facility designed by the U.S. Atomic Energy Commission to deliver a uniform dose of radiation under controlled temperature conditions has been adapted for the simultaneous measurement of respiration during the exposure period. This is achieved by attaching twelve air lines to a water-tight chamber into which the samples are placed to be lowered into the irradiation field. Air in-flow and return lines may then be attached to six containers of fruit and the respiratory rate followed with the standard Claypool–Keefer[5] technique. Temperature is normally maintained at 20° C, before, during, and after irradiation. Dose distribution as measured with Fricke dosimeters was found to be within ± 10 per cent with a dose rate of 295,000 rads per hour.

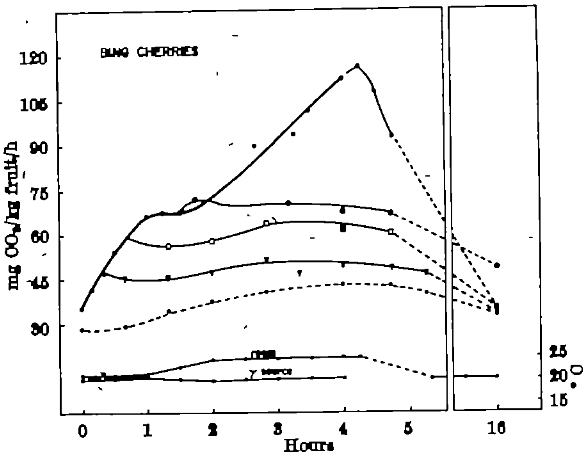

Fig. 1. Respiration of cherries during and immediately after irradiation. Simultaneous radiation and respiration period is indicated by the bold trace. ● - - - ●, Control; ▽ —— ▽, 100,000 rads; □ —— □, 200,000 rads; ○ —— ○, 500,000 rads; ● —— ●, 1,000,000 rads

An example of fruit respiration during irradiation is that elicited from Bing cherries as indicated by the bold trace in Fig. 1. The respiration rate rises with increasing dose excepting a plateau area near 500,000 rads. It is of interest to note that fruit receiving doses above the plateau-level decrease faster in post-irradiation respiration in contrast to the fruit receiving lower doses. Similar responses have been observed for oranges and lemons but with differing transition or 'plateau'-levels of irradiation. As a possible explanation of this effect one may assume that the higher doses cause irreparable damage to the respiratory mechanism, whereas at lower doses the resultant stress elicits a continued, elevated respiration commensurate with repair reactions. Experiments now in progress are directed towards the discernment of some of the intracellular changes which underlie this metabolic response.

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Increase of Strontium-90 and Caesium-137 in Japanese Leafy Vegetables due to the Russian Nuclear Tests, 1961

Fall-out radioactivity derived from tropospheric origin was detected soon after the Russian nuclear tests of September and October, 1961, in the northern hemisphere. Contamination of milk and fresh vegetables by short-lived radionuclides such as iodine-131, zirconium-95 and ruthenium-103 was reported in Japan[1].

In the case of strontium-90 and caesium-137 also, fresh leafy vegetables are fairly sensitive to reflect a change of contamination-level because of their direct foliar uptake of radionuclides besides root absorption from soil. Spinach, cabbage, hakusai (Chinese cabbage) and some other kinds of leafy vegetables were sampled from about ten locations widespread over the country in May–June (before resumption of tests) during October in 1961, January and March in 1962. These samples were analysed for strontium-90 and caesium-137 by the fuming nitric acid separation method and dipicrylamine–caesium chloroplatinate method respectively.

Table 1. STRONTIUM-90 AND CAESIUM-137 CONTENT OF JAPANESE LEAFY VEGETABLES DURING MAY 1961–MARCH 1962 (pc./kg FRESH WEIGHT)

Sample	^{90}Sr (pc./kg) May–June 1961	Oct. 1961	Jan. 1962	Mar. 1962	^{137}Cs (pc./kg) May–June 1961	Oct. 1961	Jan. 1962	Mar. 1962
Hakusai	16	63	36		44	64	31	
		10	11			24	40	
		56	10			46	19	
		32	96			29	154	
		23				24		
Cabbage	17	15	98	35	34	18	76	43
	18	19	85	61	76	25	82	64
	11	10			16	16		
	6				17			
	13				20			
	14				23			
Spinach	9	40	118	117	22	75	145	118
	136	42	75	173	64	74	154	142
	21	15	47	44	85	99	75	40
	16		102	57	58		138	25
	8		97	229	30		132	155
			90	72			126	42
Miscellaneous		41	93	54		34	70	33
		46	87	128		48	39	67
				123				37
Average	24	32	71	99	41	44	92	70

Results obtained are shown in Table 1. May–June samples in 1961 can serve as a background-level of contamination before resumption of nuclear tests in the autumn of 1961. As a rule, strontium-90 content of leafy vegetables shows considerable variation in most cases reported so far[2]. This seems to be caused by the variability of morphology, physiological conditions and environmental factors such as soil character, climate and fall-out pattern. As seen in Table 1, strontium-90 content of the May–June sample in 1961 shows fairly narrow range of about 5–20 pc./kg fresh weight except one, probably because of low strontium-90 fall-out rate during winter and spring in 1961. The October 1961 sample taken just after the resumption of nuclear tests by the U.S.S.R. shows fairly higher value and wider range of strontium-90 content (10–60 pc./kg fresh weight) than the sample in May–June caused by a sudden increase of strontium-90 fall-out in October 1961. In 1962 also, the similar tendency of increase is observed in January sample (10–110 pc./kg fresh weight) and March sample (40–230 pc./kg). Range of strontium-90 contamination-level becomes progressively larger and much higher values can be found more frequently than in 1961. Cæsium-137 content of fresh leafy vegetables in October 1961 does not seem to differ from that in May–June significantly. But, in January and March 1962, the level of cæsium-137 contamination of the sample had markedly increased when compared with that of the preceding year (Table 1). These significant increases of strontium-90 and cæsium-137 in fresh leafy vegetables should be due to the addition of the recent fall-out radioactivity which has produced direct contamination of the aerial part of these plants besides the accumulated deposition of strontium-90 and cæsium-137 in soil.

We thank members of the Prefectural Hygienic Laboratory for their help in sampling.

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BIOLOGY

Protection of the Skin Homografts by ε-Acetamide Caproic Acid

ε-AMINOCAPROIC acid has recently attracted a great deal of interest, mainly for its marked antifibrinolytic activity: it seems that it acts as an inhibitor of the transformation of profibrinolysin into fibrinolysin, or as an antagonist of the latter[1-3].

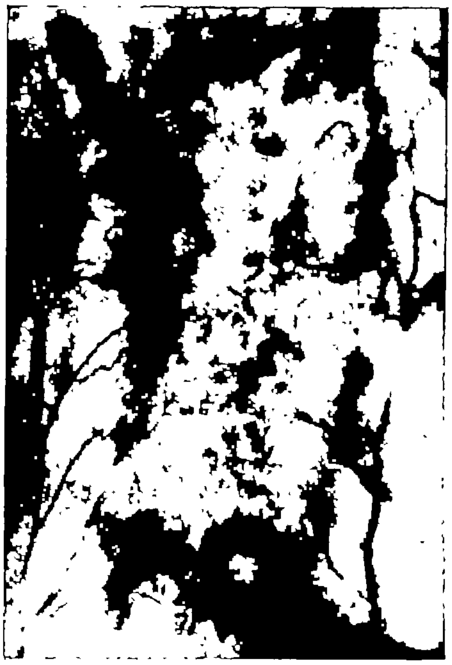

Fig. 1 Homotransplant in the seventh day in untreated control rat. Areas of diffuse necrosis and no vascularization are present in the graft

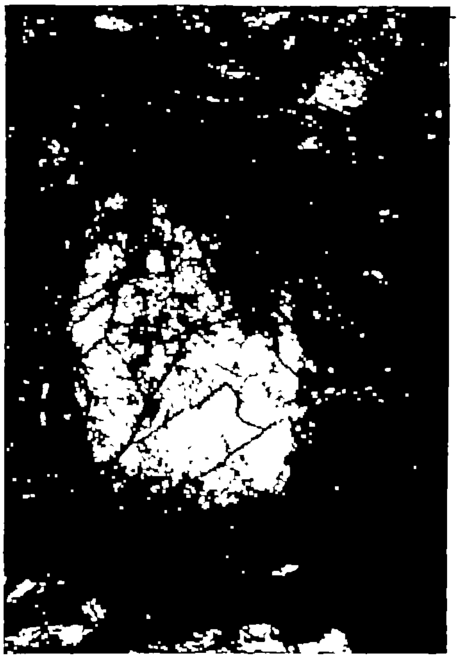

Fig. 2. Homotransplant in the fourteenth day in rat treated with ε-acetamide caproic acid. Diffused vascularization is evident and no necrosis appears

It has been shown here that ε-aminocaproic acid can prevent anaphylactic shock in the guinea pig[4], œdema from dextran, egg albumin and kaolin, agar granuloma and histamine wheals in the rat[5] and, in a particularly striking manner, the Sanarelli–Schwartzmann reaction in the rabbit[6].

One of us has been interested for some time in the problem of grafts, and especially of skin grafts, in the rat[7].

The technique used for such grafts, which is now well standardized, is as follows: careful depilation of the abdomen surface of the rat (female rats Sprague–Dawley strain, weighing about 250 g, of homogeneous strain, bred in this Institute, fed on standard diet); a rectangle of skin of full thickness measuring 2·5 × 2 cm is removed; this is substituted with another skin rectangle of identical dimensions removed from the same or from another animal. The extremities of the transplanted skin are sewn with silk No. 00. Some extra thread is left at the four angles; these threads are knotted together above a cotton wool pad, damped with physiological solution, which compresses the transplant.

The operated area is protected by raising two folds of skin from the sides and by sewing the edges with silk No. 2.

The rats are narcotized with 'Nembutal' (25 mg/kg 'Nembutal' intraperitoneally). The protecting cover may be opened whenever an inspection of the transplant is desirable.

With such a technique the homotransplants are rejected in about 6–7 days.

Practically all the autotransplants survived very well.

Different groups of rats bearing skin homografts are treated with ε-acetamide caproic acid intraperitoneally, repeating the administration twice a day. The doses were progressively increased. The starting dose was 300 mg/kg, with a daily increase of 100 mg/kg, until a final dose of 1·5 g/kg/day is reached. The animals do not seem to suffer at all for this treatment and show no signs of being inconvenienced by the drug; in any event, the quantity administered is 10 times lower than the LD_{50}.

The results are very evident: the homotransplants were observed until the 14th day and appeared healthy. The skin has a pale pink colour, soft and elastic under pressure; the adherence of the extremities is also excellent.

The veins of the homotransplant were observed after administration of China-ink in a femoral vein, and much better vascularization was observed than that of the homotransplant of a control not treated at the 6th or at the 7th day, when it was practically eliminated.

Similar results, although less striking, are obtained when ε-aminocaproic acid is used instead of ε-acetamide caproic acid.

The effects of ε-acetamide caproic acid and ε-aminocaproic acid on homotransplants of kidneys of the dog are now being investigated.

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Influence of the B Blood Group-histocompatibility Locus in Chickens on a Graft-versus-Host Reaction

PRODUCTION of nodular lesions on the chorioallantoic membrane of chick embryos after inoculation with adult fowl leucocytes has been described by Boyer[1]. These lesions, appearing as raised yellow or white foci, are considered to arise from the interaction of donor lymphocytes with foreign antigenic determinants present in the tissues of the embryonic host. The number of foci produced depends on the number of large lymphocytes in the inoculum[2] although immunization of the donor with embryonic tissue does not enhance the ability to produce foci[3]. Two independent experiments with inbred lines indicated that single gene differences between donor and host could account for chorioallantoic membrane foci production[4] as well as splenomegaly[5].

Skin-graft investigations reported from this laboratory have shown that the B blood group locus is also a major histocompatibility locus[6]. The experiment reported here was designed to determine if the B locus controls chorioallantoic membrane foci production, and if so, to make a quantitative study of its effect. Peripheral blood leucocytes from mature birds of White Leghorn inbreds (line *G*) were deposited on the chorioallantoic membrane of 14-day line *G* embryos. The technique used was similar to that described by Burnet and Boyer[7]. The inoculation consisted of 0·1 ml. of a leucocyte suspension prepared by resuspending the buffy layer of donor blood in Hanks's balanced salt solution to give a concentration of approximately $3·5 \times 10^4$ leucocytes per mm.^{3}. After four days, counts were made of the number of chorioallantoic membrane foci produced. The embryos and adult leucocyte donors had blood-group genotypes designated B^1/B^1, B^2/B^2 and B^1/B^2 so that nine donor-host combinations were used. 77 embryos from 13 hens were tested with leucocytes from eight donors. The results indicate that production of chorioallantoic membrane foci is due principally to B locus incompatibility, that is, when the embryo has a B allele (thus antigen) not also represented in the leucocyte donor. Membranes from the four B-incompatible combinations yielded an average of 65 foci. These foci were opaque and large enough to be easily recognized macroscopically. No foci of this nature were observed when donor and host were B-compatible. On close inspection, however, a number of the membranes were found to contain some small and relatively translucent lesions, presumably due to 'weak' histocompatibility antigens controlled by genes at other loci.

Further evidence for B locus control of the graft-versus-host reaction was shown by marked splenic enlargement in the B-incompatible embryos. Spleens from 10 B-compatible embryos averaged 17·6 mg compared with an average of 33·8 mg for 10 B-incompatible embryos.

The second part of the experiment was carried out by using host embryos of the same B genotypes as before (B^1/B^1, B^2/B^2 and B^1/B^2), but with two adult leucocyte donors from line *G* previously blood typed as B^3/B^3. A total of 220 membranes were examined in five replicates. All membranes exhibited the nodular foci previously described, although some low foci counts were observed in two of the five replicates. The mean foci count for B^1/B^1, B^2/B^2 and B^1/B^2 embryos was 69, 66 and 90, respectively. Mean differences due to host genotype proved to be statistically significant at the 0·05 level of probability.

An additional comparison of foci counts from 53 embryos produced by five hens is of interest. By shifting males in the breeding pens, embryos of the B^1/B^1 genotype were produced in one replicate and B^1/B^2 genotype in another. Mean foci counts (Table 1) for embryos of Dam group *a* (two hens) and Dam group *b* (three hens) appeared to be dependent on the B genotype being produced. Within each replicate the B^1/B^2 membranes yielded a considerably higher number of foci than the B^1/B^1 membranes. This difference is statistically significant at $P < 0·01$ for each donor.

Table 1. AVERAGE NUMBER OF CHORIOALLANTOIC MEMBRANE FOCI FOR B^1/B^1 AND B^1/B^2 HOST EMBRYOS OF THE SAME DAMS WITH B^3/B^3 LEUCOCYTE DONORS

	Host genotype B^1/B^1			Host genotype B^1/B^2		
	Dam group	Donor 1	Donor 2	Dam group	Donor 1	Donor 2
Replicate 1	*a*	65	113	*b*	149	175
Replicate 2	*b*	74	68	*a*	147	100

A possible explanation for the greater number of foci on B^1/B^2 membranes, following an 'elective' theory of antibody formation, is that the leucocyte inoculum from the B^3/B^3 donors contained immunologically competent cells with preformed patterns, some corresponding to B_1 and others to B_2 antigenic determinants of the chick embryo. An 'instructive' or template theory interpretation must, on the other hand, presume that the B^1/B^2 host antigens, being more foreign, stimulate proliferation of a greater number of donor cells which are analogous to each other. Further studies with chick embryos of known genetic constitution may provide evidence to support or invalidate certain assumptions basic to the two opposing theories.

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Effect of Certain Environments on the Carapace of the Freshwater Ostracod *Cypridopsis vidua* (Müller)

A FREQUENTLY occurring problem in quantitative palæontology concerns the effect of environment on the shell of an organism. In an attempt to elucidate a micropalæontological aspect of this question the effects on the carapace dimensions of *C. vidua* of the two environmental types 'lime-rich, freshwater' and 'stagnant, freshwater' were investigated by laboratory experiments, backed up by biometrical analysis. As a standard of reference observations were also made on a population kept under 'normal' conditions.

The original material of the parthenogenetic species *C. vidua* was obtained from Lake Rönningen, Parish of Täby, Uppland, Sweden, on August 7, 1958. Throughout the experiments *p*H and redox potential were controlled with a Beckman *p*H-meter (model *N*). The investigations were based on the dimensions—length, height and breadth of carapace.

The calcium carbonate of the shell of *C. vidua* is composed of calcite[1]. A chemical analysis made by F. Manheim and B. Brännström of the inorganic residue of the shells from the three environments is given in Table 1.

Table 1

Environment	$CaCO_3$	$MgCO_3$	Undetermined
Normal	89%	6%	5%
Stagnant	76%	7%	17%
Lime-rich	82%	10%	8%

An appreciable difference in the relative variability for the three dimensions for the samples from each of the environments could be established. This is here indicated by the coefficients of variation for adult carapaces (Table 2).

Table 2. COEFFICIENTS OF VARIATION FOR ADULT CARAPACES

Environment	No. in sample	Length	Height	Breadth
Normal	365	2·94 ± 0·11	4·94 ± 0·18	6·02 ± 0·22
Stagnant	308	3·78 ± 0·24	5·28 ± 0·21	5·89 ± 0·24
Lime-rich	581	6·93 ± 0·21	8·06 ± 0·24	11·32 ± 0·34

These results suggest the possibility of heterogeneity in the respective variances. This could be confirmed by *F*-tests for pairs of samples and by subjecting the trivariate covariance matrices of the adult samples and the samples of the final larval stage to a multivariate test for homogeneity of covariance matrices. The highly significant value of $\chi^2 = 1{,}128{\cdot}22$ does not support the hypothesis of equal covariance matrices. Hence, the carapace–morphologic covariance matrix of ostracoods would seem to be sensitive to environmental factors.

Highly significant *t*-values for the differences in means between the respective growth-stages of the stagnant and normal environments and the lime-rich and normal environments could be shown. The differences in means between the corresponding growth-stages of the stagnant and lime-rich environments do not give significant values. Nor are the multivariate T^2-values for differences in the mean vectors of these samples significantly different. This suggests the interpretation that the freshwater species *C. vidua* is morphologically equally affected by both an excessive calcium content in its environment as well as excessively stagnant conditions. This is expressed by a significant decrease in the carapace dimensions. (However, the 'saturation density' for the populations living in the lime-rich environment was found to be three times that for the stagnant environment.)

A reflexion of the variability in magnitude of the elements of the carapace-morphological covariance matrix is provided by the results of the principal component analysis. This discloses that there is but slight stability in the components of eigenvectors for the samples of the stages of growth from the three environments. The study suggests that not only the first principal components of a carapace–morphological covariance matrix for ostracoods is representative of the influence of environment on size, but also that in some way or other the second principal component is also correlated with environment.

The morphological closeness of the samples from the lime-rich and stagnant environments, compared with the samples from the normal environment, could be well brought out by canonical analysis of the entire data and by a generalized distance study.

The complete investigation by R. A. Reyment and B. Brännström is to be published in *Stockholm Contributions in Geology* (9, Pt. 5). The work was partially supported by a grant from the Swedish Natural Science Research Council; free computer time was made available by the Swedish Board for Computing Machinery.

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Abbreviated Pelagic Life of Chilean and New Zealand Oysters

OYSTERS of the genus *Ostrea* incubate their larvæ. In *O. edulis*, for example, veligers are released from the female when 0·15–0·17 mm long, and afterwards undergo pelagic development, usually of 6–14 days, according to water temperature and food supply, before settling and metamorphosing[1-3]. In *O. chilensis* Philippi from Chile, however, larvæ from incubating females collected by the Lund University Chile Expedition 1948–49 were examined by one of us (R. H. M.), through the courtesy of Dr. T. Soot-Ryen and Prof. H. Brattstrøm, and were found to be 0·45–0·49 mm long and to possess the eye spots and foot which other species develop only at the end of their pelagic life. The other (P. J. H.), who is publishing elsewhere a fuller account of the life-history of the New Zealand mud oyster, *O. lutaria* Hutton, has observed similar larvæ in that species. These larvæ, when released by the female in laboratory experiments, settled almost immediately. In both species this probably also happens in Nature.

Elimination of the usually wasteful pelagic phase is biologically useful, particularly in the low temperature and strong-tidal currents of areas like Foveaux Strait, New Zealand, where large oyster beds exist.

The introduction of the New Zealand species to Britain could be commercially important, as it might breed more successfully than *O. edulis* and certainly does so at lower temperatures. Repopulation may even be possible of the exhausted Scottish beds, such as those in the Firth of Forth, where formerly there may have been a local race of *O. edulis* adapted to breeding at relatively low temperatures. Experiments, including laboratory breeding, are in progress at the Marine Station, Millport with oysters flown from New Zealand, to test this possibility. Precautions are being taken to avoid the introduction of associated organisms.

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Relation of Arbutin–Hydroquinone in Pear Blossoms to Invasion by E. *amylovora*

Infection of plants by certain virus and fungus pathogens results in an increase in polyphenol synthesis and oxidation[1,2]. This has been interpreted as a possible defence reaction by host tissue. Investigations of resistance of specific varieties of pear, *Pyrus communis*, to invasion by *Venturia pirina* indicated that polyphenol metabolism might be associated with resistance[2]. A project was initiated to determine whether the location and concentration of phenolic substances in pear might be of possible significance in influencing the channels of infection and relative susceptibility of specific pear tissues to fire blight caused by *Erwinia amylovora*. The relation of the arbutin–hydroquinone complex in pear blossoms to invasion by the fire blight organism is herein reported.

Aqueous extracts of pear tissues were highly toxic to *E. amylovora*. A substance inhibitory to *E. amylovora* on bioassay plates was purified by extracting aqueous extracts with ether. The ether residues were extracted with benzene, and the benzene residues sublimed at 173° C. Final identification of the purified substance on the basis of an infra-red analysis showed it to be hydroquinone, which is toxic presumably as a semi-quinone. Hydroquinone occurs in pear principally in the form of the glucoside arbutin, which is readily hydrolysed into hydroquinone and glucose by a β-glucosidase on injury to the tissue. Chromatograms of aqueous extracts were made according to Kirkham's method[3] to determine whether other phenolic materials were present in concentrations toxic to *E. amylovora*. Strips were cut from the chromatograms and bioassayed using *E. amylovora* as the test organism. Inhibition zones were formed only at the region corresponding with the R_F value of hydroquinone.

Blossoms of *P. communis* were collected shortly after opening and tested for antibiotic activity. The stamens and petals were removed and longitudinal sections 100–150μ thick cut from the median part of the blossoms. These sections were bioassayed on potato–dextrose–peptone agar seeded with a standardized suspension of *E. amylovora*. Also bioassayed were pieces of tissue approximately equal in size cut from both the nectarial region and the exterior region of the receptacle. After cutting, all tissues were frozen for 10 min, placed on seeded bioassay plates, and incubated at 28° C for 24 h. Antibiotic activity was measured by the width of the inhibition zone adjacent to the tissue.

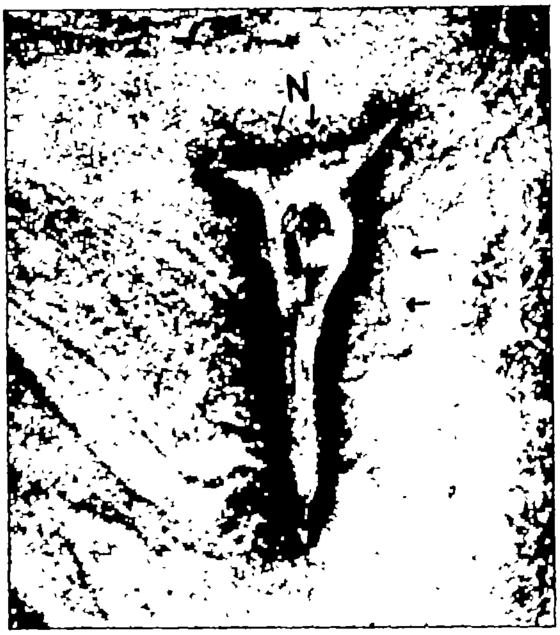

Fig. 1. Bioassay of a median longitudinal section of a pear blossom on agar seeded with *E. amylovora* showing the broad zone of inhibition except around the nectarial region (*N*). Arrows designate points of measurement used in preparing Table 1

The bioassay of the longitudinal sections of the blossom showed that the zone of inhibition was much narrower around the nectarial region than around other parts of the blossom (Fig. 1, Table 1). Often no zone could be detected around the nectarial region. The bioassay of equal-sized tissue segments cut from the nectarial and the exterior tissues of the receptacle gave results similar to those using longitudinal sections; slight or no zones of inhibition were detected around nectarial tissue pieces, whereas broad zones surrounded the exterior tissues. A chromatographic comparison of the arbutin concentration in nectarial and exterior tissues was made to determine whether antibotic activity was related to differences in arbutin concentration. No significant difference in arbutin concentration was noted. This suggests that antibiotic activity may be a function of the distribution and activity of the enzyme, β-glucosidase, rather than differences in the amount of arbutin.

Although many other natural openings occur on the blossom, *E. amylovora* usually enters through the nectaries. Here invasion and infection is so rapid that often the entrance of the organism through other natural openings is obscured[4]. This correlates with the findings that antibiotic activity is lower in nectarial tissue than in other exterior tissues. It is suggested that the arbutin–hydroquinone complex may be one of the factors which influences the progress of invasion and infection.

Table 1. INHIBITION ZONES AROUND THE NECTARIAL REGION AS COMPARED WITH THE EXTERIOR OF THE RECEPTACLE OF MEDIAN LONGITUDINAL BLOSSOM SECTIONS FROM THREE VARIETIES OF PEAR BIOASSAYED ON AGAR SEEDED WITH *E. amylovora*

Variety	Nectarial region	Receptacle exterior
D'Anjou	0·1 mm*	0·5 mm
Bartlett	0·2	0·6
Hardy	0·1	0·7

* Each value is the mean for six replications. Zones were measured at largest and smallest points and averaged. Points of measurements are indicated by arrows in Fig. 1. Mean differences are all significant at the 1 per cent level.

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Effect of Didecyldimethylammonium Bromide against Powdery Mildews and other Fungal Plant Diseases

CATIONIC surface-active agents are widely used as bactericides, and in recent years have been applied against a number of fungi of medical importance[1]. Certain agents of this type have been included in investigations at this Station of chemicals of potential value for the control of fruit diseases, especially apple mildew, *Podosphaera leucotricha* (Ell. and Ev.) Salm. Among these, didecyldimethylammonium bromide (DDAB) was outstanding both for wetting of foliage and for disease control. In all experiments, it was used as the commercial 50 per cent liquid preparation that is already widely used in the food and beverage industries, but concentrations quoted here are in terms of active ingredient for all materials.

Using a settling-tower procedure for inoculation of apple rootstocks with powdery mildew[2], we have found that protection of young foliage against this disease is related to the wetting power of the cationic surface-active agent used. Thus the well-known agent, cetrimide (cetyl tetradecyl trimethylammonium bromide), at 0·025 per cent, wets apple rootstock foliage only moderately and gives little protection against mildew. DDAB, at the same concentration, wets such foliage extremely well and provides a high level of protection against this disease.

In a comparison of DDAB with the phenolic material DNOP, obtained by hydrolysis of crude dinocap extracted from the American commercial wettable powder, DDAB was intrinsically inferior as a protective agent. However, the curative (post-infection) control given by 0·025 per cent DDAB applied 5 days after inoculation was virtually complete.

Experiments late in the season with heavily infected rootstocks have shown that one application at 0·05 per cent was sufficient to eliminate visible signs of sporulating mycelium for more than two weeks.

In the field a programme of DDAB at 0·05 per cent applied at intervals of 10–14 days until the end of June to Cox's Orange Pippin trees gave significantly ($P < 0{\cdot}01$) better control of apple mildew, as judged by secondary infections recorded in August, than one of captan plus dinocap, but produced more russet-blemish on some of the fruits.

With the settling-tower technique on potted seedlings of barley, var. Plumage Archer[2], more than 90 per cent control of mildew, *Erysiphe graminis* DC, was given without damage by protective applications of DDAB at 0·05 per cent, dinocap at 0·005 per cent, and the organophosphorus fungicide, 'W.P. 155', at 0·02 per cent.

In glasshouse tests, DDAB gave superior control of strawberry mildew, *Sphaerotheca macularis* (Wallr. ex Fries) Jaczewski, to that given by DNOP, dinocap, cetrimide, lime-sulphur, or actidione oxime. But in a late-season, small-scale field trial on the variety Cambridge Rearguard, lime-sulphur and dinocap gave more lasting control.

As sprays for control of apple mildew are usually combined with those for control of apple scab, experiments were also begun with DDAB for control of this disease. In a glass-slide test with conidia of *V. inaequalis* (Cke.) Wint., germination was inhibited almost to the same extent as by captan at the same concentrations. In a test of curative action in the glasshouse on potted MM. 109 apple rootstocks sprayed with various concentrations of DDAB 24 h after an optimal period for infection had been given, control at 0·05 per cent was fully comparable with that to be expected from 0·0015 per cent mercury as phenylmercuric chloride (PMC).

Of the compounds examined by a glasshouse technique developed for examining the action of fungicides on sporulating lesions of *Gloeosporium perennans* Zeller and Childs, DDAB has shown great promise, and at 0·05 per cent was almost equal to 0·1 per cent PMC in suppressing spore production. The field trial on apples already mentioned is designed to give evidence of field performance against mildew, scab and *Gloeosporium*.

There was no indication that DDAB damaged apple foliage in the field, and in glasshouse tests on rootstocks concentrations up to 0·2 per cent were safe; the problem raised by the russeting of apple fruits will be examined further, in view of the apparent promise of DDAB (and perhaps of related compounds) as a field fungicide.

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A Nematotoxin produced by the Nematophagous Fungus *Arthrobotrys oligospora* Fresenius

THE nematopagous fungus, *Arthrobotrys oligospora* Fres., captures nematodes by means of adhesive hyphal networks. When a nematode has been snared, the fungus pierces the animal's integument and produces a bulbous structure from which trophic hyphae grow throughout the body of the worm. Drechsler[1] attributes the death of the nematode to the partial severance of its body by the infection bulb of the fungus. This view is not shared by Shepherd[2], who found that the captured nematode becomes inactive before the infection bulb has completely developed. Duddington[3] supports Shepherd's view and suggests that a toxin, capable of paralysing or killing, may be secreted by the adhesive trapping organs and act externally, or that such a substance may be transported internally by way of the fine penetration peg through the nematode cuticle. Soprunov and Galiulina[4] claim to have detected a secretion by a hyphal network-forming species which paralyses nematodes.

In view of the conflicting reports and opinions regarding the actual cause of death of the captured nematode, it was decided to investigate this aspect of the nematode host–fungus parasite relationship.

The fungus, *A. oligospora*, was cultured in a 0·3 per cent yeast-extract amended Czapek's broth at 26° C. Seitz filtered, sterile extracts of this fungus had no apparent effect on the vitality of nematodes of *Rhabditis* sp. when they were immersed in the filtrate for periods up to 24 h. Sterile filtrates from crushed nematodes also had no adverse effect on living nematodes which were immersed in various concentrations of them for similar periods. But, when living nematodes were placed in a filtrate obtained from crushed nematodes which had been parasitized by the fungus, many of them became inactive and appeared to have been killed by some substance in the filtrate. After transferring the inactivated nematodes to tap-water a number of them revived.

In repetitions of the experiment it was found that about 30 per cent of the nematodes became inactive during a 2-h period of immersion in filtrates from fungus-parasitized nematodes and that practically all of these revived when placed in tap-water. If the nematodes were in the filtrate for 12–24 h, approximately half of them became inactive, and about 30 per cent of these were found to have died. They did not revive when placed in tap-water.

Since the osmotic pressure of the lethal filtrate was far below levels known adversely to affect nematodes[5], it is postulated that the extract contained a nematotoxic substance. It is assumed that the fungus secretes a chemical substance which paralyses or kills nematodes after they are caught by its adhesive trapping organs, because only the filtrates from fungus-parasitized nematodes contained the nematode-inactivating substance. The relatively low toxicity of the filtrates may be attributed to dilution effects. This, apparently nematotoxic, substance is probably an unstable chemical compound because a nematode population in a Petri dish culture with *A. oligospora* will usually build up again soon after the natural decline of the nematophagous activity of the fungus.

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Isolation and Maintenance of Single Spore Lines of *Peronospora tabacina* (Adam.)

While germination of conidia of the obligate parasite *Peronospora tabacina* Adam. has been effected on tobacco leaf disks[1,2], it has not previously been possible to complete the life cycle of the fungus on such disks, because of their rapid deterioration. Sporulation investigations have been possible only by taking disks from leaves which had been infected previously and in which mycelium was well established[3]. Izard[4] has described a method for the growth of *P. tabacina* on leaves and leaf disks in the laboratory, but in our experience this method has been unsuccessful with the Australian varieties of host and pathogen. Accordingly, we have devised the following technique.

Following the report by Richmond and Lang[5] on the effect of kinetin on the survival of detached leaves of *Xanthium pennsylvanicum*, leaf disks (15 mm diam.) from *Nicotiana tabacum* cv. Virginia Gold were floated, ventral side uppermost, on an aqueous solution of 1 μg/ml. kinetin. On this medium, the disks remain green and apparently healthy for approximately three weeks in the absence of infection. When kinetin is omitted from the medium, the disks show discoloration and yellowing after 5–7 days. Approximately 2×10^8 conidia of *P. tabacina* were applied in a drop of water to the centre of each disk. The disks were then incubated at 20° C under 800 ft.-candles of fluorescent illumination and a daily light regime of 16 h light and 8 h darkness.

Some necrosis occurs under the drop of conidial suspension after 2 days incubation and this afterwards extends over the leaf disk surface in an irregular manner. This necrotic reaction does not occur on control disks under a water droplet and in the absence of conidia. Sporulation occurs after 6–7 days, the number of conidiophores produced increasing to a maximum over a period of 2–3 days from their first appearance. The conidiophores appear to be confined to the green portions of the disk and have not been observed to emerge from the necrotic areas.

After 7, 8 and 9 days incubation at 20° C the average number of conidiophores produced per leaf disk was 5·6, 20·9 and 49·6 respectively (averaged over 48 disks). After incubation of a similar number of disks for 9 days at 15° C and 25° C, the average number of conidiophores per disk was 0·3 and 19·0 respectively.

When 2 μg/ml. each of *p*-aminobenzoic and nicotinic acids were added to the kinetin solution, the number of conidiophores finally produced was approximately double that occurring when these vitamins were omitted.

The infection of disks from single spores was achieved following the isolation of single conidia on agar, using a De La Rue cutter. The percentage of successful 'takes' was increased from 21 per cent to about 50 per cent when such spores were applied to leaf disks in a drop of the complete medium described by Shepherd and Mandryk[6]. Clones, isolated in this manner, have been passaged three times successfully, but some loss of viability of the organism as measured by a reduction in the number of conidiophores produced appears to occur during such serial passage on to leaf disks. This difficulty has been overcome by transferring the first leaf disk infected by the original single spore isolate to the ventral side of a tobacco leaf. If the disk is attached closely to the leaf surface by means of a thin layer of 1 per cent Difco 'Bacto' agar, the mycelium from the disk grows readily into the leaf and abundant sporulation occurs afterwards on the leaf surface. Alternatively, the first crop of conidia produced on a leaf disk following single spore isolation may be used to inoculate tobacco seedlings grown in sterile culture according to Steinberg[7].

This technique allows the ready isolation and maintenance of clonal lines of the pathogen. It opens the way to studies on the occurrence of physiological strains and to the possible isolation of biochemical mutants of this organism.

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MICROBIOLOGY

Lysogeny in *Providence* Strains and the Host-range of *Providence* Bacteriophages

Biochemically *Providence* strains are closely related to *Proteus* organisms, especially *P. rettgeri*, and on these grounds the name *Proteus inconstans* has been suggested for them[1]. The guanidine + cytosine content of *Providence* strains is very similar to those of *P. hauseri* and *P. rettgeri*[2], and recently it was found that many *Proteus* (especially *rettgeri*) phages productively lyse *Providence* strains[3]. It was decided to investigate the reciprocal relationships, that is, to test phages primarily isolated on *Providence* strains for action on *Proteus* organisms. Media used were those previously used[4]. Thirty-seven *Providence* cultures comprising 25 different serological groups were used. They are all National Collection of Type Culture strains and were kindly supplied by Miss Shona Wright of the Department of Bacteriology, University of Edinburgh. In addition, 25 *P. rettgeri* cultures (17 locally isolated, 8 N.C.T.C. strains) 17 *P. morgani* cultures (11 locally isolated, 8 N.C.T.C. strains) and 31 *P. hauseri* strains previously described[5] were used in host-range experiments. The *rettgeri* strains can all be differentiated on the basis of susceptibility to a series of *P. rettgeri* phages. The *hauseri* strains were in the *A* morphological phase and all showed a positive Dienes phenomenon when matched against one another[3].

Using each of the 37 *Providence* strains individually in an enrichment technique of sewage[5], 34 phages were isolated against a corresponding number of the strains on first attempt. The properties of these phages are still being examined, but they can all be differentiated on the basis of their host-range.

The demonstration of lysogeny in the 37 *Providence* strains was attempted by the use of the 3 methods previously described[6]. The only modification was that supernatants obtained by centrifuging the cultures were treated with 0·1 volume of chloroform instead of heating to inactivate bacteria. All 37 strains were used as possible indicators of phage activity. Twenty-three of the 37 strains (62 per cent) proved lysogenic for other *Providence* strains by one or more of the 3 methods of induction used. The 23 strains represent 19 of the 25 serological groups. Strain N.C.T.C. No. 9211 is susceptible to all 23 temperate phages. All phages were purified by repeated single-plaque propagation in broth cultures of the host organism. Phage suspensions were stored above 0·1 volume of chloroform and before use the latter was removed by bubbling the suspensions with sterile air at 45° C.

The host-range of the phages was determined by spotting drops of the suspensions on agar plates which had been layered with soft-agar seeded with enough test organism to give a lawn of growth. The phage suspensions contained about 10^7 plaque-forming particles/ml. Plates were incubated at 37° C for 4 h and then left at room temperature overnight before reading. Phages which showed reactions were then titrated simultaneously on the original host organism and on the new strain and the relative efficiency of plating calculated.

Sixteen of the 34 phages isolated from sewage productively lysed a total of 20 *P. hauseri* strains. Most of the 16 phages attacked a couple of the strains and the phage isolated on N.C.T.C. strain No. 9297 lysed 8 of the *hauseri* cultures. In contrast only one of the temperate *Providence* phages (phage from N.C.T.C. No. 9294) caused productive infection in *hauseri* strains. This phage lysed 5 different cultures. The efficiency of plating of the foregoing systems varied between 10^{-3} and unity with most near the latter value. Six of the 17 *P. morgani* cultures were productively lysed by one or more of the sewage phages. Some of the N.C.T.C. *morgani* strains were remarkably sensitive to these phages and strains Nos. 2815, 5845, 7381 and 10041 were susceptible to 28, 21, 20 and 4 of the 34 phages respectively. The efficiency of plating was often unity. None of the temperate *Providence* phages had any action on the *morgani* strains. Twenty-five of the sewage phages and 9 of the 23 temperate phages formed plaques on one or more of 21 of the *P. rettgeri* cultures. Six of the 8 N.C.T.C. *rettgeri* strains were susceptible to one or more of the temperate phages and the efficiency of plating varied between 10^{-3} and unity.

The phage relationships of the kind demonstrated here have been taken[7] to indicate close taxonomic relationships between the hosts involved and should strengthen the claim[1] for inclusion of *Providence* strains within the *Proteus* group of organisms.

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An Endotoxin-like Fraction extracted from the Cells of *Candida albicans*

ENDOTOXINS are incompletely characterized cell surface complexes which account for some of the pathogenic properties of Gram-negative bacteria. Material with similar biological characteristics has been identified in certain Gram-positive bacteria and in several plant and animal tissues[1]. The existence of a 'toxic principle' in virulent strains of *Candida albicans* has been suspected for about a decade[2]. A recent report by Hasenclever *et al.*[3] strongly supports the presence of such a fraction. Our studies of surface and subsurface antigens of this yeast suggest that several surface materials contained in the aqueous fraction after ethanol-ethyl ether[4] or phenol[5] extraction of intact yeast cells included a substance or substances with toxic activity comparable with that observed by Hasenclever *et al.*[3]. This communication presents evidence that this is indeed the case.

Antibodies produced in rabbits against intact cells of *Candida albicans* variants 1529, an old avirulent laboratory strain, and the mouse-virulent strains of *B*311 and 207 produced equivalent agglutination and cross-agglutination. (Strains *B*311 and 207 were kindly supplied by Dr. H. F. Hasenclever, National Institutes of Health, Bethesda, who also gave us details of his investigations prior to publication.) When cells which had been extracted with either ethanol-ethyl ether or phenol were used as antigens, the corresponding antibodies agglutinated homologous extracted cells to a greater degree than the heterologous strains. Antisera against untreated cells agglutinated extracted cells to the same extent as whole cells. In addition, Ouchterlony precipitin methods were used. The various antisera were reacted with the aqueous solvent-free extracts, which by themselves did not promote antibody production even with adjuvants. Antibodies against intact cells formed 2 precipitin lines with ethanol-ethyl ether as well as with the phenol extracts. These bands always displayed coalescence signifying the identity of the antigens derived from intact cells. When the immunodiffusion test was carried out with antibodies against extracted cells, 6 additional precipitin bands formed when ethanol-ethyl ether extracts were used, and 7 with the phenol extracts. Six of the ethanol-ethyl ether precipitin arcs were identical with 6 of the phenol extract bands. The phenol extract always formed an additional and distinct precipitin line with antibodies produced against the phenol-extracted cells. Chemical analysis of the extracts indicates the presence of protein and polysaccharides in proportions shown in Table 1.

The endotoxin-like nature of the complex haptens extracted from mouse virulent strains *B*311 and 207 became apparent clinically during the immunization procedures. Rabbits which received intravenous solvent-free preparations of both the ethanol-ethyl ether and phenol extracts and the extracted cells themselves manifested extensive necrosis of the skin around the sites of injection within 48 h. Similar severe skin reactions were

Table 1. COMPARISON OF EXTRACTS FROM MOUSE-VIRULENT AND AVIRULENT STRAINS OF *Candida albicans*

Property	Extraction: Virulent: Alcohol ether	Virulent: Phenol	Avirulent: Alcohol ether	Avirulent: Phenol
(*a*) Antibody precipitating (Ouchterlony)	+	+	+	+
(*b*) Antibody induction by extracts	–	–	–	–
(*c*) Carbohydrate/nitrogen	7	6	9	5
(*d*) 0·2 ml. of extract intravenously kills mice within 12 h	–	+	–	–
(*e*) Intravenous administration of extract projects against challenge 6 days later with 10^7 cells of homologous strain	+	+	–	–
(*f*) Extracts cause necrosis of skin in rabbit and mouse	+	+	–	–

+, Positive. –, negative.

observed when mice were treated with the same materials. When 0·2 ml. of the phenol extract of the mouse-virulent strains were injected into the tail veins of mice, 50 per cent died within 12 h. The survivors did not succumb when challenged intravenously with 10 million cells of the homologous strain, a uniformly lethal dose in unprotected controls. Corresponding doses of the ethanol-ethyl ether extracts were not lethal to mice; but all animals so treated were severely ill for at least 2 days and subsequent challenge with the number of yeasts fatal to unprotected controls failed to kill. The mouse-avirulent strain did not elicit any response in controls nor did extracts and extracted cells produce any ill effects. These findings, which are summarized in Table 1, suggest that material resembling endotoxin is elaborated by strains of *Candida albicans* virulent to mice.

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Cytochromes of *Pseudomonas saccharophila*

LIQUID cultures of autotrophically grown *Pseudomonas saccharophila*[1] are pink and when gassed with hydrogen the colour is intensified. On examination of spectra of cells reduced with hydrogen, using a Cary No. 11 recording spectrophotometer and the opal glass technique[2] the difference spectra showed a Soret band, flavin trough and a single peak at 550 mμ. Breaking the cells and fractionating the extract into a soluble fraction and washed small particles[1], resulted in finding a spectra characteristic of a cytochrome *c* in the soluble fraction and the presence of cytochromes of the *b* and *c* type in the particles. Comparison of the optical density of reduced-oxidized preparations at 550 mμ, shows the distribution of cytochrome *c* to be 77 per cent in the soluble fraction and 23 per cent associated with particles. Difference spectra of the particles are shown in Fig. 1. No other cytochrome peaks were ever observed in cells and extracts of *P. saccharophila*. Hand spectroscopic examination of particles in 50 per cent glycerol at the temperature of liquid nitrogen, as described by Keilin and Hartree[3], showed only three bands, one ascribed to cytochrome *b* and the other two due to the splitting of the cytochrome *c* α-band into two components. The two bands for cytochrome *c* were also observed with partially purified cytochrome *c* from the soluble fraction. Packer[4] examined another hydrogen bacterium, *Hydrogenomonas ruhlandii* and only found a cytochrome *b* and *c* in whole cells.

On fractionating the soluble extract with ammonium sulphate, 18 per cent of cytochrome *c* precipitated in the 80–100 per cent saturated ammonium sulphate fraction and the rest was soluble in saturated ammonium sulphate solution. The second fraction was dialysed and lyophilized. Absorption maxima for this fraction were 550, 523 and 417 mμ and in the case of the pyridine hæmochromogen 550, 521 and 417 mμ. Redox potential was measured using a ferrous–ferric oxalate couple[5] and E'_0 at *p*H 7·8 was + 0·08 mV. This cytochrome *c* preparation is slowly autoxidized. Only two other cytochrome *c*'s have been observed with lower redox potentials, that of *Chromatium* which has 2 hæm groups[6] and *Desulfovibrio desulfuricans* which has 2 hæm groups per molecule[7]. The other unusual property of cytochrome *c* of *P. saccharophila* is solubility in saturated ammonium sulphate solution which has also been observed with cytochrome b_3 of higher plants[8].

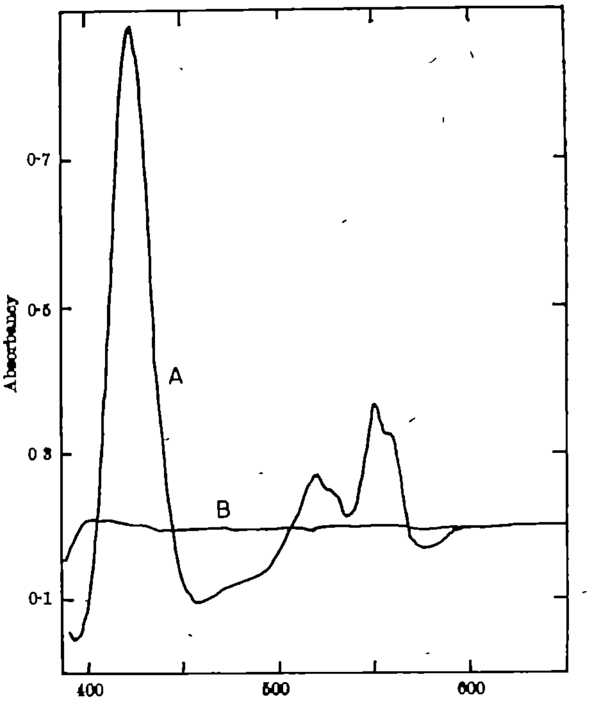

Fig. 1. Difference spectra of washed small particles. Curve *A*, particles reduced with hydrogen-oxidized particles. Curve *B*, reduced particles–reduced particles. Quantity of particles per ml. were prepared from 12 mg of dry weight of cells

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Physical and Chemical Properties of Two New Small Bacteriophages

IN recent years, several investigators have reported new bacteriophages which have a molecular weight about 100 times less than the classical *T*-series of phages[1,2]. Those small phages which have been physically characterized are roughly spherical and have a molecular weight of 4–6 × 10^6, of which 20–30 per cent is nucleic acid. The nucleic acid is either single-stranded DNA[3] or RNA[4].

We have been studying two small phages which infect only male (F^+ or *Hfr*) strains of bacteria. The phages were isolated from sewage and grown and purified using conventional methods. Both phages are resistant to DNase and RNase. One of the phages (*fr*) contains RNA and seems in many respects similar to *f*2 phage[4]. The other (*fd*) contains single-stranded DNA. It seems to have an unusual physical form.

The *fr* phage is spherical in electron micrographs, with a diameter of 200–220 Å. Its physical properties in solution are given in Table 1. Its molecular weight is calculated as

4·1 × 10⁶ using sedimentation and viscosity (assuming a perfect sphere) and 4·4 × 10⁶ using sedimentation and diffusion. The phage has 1·1 g water of hydration per gram phage from viscosity or 1·3 g water per gram phage from diffusion. Taking a mean molecular weight of 4·25 × 10⁶ the unhydrated phage would have a diameter of 210 Å. The RNA content is 28·4 per cent (orcinol method) which accounts for 97 per cent of the total phosphorus. Thus the phage contains 1·2 × 10⁶ mol. wt. RNA. The physical constants of the RNA isolated by a detergent–phenol method are shown in Table 1. The molecular weight calculated according to Scheraga and Mandelkern[4] with $\beta = 2{\cdot}25 \times 10^6$ and $\bar{v} = 0{\cdot}55$ c.c./g (c.f., ref. 5) is 1·3 × 10⁶. The RNA is therefore present as a single piece in the phage. The base percentages are: $A = 24{\cdot}3$ per cent, $U = 23{\cdot}7$ per cent, $G = 27{\cdot}1$ per cent, $C = 24{\cdot}9$ per cent. They are sufficiently distinct from those of *f2* phage to suggest that *fr* is a different phage.

Early electron micrographs of *fd* phage showed spheres embedded in a matrix of tangled fibrous material. On a macroscopic scale, infective preparations were very viscous due to the presence of those fibres. It was assumed that these fibres were pili (fimbriæ)[6] detached from the bacterial cell walls. However, during extensive attempts to clean the phage preparations by density gradient centrifugation, ion exchange chromatography, and shaking with a fluorohydrocarbon, infectivity was always found associated with high viscosity. Furthermore, it proved impossible to prepare pili from uninfected cells using methods which had been applied to prepare phage; and phage grown on pili free strains still showed high viscosity. We were therefore led to the conclusion that these fibres are in fact the phage.

To test this hypothesis we submitted mixed solutions of *fd* and *fr*, or *fd* and φ*X*174, to ultrasonics for varying lengths of time. As can be seen from Fig. 1, the infectivity of *fd* fell off extremely rapidly, while that of *fr* or φ*X*174 was scarcely affected. This sensitivity to ultrasonics is what one would expect for a long rod-shaped molecule.

Electron micrographs of carefully cleaned preparations of *fd* show long flexible rods, reminiscent of certain plant viruses[7]. (The spheres present in earlier preparations were probably contaminating ribosomes.) The rods are about 7000 Å long and about 50 Å diameter. The physical constants of *fd* in solution are listed in Table 1. For a rod-shaped molecule the mass per unit length is a function almost solely of the sedimentation constant[8]. The mass per unit length for *fd* is calculated as 1,400 mol. wt./Å. Using a length of 7000 Å as determined from electron micrographs, the molecular weight of *fd* is 9·8 × 10⁶. The molecular weight calculated from sedimentation and viscosity[4] is 11·8 × 10⁶. The total weight of infective preparations divided by their infectivity titre gives a maximum molecular weight of 16 × 10⁶ for the phage. Hence most particles are infective.

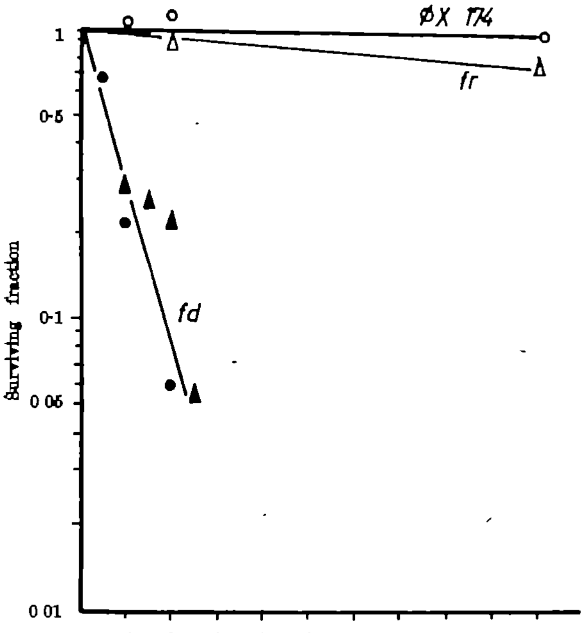

Fig. 1. Inactivation of *fd* phage by ultrasonics as compared with inactivation of spherical coli phages of comparable size. Mixtures of *fd* and φ*X*174 or *fr* (10^{11} – 10^{12} P.F.U./ml. each) were sonicated for the times indicated and plated on an F^+ sub-strain of *E. coli* *C*

Table 1. PHYSICAL CONSTANTS OF *fr* AND *fd* AND THEIR NUCLEIC ACIDS
Measurements were made at 20° C in 0·1 M sodium chloride, 0·02 M *tris* *p*H 7·3, except for *fr*-RNA, which was studied in 0·02 M sodium citrate *p*H 7·3. The estimated error is subjective and intended only as a rough guide

	fr	*fr*-RNA	*fd*	*fd*-DNA	Estimated error (%)
$S^0_{20,w}$ (Svedbergs)	79	21·0	40	24·4	± 2
[η] (dl./g)	0·044	0·80	9*	1·5	± 5
$D^0_{20,w}$ (× 10⁷ cm²/sec)	1·4	—	—	—	± 5
$\bar{v}$ (c.c./g)	0·69	—	0·71	—	± 2

* Roughly extrapolated to zero rate of shear; estimated error ± 20 per cent.

The phage contains 11·6 per cent DNA (diphenylamine method), which accounts for 83 per cent of the phosphorus; the phage therefore contains 1·3 × 10⁶ mol. wt. DNA (using a mean phage molecular weight of 10·8 × 10⁶). The physical constants for the DNA isolated by a detergent–phenol method are given in Table 1. From the Scheraga–Mandelkern equation, using β as for RNA and $\bar{v} = 0{\cdot}58$ c.c./g (ref. 9) one calculates a molecular weight of 2·3 × 10⁶. However, several considerations suggest that single-stranded DNA in solution is a more extended molecule than RNA under similar conditions; thus a larger β should be used and a smaller calculated molecular weight would result. The sedimentation constant of the DNA is similar to that found at the same ionic strength for φ*X*174 DNA[3], which has a molecular weight (from light scattering) of 1·6–1·8 × 10⁶. The measurements indicate that the DNA is present as a single piece in the phage. In low salt concentrations the DNA shows two ultracentrifuge bands of about 13*S* and 15*S*; the bands are replaced by a single band of higher sedimentation constant in high salt. This effect is similar to that found for φ*X*174 DNA. The melting curve as followed by ultra-violet absorption is typical of single-stranded nucleic acid. The base percentages are: $A = 24{\cdot}4$ per cent, $T = 34{\cdot}1$ per cent, $G = 19{\cdot}9$ per cent, $C = 21{\cdot}7$ per cent. These are clearly not consistent with Watson–Crick base pairing.

Preliminary X-ray diffraction patterns of partially oriented gels of *fd* show a strong meridional spacing at about 10 Å and a suggestion of a 60 Å layer line spacing. The diffraction patterns can be interpreted in terms of a helical structure with a 60 Å pitch and about six repeating units per turn.

We thank Dr. A. Gierer for his constructive criticism throughout this work. One of us (D. A. M.) was supported by a U.S. National Science Foundation postdoctoral fellowship.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, February 4

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. Donald W. Tull: Presidential Address.

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. L. F. Boyd: "Celestial Laboratories".*

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. M. Stacey, F.R.S.: "Recent Investigations on Immuno-Polysaccharides".

Tuesday, February 5

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. E. C. F. Bird: "Coastal Lagoons".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. C. G. Phillips: "Functions of Central Neurones".* (Eighth of twelve lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Automatic Control Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Resilience Problems in Hydraulic and Pneumatic Servo Systems".

Plastics Institute (at the Wellcome Building, Euston Road, London N.W.1), at 6.30 p.m.—Mr. R. P. Palmer: "Crystallinity in Polymers—Its Influence on Properties and Processing Techniques".

Polarographic Society (at the "Feathers" Restaurant, 36 Tudor Street, London, E.C.4), at 6.30 p m.—Discussion on "Documentation in Polarography".

Wednesday, February 6

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. A. G. Baikie: "Chromosomes and Leukæmia".*

Colour Group (Great Britain) (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 3 p.m.—Mr. R. N. Jackson: "Colour in a Television System"; Mr. R. N. Jackson and Mrs. M. L. Beeforth: "The Effects of Unmatched Taking and Reproducing Primaries in Colour Television Systems".

University of London (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Dr. F. N. L. Poynter: "Man and Nature at the Close of the Middle Ages".* (Second of three lectures on "The Passing of the Middle Ages".)

Institute of Petroleum (at 61 New Cavendish Street, London, W.1). at 5.30 p.m.—Dr. S. Reid: "Miscible Gas Injection for Flooding an Oil Reservoir—an Experimental Study of Phase Behaviour".

Society for Analytical Chemistry (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "Particle Size Analysis—Some Methods Used in the Sub-Sieve Range".

Thursday, February 7

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. Hull: "Orientation Dependence of the Deformation of Single Crystals"; Mr. K. R. Allen and Mr. K. Phillips: "Electrical Breakdown in Gases".

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 5.30 p.m.—Mr. H. S. Green: "Some Financial Aspects of Plant Selection for Industrial Refrigeration Installations".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. Marshall: "Cerebral Circulation with special reference to 'Strokes' ".* (Ninth of twelve lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Techniques of Optimization in Basic Design".

Institute of Metal Finishing (joint meeting with the Institute of Metals, at 17 Belgrave Square, London, S.W.1), at 7 p.m.—Dr. G. L. J. Bailey: "Some Characteristics of Modern Nickel + Chromium Electrodeposits".

Royal Institute of Chemistry, London Section (joint meeting with the Kingston Technical College Chemical Society, at Kingston Technical College, Fassett Road, Kingston), at 7 p.m.—Dr. C. W. Rees: "Some Aspects of Heterocyclic Chemistry".

Institution of Mechanical Engineers, Steam Plant Group (at the Royal College of Science and Technology, Glasgow), at 7.30 p.m.—Mr. G. Nicholson and Mr. E. G. White: "The Disposal of Flue Dust from a Large Generating Station".

Friday, February 8

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. B. Lennox: "Chromosomes for Beginners".*

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. C. E. Lucas: "Fisheries Research and International Collaboration".

Monday, February 11

University of London (at Senate House, London, W C.1), at 5.30 p.m. —Dr. M. F. Perutz, F.R.S.: "Structure of Proteins"* (John Coffin Memorial Lecture).

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The High-Speed Diesel Engine—Higher Performance or Lower Cost ?"

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"North America in the Making" (programme of award-winning films).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Chair of Philosophy—The Registrar, University of York, the King's Manor, York (February 8).

Turner and Newall Research Fellows in Engineering, Inorganic Chemistry, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14).

Lecturer and an Assistant in Moral Philosophy—The Secretary of University Court, The University, Glasgow (February 15).

Senior Lecturer in Education, with special reference to the teaching of geography—The Secretary, University of London Institute of Education, Malet Street, London, W.C.1 (February 15).

Senior Lecturer in Statistics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 15).

Chair of Engineering Science; and Chair of Chemical Engineering —The Secretary, The University, Exeter (February 16).

Lecturer (with a good honours degree in mathematics and experience of teaching in schools) in the Department of Education—The Registrar, The University, Liverpool, quoting CV/219 (February 18).

Lecturers or Assistant Lecturers (preferably with interests in (*a*) marine biology or freshwater biology, (*b*) ecology, systematics or physiology of mammals, (*c*) ecology of the soil fauna, or (*d*) cytology and genetics) in Zoology at the University of Ghana—The Registrar, University of Ghana—P.O. Box 25, Legon, Accra, or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (February 18)

Assistant Lecturer in the Department of Biochemistry—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 23).

Assistant Lecturer in European Prehistory in the Department of Ancient History—The Registrar, The University, Sheffield (February 23).

Lecturer or Assistant Lecturer in Pure Mathematics—The Registrar, University College of Swansea, Singleton Park, Swansea (February 23).

Nuffield Foundation Research Fellow in the History and Philosophy of Science—The Registrar, Clarendon Building, The University, Broad Street, Oxford (February 23).

Lecturer or Assistant Lecturer in Animal Husbandry at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square London, W.C.1 (February 24).

Assistant Lecturer in the Department of Philosophy—The Registrar, The University, Leeds 2 (February 25).

Senior Lecturer in Civil Engineering—The Registrar, The University, Sheffield (February 25).

Lecturer or Assistant Lecturer (preferably with special interests, teaching and research or research qualifications in one or more of the following fields: entomology, parasitology, or vertebrate zoology) in Zoology at Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

Associate Professor (preferably with medical qualifications) in Clinical Bacteriology in the Faculty of Medicine, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 1).

College Lecturer in Pure Mathematics—The Master, Emmanuel College, Cambridge (March 1).

Lecturer in Physiology—The Secretary, The Queen's University, Belfast, Northern Ireland (March 1).

Lecturers (2) (registered medical practitioners) in the Department of Pathology—The Secretary, The University, Aberdeen (March 1).

Lecturer in Atmospheric Physics—The Secretary of Faculties. University Registry, Oxford (March 2).

Chair of Geography (with special reference to Asia) at the School of Oriental and African Studies—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 4).

Lecturer in Inorganic Chemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 4).

Lecturer (preferably with a special knowledge of plant nematology) in Plant Pathology in the Department of Agriculture, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 6).

Lecturer (with a degree in science or agricultural science, and qualified in biometry, agricultural statistics or mathematical statistics) in Biometry in the Department of Agriculture, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House,. Pall Mall, London, S.W.1 (Australia and London, March 6).

Lecturer in Veterinary Medicine at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 12).

Associate Professor in Soil Mechanics at the University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 15).

Assistant Experimental Officer (graduate in a biological science (pure or applied), with an interest in chemistry or biochemistry) in the Nematology Department, to assist in work on (1) hatching factors of cyst-forming nematodes and manage the bio-assay, (2) chemical control of plant parasitic nematodes using soil sterilants and chemotherapeutants—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (March 16).

Lecturer (graduate in science or medicine) in Physiology—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 16).

PROFESSOR OF THEORETICAL PLASMA PHYSICS—The Registrar, University Registry, Oxford (March 23).

SECOND CHAIR OF BIOCHEMISTRY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

MASTER TO TEACH CHEMISTRY AND SOME BIOLOGY—The Master, Wellington College, Crowthorne, Berkshire.

MASTER TO TEACH PHYSICS to Open Scholarship level—The Headmaster, School House, Rugby.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Hat and Allied Feltmakers Research Association. Fifteenth Annual Report of the Director of Research for the year ended August 1962. Pp. 22. (Droylesden, Manchester: British Hat and Allied Feltmakers Research Association, 1962.) [1412

The British Council. Annual Report, 1961–1962. Pp. vii+107+12 plates. (London: The British Council, 1962.) 2s. 6d. net. [1412

The University of Sussex. Third Annual Report, 1961–62. Pp. 22+xii. (Stanmer, Brighton: The University of Sussex, 1962.) [1412

The Nature Conservancy. Report of the Nature Conservancy for the year ended 30th September, 1962. Pp. viii+153+5 plates. (London: H.M. Stationery Office, 1962.) 10s net. [1412

Operation Survival!—The First Step. Pp. 35. (London: Survival Books and Publications, 1962.) 2s. [1712

Proceedings of a Congregation of the University of Birmingham, 14th December, 1962. Pp. 23. (Birmingham: The University, 1962.) [1712

Proceedings of the Royal Irish Academy. Vol. 62, Section A, No. 5: Axisymmetric Solutions of the Equations of Motion of Non-Linear Viscous Flows. By S. K. Lakshmana Rao. Pp. 55–61. 1s. 6d. Vol. 62, Section B., No. 9: Noteworthy Plants of the Burren: a Catalogue Raisonné. By D. A. Webb. Pp. 117–134. 2s. Vol. 62, Section B, No. 10: Studies on Post-embryonic Growth and Development of Skeletal Muscle. By Geoffrey Goldspink. Pp. 135–150. 2s. 6d. (Dublin: Hodges, Figgis and Co., Ltd., 1962.) [1712

The Association of Universities of the British Commonwealth. Report of the Executive Council, together with the Accounts of the Association for the year 1st August, 1961, to 31st July, 1962. Pp. 36. (London: Association of Universities of the British Commonwealth, 1962.) [1912

Ministry of Housing and Local Government. Smoke Control (England and Wales) 1962–1966—Summary of Programmes submitted by Local Authorities for the Establishment of Smoke Control Areas. Pp. 18. (Cmnd. 1890.) (London: H.M. Stationery Office, 1962.) 3s. 6d. net. [1912

Other Countries

Sveriges Geologiska Undersökning. Årsberättelse for År 1961 (Tillbor Årsbok 55, 1961). Pp. 8. Översiktskartor med Beskrivningar. Ser. Ba, No. 16: Beskrivning Till Karta Over Sveriges Berggrund. Av. N. H. Magnusson, P. Thorslund, F. Brotzen, B. Asklund och O. Kulling. Pp. 290. 20 kr. Karta i tre blad 15.00 kr. per blad. Ser Ba, No. 18: Beskrivning Till Karta Över Landisens Avsmältning och Högsta Kustlinjen i Sverige. Av G. Lundqvist. Pp. 148. 10 kr. Karta i tre blad 15.00 kr. per blad. Avhandlingar och Uppsatser. Ser. C, No. 581: Phylogenetische Studien an Fossilen Invertebraten. Von Fritz Kautsky. Pp. 164+24 tafeln. 40 kr. Ser. C, No. 582: The Petrology of the Parteboda Tunnel, East of Ånge, Central Sweden. By P. H. Lindegårdh. Pp. 16. Ser. C, No. 583: Patterned Ground and Related Frost Phenomena in Sweden. By Jan Lundqvist. Pp. 101. Ser. C, No. 584: The Extension of the Serv Nappe in the Scandinavian Mountain Chain. By B. Asklund. Pp. 28+2 plates. 2.50 kr. Ser. C, No. 585: Det Svekofenniska Suprakrustalstråket Mellan Ljusterö och Rödlöga i Stockholms Norra Skärgård. Av Thomas Lundqvist. Pp. 107+tre planscher. 16 kr. Ser. C, No. 586: Vassbro Blymalmsfyndighet i Idre och dess Geologiska Inramning. Av F. R. Tegengren. Pp. 61+1 plansch. 12 kr. Ser. C, No. 587: Nya Synpunkter på Sörmlandsgnejsernas Geologi. Med Särskild Hänsyn till Stockholmstrakten. Av Goran Stålhos. Pp. 137+1 plansch. 15 kr. Ser. C, No. 589: Geological Radiocarbon Datings from the Stockholm Station. By G. Lundqvist. Pp. 23. 3 kr. (Stockholm: Sveriges Geologiska Undersökning, 1962. Distribueras genom Generalstabens Litografiska Anstalts Förlag.) [1012

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REVOLUTION IN RETAILING

FOR 'a nation of shopkeepers' the British have shown little interest in improving the quality and efficiency of their retailing services to consumers. During the past decade, however, there has been a noticeable change, and many of the growing number of little boxes which line so many urban centres are themselves beginning to give way to various types of bigger stores and supermarkets. Although such changes have profound effects on the national economy, they have hitherto attracted little notice from economists and others; lack of exact information has made it difficult to make even a general assessment of their significance.

It is encouraging, therefore, to see a book which has been produced under the ægis of the Twentieth Century Fund and represents a valuable study of distribution trends and prospects in Europe*. The authors are specialists in the field of retailing and distribution, and, although the difficulty of collecting statistical information has been immense, the material brought together and the tentative conclusions should lead to much closer investigation and control of developments in the distributive trades.

Not only is there a lack of reliable information, but also what does exist makes comparisons difficult. Butchers and bakers, for example, may be retailing establishments in some European countries but not in others, and one which includes butchers in its census of distribution may not include bakers. Further confusion arises where wholesaling and retailing activities are described together in one unit in one country while they are separated in others according to the main activity. A still greater difficulty in assessing the place of retailing in the economy of different countries is the relative absence of information on total retail sales and gross margins as well as the retail sales of individual concerns. This is largely bound up with the private nature of so many retail organizations and renders it difficult to make accurate comparison of performance and, hence, forecast trends. Nevertheless, the wind of change is blowing so strongly that a rapidly increasing number of retailers are beginning to make information available in order that they can measure their effectiveness against that of their competitors.

Figures produced by Jefferys and Knee, for example, show the overall contribution of the retailer to the European scene. In 1955, 11 per cent of the total European labour force of 132 millions was engaged in one of the distributive trades. Of the 14,500,000 engaged in distribution, 3,400,000 were concerned with wholesaling and the rest in retailing. These 11,100,000 persons were employed in 4,000,000 establishments. Not unexpectedly, the number of people employed in commodity distribution increases (13·5 per cent were so employed in the United States in 1955) as the number of those employed in commodity production decreases and, in Europe over the past forty years, the number in wholesaling and retailing has increased by about 2 per cent. Among the more important reasons why the proportion of total manpower engaged in commodity distribution has increased compared with the proportion engaged in commodity production is that the volume of goods and services distributed per man-hour in distribution has risen much less rapidly than the volume of goods produced per man-hour in commodity production. The application of scientific techniques and methods of distribution has been negligible compared with what has been done in the field of production. It is surely remarkable that, in the National Productivity Year, those retailers in Britain who wish to apply method-study to their stores and shops can find no organized course concerned with training their employees in work-study specifically applied to retailing. Nor has any of Britain's leading stores carried out any detailed investigations to improve its display, layout and methods of working even remotely comparable with the admirable work carried out by the Dillon family in the United States.

A further feature which reflects the importance of the retailer in the national life is that retail trade is increasingly becoming a source of employment for girls and women. About half the total number of people employed in retailing in Europe are female, and, equally significantly, many of them are married women who work part-time. The contribution of retail organizations to the national economy should be assessed not only by the extra purchasing power which these married women workers represent but also by the contribution it makes to their individual lives.

Although the number of people engaged in retailing has measurably increased over the past half-century, information produced by Jefferys and Knee support common observation that the number of establishments concerned with retailing has increased very little. The trend has been towards a larger average size of retail unit. In Europe between 1930 and 1955 the number of retail establishments increased by about 10 per cent. The number of persons employed during this period increased from 2·3 per retail establishment to 2·7. Of more than passing interest is the fact that, in the United States in 1954 the average number of persons engaged per retail establishment was more than double that in Europe, 5·7 persons. This may explain why, in the United States, the sales per full-time person in 1955 were, at 20,000 dollars, approximately two and a half times the average for Europe and more than twice that for the United Kingdom. On this basis, if sales per person in Europe approached the American level, the total manpower required would be about 5 million full-time persons instead of the present 10 million; and, again not without significance, there appears to be direct correlation between sales per person engaged in retailing and the level of real consumption per head in the particular country*.

Although the vast majority of individual retail establishments in Europe are small one-man businesses, the tendency is for them to decline and the larger units to grow. Jefferys and Knee extended their inquiries to see how the different types of large-scale retail organizations were increasing their efficiency and their effectiveness. Although the co-operative movement in Europe has, generally, continued to increase its share of total retail trade, its advance has, of late, been considerably slowed down and, mainly because of their method of government and direction, the Co-operative Society will probably go on losing ground to other large-scale retailers. Independent multiple-shop organizations, on the other hand, have developed rapidly since 1914 and particularly in the food trades. In the United Kingdom the number of multiple-shop branches had risen to 36,000 by 1939 against 22,000 in 1914 with a steady increase in the number employed per branch. Equally, the variety-chain-store organizations have continued to increase their number of stores throughout Europe as well as the number of persons employed. While the multiple shop retailers have followed the traditional divisions between trades—grocer, butcher, men's tailor, etc.—the variety-chain-stores have completely reversed this policy and sell a wide range of goods from food to clothes, ironmongery and, now, radio sets. This has been achieved despite intensive pressure from small and multiple retailers and even against restrictive action by Government and local authorities. The growth of the variety-chain-stores has caused the well-established and traditional department stores to examine their merchandising and retailing policies as well as their location. Traffic congestion, parking difficulties, increasing rents and rates are all adding to their difficulties, and these will be further increased by the growth of mail-order firms which sell direct to the consumer and which have shown spectacular growth since the Second World War. About 3 per cent of total retail sales in the United Kingdom are now made through mail-order companies, and, largely because of the increase of urban purchasers and the sale of branded products, this proportion is increasing.

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* *Retailing in Europe: Present Structure and Future Trends*. By Dr. James B. Jefferys and Derek Knee. Pp. xiv+177. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, 1962.) 25s.

* *Distribution in Great Britain and North America*. By M. Hall, J. Knapp and C. Winsten. (Oxford Univ. Press, 1961.)

Despite the growth of the larger retailers, however, the bulk of retailing activity is still carried out in small concerns. While their innate conservatism and desire 'to keep their business to themselves' makes change unwelcome, the threat of extinction is causing many small retailers to group together for cheaper bulk-buying and to make use of management services for mutual improvement. This co-operation, either through self-governing voluntary groups or through one of the growing number of organizations which have been set up for this purpose, has, hitherto, been largely confined to the food trades, but is now spreading to others. Its extension could well mean the preservation of many of the small-scale retailers. They have the advantage of close knowledge of their customers and of their customers' wishes, and, if they employ staff, can ensure that such staff is fully utilized. These are advantages, however, which are unlikely to stay with them long unless they develop training arrangements like those of the bigger organizations. The latter are beginning to pay not only closer attention to the selection of suitable branch managers and staffs but also are beginning to devote much greater care to determining the needs of their cusomers and what kind of service will best meet those needs.

Jefferys and Knee have not only performed a valuable service by gathering information. They have also used it to make forecasts which should be of value to all progressive retailers. So, too, has W. G. McClelland, research fellow in management studies at Oxford and still a director of a large grocery multiple group. In a recent broadcast on the Third Programme of the British Broadcasting Corporation, McClelland welcomed the spread of self-service stores and supermarkets in the food trades and hoped that these developments would spread rapidly in the non-food trades. Small margins and a high volume of sales could enable non-food supermarkets to trade as profitably as their food equivalents; they could perform an equally valuable service to the community by keeping down the cost of living. The abolition of re-sale price maintenance could also enable efficient non-food retailers to strengthen their own positions and make a marked contribution to pegging the cost of living. Price maintenance has virtually disappeared in the grocery trade because manufacturers have seen that they could not compete against other low-cost producers; unless non-food manufacturers are prepared to follow the same precept they will invite sharper competition from manufacturers in similar fields who will arrange to sell products like refrigerators direct to customers. If the latter tendency grows, the non-food retailer will find himself not only without margins but also without products to sell.

For most conclusions drawn by Jefferys and Knee there will be general approval. An overall increase of sales of up to 58 per cent by 1970 should be accounted for by a larger increase of sales in clothing, furniture, household appliances and other goods than in foodstuffs. More manpower will be required and the sales per person will increase. The number of establishments will almost certainly decrease in the next decade and the average size of them will increase; this will be even truer of foodstuff establishments than of others. The estimated increase in size of establishments will lead to a considerable new fixed capital investment in retailing, and, according to Jeffery and Knee, in Europe as a whole this may well amount to a total of 16 billion dollars. The entrepreneurs in retailing may well be forced to reconsider their traditional ways of financing capital expenditure.

But the greatest changes will have to come from the small retailers. In the next decade the multiple-shop organizations, and especially the variety-chain-store firms, will increase their share of the total trade, and small independent retailers will only survive by collaboration in merchandising and in the management of their enterprises. Before this occurs there must be radical change in the attitude of the majority of small retailers; butchers, bakers and grocers must quickly learn to become managers. The standard of retailing needs to be built up until it is accepted as a service demanding special techniques which require to be studied and developed. A number of leading retail organizations in Britain have already used the services of management consultants with considerable success in a number of ways. Instead of controlling stock by hunch, for example, operational research has been used to control the number of lines a warehouse may hold and to determine at what levels branch staffs may re-order; training courses have been devised to instruct branch managers how to keep stock-levels at an optimum. Electronic calculating machines are being regularly used to improve the information available to central buyers, while linear programming is now accepted as a means of reforming the means of distribution from factory or warehouse to shop or store. Already twenty computers are in use in the larger retailing organizations in Europe, and, as the means for hiring computing service becomes more widely known among smaller retailers, the use of statistical information will become much more common. Inter-firm comparisons will be extended to provide comparative information not only about sales per person, but also sales per man-hour and sales per linear part of the counter, if the latter continue to exist.

Before these fundamental changes come about much more attention will have to be paid to means of producing trained and competent manpower. Fundamentally, the challenge facing retail organizations is one of management, and this is daily becoming more and more apparent to many of those who control retail companies. In the next decade, two main problems will confront retail directors and these will only be surmounted by good management. The first is the supply of goods. The increasing role of the manufacturer in the branding, packing and advertising of consumer goods necessarily means much more pre-selling to the consumer and will drastically change the role of the retailer. This has already occurred with tobacconists, and is now taking place with grocers. The trend is likely to continue over a wide range of non-food goods. Instead, therefore, of grocers and ironmongers who are expert in their particular commodities, the immediate future should provide managers of grocery and hardware shops who are sufficiently informed to resist over-selling by manufacturers and overstocking by themselves.

The second problem will leave the retailer even more vulnerable. This is one of costs and margins and only those managers of retail establishments who can bring a mathematical approach to buying and distribution, a systematic approach to display and layout in their stores and shops, and a budgetary form of control to their financial accounting, can expect to attain high turnover with low margins. Moreover, unless shops and stores are organized and staffs are fully trained to provide genuine consumer satisfaction, they are still unlikely to succeed. Opening hours will have to be adjusted to make shopping possible and enjoyable, and this may mean changes in existing law. A five-day week for staff and longer opening hours are both immediately possible, and here the appropriate trade unions should see that the interests of the consumer are put first. Of education and training for the retail trades in Britain little need be said other than that what is being done is even less satisfactory than that for apprentices in industry.

England has one old-established College for the Distributive Trades, and Scotland a very new one. Adequate training for staff is provided by one leading grocery firm in England and one in Scotland. Those in control of the bigger supermarkets talk but do not act—they are mainly concerned to provide 'shelf-fillers'. Education for management in the retail trades is bedevilled by the number of proliferating bodies like the Institute of Grocers which have set up their own courses in management and so divert students from the admirable and nationally recognized arrangements first promulgated by the City and Guilds of London Institute a few years ago. In contrast to Britain's belated recognition of the need for education and training in the retail trades, there is the work of the School of Retailing, a constituent college of New York University, which was founded some forty-three years ago, and is now internationally recognized for its graduate and postgraduate work[*]. The relationship of education and training to productivity in retail organizations has been discussed by Prof. Harold F. Clark, of Columbia University, and Prof. Harold S. Sloan, of Fairleigh Dickinson University[†]. They also show that it is the trained managers who provide education and training facilities for their staffs in order that they can faithfully serve the customer. The future of retailing will depend on the quality of its management, and the experience of industry should now be used in the distributive trades to select and train individuals possessing the kind of educational background on which good management depends. Such trained managers could make a powerful contribution to the nation's productivity.

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[*] *Technical Education*, 4, No. 12 (December 1962).

[†] *Classrooms in the Stores* (Roxbury Press, Inc., Sweet Springs, Missouri, 1962).

WILLIAM HARVEY'S LECTURES ON ANATOMY

Lectures on the Whole of Anatomy

By William Harvey. An annotated translation of "Prelectiones Anatomiae Universalis", by Prof. C. D. O'Malley, F. N. L. Poynter and Prof. K. F. Russell. Pp. 239. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1961.) 64*s*.

HARVEY'S great work *De Mortu Cordis* was first published in 1628; but in the Latin manuscript of his Lumleian Lectures on anatomy delivered at the Royal College of Physicians during 1616–26 we are able to read of his earliest presentation of his views on the circulation of the blood. Indeed, in his dedication of *De Mortu Cordis* to the President of the Royal College of Physicians and to other learned physicians he writes, "on several earlier occasions in my anatomical lectures I revealed my new concept of the heart's movement and function and of the blood's passage round the body. Having now, however, for more than nine years confirmed it in your presence . . . I have yielded to the repeated desire of all and the pressing request of some, and in this small book have published it for all to see". This shows quite clearly the importance of his lecture notes, and this translation presents them in a form convenient and easy for all to read. Harvey's manuscript is in the British Museum, having been presented by Sir Hans Sloane. In 1886 the Royal College of Physicians and Royal College of Surgeons published a facsimile reproduction together with a transcription of the lecture notes; but the authors frequently refer to errors in this publication. From the reproduction of several pages of the manuscript it is clear that accurate translation and presentation of the notes must be very difficult indeed.

Apart from the interest of this first account of the circulation it is revealing to find that the College of Physicians entered the anatomical field so early (actually in 1565) and performed a function in organizing lectures that might rather be expected from the Company of Barber Surgeons, had that body been properly organized.

Harvey deals with the whole of the body, except the skeleton, but his notes contain more than pure anatomy. His interest in comparative anatomy is shown by his reference to some eighty animals. We also realize that he was a busy practising clinician and a pathologist. There are many references to the diseases of his patients, with names. Although he is presenting anatomy it is notable that his thoughts are predominantly concerned with function; he is revealed as a physiologist. Thus he states: "Since the purpose of anatomy is to know or to recognize the parts and to know them through their causes, and these in all animals, for the sake of which and therefore for this reason, (1) action, (2) use". This preoccupation with function, of course, finds its zenith in his presentation of the structure and function of the heart.

In contrast to his 'modern' grasp of so many things in the body, it is interesting to see how much he still retained of the older, empirical teachings.

The authors are to be congratulated on performing a classic task in presenting this book to the wide audience that must eagerly receive it.

One unhappy feature mars this book. This is the use as a frontispiece of a coloured reproduction of the Rolls Park portrait of William Harvey. This was unknown until it was discovered by Sir Geoffrey Keynes and described in his Vicary Lecture in 1948. The home of the Harvey family was unoccupied, derelict and severely damaged. Sir Geoffrey Keynes was able to arrange for the safe removal of the portrait and those of Harvey's brothers. They found a home in the Royal College of Physicians for some ten years and were then removed by the owner. The portrait of Harvey was fraudulently exported to the United States, where it was sold. Those responsible were heavily fined in a magistrates' court in January 1962.

Even though the portrait is of special interest in that it shows William Harvey as he was during the period he was compiling his lecture notes, it would have been more tactful not to have flaunted it so blatantly with attributions to its present American domicile and ownership in a book touching so very closely on the Royal College in which Harvey delivered his lectures and which has so recently suffered the ignominious loss of the portrait.

RUSSELL BROCK

ANTOINE FRANÇOIS DE FOURCROY

Fourcroy

Chemist and Revolutionary 1755–1809. By W. A. Smeaton. Pp. xxi + 288 + 7 plates. (Cambridge: W. Heffer and Sons, Ltd., 1962.) 40*s*.; 6.00 dollars.

THIS book is the kind of foundation on which the history of chemistry will eventually be built. There are many volumes of laborious detail in which the wood cannot be seen for trees, and many full of enticing generalizations which serious research can easily disprove; but there are not many like this one which combines thorough scholarship with a grasp of how the particular subject fits into the general picture. It is common to find the history of chemistry presented in isolation, without reference to the industry, politics, society or educational system around it. Yet chemists are certainly not cut off from the world, and this book has the great merit of showing the life of a distinguished chemist as a whole, with all his varied activities and their influence on his scientific work.

Fourcroy is, indeed, a particularly attractive subject—active both in chemical research at the crisis of the chemical revolution and in politics in the France of Robespierre and Napoleon. His election to the Convention seems to have been against his will, but he served on it and on the Committee of Public Instruction during the Terror, and then on the Committee of Public Safety, returning after an interval to serve on the Council of State under Bonaparte. As the director-general of public instruction who planned the Imperial University to control all education he might well have become its first Grand Master, but was rejected probably because the Emperor needed the Church's co-operation, and Fourcroy was a freethinker. (So was the Emperor, unofficially.)

Fourcroy has often been held responsible for Lavoisier's execution, or at least for not having done all he could to save him. Dr. Smeaton argues, however, that the evidence on which Lavoisier was condemned was the same as that against all the Farmers-General of Taxes, and shows that Fourcroy actually risked his life in speaking for Lavoisier at a meeting of the Committee of Public Safety, to which he had then no right of entry. There is no proof that the incriminating letters found among Lavoisier's papers, which have been published by Prof. McKie, were ever reported to the authorities.

Unfortunately, little is known of Fourcroy's private life and character, and the author will no doubt be criticized for not having 'brought his subject to life'. However, it is not a scholar's part to produce imaginative reconstructions without evidence. What he does bring to life most vividly is the world of chemical teaching and research in which Fourcroy was a leader. To understand the processes of formation of scientific theory, it is necessary to understand not only the few contemporary ideas which have survived but also all the concepts and attitudes, since forgotten, which seemed important at the time and were the context in which advances were made. It is in this that the author is particularly successful.

Fourcroy was a distinguished teacher and writer of text-books, and did valuable work, generally in collaboration with Vauquelin, on plant and animal chemistry, the application of chemistry to medicine, and on inorganic salts. He produced what may have been the earliest systematic scheme of inorganic analysis, and, like most French chemists of the time, was often called on to apply his knowledge for practical uses. This book, which has good indexes, notes and copious bibliography, will certainly become the standard work on its subject.

A. M. DUNCAN

GEOPHYSICAL SCIENCES

Applied Geophysics U.S.S.R.

Edited by Dr. Nicholas Rast. Pp. 429. (London and New York: Pergamon Press, 1962.) 100*s*. net.

Fundamental Problems in Turbulence and Their Relation to Geophysics

Proceedings of a Symposium held at the Faculty of Sciences and at the Institut de Mécanique Statistique de la Turbulence of the University of Aix-Marseille, September 4–9, 1961, Marseilles, France. Edited by Francoise N. Frenkiel. (International Union of Geodesy and Geophysics and International Union of Theoretical and Applied Mechanics. Reprinted from the *Journal of Geophysical Research*, Vol. 67, No. 8.) Pp. iv + 234. (Washington, D.C.: American Geophysical Union, 1962.) 5 dollars.

Earth, Sea, and Air

A Survey of the Geophysical Sciences (Addison-Wesley Series in the Earth Sciences.) By Prof. Jerome Spar. Pp. vii + 152. (Reading, Massachusetts and London: Addison-Wesley Publishing Co., Inc., 1962.) Paperback 14*s*.; Hardbound 23*s*.

WE still know too little about geophysical studies in the U.S.S.R., and Dr. Rast's translation of a selection of Soviet papers on applied geophysics is to be welcomed. There are four papers on seismic methods, two on gravity, five on electrical methods and six on bore-hole logging methods. Undoubtedly, the most useful articles for Western readers concern the methods of electrical prospecting, on which the literature in Western countries is sparse.

The papers presented at a symposium held in Marseilles during September 4–9, on "Fundamental Problems in Turbulence and Their Relation to Geophysics", and originally published in the *Journal of Geophysical Research*, have now been published together in book form under the editorship of F. N. Frenkiel. The value of this publication need scarcely be emphasized.

Earth, Sea, and Air is a survey of geophysics intended for a course in a liberal arts college in the United States. Prof. Spar feels that the arts man needs some knowledge of science if he is to be effective in the teaching or administrative work in which he will later be engaged. This notion is, of course, still quite foreign to the English university or to the grammar or public school sixth-form on the modern or classical side. The book is quite a good one, emphasizing well the exciting human quest which any scientific field, if treated historically, represents. It would be an interesting experiment to use such a book as the basis of a course in an arts department of an English university.

Slightly irritating Americanisms crop up; for example, on p. 41 we read of the founder of geology William (Strata) Smith. It is also rather out of date and treats as an article of faith that the Earth was once molten, but then almost every geologist believes this, in spite of strong geophysical and geochemical evidence to the contrary. This, however, is poetic justice, as geologists are fond of quoting, for the discomfiture of the geophysicist, Kelvin's celebrated 'proof' that the age of the Earth was only some tens of millions of years. While rejecting this, they seem notwithstanding to have swallowed whole Kelvin's idea of a cooling Earth. Only in recent times does this idea seem to have been effectively challenged.

S. K. Runcorn

NON-LINEAR OSCILLATIONS

Nonlinear Oscillations

By Dr. Nicholas Minorsky. Pp. xviii + 714. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1962.) 107s.

THIS encyclopaedic volume, of more than seven hundred pages, is the most extensive treatment so far published in book form on the theory of non-linear oscillations. It will undoubtedly become the standard reference work on the subject and no applied mathematician can afford to be without it. The book, containing thirty-one chapters, is divided into four parts and only a brief outline of the contents can be given.

In Part 1 (eight chapters) topological methods are discussed, and the theory is based largely on that of Poincaré. The theory of stability is developed, especially in relation to the ideas of Liapounov. Part 2 (nine chapters) deals with quantitative methods and is largely concerned with the classical theory of approximations. The asymptotic methods of Krylov–Bogoliubov–Mitropolsky, both for autonomous and non-autonomous systems, is presented in detail. The highlight of Part 2, however, comes in Chapter 16, which is concerned with the stroboscopic method. This method was developed by the author himself who, in 1953, was awarded *Le Prix Montyou* by the French Academy of Sciences for this work. Part 3 (eight chapters) is given to the study of the oscillations of nearly linear systems, and it is based almost entirely on the stroboscopic method. There are chapters on synchronization, non-linear resonance, parametric excitation and oscillations caused by retarded actions. The next chapter introduces the concept of a parameter space and discusses, in this space, the topology of Lienard's equation:

$$\ddot{x} + f(x)\dot{x} + g(x) = 0$$

where $f(x)$ is a polynomial. Part 4 (six chapters) is concerned with relaxation oscillations, a subject where a wide departure from the near-linearity of Part 3 frequently holds. Because of this departure entirely different methods from those of analytic approximations are necessary. The discontinuous theory of relaxation oscillations is presented and it is applied both to electrical and to mechanical problems. Asymptotic methods are then discussed, including that of Cartwright–Littlewood and that of Dorodnitzin. The book concludes with a chapter on piecewise linear idealization.

A feature of the present work is the detailed exposition of the outstanding Russian contributions to the subject over recent decades. There are many references to the original papers and every research student in this field will need to use this book. The applications of the theory are chiefly in mechanics and electricity, fields where an immense amount of experimental evidence has been brought to bear on the theory. It has happened that the mathematical models that have been constructed in these fields have been particularly robust. This has not so far been the case with model making in the fields of biology and econometrics, where many non-linear phenomena also occur. However, the applications of the theory to biological fluctuations and to econometric recurrent processes are discussed and the limitations of the models are pointed out.

L. S. Goddard

ENGINEERING ASPECTS OF SUPERSONIC AIRCRAFT

Supersonic Engineering

A Symposium held at the Royal College of Advanced Technology, Salford. Edited by J. T. Henshaw. Pp. viii + 264. (London: William Heinemann, 1962.) 63s.

IN this book Heinemann, with the assistance of Mr. J. T. Henshaw, who has edited the contributions, have bound within one volume a series of lectures delivered at a conference which was held in May 1961 at the Royal College of Advanced Technology, Salford. Having myself spent tedious hours trying to obtain scientific papers presented at similar conferences and made available only to those present, I am always pleased when I see that a publishing house has been bold enough to make a book from this kind of material.

The subject-matter of this book is far more specific than its title would suggest, for it deals almost exclusively with the engineering aspects of the supersonic transport aircraft and, so far as one can expect within its 264 pages, provides a comprehensive survey of as widely varying topics as the basic aerodynamics, the structure and materials likely to be used in its design, the noise problem, the systems engineering, the safety and airworthiness and the power plants and their aerodynamics. The book is introduced by a chapter which outlines the problems posed by the supersonic transport and there are sections dealing with the stability and control and with the economics of operation. To my knowledge it is the first work to be published which attempts to cover the complete subject in this way.

In preparing this book, no attempt has been made to produce an academic text-book, something clearly impossible within the few pages, but rather its objective has been to present a synopsis of the many problems facing the engineer and by so doing, to stimulate the interest of those, as the book says, "experienced graduates", who are at present in the aircraft industry. This it probably will do quite successfully for the book is very readable and is almost devoid of mathematical analysis. However, I would criticize it on the grounds that it probably has gone too far in its attempt to make the exceedingly complex problems appear simple if tackled with sufficient common sense. I also find that the lack of rigour in most of the contributions makes it very hard to decide when an author is stating a well-established fact or his own point of view, valuable though that may be. To this end, liberal use of references would have been most welcome, but unfortunately they are completely absent from more than half the contributions. This is even more regrettable when one considers that the aim of the volume was to stimulate the interest of engineers, which implies that they will follow up with a more detailed study in their own field if they know where the information can be found.

J. K. Harvey

HISTORY OF VISCOSE RAYON

The Beginning of Rayon

By Edwin J. Beer. Pp. 206 + 13 plates. (Paignton, Devon: Phoebe Beer, Shorton Manor, Shorton, 1962.) 20s.

VISCOSE rayon was the first man-made fibre to reach the mass market. The pioneering work, which made this possible, began 60–70 years ago and is associated with the names of Cross, Bevan, Stearn, Beer, Topham, Tetley and many others. With the exception of Beer, these pioneers have vanished from the stage, and such accounts of the beginnings of viscose rayon as have appeared are based on the recollections of the generation succeeding Beer and his co-workers, that is, they are based to a considerable extent on hearsay. The author, Mr. Beer, has made available in his book, his own recollections, reinforced by extracts from his diaries and old letters, over the period covering the last few years of the nineteenth century at Kew until a year or so after Courtaulds had set up a plant at Foleshill, then just outside Coventry, in 1905.

The author's laboratory notebooks were handed over to Courtaulds many years ago and have disappeared. I myself do not think that this is very important except in so far as it would lead to greater precision in allocating priorities to ideas and the people responsible for them. After reading the book, one is left with a feeling of respect for Mr. Stearn, "Mr. Stearn's chemist", that is, the author, and their colleagues at having succeeded in developing a technology for producing a fibre from viscose, a liquid material which varied in colour, viscosity and spinnability, according to the source and nature of the wood pulp, the purity of the chemicals available to them and the ambient temperature.

The book is, therefore, a valuable source on the early history of viscose rayon. The information presented is not interpreted, that is, the author makes quite clear when he is quoting from his diary and letters and when he is commenting on other sources of information or hearsay. However, judged from the point of view of the book's stated purpose, it is too long and discursive, containing many interpolations of the author's experiences, particularly in India, after leaving viscose. I consider that the ease of following the development of viscose rayon would have been considerably improved if the author had organized his material better. On the other hand, if he had, the reader might have missed much information about social conditions at the turn of the century and about the experiences of the author, who certainly is in many ways an exceptional man.

L. A. WISEMAN

PROGRESS IN BIOCHEMISTRY AND PHYSIOLOGY

Annual Review of Biochemistry

Vol. 31. Edited by J. M. Luck and Associate Editors: F. W. Allen and G. Mackinney. Pp. vii+731. (Palo Alto, California: Annual Reviews, Inc., 1962.) 8.5 dollars.

Advances in Comparative Physiology and Biochemistry

Vol. 1. Edited by O. Lowenstein. Pp. xii+392. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 86*s*.

VOLUME 31 of *Annual Review of Biochemistry* begins with an autobiographical chapter by Dr. Mansfield Clark, whose studies on hydrogen ions and oxidation-reduction have contributed so much to biochemistry. Most of the 21 reviews in this volume are on subjects usually covered each year, but there is at least one surprise: an article on germ-free animal research (O. Mickelsen). This deals with the subject from its beginnings, which seem to go back to experiments designed to test Pasteur's suggestion that an animal could not live in the germ-free state. The suggestion proved to be wrong, and to-day there are about a dozen centres, most of them in the United States, where colonies of animals are maintained in a germ-free condition, that is, free from all detectable organisms, whether pathogenic or not. Germ-free rats and mice have been shown to have practically normal life-spans, and chickens, monkeys and guinea pigs have also been successfully maintained in a germ-free state for long periods. The technique can be used to throw light on many problems; for example, the effect of a germ-free upbringing on wound healing or on sensitivity to subsequent infection, or the role of the intestinal flora in animal nutrition, particularly as regards vitamin requirements. Other reviews of general interest deal with polypeptide hormones (K. Hofmann), inborn errors of metabolism (J. Larner), and insect nutrition (H. L. House). There are three chapters on nucleic acids and two on different aspects of immunology.

Advances in Comparative Physiology and Biochemistry is a new serial publication. Volume 1 contains reviews on the following subjects: digestive enzymes (E. J. W. Barrington), the amine oxidases of mammalian blood plasma (H. Blaschko), temperature receptors (R. W. Murray), neuromuscular physiology (G. Hoyle), animal luminescence (J. A. C. Nicol), and respiratory mechanisms and their nervous control in fish (G. M. Hughes and G. Shelton). The reviews are illustrated with many figures. Most of the contributions in the present volume come from university departments of zoology in Great Britain. In planning future volumes, the editor will, no doubt, be looking to authors from other departments and other countries to write some of the reviews. This new publication has been planned to keep physiologists and biochemists abreast of present-day work in the comparative branches of their subjects. It has made a good beginning.

D. C. HARRISON

CANCER CHEMOTHERAPY

Biological Approaches to Cancer Chemotherapy

A Symposium held at Louvain, June 1960, under the auspices of Unesco and the World Health Organization. Edited by R. J. C. Harris. Pp. x+431. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1961.) 96*s*.

THE failure of the organizers of the Louvain Symposium to define the meaning of chemotherapy in relation to cancer enabled the contributors to this book to range over a wide variety of biological and biochemical topics related to cancer therapy. This has the advantage that the reader is stimulated by many theoretical approaches to the alleviation or cure of cancer.

Most of the thirty-one contributions are directed towards answering one or more of the following questions. What are the differences between normal and cancerous tissue and which cellular functions are most suitable for therapeutic attack? What considerations affect the design and transport of drugs having a specific effect on malignant cells and can any steps be taken to protect the organism against undesirable side actions? What methods, other than surgery and externally applied radiation, may be used in the treatment of the disease? Because relatively little progress has been made in the chemotherapy of most forms of cancer, the answers to these questions are necessarily speculative. Danielli, in the introductory paper, suggests that the differences in properties between normal and malignant tissue are such that a drug, to be effective, will have to act on two or more of these differences.

Several papers are concerned with aspects of cell or tissue life which are potentially useful as points of chemotherapeutic attack. Thus homeostatic (or feedback) mechanisms, steroid hormone–pyridine nucleotide interaction, the dependence of epithelial tissue (from 5-day-old chick embryos) on the underlying mesochyme in tissue culture, and lysosomes are subjects for discussion. De Duve speculates on the induced rupture of the lysosome as a method for destroying malignant cells.

It appears from the articles by Bergel and by Larionov that they and their colleagues are concentrating on the problem of how cytotoxic chemicals may be transported specifically to their site of action. Both are synthesizing drugs in which a nitrogen mustard moiety is attached to a peptide chain, and both claim successes against transplanted tumours in experimental animals. Other papers concern the metabolism of drugs in general, their introduction into normally impermeable cells by pinocytosis or ion transport, and the way in which drug resistance arises. Jacobson reviews the inactivation of clinically useful antifolic acid antagonists by leukæmic and other cells and suggests ways by which this inactivation may be circumvented.

Four potential methods of tumour control, which can only loosely be called chemotherapy, are also discussed. The manipulation of the hormonal environment in the control of tumours of the endocrine glands and their dependent tissues is practised widely in clinical medicine. Furth and Mühlbock detail some of their experimental work, the former using primary mammary tumours in the rat and the latter transplanted ovarian tumours in the mouse. Secondly, the existence of specific antigenic groupings in cancer cells is reviewed. It is accepted that chemically induced tumours of rodents contain antigens not demonstrable in the corresponding normal tissue, but the position with 'spontaneous' tumours is less certain. Thirdly, the induction of cancer by viruses and the possibility that viruses will be found which specifically destroy tumours is discussed. Finally, Kinosita and Maisin both review the inhibition of azo dye cancer in the rat liver.

It is noteworthy that one of the most pressing needs in the investigation of cancer chemotherapy, that is the lack of an adequate method for the biological testing of drugs, is discussed in only one paper. Garattini and Palma are more concerned with the determination of general toxicity than with the investigation of the reactions of a wide range of tumours.

There is a considerable variation in the scope and standard of papers in this Symposium. Some describe their subject in the most general terms, others report the results of experimental work. They all combine to produce a fascinating picture of the difficulties of the problem and of the methods used to overcome them.

The editing of these proceedings is not of the standard normally expected in a book of this quality. There are several typographical errors, a lack of consistency in the use of abbreviations for chemical and biochemical substances and sometimes a failure to record the meaning of these abbreviations. The lack of firm editing and careful grouping and linking of the contributions has resulted in a collection of papers rather than in a useful guide to present trends in cancer chemotherapy.

D. B. CLAYSON

ECOLOGY FOR STUDENTS

Introduction to the Study of Animal Populations

By H. G. Andrewartha. Pp. xvii+281. (London: Methuen and Co., Ltd., 1961.) 30s. net.

It is scarcely possible to cover the whole field of animal ecology in a text-book of moderate size and cost; authors are practically obliged to develop selected sectors of it, and omit others altogether. Their purpose must be to convey to the student the principles underlying the inter-relations, distribution and numbers of animals, and to give him at the same time an introduction to the methods by which knowledge can be advanced. To a considerable extent these objects can be achieved independently of the actual material and context chosen for illustration.

Dr. Andrewartha's book is based on the well-known and much larger work of Andrewartha and Birch, *The Distribution and Abundance of Animals* (1954). It is divided into a section on "Theory", which occupies about two-thirds of the book, and a "Practical Course", consisting of twenty interesting and varied experiments to be carried out in the laboratory. The main emphasis in the theoretical section is on the environment; and for the most part the context chosen is the kind of terrestrial environment that man has influenced in varying degrees for purposes of crop and animal husbandry. It is a world that comes closer than most to the doors of the average university department; its predominant inhabitants are insects, and its 'natural' populations are those that are not being directly subjected to human intervention or control, though they may be living in fields, orchards or cultivated soils.

Against this background the environment is broken down into four major components, namely, weather, food, other animals and a place in which to live. A chapter is devoted to each of these; they occupy respectively 43, 32, 13 and 8 pages. In this case, doubts are likely to arise as to whether the picture they convey does give the student real insight into the basic attributes of environments in general, as they affect the individual. If he tried later to apply the same principles to analysing quite different environments, let us say those of a whale, a termite or a tapeworm, Dr. Andrewartha's components might be found lacking in realism, and not always mutually distinguishable. For a carnivorous cetacean, Where is the line drawn between food and other animals; or, for an internal parasite, between weather, food and a place in which to live? The last component especially seems to provide an umbrella for miscellaneous factors not analysed at all, or not readily assignable to the other three. In one place (p. 16) the environment as a whole is said to comprise "everything that may influence [the animal's] chance to survive and multiply", a definition that gives the appearance of overlooking the great complementary influence that heredity and physiology are likely to have on survival and reproduction.

In the practical course each experiment is worked out in full; tables of actual measurements are given, and where appropriate the latter are subjected to a useful range of statistical tests; permissible conclusions are drawn and commented on. Use is made of populations of such common laboratory animals as *Paramecium*, *Tribolium*, *Calandra*, and mice; or of snails, wood-lice and insects collected out of doors and brought into the laboratory; with a little improvization the experiments could be adapted for use almost anywhere. They will give valuable experience in laboratory methods. Quantitative observation and experiment in the field, however desirable as a supplement, often present almost insuperable difficulties at this introductory level.

V. C. WYNNE-EDWARDS

PLANT CHEMOTAXONOMY

Chemotaxonomie der Pflanzen

Eine Übersicht über die Verbreitung und die Systematische Bedeutung der Pflanzenstoffe. Band 1: Thallophyten, Bryophyten, Pteridophyten und Gymnospermen. Von Prof. R. Hegnauer. (Lehrbücher und Monographien aus dem Gebiete der Exakten Wissenschaften. Chemische Reihe, Band 14.) Pp. 517. (Basel und Stuttgart: Birkhauser Verlag, 1962.) 96 D.M.

This book intends to supply information of mutual interest to workers studying the morphology of plants and those concerned with phytochemistry, who have kept apart because their methods and terminology are different.

The author, who uses the classifications of Gilbert M. Smith, and partly of A. Engler, points out that the presence of similar compounds in a variety of plants does not necessarily imply a close phylogenetic relationship because a substance may owe its origin to entirely different metabolic processes. For example, some fungi develop cyanic acid but they do not produce it by enzymatic liberation from glycosides, as do higher plants (p. 138).

The chapters dealing with the chemistry of each group are preceded by brief anatomical descriptions and followed by comments in which the findings are surveyed and correlated with those reported for other groups of plants. Frequently, addenda contain information published in recent papers, up to 1961.

The chemical parts are divided according to compounds like pigments, volatile oils, carbohydrates and fats, and are subdivided according to localization, for example, carbohydrates in membranes and in the extracellular space, or according to chemical principles, such as derivatives of phenol, resorcin, hydroquinone, pyrogallol. The great seasonal variation in the concentration of active substances is mentioned for *Taxus*, *Ephedra* and some other plants. Often, structural formulæ are given and metabolic pathways outlined. Occasionally, the presence of a compound and its derivatives in various groups of plants is tabulated, for example, for chrysophanol. Biological actions are noted, some not well known, like the anthelmintic effect of algae (p. 86 ff.), the antibiotic activity of purine derivatives (p. 143) and of certain kinds of moss (p. 189), and the fall in blood pressure caused by a chemically unidentified alkaloid from the root of *Ephedra* (p. 459).

Both morphological classification of plants and the grouping of phytochemical data meet with great problems. For example, Cronquist considers that the classification of the plant kingdom adopted in this work has outlived its usefulness and is now disappearing from the text-books. The relevant chemical literature, too, varies in its approach as the biogenesis of plant constituents is widely studied and the concept of chemical races developed. This book is a welcome source of information to those interested in botany, pharmacognosy, pharmacology and biochemistry.

L. WISLICKI

A SURVEY OF PALESTINE'S VEGETATION

Plant Life of Palestine

Israel and Jordan. By Prof. Michael Zohary. ("Chronica Botanica": New Series of Plant Science Books, No. 33.) Pp. xi+262. (New York: The Ronald Press Company, 1962.) 8 dollars.

TO write a balanced and succinct account of the vegetation of Palestine is probably one of the most difficult tasks that can confront an ecologist. Even in its pristine, undisturbed condition, a country with such diverse topographic, edaphic and climatic features must have exhibited complex vegetation patterns; but when one super-imposes roughly ten thousand years of varied and intermittent interference by man and his beasts, then Nature and artifice combine to produce a situation which almost defies analysis.

What the pristine vegetation of Palestine was really like will probably never be known with certainty, but this in no way derogates from the value of an observant naturalist's deductions. For this reason, everyone who is interested in the vegetation of the eastern Mediterranean and the Near East will welcome Prof. Zohary's book, which, it must be added, fully acknowledges all the difficulties, and outlines the present characteristics of Palestine plant-life, and its possible antecedents, with commendable impartiality and restraint. It is also very much to the author's credit that he has been able to view the subject from so many aspects, in the space of so few pages, without loss of clarity.

Topography, soils, climate, floristics, life-forms and all the more important plant-societies of Palestine are dealt with in considerable detail, and, for good measure, we are then offered valuable, and very instructive, chapters on ecological behaviour, and on the relationships of man with the vegetation, all this rounded off with an excellent list of references and index. One might wish, perhaps, that Prof. Zohary had relaxed a little, and freed himself a little more from the strait-jacket of Continental phytosociology. He is at his best when recording plain facts and personal observations—the unexpected dormancy of deciduous trees during the most favourable season for growth; the anomalous behaviour of the carob tree, or the failure of tall trees and bushes to invade protected areas of batha and garigue—and one hopes that his successful completion of this book will now encourage him to set about writing a more leisurely, contemplative one, with ample digressions on the quirks and oddities of plant behaviour in Palestine, and speculations on the history and relationships of Palestine plants and plant associations. Such scientific ruminations would be a valuable supplement to the factual austerities of the present volume, and, coming from one with such extensive field experience in the Palestine area, would be an important contribution to that remote but most alluring of goals—an all-embracing survey of Mediterranean vegetation and its history.

R. D. MEIKLE

MYTHOLOGY OF THE ORIENT

Oriental Mythology

The Masks of God, Vol. 2. By Joseph Campbell. Pp. x+561+23 illustrations. (London: Secker and Warburg, 1962.) 50s.

IF Volume 1 of *The Masks of God*, adversely reviewed in *Nature* (187, 7; 1960), was an unsatisfactory hotch-potch, with this second volume Dr. Campbell executes a *tour de force*. Concerned now with the mythologies of the great civilizations of India, China and Japan, Dr. Campbell has plenty of literary, historical and archæological evidence to guide him in identifying what he most happily calls the mythological 'signatures' of these civilizations. No longer basing his argument on supposition, pseudo-biology, and inadequate ethnographic reports, he moulds his analysis consistently on the firmer ground of 'social representation'. His main point is to show how, as much to-day as in the past, the East is dominated by monistic thought: the individual, a monad or parcel of monads, is but an illusory projection of the social or moral order on one hand, and the divine order on the other. Indeed, the individual only begins to be relevant when merged with the void, when nothing becomes nothing.

In Western eyes this last is more than a paradox: it is a contradiction without meaning. 'Quite so!' the Easterner might comment: and the gulf between different modes of understanding remains. In contrasting this Eastern 'signature' with the Western—where the notion of the individual bestrides the world—Dr. Campbell seems to attempt, rather uneasily, to bridge the gulf. Since both modes of thought had a common source—in Sumer, that first of all flights into the civilized condition—he seems to say, and since, through several millennia, the civilizations of the East have flowered because of fertilizing influences from the West, a *rapprochement* is possible. Perhaps. Certainly this volume in itself could be a step away from Kipling's dictum.

One says 'could be' rather than 'is' because, though the reader will find this book a mine of information, not only are syntheses such as this two-edged and inherently vulnerable to attacks from many quarters, but also this particular volume is both too long and too short. The former because the central statement is apt to be lost in a plethora of detail and a rather diffuse style, and the latter because specialists will quarrel at the use and interpretations of their particular contributions. Nevertheless, here is a book worth reading and worth keeping for dipping into. One looks forward to Volume 3, which is to consider Western mythologies, and then—may one hope for a brief and concise statement?

K. O. L. BURRIDGE

THE INTERNATIONAL RESEARCH AND DEVELOPMENT CO., LTD.

THE International Research and Development Co., Ltd. was formed in December 1962 as an independent organization undertaking a wide range of scientific and technical operations for industry and Government agencies in the United Kingdom and overseas. The directors are: Lord Fleck (chairman), Dr. H. M. Finniston (managing), G. M. Baker, Dr. A. T. Bowden and Sir Harold Mullens. The secretary is W. A. Ranson.

The company was first established in April 1959 as the Nuclear Research Centre of C. A. Parsons and Co., Ltd., at Heaton, Newcastle upon Tyne. At that time it was principally concerned with special aspects of the research and development required by one of the consortia authorized to build nuclear power stations in Britain. It soon became apparent, however, that the trained manpower, disciplines, facilities and techniques appropriate to nuclear investigations could be applied to a wider spectrum of established and advanced industrial activities. To assist industry to maintain itself in the van of technological progress, the Centre extended its services to include such interests as masers, magnetoplasmadynamics, superconductivity and solid-state physics.

International Research and Development Co., Ltd., places a staff of 500 at the disposal of organizations which require technical assistance in the fields of engineering, metallurgy, physics and chemistry. This staff includes 230 graduate scientists and technical personnel with professional qualifications.

The laboratories are on a six-acre site within two miles of the centre of Newcastle. The main block of laboratories and offices contain metallurgical, physics, computing and basic engineering facilities as well as administrative and supporting services (Fig. 1). In separate buildings are a materials fabrication laboratory, an engineering laboratory and a 140-ft. test tower. The floor space totals more than 130,000 sq. ft.

The laboratories were formally opened by the Right Hon. F. J. Erroll, President of the Board of Trade, on January 21.

The following are general descriptions of some of the activies and facilities of International Research and Development Co., Ltd.

Metallurgy

Metallurgical research is carried out in laboratories well equipped for mechanical creep and corrosion testing, metallography, electron microscopy, X-ray diffraction and materials fabrication. Rare and refractory metals and alloys with nuclear and non-nuclear applications, as well as the common metals, are the concern of the Metallurgy Group.

Physical metallurgy. An important part of the metallurgical effort has been concentrated on materials for fuel elements for the natural uranium/magnox gas-cooled type of nuclear reactor. Investigations of uranium have been directed to the understanding and prediction of its growth and swelling under irradiation, its response to heat treatment when alloyed, its creep behaviour when subjected to cyclic temperature variation and its behaviour when transformed to the higher temperature β-crystalline state. As the addition of small amounts of alloying elements may improve various properties of uranium, assessments are being made of different binary and ternary additions. This Group is equipped to prepare thermal equilibrium diagrams.

As the can containing the uranium in a reactor must maintain its integrity under arduous operating conditions, the mechanism of intergranular cavity formation in magnesium alloys under slow rates of strain has been examined. Other investigations have been concerned with the effects of heat treatment and alloy composition on grain size, creep strength and ductility in magnesium-base alloys.

For reactor systems other than the magnox type, various aspects of the metallurgy of beryllium, zirconium and appropriate stainless steels have been studied.

Outside the nuclear field, investigations have been made of brazed joints, of welds in ferrous and non-ferrous metals, of non-ferrous alloys with niobium additions and of superconducting materials such as niobium–zirconium, vanadium–gallium and niobium–rhodium.

Engineering metallurgy. The greater part of the engineering metallurgical work concerns the examination of materials for large-scale engineering applications.

The present trend in heavy engineering designs to increased size and to wider working ranges of temperature and pressure has added to the importance of the selection and assessment of materials, and in the Company many investigations are devoted to high- and low-temperature materials in a variety of atmospheres (steam, air, carbon dioxide, etc.).

General investigations are made of processing techniques, including assessments of new methods of mechanical and heat treatments, such as ausforming in modern steels. Operations investigated range from the specialized brazing of small components to the welding of large sections of heavy electrical plant and large diameter pipework for marine use. Among other investigations are those relating to frictional wear in large bearings and gas seals, erosion of turbine blades by impact with condensed-steam droplets, surface breakdown during electrical discharge and factors affecting iron loss and magnetic permeability of various materials.

The extensive test facilities include equipment for the examination of stress rupture, for the selective high-sensitivity measurement of creep up to 750° C and for measuring mechanical properties down to $-200°$ C. Among the methods used for investigation and non-destructive testing are eddy currents, hot stage microscopy, magnetic behaviour, ultrasonics and potentiostatic measurements.

Non-Metallic Materials

Among the more useful ceramic materials, which can be loosely defined as high melting-point inorganic compounds, are the metal oxides, carbides and nitrides. Ceramics are of interest in modern technology mainly because they can be used at high temperatures, but their hardness, low electrical conductivity and, in the case of nuclear ceramics, good stability under irradiation are also important properties.

The fabrication and properties of uranium dioxide, uranium carbide and uranium oxide/stainless steel cermets have been investigated in the Company's laboratories. These materials were prepared from powders by either sintering, melting, a mechanical hot-working technique such as swaging or rolling, or a combination of these processes. Properties such as thermal conductivity, thermal expansion and mechanical strength have been measured up to 1,000° C. In some high-temperature applications, for example, the magnetoplasmadynamic generator, the volatility of ceramics becomes appreciable. The volatilities of a large number of oxides have been measured at temperatures up to 2,300° C.

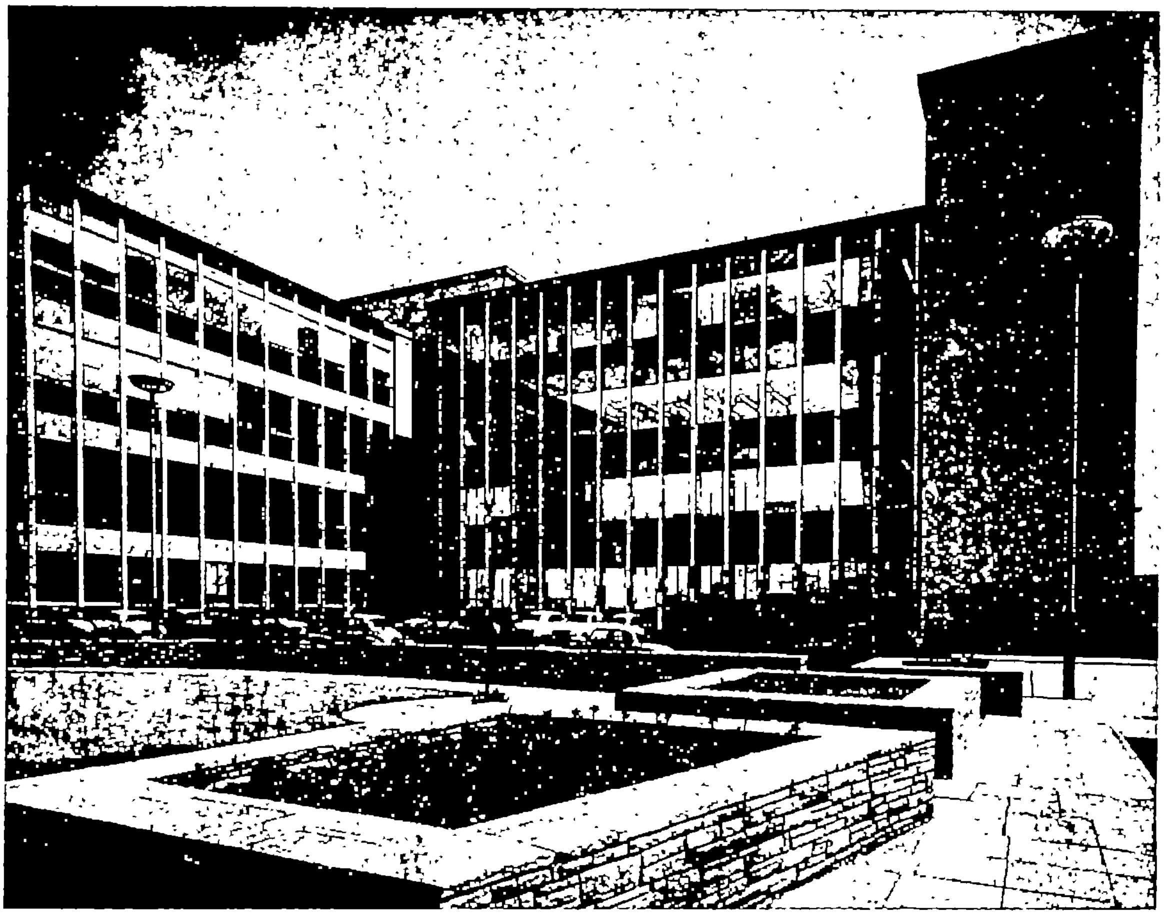

Fig. 1. The International Research and Development Co., Ltd., Newcastle upon Tyne

Other work includes the measurement of the electrical and thermal properties of magnesia powders for mineral-insulated cable, the growth of ceramic single crystals suitable for laser operation and the measurement of the thermionic emission of some materials stable at high temperatures.

Oxide semiconductors, especially materials with large positive and negative temperature coefficients of resistance, are being examined over certain temperature ranges. These materials are expected to have many technological applications in temperature measurement, control circuits and switching.

Chemistry

The corrosion behaviour of metals, alloys and non-metallic materials is especially important in engineering. An examination of the corrosion reaction between gases and solids, particularly those encountered in nuclear and conventional power plants (including gas turbines), is under way.

Mild, low-alloy and austenitic steels are being subjected to extremely long-term (up to 15,000 h) corrosion tests in carbon dioxide/monoxide mixtures in the ranges 150–300 lb./in.^{2} and 450°–750° C. Similar tests in steam at atmospheric pressure and up to 650° C are being made on low-alloy and high-chromium ferritic steels, austenitic stainless steels, nickel–chromium alloys and various hard metals towards the development of new and improved materials for advanced steam-power generating plant. Steam corrison experiments are also being carried out on newly developed zirconium alloys. Advanced techniques used in these experiments include interference microscopy for surface profile effect and radioactive tracers for diffusion measurements.

The corrosion of steels in flowing water of controlled *p*H and oxygen contents, with and without γ-irradiation, is also being examined.

Facilities are available for the detailed examination of the many types of reaction between solids, liquids and gases. A section is devoted to research and development on advanced chemical analytical techniques, including infra-red absorption spectroscopy, flame spectrophotometry, gas chromatography and the use of radioactive tracers. In addition, facilities exist for the routine chemical analysis of many industrial products.

The mechanisms and kinetics of the oxidation of graphite in air and the inhibiting effect of various compound additives on such oxidation are being studied. The aim of these experiments is to provide a volatile compound suitable for injection to extinguish a graphite fire under accident conditions. An investigation is also being made of the corrosion/erosion processes which occur at graphite anodes as used in the electrolysis of brine.

Investigations have been made of the thermal decomposition of carbon monoxide in carbon dioxide/monoxide mixtures, especially of the characterization of certain iron-bearing catalysts and the conditions under which they are effective. The carbon deposited in this catalytic decomposition of carbon monoxide at about 550° C is graphitized to a degree equivalent to that obtained with a thermal graphitization temperature of about 2,000° C. Simulation of carbon monoxide breakdown under irradiation has been achieved using an electron discharge, and the resultant carbon sub-oxides are being characterized using infra-red spectroscopic analysis.

Development work is being conducted on the production of graphitized carbon by the catalysed decomposition of carbon monoxide and on water purification by ion exchange and electrodialysis.

Engineering

Engineering covers a wide range, and varies from the construction of a large reactor test core to the investigation of sticking fuel element end fittings, and from the cooling of power transformers to the performance of gas-lubricated bearings.

Reactor projects. The building costs and safety requirements of nuclear reactors are such that before any reactor is built the satisfactory long-term behaviour of all components must be proved. This testing, sometimes of complete circuits, at other times of individual components, requires a large amount of specialized experimental plant.

Dragon reactor. The high-temperature gas-cooled reactor concept, on which the O.E.C.D. 20-MW *Dragon* project is based, involves radically new designs of core and fuel elements. The fuel elements are of highly enriched uranium dispersed in graphite and are clad in impermeable graphite. The test plant, designed and constructed in the Company, represents a section of the reactor core and consists of seven elements, each of seven hollow graphite rods. The elements are electrically heated and subjected to the conditions of gas flow, temperature, pressure and heat rating expected in the reactor; the total electrical heat input to the test core exceeds 3·5 MW. Extensive measurements of gas and surface temperatures, gas flow and pressure, vibrations and strains will give information on the thermal and mechanical behaviour of the reactor core. The continuous removal of oxidizing impurity gases from the helium is also being investigated. The information obtained should help to solve many other problems associated with the use of high-pressure helium in large vessels and helium in contact with graphite at high temperatures.

Steam-generating heavy-water-moderated reactor (*SGHW*). An alternative to the graphite-moderated gas-cooled reactor of the present British programme is the water-moderated steam-generating reactor with its many variants of moderator arrangement. The proposed fuel element for the heavy water (*SGHW*) reactor is a closely spaced multi-rod cluster. As the design is largely based on experience with single rods the experimental testing and determination of burn-out flux are of great importance. The Company has designed and built a test rig to simulate the operation of one complete fuel channel at reactor conditions of flow, pressure and temperature. The test section, containing an electrically heated multi-rod fuel element delivers a steam-water mixture into the main circulation loop. The heat input of more than 1 MW is supplied to all the rods except one, which is designed to allow a hot patch to be operated up to 'burn-out' heat fluxes.

Fuel element development. Engineering aspects of fuel element design fall into two sections: thermal and mechanical. In both cases important contributions have been made in the Company's laboratories, notably in the introduction and development to an advanced stage of the polyzonal spiral fuel element now being used in nuclear power stations in the United Kingdom.

The output of any reactor fuel element is in general subject to the two major limitations of maximum permissible cladding temperature and maximum permissible fuel temperature. Excluding metallurgical problems, the first of these limitations is largely governed by the efficiency of the heat transfer mechanism between cladding and coolant. Investigations have been made of the heat transfer characteristics of radial and longitudinal finning and, in particular, a form of spiral finning in which the cooling gas is continually being forced to leave the space between the fins and mix with the main coolant stream. New surface configurations are investigated in open-circuit air rigs or in high-pressure carbon dioxide closed loops to simulate design operating conditions of temperature, pressure and power rating. The results are processed by the Company's digital computer to yield the Stanton number, friction factor and Reynolds number which represent non-dimensionally the heat transfer, pressure drop and mass flow respectively.

Full facilities exist for the manufacture and mechanical testing of prototype fuel elements. Investigations in progress include the examination of the modes of failure of end assemblies under excessive compressive or tensile loadings, the relaxation of the can with respect to the uranium rod during thermal shock and cycling, the possibility of bowing of the element during its lifetime and the sticking of element end fittings during operation. The test furnaces vary in size from those required for a fuel end assembly to others capable of containing a full 30-ft. long stack of elements with the temperature varied along the length to simulate a reactor channel. To examine the rattling and vibration of fuel elements in coolant gas flow, single and stacked elements can be exposed to circulating cold carbon dioxide in a three-column vibration rig in the test tower. In this rig gross movements are observed through transparent windows between the mild steel duct sections, while fine movements are measured with capacity probes, accelerometers and strain gauges.

The continued action of reactor gases on hot elements may in time produce distortions which affect stability of heat transfer characteristics. To investigate this, stacks of fuel elements in vertical graphite channels are subjected to endurance tests, usually in carbon dioxide, under reactor gas-flow conditions. In an associated loop the loading, separation and discharge of strings of connected and thermocoupled elements are simulated under reactor operating conditions.

Gas-lubricated bearings. Although small gas bearings have been used for a number of years in various mechanisms such as wind-tunnel balances and electronic computers, little interest was taken in their application to heavily loaded shafts in large machinery until an incentive was provided by the development of gas-cooled reactor techniques, especially those using helium coolant.

The two major advantages of hydrodynamic gas bearings in gas circulators for reactor circuits are the elimination of oil lubrication with its attendant problem of oil leakage into the coolant and the elimination of gas seals to prevent the leakage of possibly radioactive coolant into the atmosphere. One great disadvantage is that gases have very low viscosities and during stopping and starting do not provide adequate lubrication. Thus for large bearings the selection of two compatible bearing materials, which have good wearing properties and are not subject to pick-up, is of importance. The low viscosities also require extremely fine clearances between the bearing surfaces to provide the requisite lifting forces. Test equipment has been built and operated in the Company for the study of all these problems.

Instrumentation. To meet the requirements of power stations, the Company has undertaken the design, development and supply of many instrumented systems of recording and control.

The Company has provided a large proportion of the instrumentation for nuclear reactors in the United Kingdom and overseas, having undertaken all phases from design to on-site maintenance. Typical installations include in-pile temperature, neutron-flux and gas-monitoring systems to take detailed measurements without interference with the normal charge and discharge of fuel elements. The associated data handling and overall automatic control equipment has also been built by the Company.

Instruments designed for a wide field of applications range from a simple fail-safe water detector to a capacitance-operated displacement indicator measuring to an accuracy of 0·001 in. in an environment of 1,000° C and 1,000 lb./in.^{2}.

Solid-state devices are being used in the equipment as much as possible to increase reliability and reduce maintenance. Control systems for d.-c. machine-tool motors of up to 10 horse-power have incorporated silicon-controlled rectifiers, which have also been used in alarm annunciator systems and in a.-c. power regulators. Regulators of up to 20-kW rating have been manufactured, and design studies completed for ratings up to 500 kW.

The many items of electronic equipment designed and built for specific purposes have included simulators for the study of reactor control problems and for the training of reactor operators.

Physics

The work of the Physics Group includes some of the more fundamental research and more novel investigations being made by the Company, including magnetoplasmadynamic conversion of heat directly into electricity and the superconductivity of materials at low temperatures.

Direct generation of electricity. Anticipating the eventual development of large-scale steam turbo-alternator plant to overall thermal efficiencies of about 45 per cent, it follows that any future performance improvement must be achieved through a radical change in technique. It appears impossible to better the performance of the steam plant at the temperature range over which it operates; consequently, interest must be directed towards utilizing higher temperature heat sources in conjunction with energy conversion systems which do not require highly stressed rotating parts.

The magnetoplasmadynamic generator, in which electrical energy is extracted from a plasma flowing through a transverse magnetic field, falls into this category and, of the presently known energy conversion techniques, appears to offer the most attractive possibilities for multi-megawatt power requirements. Therefore, the magnetoplasmadynamic generator has been selected for study as a long-term project and an experimental closed-cycle plant has been built by the Company. This has a mixture of helium and caesium as the working fluid and will operate at temperatures of 1,500°–2,200° C. As helium, or other chemically inert gas, would probably be used as the coolant in very high temperature nuclear reactors, such as might be developed from the O.E.C.D. *Dragon* project, this research is closely related to nuclear fission power.

A number of supporting experiments to evaluate plasma basic parameters are in progress, and diagnostic techniques include microwaves of 3 cm, 8 mm and shorter wave-lengths, spectroscopy and the use of Langmuir-type probes.

Experimental investigations are being made on the caesium-neutralized diode and also on a type of thermionic power generator in which space charge neutralization is produced by an auxiliary plasma discharge. Such thermionic generators are suitable for specialized applications in which powers from a few watts to a few kilowatts are required. Thermoelectric power generators for outputs up to a few hundred watts are being designed and constructed for applications in which their silence and reliability characteristics are particularly attractive.

Applied physics. The measurement of the physical properties of materials and a better knowledge of the solid state are important spheres of activity in applied physics. Work includes investigations of superconductivity, lasers and materials with large positive temperature coefficients of resistance, and measurements of thermoelectric properties, thermal conductivity and expansion, radiation emissivity, magnetostriction, hysteresis loss and electrical resistivity. Emphasis is placed on relations between the physical properties, examples being the correlation of thermal expansion with irradiation grain growth in uranium and the correlation of changes in Seebeck coefficients of metals and alloys with cold work. The application of physical principles to the development of modified eddy current techniques is under way, as are other non-destructive methods of testing for general use.

The existence of metals which show zero electrical resistance at temperatures near the absolute zero (usually in liquid helium which boils at about 4·2° K) has been known for many years. However, it is only recently that compounds and alloys have been discovered which have practical use in the construction of superconducting solenoids for producing high magnetic fields (greater than 50 kilogauss). Since Joule losses are eliminated and current desities can be extremely high, these promise to be more compact and economical than similar equipment using conventional techniques.

Many aspects of applications of superconductivity are being studied, including the design of refrigeration systems to produce the necessary low temperatures, feasibility studies of superconducting transformers, and the physical factors associated with the superconducting properties of selected materials produced by the Company. Prototype superconducting magnets and transformers have been constructed and tested.

Crystallography. Crystallographical research in the Company mainly concerns microstructural problems related to the performance of materials.

One major project is aimed at understanding the basic mechanism of the oxidation of graphite. Flakes of natural graphite have been examined in the electron microscope, both before and after oxidation, to determine the part that lattice or surface imperfections play as sites of active oxidation. The frequent concentration of oxidative attack in long narrow channels, changing direction in a crystallographic manner and with an impurity particle at the head of each channel, seems to indicate that catalytic oxidation is taking place. In this work an important facility is a specimen stage which permits the heating of graphite, or other materials, in a controlled atmosphere inside the electron microscope at temperatures up to 1,300° C, so enabling the oxidation process to be observed directly. The electron microscope is also used for the monitoring of crystallite size during the graphitization of thin films of amorphous carbon prepared by vacuum evaporation.

Another typical investigation has been the relating of microstructure to magnetic properties in evaporated films of iron–3·25 per cent silicon, the type of alloy used in transformer core sheet.

The X-ray laboratory includes diffractometer and microfocus units. Subjects examined have included the evaluation of lattice parameters for uranium carbides with different carbon contents, the determination of preferred orientation in uranium and beryllium, and the examination of various corrosion products and deposits in oils.

Irradiation. Experimental irradiation equipment has been developed and built by the Company for operation in research reactors of various organizations at home and abroad.

A typical project is the undertaking, in conjunction with the Nuclear Power Group and the Italian organizations Agip Nucleare and C.N.E.N., of a series of irradiation tests in the Euratom high-flux research reactor at Ispra. Specimens of enriched uranium and uranium alloys, manufactured by the Company, are irradiated to high burn-up in rigs, each of which consists essentially of a water-cooled assembly containing ten doubly canned stainless steel capsules in a helium atmosphere. Each capsule is capable of accommodating up to three specimens which are immersed in liquid sodium for improved heat transfer. Temperatures are carefully monitored and a 'thermal-history' rig outside the reactor identifies effects due to temperature alone. Theoretical studies include the evaluation of the uranium-235 enrichment necessary to achieve a given burn-up of fissile material in a fixed irradiation time, and the assessment of nuclear changes in the specimens as a function of irradiation dosage. Radiation shielding requirements and the radioactivity of rig components after irradiation are also calculated.

Health physics. All work with radioactive and toxic materials in the laboratories is constantly monitored by the Health Physics Section. This Section also advises on safety procedures for new techniques or materials and, as a public service, assists external organizations.

Theoretical Services

The Theoretical Group carries out mathematical investigations of problems which arise during practical research, and provides a service for the performance of routine calculations. It has a variety of analogue computers and a Ferranti *Pegasus* digital computer.

One of the major theoretical contributions has been an analysis of the creep of uranium, under thermal cycling and under irradiation, based on the assumption that deformation is locally homogeneous and that the difference in the expansion along the three crystal axes causes internal stresses which accelerate the creep rate. The theory has been applied to predict the rate of collapse of hollow fuel elements under external pressure, the rate of bowing of stacked fuel elements, and the swelling of uranium caused by the formation of bubbles of gaseous fission products.

There is an exceptionally flexible analogue computing service, using computers designed and built in the Company. These are used to simulate many operations and can be readily programmed to study overall automatic control equipment and the dynamic behaviour of engineering plant and systems, such as boilers and turbines.

The computing services are frequently used in design studies. These have included investigations of vibrations in large bearings and stresses in bodies of unusual shape, and the design of transformers, dry cooling towers and magnetoplasmadynamic power generators.

High-Voltage Laboratory

At present most of the high-voltage work at the Company concerns corona investigations. Both fundamental and practical studies are being made of corona discharge effects in solid insulation and liquid-immersed insulation assemblies. Electrical measurement aspects of corona discharges are also examined to evolve diagnostic techniques to determine the nature and extent of possible discharges and discharge damage, not only in small samples but also in complete assemblies. An extensive investigation has been made of the breakdown mechanism in high vacuum.

A typical investigation is that of the parameters affecting gas evolution in an electrically stressed oil gap, leading to the eventual breakdown of the oil. Other projects include investigations of conduction currents and the assessment of practical aspects of insulation processing.

To facilitate low-level discharge measurements the main Corona Laboratories have been screened, reducing the external radiofrequency disturbances from 40,000 μV to 6 μV at 1 Mc/s, and a corona-free high-voltage busbar system has been developed to avoid direct interference from the test equipment.

Equipment is available for experimental techniques involving direct recording of corona pulses by ultra-fast oscilloscopes and for pulse amplitude/frequency analysis using pulse counters. High-voltage bridges, dispersion meters and equipment for the calibration of all instruments is also available. Voltage sources include a 300-kV insulated tank transformer, noise free up to 150 kV, a 150-kV stabilized d.-c. supply and several smaller high-voltage sets.

Production Facilities

The specialized engineering facilities and the experience gained in the preparation of test and experimental equipment enable the Company to manufacture prototype and early production models of many components and instrumentation units for outside organizations.

Among the items the Company produces for nuclear purposes are irradiation rigs and instrumentation, nuclear fuels (including high-density uranium carbides), reactor simulators, experimental test rigs and many other reactor components. Products with an engineering bias include silicon-controlled rectifier units to control furnaces and motors, machines to measure the creep of materials in vacuum, water-cooled heaters of ratings up to 25 kW/ft.-length and vacuum tantalum furnaces operating at up to 2,200° C. More specialized products, usually designed for particular requirements, include arc jets. devices based on lasers, semiconductor devices, rare metal alloys (including superconducting materials) and equipment for plasma physics and cryogenics.

Client Relationship

All contracts are initiated by 'Project proposals', which establish the work required, suggested approach, staff assignment, estimated time and cost, and reporting arrangements. Unless an especially detailed preliminary study is required, this proposal is submitted to the client without financial obligation.

All sponsored work is confidential and senior staff have Government security clearance appropriate to their work. Any patent arising from a project is the property of the client.

NATURE OF STRONG RADIO SOURCES

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IN a recent paper[1] we have considered the possibility that masses of gas, $10^5 M_\odot$ to $10^8 M_\odot$, may accumulate at the centres of galaxies, and may behave as stars. The existence of such objects is supported theoretically by the energy requirements of strong radio galaxies, $\sim 10^{60}$ ergs or more[2,3], which seem to demand large aggregations which either draw on nuclear energy or on the very considerable gravitational energy that can arise in a massive body of stellar dimensions. The reason why gravitation can play a decisive part in the evolution of a very massive body is that the gravitational energy depends on $|\Omega| = GM^2/R$, where G is the gravitational constant, M the mass, and R a characteristic dimension. Since gravitational energy depends on the square of the mass, it can be of decisive importance for bodies in the range $10^5 M_\odot$–$10^8 M_\odot$, whereas it is in general unimportant in ordinary stars.

The view that such objects can exist is supported observationally by the Seyfert galaxies[4], which possess nuclei having dimensions of ~ 100 parsec containing gas in violent dynamical motion with velocities from $\sim 1{,}000$ km sec^{-1} to $\sim 5{,}000$ km sec^{-1}, the total dynamical energy of the gas being of order 10^{54} ergs. The energy in emission lines amounts to $\sim 10^{43}$ ergs sec^{-1}, corresponding to an astronomical magnitude of about -19. In ref. 1 our consideration of the hydrogen-burning phase for a star of mass $\sim 10^{8}\ M_\odot$ showed that the magnitude is in the region of -18. Moreover, the total energy output from the whole hydrogen-burning phase is $\sim 10^{57}$ ergs, which is of the order observed in the Seyfert galaxies.

We may add that although Seyfert galaxies appear at first sight to be uncommon objects, comprising perhaps 1 per cent of all major galaxies, their very short lifetimes, as galaxies with nuclei in eruption, imply that their number taken over a cosmological lifetime of $\sim 10^{10}$ years is high. To explain the observed frequency of Seyfert galaxies it is necessary that the average major galaxy (mass $> \sim 5 \times 10^{10}\ M_\odot$) shall undergo an outburst 10–100 times during its lifetime.

Two or three of the Seyfert galaxies are radio sources of intensities intermediate between normal galaxies and the strong sources. Our suggestion is not therefore that the observed activity explains the strong sources, but rather that the observed activity in Seyfert galaxies is a weak example of the process that gives rise to the strong sources—explicitly that masses of $\sim 10^{8}\ M_\odot$ are involved in the strong sources rather than 10^{5}–$10^{6}\ M_\odot$.

In our former paper[1] we considered evolution beyond helium burning, up to temperatures of $T_9 = 1$ or 2 (T_9 is in units of 10^9 K). We were concerned with comparing the loss of energy due to neutrino emission and the energy released by the burning of oxygen-16. Our aim was to show that the latter was greater than the former, and that an explosion could ensue from catastrophic oxygen burning, in analogy to what we have found[2] for stars with masses of $\sim 30\ M_\odot$. Our conclusion was that such explosions could occur, and we termed them super-supernovae. However, it has appeared on further investigation that this conclusion, in the form in which we stated it, was erroneous. The energy required for the creation of electron-positron pairs, which we had found at $M \simeq 30\ M_\odot$ to be less than the yield of energy from burning oxygen, turns out to play a dominant part at very large masses. It entirely overwhelms any form of nuclear energy generation, which therefore cannot lead to an explosive outburst of a really massive star. This is not to say that very massive stars cannot undergo violent disruption, but that the cause of disruption does not lie in a sudden nuclear energy generation at $T_9 \simeq 2$, as it probably does in ordinary supernovae.

During burning of hydrogen and helium the star is entirely convective (it being assumed that convection is not inhibited by a magnetic field). During these phases the ratio β of gas pressure to total pressure is $\ll 1$, and the density is proportional to $M^{-1/2}T^{3}$, both for a particular element of material as the star evolves, and also from one element to another at any moment. The radius R of the star is proportional to $M^{1/2}$ and to the reciprocal of the central temperature T_c; in fact:

$$R \simeq \frac{5{\cdot}8 \times 10^{9}}{(T_9)_c}\left(\frac{M}{M_\odot}\right)^{1/2} \text{ cm} \tag{1}$$

where $(T_9)_c$ is in units of 10^9 deg K. During burning of hydrogen $(T_9)_c \simeq 0{\cdot}07$, so that $R \simeq 8 \times 10^{10}\ (M/M_\odot)^{1/2}$ cm. The radius is about seven times smaller than this during burning of helium when $(T_9)_c \simeq 0{\cdot}5$.

After burning of helium to oxygen-16, which lasts for $\sim 10^{4}$ years or less, the central temperature rises. As $(T_9)_c$ approaches unity, neutrino losses shorten the evolution time to about a day. This, however, is the main physical effect of the neutrino losses, since pair creation then dominates energy considerations. With pair creation dominant we have the following formulæ, deduced on the basis that the star is still in approximate hydrostatic equilibrium as a polytrope of index $n = 3$:

$$\beta \simeq \frac{180}{\pi^4}\bar{K}_2\left(1 + \frac{180}{\pi^4}\bar{K}_2\right)^{-1} \tag{2}$$

$$\mu \simeq \left(\frac{180}{\pi^2}\right)^{1/2}\left(\frac{M_\odot}{M}\right)^{1/2}\left(1 + \frac{180}{\pi^4}\bar{K}_2\right)^{3/4}\left(\frac{180}{\pi^4}\bar{K}_2\right)^{-1} \tag{3}$$

$$\mu\beta \simeq \left(\frac{180}{\pi^2}\right)^{1/2}\left(\frac{M_\odot}{M}\right)^{1/2}\left(1 + \frac{180}{\pi^4}\bar{K}_2\right)^{-1/4} \tag{4}$$

$$\rho \simeq 1{\cdot}30 \times 10^{[illegible]}\left(1 + \frac{180}{\pi^4}\bar{K}_2\right)^{3/4}\left(\frac{M_\odot}{M}\right)^{1/2} T_9^{3} \text{ g cm}^{-3} \tag{5}$$

and equation (1) becomes:

$$R \simeq \frac{5{\cdot}8 \times 10^{9}}{(T_9)_c}\left(\frac{M}{M_\odot}\right)^{1/2}\left(1 + \frac{180}{\pi^4}\bar{K}_2\right)_c^{-1/4} \tag{1'}$$

Here ρ = density, β = ratio of gas pressure to total pressure, μ = mean molecular weight, $\theta = m_e c^2/kT$ and $\bar{K}_2(\theta) = \frac{1}{2}\theta^2 K_2(\theta)$, K_2 being the modified Hankel function of order two. Note that $\bar{K}_2(\theta)$ is normalized to unity as $\theta \rightarrow 0$, $T \rightarrow \infty$. At low temperatures $\bar{K}_2(\theta)$ approaches zero. In equation (1′), c designates centre. The foregoing expressions have been derived on the assumption that the electron–proton pairs are much more numerous than the electrons associated with nuclei but are still strictly non-degenerate. More accurate expressions for pairs dominant but not necessarily non-degenerate can be derived on the assumption that the chemical potential is zero for electrons and positrons in which case $\bar{K}_2(\theta)$ is replaced by various series expansions involving $\bar{K}_2(n\theta)$, $n = 1, 2, 3, \ldots$. At $T_9 \sim 1$ the foregoing expressions are sufficiently accurate for $M/M_\odot \gtrsim 5{,}000$ and at $T_9 \sim 2$ for $M/M_\odot \gtrsim 100$. The expression for β is independent of the assumption that the star is a polytrope of index $n = 3$. The numerical coefficients in the remaining expressions are not too sensitive to this assumption.

As T_9 approaches 2 the energy necessary for pair creation must be supplied by gravitation—it cannot be supplied by oxygen burning, as it is in stars with $M \simeq 30\ M_\odot$. This leads to a departure from hydrostatic equilibrium and the above formula relating ρ and T is no longer valid, the density rises without the temperature keeping step.

In this connexion we have derived the following curious result. For a normal star all equilibrium states possess negative total energy, that is, less energy than a dispersed state at infinity. But in the case of very massive stars, with T_9 high enough for pairs to play a critical part, the equilibrium state has positive total energy. We find the excess of energy, Q, compared with the dispersed state, is:

$$Q = \tfrac{1}{2}\,|\Omega|\, < \beta \,d\ln\bar{K}_2/d\ln T > \tag{6}$$

where again $|\Omega|$ is the absolute magnitude of the total gravitational energy of the star. The average must be taken throughout the star. But, since β and $d\ln\bar{K}_2/d\ln T$ are everywhere positive, the average is positive. In deriving this result we included the rest mass of the pairs. If this had not been included we should have obtained a negative value, as in the normal case. However, since pairs annihilate on dispersal our procedure would seem to be correct. At $M = 10^{8}\ M_\odot$ the rest mass of the pairs is comparable with that of the original nuclei when high temperatures are reached.

It is emphasized that hydrostatic equilibrium was assumed in obtaining the above result. An actual star need not be in hydrostatic equilibrium—indeed, the obtaining of a positive value demands that an actual star cannot be in equilibrium once pairs dominate over the electrons that were originally present. For the total energy cannot become positive, except possibly in the case of an explosive release of nuclear energy—and for very massive stars this is not the case. Hence an actual star implodes.

Lack of mechanical equilibrium implies that dynamical velocities are generated. For the masses under consideration, these velocities are not greatly below the speed of light. Thus, the velocities are of the order of, but less than, $(GM/R)^{1/2}$. Using the equation (1′) for R:

$$\frac{GM}{R} \simeq 2\cdot3 \times 10^{15}\left(\frac{M}{M_{\odot}}\right)^{1/2}\left[T_{s}\left(1+\frac{180}{\pi^{4}}\overline{K}_{s}\right)^{1/4}\right]_{c} \tag{7}$$

For $M/M_{\odot} = 10^{8}$, $(T_{s})_{c} = 2$, $\theta \simeq 3$, $\overline{K}_{s} \simeq 0\cdot28$, $(GM/R)^{1/2} \simeq 7 \times 10^{9}$ cm sec^{-1}, while for $M/M_{\odot} = 10^{9}$ the velocity of light is approached closely.

Two possibilities now arise. First, an idealized case of spherically symmetric implosion to the relativity limit $2|\Omega| \rightarrow Mc^{2}$. As this limit is approached general relativity must be used, and the effect of radiation on the gravitational field must be considered.

A more likely practical situation is one in which the star is rotating or does not possess strict symmetry and in which portions of the star break away from a central mass. The simplest case is one in which two pieces break away, moving in opposite directions, leaving a central mass that ultimately condenses to the relativity limit. Such a central mass must always be present, since energy considerations demand that the energy of the dynamically ejected portions be compensated by the gravitational binding of the remaining mass.

We see that masses perhaps of order 10^{7} $M_{\odot}$ can in this way be ejected at speeds comparable to c. The dynamical energy is less than, but comparable to c^{2}, say $\sim 10^{20}$ ergs g^{-1}. For $M = 10^{7}$ $M_{\odot}$ this implies an outburst of $\sim 10^{60}$ ergs. This we regard as the energy of outburst of a strong radio source. Our point of view is that the energies demanded by the strong sources are so enormous as to make it clear that the relativity limit must be involved. Normal dynamical motions within a galaxy give kinetic energies of no more than 10^{16} ergs g^{-1}, and this falls far short of the radio source requirement on any reasonable basis for the total mass involved. Even nuclear energy, yielding 6×10^{18} ergs g^{-1} in the case of hydrogen burning[6], requires an outburst involving 10^{8} $M_{\odot}$. While this mass is not impossibly high, hydrogen burning is not an explosive process, as has formerly been pointed out[7]. The light elements, carbon and oxygen, are the best materials for providing a sudden outburst, but these materials yield only 5×10^{17} ergs g^{-1}, and the necessary mass is then increased to $\sim 10^{9}$ $M_{\odot}$. The situation is made worse by the circumstance that upwards of 10^{60} ergs is very likely required for the most powerful radio sources. On any reasonable basis as to the efficiency of production of radio waves, the energy required lies at maximum in the range 10^{61}–10^{62} ergs, and this seems too much to attribute to the burning of the light elements. Our present opinion is that only through the contraction of a mass of 10^{7}–10^{8} $M_{\odot}$ to the relativity limit can the energies of the strongest sources be obtained.

Finally, the materials ejected have an important bearing on nucleosynthesis, particularly in their relation to what we have called the r-process[6]. There is strong evidence that the r-process nuclei were built from seed nuclei immersed in a sea of neutrons: to build the heaviest nuclei at least 200 neutrons per seed nucleus are needed. The temperature must have been about 10^{9} deg. K, as was shown by Burbidge, Burbidge, Fowler and Hoyle[6]. At this temperature, light nuclei, magnesium in particular, are strong neutron absorbers, and hence there cannot have been large numbers of light nuclei present (as there can be in the case of the s-process, which operates at lower temperature). This led us to propose[7] that the r-process arises from a mixture of neutrons and α-particles, a mixture given by a phase change break-up of iron-group nuclei, the phase change occurring at $T_{9} \simeq 5$. Such material on being ejected from a star cools due to adiabatic expansion, and as it does so the tendency is to rebuild heavy nuclei. However, the first step of the rebuilding process 3 helium-4 → carbon-12 is slow. We pointed out that for sufficiently low density only a very small fraction of the helium is able to build to carbon-12, and this small fraction we took as our seed nuclei. But we were not really able to keep the density low enough for the case $M \simeq 30$ $M_{\odot}$, which we then considered. We wish now to point out that at much larger masses the density is significantly lower (see equation 5) and the process works without difficulty.

A further satisfactory feature is that a difficulty concerning the absolute amount of r-process material is also resolved. So long as we took supernovæ occurring at a frequency of 1 per 300 years as the seat of origin of the r-process, the amount was grossly too large, at least by two orders of magnitude[8]. We now estimate that the amount of r-process material produced per outburst is $\sim 10^{2}$ $M_{\odot}$, approximately independent of M. With about 100 such occurrences in the lifetime of the Galaxy, the total production $\sim 10^{4}$ $M_{\odot}$, in good agreement with the observed absolute abundances.

Our previous estimates of the age of the Galaxy are changed by these considerations. On the basis of the observed abundances of thorium-232, uranium-235 and uranium-238 in solar system material and the calculated production rates in r-process events, we showed[8] that nucleosynthesis occurred over an interval 7 to 8 × 10^{9} years in duration prior to the formation of the Solar System 4 to 5 × 10^{9} years ago. This sets a low limit on the age of the Galaxy equal to 12 × 10^{9} years with an uncertainty of the order of 2–3 × 10^{9} years. We placed the r-process in stars with masses just above $1\cdot5$ $M_{\odot}$ for which the evolution time is $\sim 3 \times 10^{9}$ years and thus arrived at a galactic age $\sim 15 \times 10^{9}$ years. In a later paper Fowler[9] placed the r-process in stars of somewhat lower mass and arrived at an age $\sim 20 \times 10^{9}$ years. For the reasons advanced in this work we now assign the r-process primarily to very massive stars with very short lifetimes which lie in the centre of the Galaxy and hence believe that the best present estimate for the age of the Galaxy, or more specifically the galactic nucleus, is:

$$t_{GN} = 12^{+3}_{-2} \times 10^{9} \text{ years}$$

Small stars which formed early in the history of the Galaxy and which still exist because of long evolutionary lifetimes will contain heavy elements previously produced in the rapidly evolving very massive stars. The abundance distribution among the heavy elements and their isotopes in these old stars will differ considerably from that in young stars. Elements and isotopes produced in the r-process will be relatively over-abundant compared with those produced in the s-process[10,11]. The e-process elements near iron will be peculiar in that a short time scale e-process leads to abnormal ratios of chromium, manganese, iron, cobalt and nickel and especially among the isotopes of these elements. Comparisons of old-star spectra and young-star spectra should prove to be most significant in this respect.

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EVIDENCE FROM ISLANDS ON THE SPREADING OF OCEAN FLOORS

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THE question whether the continents have been fixed in approximately the same relative position since their creation, or whether they have moved has been debated for fifty years. Perhaps the reason that this has never been settled is that much more is known about the continents than about the ocean floors, where the decisive evidence probably lies. If the Earth has been rigid, the history of the ocean basins should parallel that of the continents and large parts of the ocean floors should be old; but, if continents have moved, those parts of the floors exposed by the motion should not be older than the time of drifting. Explorations of the ocean floors may settle this matter, but in the meantime information has been sought from the literature published about ocean islands. This article deals with the evidence so obtained of possible horizontal motions of islands. Vertical motions have been discussed elsewhere[1]. Only those islands have been considered which lie in the main ocean basins, beyond continental shelves, island arcs and the andesite line[2]. This excludes such islands as New Zealand, Fiji and the West Indies. Consideration has been given to the possibility that there may or may not have been continental drift, but that if it has occurred it could have done so in an infinite number of ways. Special attention has been directed to the hypothesis that, if continents have moved, the motion may have been due to the action of convection currents rising under mid-ocean ridges and descending under the system of young mountains and islands arcs.

Table 1 lists islands for which fossiliferous limestones provide ages and a few islands dated by other means. The oldest dates reported for each island are given, but they are, of course, only minimum ages for the islands. J. L. Kulp's time-scale has been used[3]. Madagascar, Seychelles and Falkland Islands, which are well known to be atypical, will be omitted from this discussion.

Fig. 1 is a frequency diagram of the results. It makes a remarkable contrast with a similar diagram for continents which would be close to the dotted line shown on the diagram[42–44].

One immediate reaction to this apparent youth of the islands is to suggest that the ocean basins are nevertheless permanent and old, but that the study of islands is a poor method of sampling the floors. Another possibility is that continental drift has occurred as suggested by A. Wegener and A. L. Du Toit; that F. A. Vening Meinesz, A. Holmes, D. T. Griggs, R. S. Dietz, H. H. Hess, S. K. Runcorn and others have been correct in their advocacy of convection currents; and that the youth of the islands reflects the youth of the ocean floors.

To help make a choice between these alternatives, the distance of each island to the nearest point on a mid-ocean ridge was measured. The results for the Atlantic and Indian Oceans are given in Table 1 and plotted in Fig. 2.

Fig. 2 shows that the distance of islands from the ridge increases with the age of the islands. Although the correlation is not perfect it seems adequate to suggest as one possible explanation that many ocean islands have originated over the mid-ocean ridges and that the older ones have been steadily carried away from the ridge. In the Atlantic Ocean it is noticeable that six active or very recently active volcanic islands lie along the ridge. They are Jan Mayen, Iceland, the Azores, Ascension, Tristan da Cunha and Bouvet. On the other hand, islands with Cretaceous rocks, the Bahamas, Fernando de Noronha and Cape Verde, lie close to continents, while islands of Miocene to Eocene age, like the Faeroes, Bermuda, Saint Helena and Madeira, lie at intermediate positions. All these are inactive.

One interpretation of the cause of this observation is that convection currents are rising in the mantle under the mid-ocean ridge, that greater heat there tends to produce volcanoes, but that horizontal motion carries the volcanic islands away from their source in the ridge so that they become extinct and progressively older with distance from the ridge.

The distances for the Faeroes and Iceland are short, but this may be due to the fact that, if the Atlantic Ocean spread, it must have done so by the least amount in the north. Another contributing factor was perhaps the opening of Baffin Bay, which it is intended to discuss elsewhere. Another exceptional island is Christmas Island (Indian Ocean), which has moved far considering the age of its oldest exposed rocks, but, of course, its real age may be greater, and Andrews[45] has suggested that this may be the case.

Table 1. THE OLDEST DATES, DISTANCES TO THE MID-OCEAN RIDGE, AND APPARENT MAXIMUM RATES OF MOTION FOR SOME OCEAN ISLANDS

Island(s)	Oldest ages Period	m.y.	Distance to ridge (km)	Apparent maximum rate of motion (cm/yr)	Refs.
	Atlantic Ocean				
Jan Mayen	Pliocene	10	0	0	4
Iceland	Eocene	50	150	0·8	5
Faeroes	Eocene	50	600	1·2	6
Azores	Miocene	20	300	1·5	7
Madeira Is.	Miocene (Cret. ?)	20 (90 ?)	1,500	7·5 (1·7)	8
Canary Is.	Oligocene	32	1,900	6·0	9
C. Verde Is.	L. Cretaceous	120	2,200	1·8	10
F. de Noronha	L. Cretaceous	120	2,000	1·7	11
Bermuda Is.	Eoc. or Olig.	36	2,200	6·1	12
Bahamas	L. Cretaceous	120	3,000	2·5	13
Ascension	Pleistocene (?)	1	150	1·5	14
Saint Helena	Miocene (?)	20	700	3·5	15
Tristan da Cunha	—	18	0	0	16, 17
Bouvet	Pleistocene	1	0	0	18, 19
Falkland Is.	Pre-Devonian	1,100 (?)	3,100	—	20
	Indian Ocean				
Kerguelen	Miocene	20	1,200	6·0	21
Heard	Paleocene	60	1,500	2·5	22, 23
Saint Paul	Recent	1	0	0	24
Madagascar	Precambrian	2,700	1,800	—	25
Seychelles	Precambrian	600	700	—	26
Providence	Eocene–Oligocene	36	1,600	4·4	27
Christmas	Eocene	50	3,700	7·4	28
	Pacific Ocean				
Sala-y-Gomez	Pleistocene	1			20
Nasca Seamount	Miocene	20			29
Erben Seamount	Miocene	20			30
Hess Seamount	Middle Cretaceous	90			31
Cape Johnson Seamount	Middle Cretaceous	90			31
Daito	Upper Oligocene	30			32
Truk	Miocene	20			33
Sylvania Seamount	Eocene–Paleocene	60			34
Jaluit	Middle Tertiary	32			35
Eniwetok	Upper Eocene	40			36
Bikini	Lower Miocene	25			37
Ocean	Upper Tertiary	15			38
Mangaia (Cook Is.)	Miocene–Oligocene	25			39
Atiu (Cook Is.)	Pliocene	10			40
Makatea (Tuamotu Is.)	Eocene	50			41

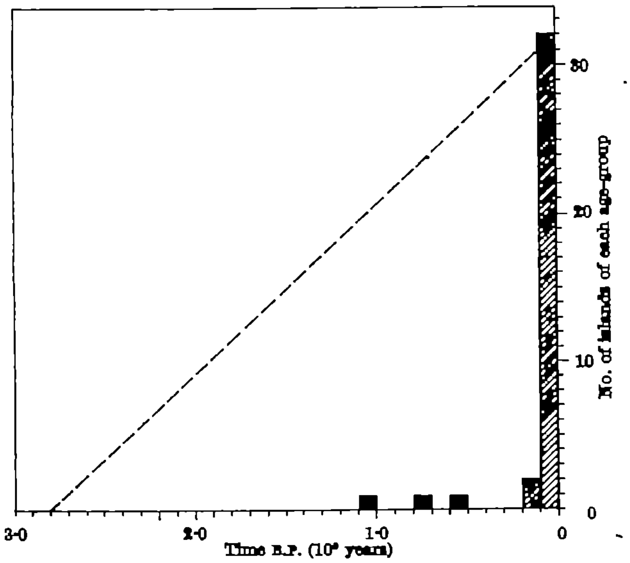

Fig. 1. Frequency diagram for ages of islands in main ocean basins. The dotted line is the corresponding curve for continents

Our method of dating islands is not precise, nor is it to be supposed that if drift occurred that all islands would have travelled at the same rate. Since the discovery of older rocks on islands would reduce their apparent rates of motion, the rates calculated and shown in Table 1 are maximum values. The average value found for the maximum possible rate of drift is 3·5 cm per year. It is interesting that this is of the same order of magnitude as that usually given for the rate of continental drift. S. K. Runcorn[46], after discussing various estimates of the possible rate of motion, concludes that 3 cm a year is the most probable value. Having regard to these uncertainties, the analysis suggests that, the farther an ocean island is from a mid-ocean ridge, the older it is likely to be, which supports the view that the floors of oceans may have grown by spreading from the mid-ocean ridges.

No such simple pattern is apparent in the Pacific islands. The explanation may lie in some combination of the following factors: (*a*) the continents are advancing over the borders of the Pacific Ocean; (*b*) H. H. Hess[47] has suggested a recent change in the pattern of mid-ocean ridges in the Pacific; (*c*) the data for the Pacific are poor, there being less dates per unit area than elsewhere, and most of those which are available were obtained by drilling or dredging; (*d*) another complexity is discussed elsewhere which suggests that some Pacific islands did not start at the mid-ocean ridge[48].

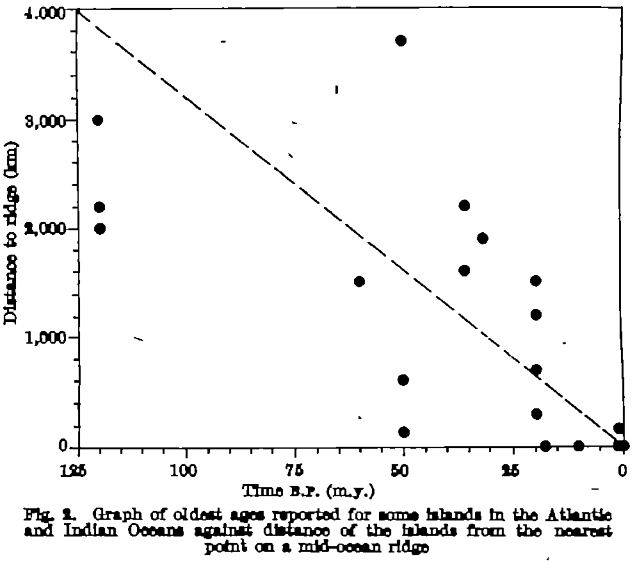

Fig. 2. Graph of oldest ages reported for some islands in the Atlantic and Indian Oceans against distance of the islands from the nearest point on a mid-ocean ridge

Examination of bathymetric charts shows that islands occur most commonly on the mid-ocean ridge at points where the ridge both changes direction and intersects a branch ridge. Iceland on the Wyville–Thomson Ridge, the Azores on the Azores Rise, Gough Island on both the Walvis Ridge and on the Rio Grande Rise and Easter Island at the point of intersection of several ridges are examples of islands so situated.

If one regards the ocean basins as permanent, one can take the view that all the ridges have formed over fractures and that places where fractures intersect are likely to be particularly favourable for extrusion. This could explain the growth of volcanoes and islands at those places. This theory has the disadvantage that it does not explain why the branch ridges are aseismic or why they are quite different in character and appearance from the mid-ocean ridge. Some branch ridges, like the Azores and the Nazca Ridge which passes through Easter and Sala-y-Gomez Islands, seem to be chains of volcanoes and seamounts quite unlike the mid-ocean ridges.

If, on the other hand, convection is occurring, it is reasonable to believe that bends in the mid-ocean ridge are associated with deflexions in the current system and that at such places reduced pressure or deeper fracturing may cause extrusion of more lava than elsewhere on the ridge. This could determine the location of volcanic islands on the ridge.

Convection also introduces horizontal motion. This would slowly move volcanoes away from their sources on the mid-ocean ridge, so that those volcanoes would cease to be active. The sources would remain fixed over the vertical currents and would produce fresh volcanoes. Thus, in time, each source on the mid-ocean ridge would produce a chain of progressively older extinct volcanoes, or perhaps two such chains, one on either side of the mid-ocean ridge (Fig. 3). Here is a possible explanation for ridges like the Walvis and Rio Grande. This proposal could be tested because the oldest seamounts on the branches should lie farthest from the mid-ocean ridge. All such branches might be flow lines. This hypothesis could explain the general parallelism of all the rises on either side of the Atlantic. The well-known herringbone pattern of the ridges could be an expression of the fact that Africa and North America did not move directly apart, but that each had a northward component of motion as well. For Africa at least palæomagnetic evidence has already suggested this[49].

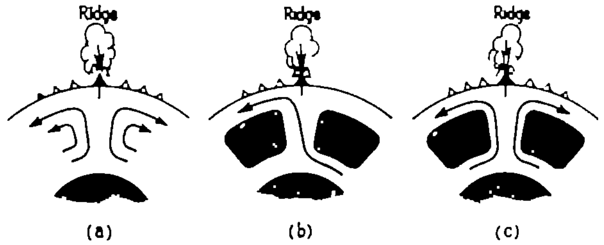

Fig. 3. Some possible patterns of convection, showing how chains of extinct volcanoes might be formed. The shaded areas represent stable cores of cells

The pattern of the Pacific is much less clear, but some suggestions can be made for the eastern part of the Pacific basin. The line of seamounts which extends east and then north-east from Easter Island may be its flow line. Part of it is called the Nazca Ridge. H. W. Menard[50] has shown it as a fracture zone but R. L. Fisher[51] has called it a line of seamounts. The Cocos Ridge off Colombia is nearly parallel with the Nazca Ridge and may be part of another flow-line. On the other hand, the West Chile Rise which joins Easter Island to the great fault through the Strait of Magellan may be a fault scarp.

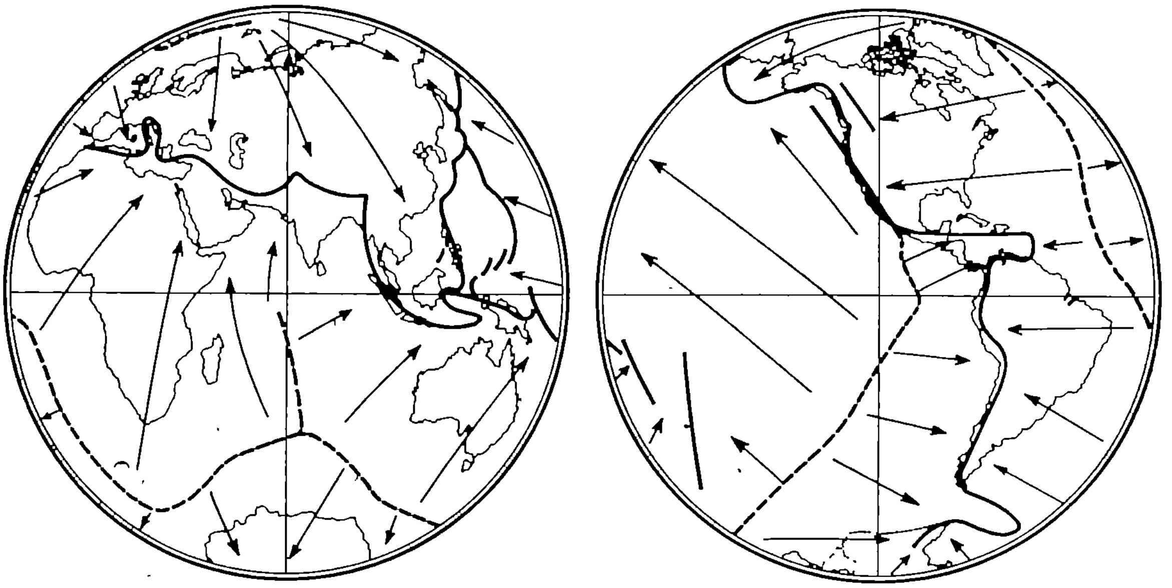

Fig. 4. Sketch of assumed convective flow from the mid-ocean ridge to the continental mountain system. The convection cell is a folded, irregular, cylinder with both ends west of North America

Thus the pattern of distribution of mid-ocean islands of different ages is considered to favour the hypothesis of spreading apart of some ocean floors and the pattern of aseismic ridges may be flow-lines indicating directions of recent movement. It is considered that at the contacts of mid-ocean and aseismic ridges with continents there are often areas of flood basalts, as in the Parana basin, Mexico, Ethiopia, Greenland and Scotland. This is probably not coincidental and may provide a means of dating the start of continental separation in various localities.

On the assumption that convection currents rise under mid-ocean ridges and sink under continental mountains, a map attempting to portray the probable pattern of horizontal flow has already been published[51]. If the aseismic ridges are flow-lines, more control is available and a more precise map (Fig. 4) based on this assumption has been prepared[48]. The chief change is in the north-eastern part of the Pacific Ocean. The new pattern of convection is not a torus, but one long cylindrical cell twisted about the Earth. Both ends of this cell lie off the west coast of North America. One end pinches out off Mexico where the East Pacific Rise reaches the Cordillera, the other end is broad and sheared off against the Cordillera of United States and Canada. The San Andreas fault and other structural features striking north-west may be consequences of this shearing.

If this view is adopted it seems likely that the Austral, Society, Tuamotu, and Hawaiian Island chains also lie along stream lines and that the progression in age of the islands along each chain is due to this cause. The Hawaiian Islands and some of the other chains are exceptional in that their source does not lie on the mid-ocean ridge. A fuller discussion of this[48] suggests that chains of islands could still be formed if the source of lava lay within the stable cores of convection cells of the type shown in Fig. 3c.

The recent discovery[52] of Miocene rocks on Oahu supports, but does not prove, the view that the Hawaiian Islands have been formed successively over a common source beneath the present position of the Island of Hawaii, and that they have been successively borne away toward the west. The dates given by Menard *et al.* indicate that the rate of movement is not greater than 10 cm a year.

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OBITUARIES

Dr. J. L. Pawsey, F.R.S.

Dr. Joseph Lade Pawsey, Australia's pioneer in radio astronomy, died in Sydney on November 30, aged fifty-four. Dr. Pawsey was assistant chief of the Division of Radiophysics of the Commonwealth Scientific and Industrial Research Organization, which he had joined early in 1940 and where he directed the Division's radio astronomy work during the seventeen years preceding his death. During 1962 he was to have taken up a new appointment in the United States as director of the National Radio Astronomy Observatory at Green Bank, West Virginia. It was during a preliminary visit to the United States early in 1962 in preparation for this appointment that he became seriously ill, and it was only due to the medical skill and devotion given him through the efforts of his American colleagues that he lived long enough to return home to his family and indeed to accomplish a final work—the editing of a large collection of radio astronomical works shortly to be published. He is survived by his wife, two sons and a daughter.

Dr. Pawsey's interest in radio astronomy began while working on radar developments during the Second World War when confidential reports became available of the discovery of radio-frequency radiation from the Sun. After the War he began exploring these emissions and soon discovered that metre waves from the Sun consisted of two components—a basic thermal component from the Sun's million-degree corona, and a much more intense variable component which he accurately located in the vicinity of sunspots. From the impetus of these observations he began to form around him a group of physicists and engineers which was to play a leading part in the evolution of the new science of radio astronomy. Dr. Pawsey devoted most of his time to encouraging and stimulating this group, guiding them with inexhaustible patience and skill into a series of crucial experiments which helped to reveal the nature of the radio universe. The first unambiguous demonstration of the existence of radio stars, their first identifications with visible objects (nebulæ and galaxies as we now know), the mapping of the sky over a great range of frequencies, including the 21-cm emission of hydrogen, the detailed exploration of the complex emissions from the Sun, quiet and disturbed, and the interpretation of solar bursts in terms of matter expelled from solar explosions—these are a few of the contributions made by his group during his years of directorship. His interest in the astronomical results was matched by his interest in the development of the techniques required for obtaining them (he had, during the five years before the War, been intimately connected with the development of the original television system at Alexandra Palace), and again his group became known for novelty of approach to instrumental problems—various types of interferometers, the solar radio spectrograph, the grating aerial array, the Mills Cross and the multi-channel hydrogen spectrograph were all developed under his leadership. In the stimulus he gave his group for methods of obtaining higher and higher angular resolution, his interest lay more in the application of the principles of interferometry than in the conventional 'big-dish' approach; but when the decision was made to build a 210-ft. radio telescope for the Laboratory, he threw his energies into organizing the preparation of first-class receiving equipment needed to ensure its success.

Dr. Pawsey will be particularly remembered by his group for his ability to simplify complex problems and situations by pointing to the heart of the matter, for his willingness to discuss any aspect of a problem at all times at any level, and for his decisive and positive criticism of any manuscript or project put before him. He will also be remembered by his many friends across the world for his absolute integrity and honesty throughout his work and personal life.

Dr. Pawsey was made a Foundation Fellow of the Australian Academy of Science in 1954, and was elected Fellow of the Royal Society in the same year. He was awarded the Lyle Medal of the Australian Academy in 1954, and the Hughes Medal of the Royal Society in 1960. International recognition of his work was signified by his election as president of the Radio Astronomy Commission of the International Astronomical Union between 1952 and 1958.

His publications, though extensive, give no indication of his real contributions unless one reads the acknowledgments at the conclusion of scores of papers written by other authors. His most comprehensive work, in collaboration with Prof. R. N. Bracewell, is the classical text-book on *Radio Astronomy*.

Dr. Pawsey was a graduate of the University of Melbourne, where, in 1931, he won an 1851 Exhibition Scholarship to take his Ph.D. at the Cavendish Laboratory under Lord Rutherford. One may sense something of Rutherford's influence both in his love of the simple direct experiment and in his achievement of establishing the kind of group in which every member acquires the confidence and individuality to play his part in international research.

J. P. Wild

Prof. John J. Bittner

Experimental mammary tumour pathology suffered a great loss by the death in Minneapolis, on December 14, 1961, of Prof. John J. Bittner.

Born on February 25, 1904, in Meadville, Pa., he was educated at St. Stephen's College (now Bard), Annandale-on-Hudson, New York, where he earned his A.B. degree in 1925. He received his M.S. in 1929, and Ph.D. in 1930, at the University of Michigan.

Bittner was assistant in cancer research (1927–30) in the University of Michigan; research associate (1930–40) and assistant director (1940–42) at Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine; George Chase Christian professor of cancer research and director of cancer biology (1942–61) and professor of experimental pathology (1957–61) in the Medical School of the University of Minnesota; consulting associate scientist of the Sloan-Kettering Institute (1956–61).

Until about thirty years ago, it was generally agreed that mammary cancer in mice developed as the result of hormonal stimulation acting on susceptible tissues having the hereditary factor. In 1933 the situation of the causative factors of mammary tumours in mice began to change because the staff of Roscoe B. Jackson Memorial Laboratory announced the discovery of a non-chromosomal influence of maternal origin, which played a decisive part in the development of spontaneous mammary tumours in mice.

In 1936, Bittner wrote in a famous preliminary report: "Should further study demonstrate that the incidence of mammary gland tumours in mice may be affected by nursing, an explanation may be offered for the so-called extrachromosomal influence as a cause in the development of this type of neoplasm".

Bittner's major discovery (1939) was to identify this extrachromosomal influence with nursing, that is, with the milk factor.

So it was shown that spontaneous mammary cancer in mice usually resulted from the interaction of three causative factors: hormonal stimulation, inherited susceptibility, and the milk-borne mammary tumour agent.

Bittner also contributed to the discovery of an inherited hormonal influence. In 1944, when reciprocal matings were made between animals of the cancer-susceptible *A* (low incidence in virgins) and *C3H* (high incidence in virgins) strains, it was demonstrated that the hormonal pattern associated with the genesis of mammary cancer in virgin females was transmitted to the F_1 females, maintained as virgins, which had high incidences of mammary cancer. Bittner (1945) termed the hormonal mechanism responsible for the induction of mammary cancer in virgins "the inherited hormonal influence".

"The inherited hormonal influence . . . is probably the result of the action of multiple genes. The same genes probably do not produce the inherited susceptibility and the inherited hormonal influence."

Apart from these contributions, which opened entirely new fields, he wrote many other papers—245 in all—which made valuable contributions to the knowledge of mouse mammary tumours.

As may be expected, Bittner was the recipient of many honours and he was much sought after as a lecturer: among these were the Alvaranga Prize, College of Physicians of Philadelphia (1941); Comfort Crookshank Award and Lecture, London (1951); Bertner Award and Lecture, University of Texas (1957); honorary D.Sc. from Bard College (St. Stephen's) (1950).

In 1957 Bittner visited Perugia to participate in the Second International Symposium on Mammary Cancer. It was a great satisfaction to him to be the recipient (together with Alexander Haddow and Leonell C. Strong) of an honorary degree in medicine and surgery, from the University of Perugia, on July 29 of that year.

On February 23, 1961, when Dr. O. Meredith Wilson was inaugurated president of the University of Minnesota, Bittner was in a position of honour. In fact, he participated on behalf of one of the two oldest institutions represented, leading the academic group by wearing the gown of the Perugia University Medical School, incidentally with a colleague dressed in an Oxford (England) robe. On Thursday, February 16, 1961, *The Minneapolis Star* published a three-column article illustrated with coloured photographs of Bittner and other doctors in their academic gowns as they would appear at the inauguration.

Bittner returned to Perugia in June 1961 to participate in the Ciba Foundation Symposium on Tumour Viruses of Murine Origin (June 21–23) and the International Conference on the Morphological Precursors of Cancer (June 26–30), and to attend the centenary celebrations of the chair of morbid anatomy in the University of Perugia, as the representative of the University of Minnesota.

I would like also to recall the exceptional brightness and intelligence of his eyes and his inimitable charm. He was fond of his house and garden and was an enthusiastic photographer.

He is survived by his wife, formerly Mary Esther Mahaffy, two daughters, Mary Margaret (Mrs. Merlyn C. Bowker) and Elizabeth Ann (Mrs. Edwin H. Loague), two sisters, Katherine (Mrs. Leonell C. Strong) and Josephine (Mrs. George E. Turnbull) and a brother, Mr. Guy C. Bittner, to all of whom their many friends extend their deepest sympathy.

LUCIO SEVERI

His family, his numerous professional associates and friends, others who liked him personally, and many more who were stimulated by his writings learned with sorrow that Prof. John J. Bittner had died on December 14, 1961. Some of Bittner's fundamental contributions to cancer research and his background have already been summarized by Dr. Leonell C. Strong in the April 1962 issue of *Cancer Research*. Added emphasis of deserved international scope is placed on Bittner's contributions to cancer research by Prof. Lucio Severi in the preceding obituary.

To-day's investigators in cancer biology must realize indeed that the foundations of their discipline were laid by a few gifted researchers, such as Bittner, making a series of basic discoveries with keen intuition and painstaking observation. Bittner's important findings do not depend on the extensive instrumentation now so much used in oncology. His achievements demonstrate the pioneering application of uncomplicated experimental technique to clearly formulated problems, even in a field as complex as is the inquiry into the nature of cancer. The impact of Bittner's findings cannot be positively correlated with the cost of his instrumentation or with his laboratory space in square feet.

Bittner's long series of studies on the development of mammary tumours in mice has probably stimulated cancer research on a world-wide scale as much as any other single series of observations. His long-continued and scarcely fashionable insistence, particularly during the past decade, on a mammary tumour 'agent' rather than a 'virus' reflected his sound caution when faced with tempting premature extrapolation. While documenting painstakingly, throughout a lifetime, the part played by what we now call the Bittner agent, he also devoted careful observation to the factors interacting with his agent.

In a virus-conscious era of oncology, it is scarcely surprising that the question whether the Bittner agent is indeed a virus or a cytoplasmic 'gene' or 'particle' is often posed. Such semantic controversy revolving around as yet inconclusive data must not detract, however, from Bittner's important disclosure of the part played by an infective agent in the genesis of a mammalian neoplasm.

We may remember, further, that the Bittner of Bittner-agent fame consistently concerned himself with the part played by endocrine and metabolic factors in mammalian carcinogenesis. Two points are particularly pertinent. First, he emphasized that mammary cancer in the mouse can develop in the absence of a demonstrable agent, and that the burden of proof for assuming that the presence of an infective agent constitutes a *sine qua non* for mammary carcinogenesis in the mouse rests with those who make this claim. Secondly, he devoted much research to the question as to whether the agent exerted its effect via the endocrine.

Basic contributions by Huseby and Bittner related in this connexion to endocrine mechanisms of breast cancer in the mouse. Many others, too many to name, are indebted to Bittner for the opportunity to co-operate with him, as I am.

Bittner's scholarship in the field of inquiry into the nature of cancer was indeed an invaluable asset not only to those privileged to work with him, but also to many others who came or wrote for his advice. He was open-minded to new approaches and willing to lend his unreserved support to explorations of unconventional fields. The resolution of several temporal parameters of physiology and pathology at different levels of mammalian organization would not have been possible without his guidance and help. In later years, Bittner's activities also included the support of the work of others by making available his inbred strains of mice. He kept up with the literature in a rapidly developing field which he had so greatly stimulated and remained active in the laboratory for seven years following a first coronary occlusion, which preceded the second, fatal one. He died on his way to work. He lives in the history of biology and medicine with those endeavouring to understand organisms and their diseases by dint of painstaking observation.

FRANZ HALBERG

NEWS and VIEWS

The Geological Society of London: Awards

The following awards for 1963 have been made by the Geological Society of London: *Wollaston Medal*, to Dr. F. A. Vening Meinesz, emeritus professor, University of Utrecht, for his pioneer researches in geophysics, notably in measurements of gravity at sea, and his studies of isostasy and thermal convection in the Earth; *Murchison Medal*, to Mr. N. L. Falcon of the British Petroleum Co., Ltd., for his application of geology to exploration for petroleum throughout the world; *Lyell Medal*, to Prof. T. N. George, University of Glasgow, for his researches on carboniferous stratigraphy and palæontology and work in the field of evolutionary palæontology; *Bigsby Medal*, to Prof. W. S. Pitcher, University of Liverpool, for his work on igneous and metamorphic rocks, particularly in north-west Ireland; *Wollaston Fund*, to Dr. G. M. Brown, University of Oxford, for his contributions to petrology and mineralogy; *Murchison Fund*, to Mr. E. H. Francis, Geological Survey of Great Britain (Scottish Office), for his contributions to stratigraphy and volcanism, especially in the Midland Valley of Scotland; *Lyell Fund*, a moiety to Dr. G. P. L. Walker, Imperial College of Science and Technology, for his researches on the volcanic rocks of Iceland and Northern Ireland; and another moiety to Dr. A. E. M. Nairn, Turner and Newall Fellow, King's College, Newcastle upon Tyne, for his researches in the field of palæomagnetism and palæoclimatology.

The Institute of Physics and the Physical Society: Appointments and Awards

The following appointments and awards for 1963 have been made by the Institute of Physics and the Physical Society: *Guthrie Lecturer*, Prof. L. F. Bates, Spencer professor of physics at the University of Nottingham. The Lecture will be delivered during the International Conference on Magnetism to be held in Nottingham during September 7–11, 1964; *The Thomas Young Medal and Prize*, jointly to Prof. C. H. Townes, of the Massachusetts Institute of Technology, and Prof. A. L. Schawlow, of Stanford University, California, for their work in originating the principle of the optical maser; *The Duddell Medal and Prize*, to Prof. B. N. Brockhouse, of McMaster University, Ontario, Canada, for his work on the lattice dynamics of crystals and the development of the three-axis crystal spectrometer used in this work; *The Charles Chree Medal and Prize*, to Dr. M. N. Hill, of the University of Cambridge, for his work in marine geophysics, particularly in connexion with the application of the seismic method in the deep ocean; *The Charles Vernon Boys Prize*, to Dr. K. D. Froome, of the National Physical Laboratory, for his contributions to experimental physics, particularly his determination of the velocity of light, his invention of a source of continuous short waves and his method for accurate measurement of length.

Mining and Mineral Technology at the Imperial College of Science and Technology:

Prof. J. C. Mitcheson, C.B.E.

James Cecil Mitcheson, who retires on September 30, has been professor of mining and head of the Department of Mining and Mineral Technology at the Imperial College of Science and Technology since 1952. This critical period has coincided with the contraction of many of the mining schools in the Western World, and it is in a large measure due to Mitcheson's leadership that the Royal School of Mines has strengthened its position, modernized its outlook and taken a full part in the expansion of the Imperial College. A man of absolute integrity, he combines firmness of purpose with kindness of nature in a manner which has earned the respect and affection of both his colleagues and his students. He came to the Royal School of Mines with an established reputation as an administrator and expert in the economics of coal mining and quickly won the confidence of the metalliferous mining industry. A past-president of the Institution of Mining Engineers, he is now vice-president of the Institution of Mining and Metallurgy. To his academic and professional duties have been added many public responsibilities, including that of chairman of the Safety in Mines Research Advisory Board and, since 1961, chairman of the Geological Survey Board. However, his principal interest has long been mining education in all its forms and, characteristically, his activities in this field have extended far beyond the Imperial College. For these and other public services—all generously and modestly given—he was awarded the C.B.E. in 1961. Mitcheson was Dean of the Royal School of Mines from 1959 until 1962, during which time the rebuilding of the School was planned and initiated. His experience, judgment and rare human qualities have had an important influence on the course of mining education in Britain.

Prof. R. A. L. Black

Prof. R. A. L. Black, who is to succeed Prof. J. C. Mitcheson, has spent most of his professional life in South Africa. He was, however, born and brought up in England, being educated at Stowe and the Royal School of Mines (Imperial College of Science and Technology). His university course was interrupted by the outbreak of war when he volunteered for the Air Branch of the Royal Navy, in which he served as a member of air crew and was awarded the Distinguished Service Cross. At the earliest moment after hostilities ended he returned to the Royal School of Mines and, graduating with honours in 1946, he immediately joined the staff of the Central Mining and Investment Corporation in Johannesburg. This large finance house controls a number of important mines, and Black was given a wide practical experience of underground mining operations before being called to head office. Here he was appointed assistant to the consulting engineer with responsibility for the development of a research programme covering rock mechanics and ground control. Following a short period of actual mine management, he was in 1956 appointed to his present position as Chamber of Mines professor in mining engineering in the University of the Witwatersrand, Johannesburg. Since his appointment, Black has reorganized the undergraduate courses, changing the emphasis away from the teaching of applied technology towards a better grounding in the sciences and basic engineering principles. At the same time he has developed a postgraduate school together with a research unit largely engaged in studying problems arising out of mining at great depth. His own especial fields of interest are in rock mechanics and explosives engineering. In addition to his academic activities Black has been dean of the University Residence, where Mrs. Black and he have most successfully provided a 'home' for some 160 students and 20 members of staff for the past four years.

The U.S. National Bureau of Standards

Dr. Ernest K. Smith has been appointed chief of the Upper Atmosphere and Space Physics Division and Robert W. Knecht chief of the Ionosphere Research and Propagation Division at the Boulder Laboratories of the

National Bureau of Standards, U.S. Department of Commerce. These divisions are part of the Central Radio Propagation Laboratory.

Dr. E. K. Smith

DR. SMITH succeeds C. Gordon Little, who was recently appointed chief of the Central Radio Propagation Laboratory. Since joining the Bureau's staff in 1951, Dr. Smith has investigated such ionospheric phenomena as sporadic-E, F-region anomalies and studies of the atmospheric refractive index for non-ionized gases.

Mr. R. W. Knecht

MR. KNECHT succeeds Dr. Smith as chief of the Ionosphere Research and Propagation Division. He joined the National Bureau of Standards in June 1949 immediately on graduation in physics from Union College in Schenectady, New York. After an initial assignment in Washington, he was transferred to Alaska, spending eight months at Barrow Radio Propagation Field Station and, afterwards, two years at Anchorage as physicist-in-charge of the North Pacific Radio Warning Service. Since his return from Alaska in 1954, Mr. Knecht has worked primarily in the fields of ionospheric physics and solar-terrestrial relationships. He was formerly chief of the Sun–Earth Relationships Section. After exploring the possibilities of a satellite-borne top-side sounding experiment for investigating the ionosphere, Knecht promoted the development of *Topsi*, the U.S. fixed-frequency top-side sounding satellite scheduled for launching in the first half of 1963.

Scientific and Technical Personnel in the Federal Government

ACCORDING to *Scientific and Technical Personnel in the Federal Government, 1959 and 1960*, the professional scientific and technical staff in the Federal Government increased by 26 per cent to 156,000 between 1954 and 1960 (*NSF* 62–26. Pp. vi+79. Washington, D.C.: Government Printing Office, 1962). The Department of Defence employed 37 per cent of these, 56,100 in 1959 and 57,400 in 1960, more than two-thirds being engineers. The Veterans Administration employed about 29,000 in 1960, mostly in health occupations. The total included about 27,500 women in 1959, mostly in health professions where about 58 per cent were women, compared with 3,900 or almost 8 per cent in science and less than 1 per cent in engineering. The number engaged in research and development was relatively stable during 1958–60, and in 1960 about 44,000 were in research and development, about half in the Department of Defence. Of the 53,000 scientific personnel in 1960, 18,000 were in the physical sciences and nearly 22,000 in biological sciences. Non-professional scientific and technical staff in the Government increased by 11 per cent over the period 1958–60 from 75,200 to almost 83,500, more than 35,800 in 1960 being in engineering and 11,500 in mechanics. Almost 36,000, or 43 per cent of the total, were in the Department of Defence and only 14,200, or 17 per cent, in research and development.

Problems in the Communication of Scientific and Technical Information

IN September 1962 the Department of English and Liberal Studies of the Welsh College of Advanced Technology began an investigation into the problems involved in the communication of scientific and technical information. A circular letter was sent to a group of fifty-four research centres and companies, inquiring what forms of writing they required and what difficulties and weaknesses, if any, they encountered. All the thirty-one companies which replied expressed their concern with the present standards in technical writing, and nineteen suggested a visit to discuss the subject, while seven submitted detailed opinions or documentary evidence. All the companies welcomed the Department's positive measures to train undergraduates in written and spoken communication and were willing to co-operate in the investigation. Some were interested in suggestions for short courses for their graduate personnel, either in the college or in the companies themselves. A circular letter inquiring about the prevalence of communication problems in science and engineering departments in universities and colleges of advanced technology is being sent out to all such colleges and to selected universities, and the writing of reports has been discussed with the professors of chemical, civil and electrical engineering in the University of Nottingham, where much interest in standards of writing was shown. Staff in scientific departments at the Universities of Leeds, Liverpool and Reading has also been approached, and it is proposed to make inquiries of a number of national research centres and institutes and of more companies. Experiments have already been made with testing methods in recent college courses and examinations, and consideration of the philosophical problems involved in the processes of written communication will commence shortly.

Mental Health Book Review Index

THE *Mental Health Book Review Index* contains a list of books relating in some way to mental health, and the title of each book is followed by references to reviews of the book which have appeared in the appropriate journals. Volume 7 deals in this way with some 300 new books and there are also included new reviews of books which have been referred to in previous volumes (Pp. xiii+66. Flushing, N.Y.: Miss Lois Afflerback, Paul Klapper Library, Queens College, 1962. Annual subscription, 3 dollars). The field of mental health is widely interpreted and covers not only psychology and psychiatry and sociological aspects, but also a good many books of general cultural interest. For example, the present volume includes Sir Charles Snow's Rede Lecture on *The Two Cultures and the Scientific Revolution*, J. P. Sartre's *Being and Nothingness* and Teilhard de Chardin's *The Phenomenon of Man*. In these days when it is increasingly difficult for workers even to know what is published in their own field, not to mention in allied disciplines, this publication serves a very valuable purpose. The *Index* is sponsored, among others, by the World Federation for Mental Health.

Animals

THE aim of the new magazine, *Animals*, published by Knowledge Publications, Ltd., at 2*s*. weekly, commencing January 8, is expressed by the editor-in-chief, Armand Denis: ". . . to interest you in every aspect of the animal world". The main appeal is visual, well over half the space being given to pictures, four-fifths of which are superb colour photographs. Sir Julian Huxley and Sir Solly Zuckerman are patrons, and there seem to be links with the World Wildlife Fund, although what these may be is not made precisely clear. The editorial board includes the Duke of Bedford, Gerald Durrell, Bernard Grzimek, Nicholas Guppy, Gavin Maxwell, Alan Moorehead, Peter Scott and Niko Tinbergen. The editor is John Chancellor, and the consulting editor Michael Tweedie. The text is less informative than should be expected for the price, an outstanding instance being an 800-word article on the giant panda, which seems mainly concerned with how little we know of this species while failing to give as much as is known. (Perhaps somebody will one day explain why the giant panda was not named as one of the world's rarest animals until it was adopted as the symbol of the World Wildlife Fund (British Branch).) There is also a tendency for error and misstatement, not always trivial and sometimes needless, to creep in, and to maintain our interest in the animal world it may be necessary to bring the textual matter more closely into accord with the high standard of the art work.

Estimation of Calcium in Biological Material

The adaptation of the flame-photometer to the estimation of calcium in biological material has proved difficult because of serious interference from other ions commonly present. Sodium and phosphate ions are specially troublesome, sodium enhancing and phosphates inhibiting the intensity of the calcium flame. Other ions also interfere in varying degrees of severity. The intrinsic simplicity of flame-photometry, however, has made worth while the development of several techniques for isolating calcium ions free from interfering material. G. E. Trout, of the University of the Witwatersrand, Johannesburg, has recently described an improved technique whereby the calcium is purified by means of ion-exchange chromatography on fine-mesh resins (*South Afric. J. Med. Sci.*, 27, Nos. 1 and 2; July 1962) It is particularly suitable for the estimation of calcium in tissue ashes. The method has been extensively tested with a synthetic mixture of sodium, magnesium, potassium and calcium salts.

Control of Ticks on Livestock

In 1958 an expert panel on tick-borne diseases of livestock was established by the Food and Agriculture Organization, the first meeting of this body being held in November of that year. The recent publication, *The Control of Ticks on Livestock*, is the first to be provided by the panel (*FAO Agricultural Studies No. 54*. Prepared by S. F. Barnett. Pp. vii + 115. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1961. 5*s*.; 1 dollar). The whole subject of tick control is reviewed very thoroughly, and this booklet can claim to cover the whole of the subject from the angle of the field veterinary officer. It contains a select reference list of titles dating from 1941. The nine chapters deal with reasons for controlling ticks; control of parasites off the host; control of parasites on the host; timing of ixodicidal applications; ixodicides; deterioration and depletion of ixodicidal washes and estimation of their concentration; resistance of ticks to ixodicides; toxicity of ixodicides; and a summary. Eight very important pages were left unprinted in the copy received for review; this may also apply to two other pages at the end of the book. It is to be hoped that this has not happened with other copies.

Courses supported by the U.S. National Science Foundation

Under the title *Science Course Improvement Projects* the National Science Foundation has issued a booklet listing and describing the courses designed to improve the teaching of science and mathematics as well as engineering supported by the National Science Foundation (NSF 62–38. 1: *Courses, Written Materials, Films, Studies supported by the National Science Foundation*. Pp. 64. Washington, D.C.: National Science Foundation, 1962). In all, some 132 courses and studies are listed. The projects are arranged in four main groups, concerned first with elementary schools, secondly with secondary schools, thirdly with technical institutes, and fourthly with college and university projects. Under these headings, they are arranged according to disciplines. The information covers courses, written material, films and investigations.

The Simon Population Trust

The Simon Population Trust was formed following a bequest of £15,000 from the late Lord Simon of Wythenshawe. The objects of the Trust, designedly formulated in broad terms, are: (1) to promote a better understanding of the problems of world population and resources; (2) to encourage such research, education or other actions in any part of the world as may contribute to the adjustment of population to resources. The need had long been felt for an objective and independent body, non-partisan and of broad sympathies, which could give encouragement and adequate financial support to promising initiatives from any quarter, and which could enable new growing points to develop until a comprehensive and well-balanced provision existed for meeting this important need of modern civilization. By the terms of the Trust, the Trustees are enabled to give support to a wide range of projects. However, as yet they are not in a financial position to subsidize basic researches such as have yielded the oral methods of contraception which are now on trial. The objectives which the Trustees think are at present worthy of support include the following: birth control in place of abortion; use of midwives; improvement of methods acceptable to Roman Catholics; local manufacture of contraceptives; reversible sterilization in males; travelling fellowships. Further information is obtainable from the Simon Population Trust, 69 Eccleston Square, London, S.W.1.

Announcements

Prof. W. M. Fairbank, of Stanford University, has been awarded the 1963 Oliver E. Buckley Solid-State Physics Prize of the American Physical Society, for his work on the properties of helium-3, and especially for his part in the discovery of flux quantization in superconductors.

The Office of Documentation has issued a booklet surveying the scientific information activities of the National Academy of Sciences–National Research Council (Publication 1031. Pp. vi + 49. Washington, D.C.: National Academy of Sciences–National Research Council, 1962). These are described under the divisional subject heads and, besides listing the various Committees concerned and information services, the booklet lists the various publications available.

The fifteenth international symposium on "Crop Protection" will be held at the State Agricultural College. Gent, on May 7. Further information can be obtained from Prof. J. van den Brande, State Agricultural College, Coupure Links, 233, Gent.

The Plant Phenolics Group is organizing a symposium on "Methods in Polyphenol Chemistry" at the Dyson Perrins Laboratory, Oxford, during April 2–4. Further information can be obtained from the honorary secretary. A. H. Williams, Research Station, Long Ashton, Bristol.

An international symposium on "Physical Processes in Radiation Biology" will be held at Michigan State University during May 5–8. Further information can be obtained from Mr. Bruce Alderman, Room 24, Kellogg Center, Michigan State University, East Lansing, Michigan.

The spring meeting of the British Society of Rheology will be held at the College of Aeronautics, Cranfield. during April 17–19. The subject of the meeting will be "Ductility in Metals and its Relation to Fabrication Processes". Non-members of the Society are invited to participate in the meeting. Further information can be obtained from Mr. R. Pearce, Department of Materials, Cranfield, Bletchley, Bucks.

The British Radio Spectroscopy Group is organizing a conference on "Ions, Masers and Irradiation" at the Physics Department, University of Keele, during April 8–10. The topics covered will include the experimental and theoretical investigations of both bound and free ions; the relation of such investigations to optical, infra-red and microwave masers; the use of microwave spectroscopy to detect effects produced by irradiation at radio, microwave or optical frequencies. Further information and registration forms are available from Dr. D. C. Lainé, Physics Department, University of Keele, Staffs.

GEOLOGICAL ASPECTS OF COMMONWEALTH ECONOMY

THE eleventh Inter-University Geological Congress was held in the Department of Geology, University College, Cardiff, during January 3–5. The subject of the congress was "Geological Aspects of Commonwealth Economy" and more than 120 delegates from at least 20 universities and colleges attended.

The first speaker was J. H. Bean, of the Geological Survey of the Federation of Malaya, who described the iron deposits of Malaya. He pointed out that iron ore has been worked on a commercial scale in Malaya since 1921, but since the Second World War the industry has been greatly expanded and now makes a significant contribution to the national economy. About eighteen different iron ore deposits are being worked at the moment, nearly all being located in the proximity of the coast. The iron deposits are found in a wide variety of host rocks—quartzite, schist, quartz porphyry, limestone, andesite, agglomerate, shale, etc.—but one feature common to most of them is that they are associated with granite. The primary iron ore mineral is usually magnetite (Fe_3O_4), less frequently hæmatite (Fe_2O_3), and in one deposit it is siderite ($FeCO_3$). Mr. Bean described several of the important deposits and then went on to consider the genesis of the iron minerals. He thought the fact that iron ore deposits almost invariably lie close to granite masses indicates a genetic relationship. In several areas magnetite and hæmatite are associated with different phases of mineralization. Thus, in the Kedah Peak area, on the north-west coast of Malaya, magnetite is associated with the pegmatites while the hæmatite appears to be of a later hydrothermal origin. In the Ipoh deposit, 100 miles farther south, magnetite is associated with high-temperature silicate minerals, such as garnet and epidote, while the hæmatite is devoid of such minerals, presumably indicating formation at a lower temperature. Probably, the mineralogy of any particular deposit depends on the time of concentration of the mineralizing solutions in relation to the magmatic cycle, with magnetite forming from early solutions and hæmatite from later ones. Finally, Mr. Bean pointed out how the lithology and structure of country rocks control the location and form of ore bodies. Thus, tabular ore bodies are formed in well-bedded shales, but where the rock is of a structureless nature the ore body is irregular. Again, in folded areas ore is usually located along the limbs of folds but rarely along the crests.

The second paper was given by D. Ostle, of the Atomic Energy Division of the Geological Survey of Great Britain. Mr. Ostle discussed prospecting for atomic energy minerals. He pointed out that in recent years most attention has been directed at uranium, thorium and beryllium-bearing minerals, and that some work has been done on the geological environments in which helium can be expected to occur in economic concentrations, since this element is of potential value in gas-cooled reactors. Mr. Ostle discussed in detail methods of radiometric prospecting, particularly stressing the role of the aeroplane in this respect. Aeroradiometric surveys can log radioactivity from a height of 500 ft. above ground-level and, properly used, this technique is an invaluable time saver. Where an area is covered by drift normal radiometric survey techniques are useless, and one means of overcoming this problem is by determining the amount of radon in ground air. Mr. Ostle described other geochemical techniques of prospecting for atomic energy minerals, including systematic sampling and analysis, by fluorimetric and colorimetric means, of uranium in natural ground waters. He also remarked on the selection of geologically favourable areas where these prospecting techniques can be most profitably applied. Thus, it has been found that in igneous rocks, uranium and thorium are largely concentrated in rocks of acidic composition. No deposits of any significance have ever been found in basic igneous rocks.

In the afternoon session, Prof. J. G. C. Anderson, of University College, Cardiff, dealt with the role of geology in hydro-electric development. He began by saying that although civil engineering excavations have greatly assisted geologists, it was not until comparatively recently that widespread use was made of geological information in the design and structure of major engineering works. Yet the engineering geologist has probably just as important a part to play as the mining and the oil geologist. The use of geology in saving money and ensuring safety in the carrying out of major works can be just as important as in locating mineral and energy resources. In the past 25 years this has become more widely recognized. and in the United States an Association of Engineering Geologists has been founded. Prof. Anderson continued by saying that geological knowledge has three main applications in the construction of major hydro-electric developments. The first is in choosing dam and power station sites where the necessary structure can be built with minimum cost and maximum long-term safety. Where the geology of a dam site is not taken into account the result may be the collapse of the dam. The disaster at Dolgarrog can be cited as an example where only a very superficial examination of the geology of the dam site was made, leading to serious weaknesses in the foundations and abutment. The second application of geology to hydro-electric development is in choosing tunnel routes which afford the optimum geological conditions and avoid structures likely to cause special difficulty and even danger. Prof. Anderson cited many examples, including the Loch Sloy and Rheidol hydro-electric schemes, where a geological investigation had indicated alternative tunnel routes of greater potential efficiency and safety, at no extra cost, other than that originally planned. He concluded his address by pointing out that the third application of geology to hydro-electric development lies in the location of suitable constructional materials as close to the major works as possible.

In the evening, Dr. P. G. Harris, of the University of Leeds, dealt with recent volcanic eruption on Tristan da Cunha. Dr. Harris, a member of the expedition sent by the Royal Society to the island, outlined the volcanic history of the island, and with the aid of slides traced the evolution and effects of the recent parasitic volcano. A short film of the volcanic eruption was also shown.

The congress continued the next morning, January 4, with a paper by Dr. W. Idris Jones, director-general of research at the National Coal Board, on fuel gases in the coal measures. Dr. Jones said that the presence of methane in coal-bearing strata has been a constant hazard to coal-mining throughout its history. A complete understanding of how methane is stored and emitted by coal and its surrounding strata is still lacking, and research is now being directed to increase fundamental knowledge of the coal/methane relationship. In recent years, techniques have been developed that make it possible to drain gas from seams or worked-out areas, and pipe it to the surface as fuel. Dr. Jones claimed that Britain led the world in this technique, and said that possibly, in the near future, methane drainage would become part of standard mining practice. He thought the amount of gas which was being wasted nowadays presented a great challenge. Up to 150,000 million cubic feet is lost into the atmosphere every year. Ultimately, coal-mines may provide about 10 per cent of Britain's present gas requirements. Dr. Jones also reported on fuel gases which can be derived from coal seams by gasification *in situ*, the gas produced being led to the surface through boreholes. This involves carbonizing the coal by transmitting heat through it under close control, a technique which has been very successfully used in the U.S.S.R. Dr. Jones concluded by pointing out that both techniques require a knowledge of the geometry and physical properties of the strata in order to exploit natural conditions and obtain maximum yield of gas.

A comparison of oil accumulation conditions in Trinidad and the Canadian Prairies was jointly given by P. Fitzgerald-Moore and Dr. J. Weeda of the Shell International Petroleum Co. Dealing first with the Canadian oil fields, Mr. Fitzgerald-Moore pointed out that oil had accumulated mainly in Upper Palæozoic and Mesozoic sediments. The oil basin lies between a mobile belt and a shield. The general geological history is of geosynclinal warping of the crust, followed by mountain building and foredeep sedimentation, which has migrated shieldwards in time. Oil was found in epicontinental deposits bordering the miogeosyncline, and in quartz sandstones of the early foredeep phase which border, and are derived from, the shield. This latter oil becomes heavier towards the shield and forms an important tar belt. Mr. Fitzgerald-Moore stated that other accumulations of oil have been found in transgressive sands of the foredeep, derived from the craton, and regressive sands of the foredeep, from a Cordilleran source. He also described the tectonic structure of the area, in particular, some remarkable flat-lying thrusts in Palæozoic strata. Mr. Fitzgerald-Moore pointed out that the deeper structures of the Cordillera are unknown but he thought there had possibly been a *décollement* between the crystalline basement and the overlying Palæozoic rocks.

Dr. J. Weeda turned to the Trinidad oil field but first placed it within the framework of the Orinoco Basin. This is essentially Tertiary in age and has followed the same general geological history as the Canadian Basin, although the latter involves far less crustal downwarping. Dr. Weeda said that oil was found in the transgressive and regressive sands in the Orinoco Basin, but in this case the transgressive sands derive from the Cordillera, and the regressive sands from the craton. There was also an extensive tar belt bordering the shield, he added, but only insignificant oil accumulations occurred in the epicontinental deposits. Dr. Weeda concluded by describing the tectonic structure of the Trinidad oil field. This is extremely complex and has been described as "the graveyard of geologists' reputations". The shelf area is broken by numerous powerful faults and the incompetent Tertiary sediments of the mobile belt exhibit flowage and diapiric structures.

The first lecture in the afternoon session was given by L. C. Noakes, the British Commonwealth Geological Liaison Officer, on underground water in Australia. Three-quarters of the Australian continent, said Mr. Noakes, has been described as an 'arid zone'. Although the zone supports only 2 per cent of the population, it provides 40 per cent of Australia's mineral wealth, and 30 per cent and 25 per cent of the beef cattle and sheep population respectively. Underground water supplies have played an extremely important part in the exploitation of mineral resources and the development of pastoral farming. Mr. Noakes commented that the geology of the arid zone divides it into well-marked areas of differing water potential. A quarter of the zone overlies compact, crystalline Pre-Cambrian rocks of low ground-water potential. In this area water accumulates mainly at the interface between weathered and unweathered rock, and in joints and fault zones. A sixth of the zone is underlain by the more permeable Upper Pre-Cambrian rocks, which thus possess a higher ground-water potential. More than half the area overlies major sedimentary basins, such as the great Artesian Basin, which have a high water potential. An appreciable proportion of the underground water in these areas, Mr. Noakes suggested, may be 'fossil water', derived from pluvial periods hundreds of thousands of years ago, and retained in the rocks ever since. Finally, there are small areas where crystalline Pre-Cambrian rocks are overlain by recent unconsolidated sediments, which act as aquifers. The speaker pointed out that water problems in Australia may be common to a number of States, and this has resulted in the recent founding of the permanent Inter-state Underground Water Conference. Finally, Mr. Noakes emphasized one of the most important and interesting problems in hydrogeology, namely, the establishment of a 'water balance' in artesian water supplies. This ensures, first, that these supplies are not depleted by excessive usage, and secondly, that they are used to their maximum capacity compatible with natural replenishment.

Mr. J. W. Pallister, commissioner of the Geological Survey of Tanganyika, delivered the final lecture of the congress on "The Geological Survey and Economic Development in East Africa". He said that the social and economic development of the East African territories can be divided into four phases, and in each the official Geological Surveys have played an important and distinctive part. The first phase broadly occupies the early 1920's, when the Surveys were established, to the onset of the Second World War. During this period the Surveys were mainly assisting and stimulating the small mining industries which were largely interested in exporting such minerals as gold, tin, mica, copper, lead and diamonds. Mr. Pallister emphasized that the Surveys were busy with many *ad hoc* investigations, and there was little opportunity for systematic geological mapping. During the Second World War—the second period of economic development—there was a demand for special strategic materials such as wolfram, graphite, kyanite and niobium minerals. Local industries, cut off from normal sources of supply, also developed local raw materials. The third period, which occupies the post-War years up to independence, saw an increased interest in the natural resources of Africa by both the British Government and the local population. During these years the Surveys embarked on long-term systematic mapping. After some years of prospecting, the partial withdrawal of interest by large outside mining concerns compelled the Surveys to undertake the prospecting and assessing of potential mineral deposits beyond the point when private interests would normally take over. This also coincided with a tendency for the local population to take an increasing part in the prospecting and exploiting of smaller deposits. With independence, Mr. Pallister pointed out, the new countries are eager to develop their natural resources. This development is dependent on outside Governmental or international agencies for funds, and the basic geological information required by these kinds of organizations has stimulated systematic mapping of the geology. Mr. Pallister concluded by saying that the Geological Surveys have always played an important part in developing water supplies. Not only have new water supplies been developed in areas where existing sources are inadequate, but also in areas where sources, although sufficient in quantity, are badly contaminated and seriously affect the health of the local people.

On the last day of the congress, field excursions were arranged to two local coal-mines—the Cwm and Nantgarw Collieries—and also to the Llanharry Iron Ore Mine. Other field trips which were arranged had to be cancelled because of poor weather. M. J. Wilson

PALÆOMAGNETIC STUDIES IN THE U.S.S.R.

IN the U.S.S.R. intensive palæomagnetic studies began in 1953. Since that year three palæomagnetic conferences have been held and a special Palæomagnetic Commission formed at the Institute of Physics of the Earth. A special task entrusted to this Commission is the collection of palæomagnetic data for further studies and generalizations. One of the first comprehensive and detailed works on palæomagnetism in the U.S.S.R. was the memoir by Khramov[1] on the palæomagnetic correlation of sedimentary formations in Western Turkmenia, ranging in age from the Lower Jurassic to the Quaternary. Besides its main object this study opened up other problems, such as geochronology on a wider scale, the rate of sedimentary accumulation and the problem of the continental drift. In the introduction and in the first chapter Khramov gives a brief history of palæomagnetic research and a detailed description of the methods used. The last-mentioned subject has provided material for a special book written by Khramov and collaborators[2]. Another important publication is that by Kalashnikov[3], who begins by providing the general setting of the problem (pp. 1242–1257) and by giving a succinct review of the achievements in this field outside the U.S.S.R. This study, Kalashnikov claims, demonstrates beyond any doubt that there exists a definite functional relation between the orientation of magnetic vectors of rocks and their age. Many problems, however, such as the cause of reverse polarity and of the part played by the continental drift, remain still unsolved.

In the second part of this work (pp. 1257–1279) the author gives a most valuable summary of the results of palæomagnetic studies in the U.S.S.R. during the past decade. This summary consists of the tabulation of data relating to 111 collection localities, involving more than 5,000 oriented and measured specimens of rocks ranging in age from the Cambrian to the Quaternary. Against each locality item are given the values of declination and inclination of the magnetic vector and the calculated values of the co-ordinates of the North Pole. These data are then represented on eleven polar projections of the northern hemisphere, one for each geological period. The average values of the positions of the North Pole during each period are used to provide a background for the construction of the polar migration curve. The greater scatter of points relating to the polar position during the Lower Palæozoic era necessitates the separation of the migration curve into two widely separated sectors, one based on the data of the European part of the U.S.S.R. and the other of the Asiatic part. The main migration curve with its European sector extension is in general alignment with the migration curve constructed on the basis of data from West European and British localities, but, as the summary diagram shows, differs widely from the migration curves based on American and Chinese data. This suggests the influence of continental drift. Such a world-wide marshalling of palæomagnetic data and comparison of the polar migration curves is a great achievement. At the same time Kalashnikov points out that to be fully acceptable these results must agree with the results of palæoclimatology and palæogeochemistry. He does not discuss the data of palæogeochemistry, but with regard to the data of palæoclimatology he claims that so far as the U.S.S.R. is concerned such a correlation has been achieved, as shown by the material published by Forsh and Khramov[4], Rukhin[5] and Strakhov[6]. Among problems still remaining unsolved are the problem of the continental drift, of the possibility that the Earth is increasing in size in time, of the velocity of polar migration and its variation in time (the average velocity of polar migration as calculated by Kalashnikov for the span of 500 m.y. is about 2 cm per annum). S. I. TOMKEIEFF

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TROPICAL HÆMATOLOGY

AN international colloquium on "Tropical Hæmatology" was held at the Prince Leopold Institute of Tropical Medicine, Antwerp, during November 30–December 2.

The inaugural academic session was presided over by the Governor of the Province of Antwerp, president of the Administrative Council of the Institute, who welcomed the participants among whom were many foreign hæmatologists.

In his opening lecture, Prof. P. G. Janssens, director of the Institute, dealt with the perspectives offered by the proposed installation at Antwerp of university faculties; on the other hand, he emphasized the responsibilities of official bodies in the particular problems raised by the medical aid given by Belgium to the developing countries.

The Academic Lecture was given by Prof. J. Van Riel, of the Institute of Tropical Medicine, the subject being "The Great Steps of Progress in Public Health".

The first working session, presided over by Prof. J. Schneider (Paris), was concerned with tropical hypereosinophilias. This was discussed by Dr. A. M. Smit (Amsterdam), Dr. M. Fernex (Basle), and Profs. W. Mohr (Hamburg), A. F. Essellier (Zurich) and P. G. Janssens (Antwerp). From the discussion it appeared that though many of the hypereosinophilias contracted in the tropics can be explained by helminth infestations, many cases remain enigmas. Infestations by parasites of which natural hosts are animals are responsible, at least in part, for the appearance of the syndrome. It was also emphasized that the eosinophilia of helminthiasis is a reflexion of the tissue mastocytosis found in these cases, in which allergy seems to play only a minor part (so-called 'resorption eosinophilia').

The second working session, with Prof. Mohr (Hamburg) in the chair, discussed blood groups and blood transfusion. Prof. J. Schneider (Paris) dealt with tropical diseases transmitted by blood transfusion; of these, malaria is the most frequent, the other tropical diseases being seldom or only very rarely transmitted by this procedure. Prof. J. Jadin (Antwerp) dealt with blood groups in Congolese. The pygmoids possess genetic ABO frequencies very different from the negroids. Certain negroid tribes seemed to have cross-bred with the pygmoids, especially in Kivu and in the north of Equateur Province.

The third working session, under the presidency of Prof. Lambin (Louvain), was devoted to the serum proteins of hæmatological importance. Profs. G. Charmot (Marseilles) and R. Vargues (Tours) dealt with African macroglobulinæmias. They showed that, in Africa, macroglobulinæmia secondary to first stage trypanosomiasis, tuberculoid leprosy and to malaria is not uncommon. All their cases of macroglobulinæmia were confirmed by ultracentrifugation. Drs. G. Van Ros and M. Van Sande (Antwerp) and R. Druet (Usumbura) then discussed the serum haptoglobin and transferrin groups in White and Negro peoples as demonstrated by electrophoresis on starch gel. The frequencies of the genes determining these groups has been established as well as the existence of certain uncommon types in Negro peoples. It was also stressed that anhaptoglobinæmia was common in carriers of the sickle-cell trait. Finally, Drs. Van Sande and Van Ros also dealt with the value of agar-gel electrophoresis in hæmatology, especially in the determination of the types of haptoglobin and transferrin, and in the detection of abnormal hæmoglobins.

The fourth session, with Prof. J. H. P. Jonxis (Groningen) in the chair, was concerned with the investigation of fœtal hæmoglobin. Prof. A. B. Raper (Bristol) mentioned the factors delaying the passage of fœtal erythropoiesis to that found in adults. Prof. J. Sonnet and Dr. M. Michaux (Leopoldville) described in detail a case of persistence of fœtal erythropoiesis in an adult, and Dr. G. Helderweirt (Antwerp) demonstrated that the detection, by a personal method, of Rh + fœtal red cells in the blood circulation of pregnant and delivered Rh – women enables us the better to understand and supervise Rh immunization during pregnancy.

The fifth session, presided over by Prof. A. C. Allison (London), discussed tropical anæmias. Prof. T. A. J. Prankerd (London) described two methods of detecting folic acid deficiency. One of these determines the concentration of folic acid in the serum using *L. casei*, the other measures the excretion of formimino-glutamic acid in the urine after the administration of histidine. Dr. H. R. Marti (Basle) reported observations made on the symptomatology of thalassæmia in 200 patients showing either the alpha- or beta-forms. Dr. H. Knüttgen (Hamburg) gave a report on the phagocytic activity of the cells of the bone marrow in malaria. Finally, Dr. H. M. Gilles (Liverpool) dealt with the epidemiological and clinical aspects of the anæmia caused by hookworms.

The sixth and last session, under the presidency of Prof. A. Dubois (Antwerp), considered the abnormal hæmoglobins (Hb). Prof. J. H. P. Jonxis (Groningen) described the molecular structure of these Hb's, and Prof. P. Boivin and L. Hartmann (Paris) their antigenic specificity. Prof. A. C. Allison (London) dealt with the relationship between the hæmoglobinoses and deficiency in erythrocytic glucose-6-phosphate dehydrogenase. Finally, Profs. E. Silvestroni and I. Bianco (Rome) described the varieties of human Hb and of thalassæmia reported so far from Italy.

G. VAN ROS

QUASI-CONTINUOUS OUTPUT FROM A RUBY OPTICAL MASER

By M. S. LIPSETT and DR. L. MANDEL

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SEVERAL workers[1,2] have observed that reducing the crystal temperature may suppress the relaxation oscillations (or spikes) in some ruby optical masers. As the fluorescence line-width narrows the energy is made available to fewer resonator modes, and the interval between successive spikes is progressively shortened until they merge and quasi-continuous output results.

We wish to report observations of a similar effect, but at room temperature and for quite different reasons. We have observed quasi-continuous output from a ruby optical maser oscillating with a very non-uniform distribution of energy along the length of the maser rod.

Our observations were made on a ruby rod 2·5 mm in diameter and 3·76(7) mm long, faced with spherical surfaces in what was to have been a near-confocal configuration. The radii of curvature of the reflecting surfaces were found to be 3·84 mm and 3·69 mm. The detailed optical properties of this crystal are not known. Such maser crystals are, of course, far from optically perfect[3-5]. The ruby was excited in the usual way by a linear flashtube in an elliptical cylinder reflector. Part of the emerging maser light beam was sampled by means of a beam splitter and directed to a fast-response photocell from which the temporal development was recorded. At the same time a magnified image of each face was photographed in turn (through a red filter to remove background light) with a single-lens reflex camera.

Fig. 1 illustrates the kind of temporal behaviour that was observed at room temperature, while Fig. 2 shows a corresponding set of photographs of the reflectors. It should be noted from Fig. 1 that, although the maser light fluctuates fairly strongly, its intensity does not approach zero for several hundred μsec. Similar behaviour was observed both for input energies near threshold and well above threshold. Near threshold, where a few well-defined spikes might have been expected, the output lasted for 50 μsec or less.

The photographs of Fig. 2 show the mode pattern at both ends of the maser rod. Maser light diverges from tiny areas on one surface to a broad area on the other surface and re-converges to conjugate points on the first surface. The maser modes are therefore cone shaped. Fig. 3 illustrates the situation.

Some features of these modes are somewhat puzzling. For example, the bright pairs of spots always occurred at the reflector of smaller radius of curvature, even for quite a variety of reflecting coatings. A possible reason for this preferred direction is that the reflector of greater radius of

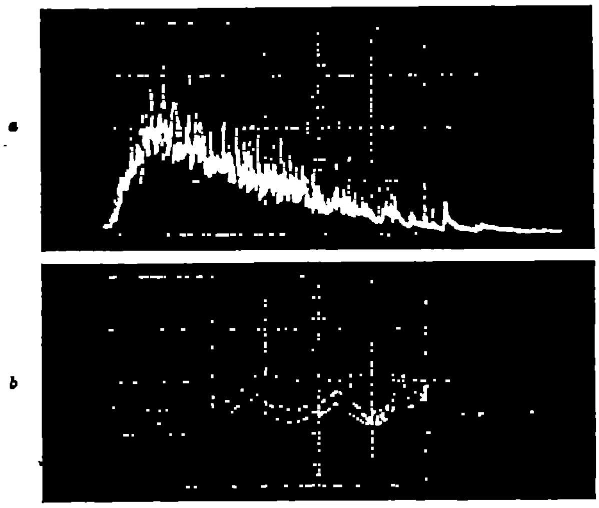

Fig. 1. Time development of the output from the maser. *a*, Vertical scale arbitrary, horizontal scale 50 μsec per division. The signal is due entirely to the maser radiation. The input to the flashlamp is 160 joules (15 per cent above threshold); the pulse of pumping light lasts about 1 m.sec. *b*, Portion of trace under the same conditions as *a* expanded to 1 μsec per division

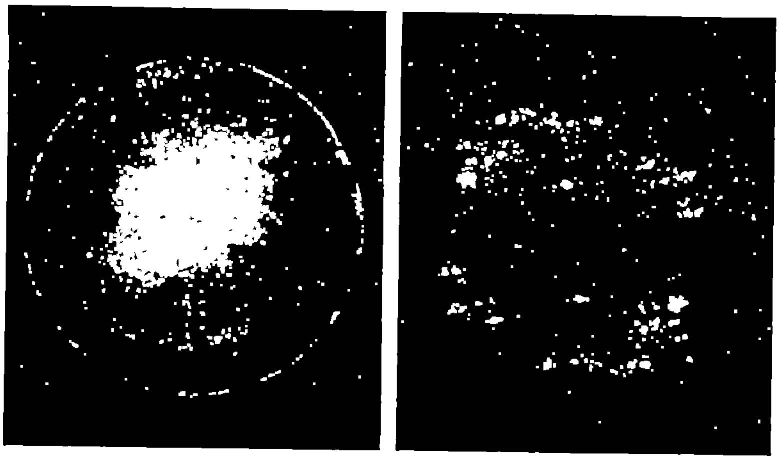

Fig. 2. Photographs of both ends of the maser for the same input conditions as in Fig. 1. Note the symmetry about any diameter

curvature more nearly matched the spacing of the surfaces[6]. Our measurements were not sufficiently accurate to confirm this possibility.

To understand why the output might be quasi-continuous for these conical modes let us consider how the rate of stimulated emission varies along the length of the resonator. We note from Fig. 2 that the energy density associated with the mode varies from one surface to the other by a factor of about 50. Since the probability of stimulated emission is proportional to the energy density, the rate of depletion of the metastable level—and therefore the relaxation cycle—varies appreciably along the crystal. In any elementary region of the crystal typical relaxation oscillations may be occurring, but the sum of all such contributions from the crystal leads to a fairly continuous output. Burch[7] has analysed the convergent wave-front kind of resonator and has predicted the possibility of quasi-continuous maser output for certain energy distributions (see also Pao[8]). These conditions appear to have been met in our observations.

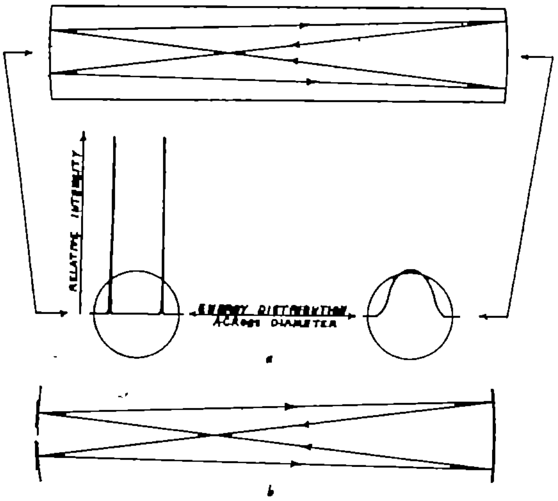

Fig. 3. Mode behaviour suggested by Fig. 2; b, equivalent conical resonator

The observed modes were much more asymmetric than one might have expected from the geometry of the ruby. However, the resonator dimensions were such that the reflector spacing was intermediate between the two radii of curvature. It has been shown by Boyd and Kogelnik[6] that this is one condition on the resonator leading to modes with high diffraction losses and, as will be seen from Fig. 2, the diffraction losses are indeed high at one face. An equivalent conical resonator[9] can be formed by a spherical reflector and a pair of plane reflectors arranged as shown in Fig. 3b. Evidently a spherical reflector can behave as a large number of such pairs of planes—at least to a first approximation of small areas—when the maser prefers to oscillate in highly asymmetric modes.

One can probably obtain similar behaviour by other reflector configurations. The most obvious choice is a maser rod faced at one end with a plane reflector and at the other with a spherical reflector having a radius of curvature equal to or slightly greater than the length of the crystal. Exact matching of the dimensions may lead to excessive energy densities at the plane reflector. Burch[7] has suggested an alternative scheme with one concave and one convex reflector. If the foregoing explanation of the quasi-continuous output is correct in principle, any configuration giving rise to modes with a non-uniform distribution of energy might be expected to reduce the tendency of maser crystals to exhibit spikes. This point should not be too difficult to investigate and might provide a clue to the design of ruby optical masers for producing continuous oscillations.

We are grateful for technical assistance from Mr. B. Bates and Mr. R. V. Shack. The work was supported financially by a grant from the Department of Scientific and Industrial Research.

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LOW-GRADE METAMORPHIC ROCKS FROM THE PRINCE CHARLES MOUNTAINS, EAST ANTARCTICA

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WITH few exceptions the rocks so far described from the East Antarctic Shield are high-grade metamorphics, predominantly gneisses, granites and granulites, in the granulite or the almandine amphibolite facies of metamorphism as defined by Fyfe, Turner, and Verhoogen[1]. Small exposures of low-grade metamorphic rocks have been described from Western Dronning Maud Land (long. 0°) (ref. 2), from Mount Sandow (long. 101° E.) (ref. 3), and from Oates Land (long. 156° E.) near the boundary of the Shield[4].

In the southern ranges of the Prince Charles Mountains low-grade metamorphic rocks have recently been discovered by the Australian National Antarctic Research Expeditions. The rocks crop out within an area of 2,000

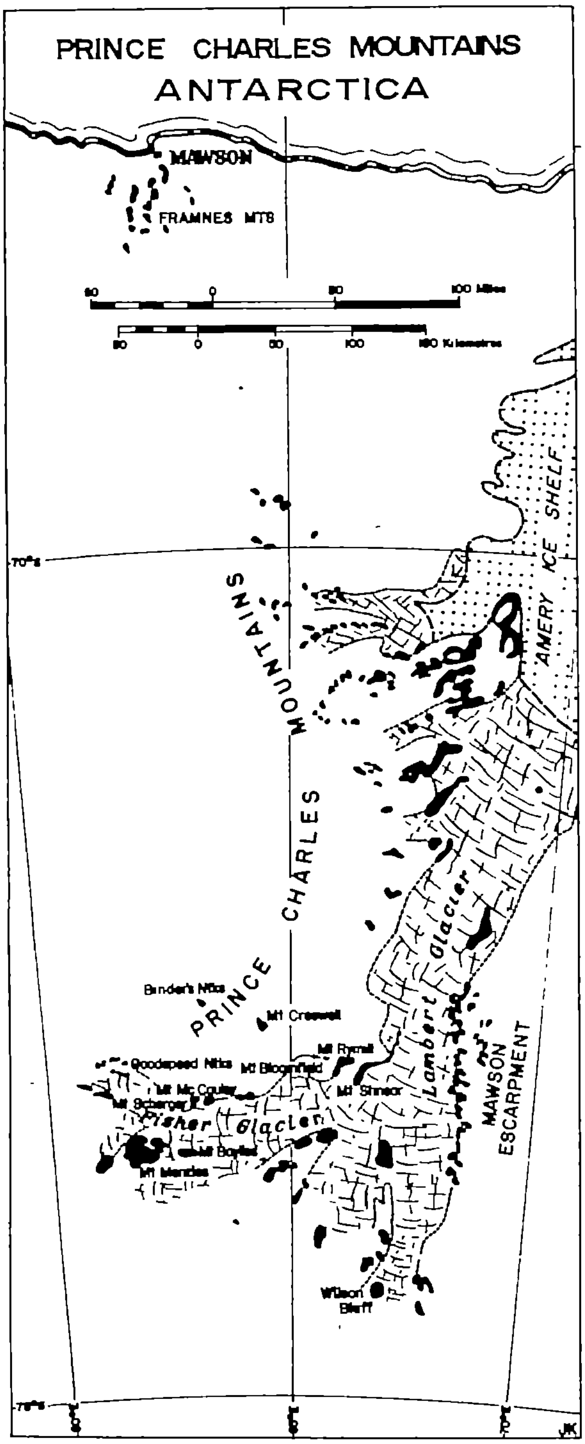

Fig. 1

sq. miles (5,000 km^2) around the Fisher Glacier. They may be continuous with similar rocks at Wilson Bluff, at the head of the Lambert Glacier.

High-grade metamorphic rocks form the outcrops along the coast of East Antarctica between 45° E. and 80° E., and the numerous nunataks within 100 miles of this coastline[2,3]. These rocks are gneisses, granulites, and foliated granites, with biotite, garnet and pyroxene as their principal ferromagnesian minerals. The northern ranges of the Prince Charles Mountains are formed by similar high-grade metamorphics; they also contain a low-lying area of Permian sediments[4].

Low-grade metamorphic rocks were first discovered in this sector in 1957 when M. Fisher, an Expeditions surveyor, was landed at Mount Stinear, in the southern ranges of the Prince Charles Mountains, where he collected slate and mica schist from moraine fringing the mountain. M. Mellor, an Expeditions glaciologist, collected metamorphosed sandstone from moraine in the Goodspeed Nunataks in 1958 [4]. Later in 1958, I. McLeod was landed at Wilson Bluff, near the southern termination of the Prince Charles Mountains, where he found outcrops of mica schist, quartzite and amphibole-bearing rocks, quite unlike the high-grade metamorphics typical of the East Antarctic Shield[4].

In 1960, R. Ruker reconnoitred the mountains along the north side of the Fisher Glacier[7]. The outlying nunataks—Binder's Nunataks, Mount Creswell and Mount Bloomfield—are composed of gneisses in the almandine amphibolite facies of Fyfe, Turner and Verhoogen[1]. The large mountains fringing the Fisher Glacier are dominantly made up of quartzites and mica schists in the greenschist facies. These low-grade metamorphic rocks are intruded by large bodies of massive biotite granite and by basic dykes. Moraine near these mountains contains fragments of dolomitic marble and large blocks of jaspilite and hæmatite.

D. Trail extended the investigation south of the Fisher Glacier in 1961. Mount Menzies, a massif 18 miles across, is composed of greenschist facies rocks, dominantly chloritoid quartzite with bands of micaceous quartzite and mica schist.

At Mount Bayliss, marble, quartzite and amphibolite, originally in the greenschist facies of metamorphism, are interbanded with granite and have been thermally metamorphosed. Later intense shearing has imposed a strong sub-horizontal foliation on both the granite and the metamorphosed rocks. Slate and phyllite are common in the moraine on both mountains.

The structure of Mount Bayliss suggests that a major thrust-plane may separate low-grade and high-grade metamorphic rocks. In the interpretation of seismic traverses between Mawson and the Goodspeed Nunataks, Goodspeed and Jesson[8] show a deep rock-surface depression between the gneiss at Binder's Nunataks and the quartzite at the Goodspeed Nunataks. This depression may be occupied by a major tectonic discordance.

The low-grade quartzites and schists are the metamorphosed equivalents of quartz-rich sands and silts which would be produced by the erosion of terrain composed of the dominantly acid, high-grade gneisses.

Evidence for two episodes of metamorphism has been obtained from the coast of Kemp Land between long. 60° E. and 55°. E (100–200 miles west of Mawson). Here, basic dykes, which sharply cut well-foliated gneisses, are themselves metamorphosed; some have developed garnet and have a faint, discordant foliation[9].

Starik *et al.*[10] have obtained values between 400 million and 1,500 million years for the absolute ages of various high-grade metamorphic rocks from the East Antarctic Shield. From determinations of both high-grade and low-grade metamorphic rocks, Starik *et al.*[10] and Sarah Deutsch *et al.*[11] have obtained an age between 400 million and 500 million years for the latest metamorphism in East Antarctica.

The low-grade metamorphic rocks have probably been involved only in the latest, Lower Palæozoic episode. They may be considerably younger than the high-grade metamorphics, many of which have been repeatedly metamorphosed in successive orogenies throughout the Proterozoic era.

In moraine at Mount Rymill, Ruker[7] discovered fragments of unmetamorphosed sandstone and shale with *Glossopteris* and other fossil plants, referred to the Permian by Mary White[12]. These unaltered rocks may be exposed *in situ* in mountains at the head of the Lambert Glacier.

I thank I. McLeod for guidance and R. Ruker for information. I also thank W. R. McCarthy, of the Australian Mineral Development Laboratories, for the petrographic determinations of rock type and metamorphic facies on which this work is based.

My colleagues and I are grateful to fellow members of the Australian National Antarctic Research Expeditions for their support and assistance to geologists in the field.

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STRUCTURE OF RIPPLE MARKS

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A VALUABLE new classification has recently been proposed by Allen[1] for asymmetric ripple marks, based essentially on their external form and scale. Allen has also offered an explanation of how straight and trough (*sic*) cross-stratification is generated. This is based on his own and other selected observations of straight and linguoid asymmetric ripples in shallow seas, estuaries and rivers. Allen's mechanism involves overstep by the advancing foresets of a ripple on to the upstream (stoss) slope of the succeeding downstream ripple, which itself is being synchronously eroded up to the moment of its burial. His figures (*A* and *B*) show that if this mechanism is correct, the erosion surfaces, which form the boundaries of the sets of cross-strata generated from a society of aggrading ripples, must always be oblique to the regional depositional dip of the deposit. Furthermore, if his suggestions relating the depth of water to the thickness of individual sets or units or cross-strata are to be accepted, the dip of the upper boundary of the set must always be greater than, or equal to, the dip of the stoss side of the ripple or dune.

In making his assumptions Allen appears to have disregarded two commonly observed phenomena: (*a*) the ubiquitous field observation, that in both large and small scale cross-stratification the lower boundary of the cross-stratified unit or set is generally parallel and not generally oblique to the regional depositional dip, as evidenced by preceding and succeeding plane-concordant stratified formations; (*b*) the phenomenon referred to as 'kolking' by Blasius[2] and 'pot-holing' by Simons *et al.*[3] and what, following Simons *et al.*[3], may be termed 'toe-blasting' of the front of an advancing dune or asymmetric ripple.

When the net effect of deposition and erosion in a fluvial or distributary dune system is positive (for example, the system is aggrading) any explanation of the resulting bedding structures must therefore take account of these two phenomena.

The results of preliminary examinations of both straight and festoon types of cross-stratification on the north-east coast, in the Jurassic at Whitby (N.G.R. *NZ* 898115) and elsewhere, and the Carboniferous at Seaton Sluice (*NZ* 3476) and Hawick (*NU* 2617) and in numerous other exposures in other systems, may be represented as follows:

(1) *Festoon cross-stratification* (Fig. 1). The morphological element responsible for festoon cross-stratification appears to consist of the lower segment of a nearly circular trough the axial line (that is, the line formed by the intersection of the axial plane with the surface of the trough) of which may be inclined at various angles in the plane of the regional dip. The erosional furrows appear to be

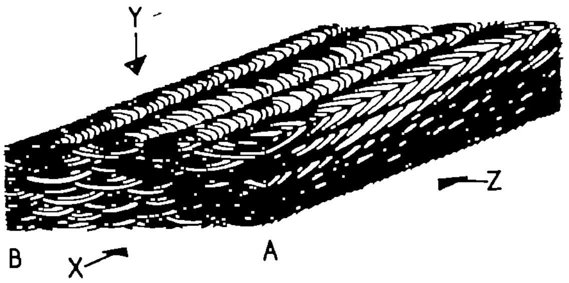

Fig. 1. Block diagram of festoon bedding, showing surface form of plane of truncation (Y) parallel to regional depositional dip

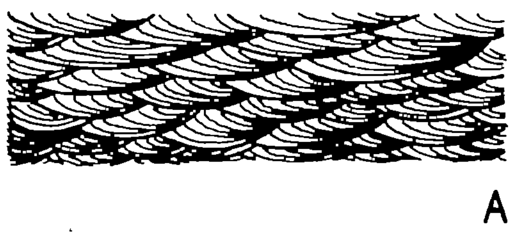

Fig. 2

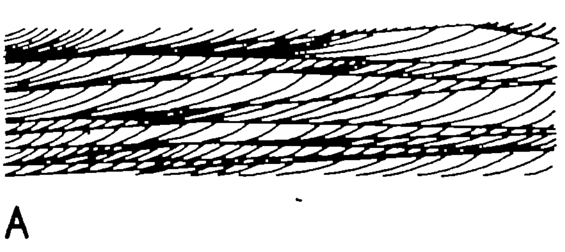

Fig. 3

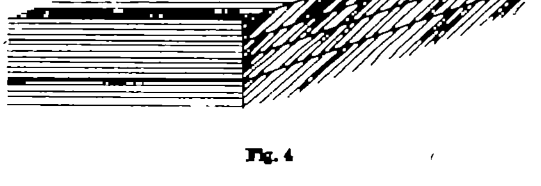

Fig. 4

formed by the trace of this inclined trough across an earlier surface parallel to the regional dip. Allen has already directed attention to the offset super-position of the furrows as a consequence of the surface pattern of a society of linguoid dunes.

The foreset deposits filling the furrow consist of a conformable series with the same shape and inclination as the trough element responsible for the furrow. Thus the foresets are seen to be conformable with the base of the furrow when viewed perpendicular to the main current direction in Fig. 1. The section parallel to the main current direction *Z* in Fig. 1 illustrates one of the causes of the apparent irregularity of thickness displayed in those exposures of festoon cross-stratification in which the unit boundaries are parallel. It also shows one of the causes of the apparent concavity of much of the cross-strata in such an exposure.

Oblique sections across sets of festoon trough cross-stratified units reveal a wide range of forms. The section at approximately 20° to *AB* (Fig. 2) is similar to a type of trough cross-stratification recently described by Allen[4] from the Senni Beds of the Lower Old Red Sandstone of the Black Mountains, and by Zhemchuznikov *et al.*[5] from the Carboniferous of the Donetz Basin and in contemporary alluvial sediments by Shantzer[6]. Zhemchuznikov *et al.*[5] ascribe this type to the upper part of the main channel in shoal and braided portions (Table 2).

Basin-like (*sic*) bedding similar to that seen in sections perpendicular to the main current direction festoon cross-stratification is attributed[5] to inshore marine conditions as expressed in sandstones and coarse siltstones (Table 3). Fig. 3 shows a vertical section at approximately 70° to *AB*: this also appears to have been considered as having a distinctive environmental significance by Zhemchuznikov *et al.*[5].

Botvinkina[7] has also given a detailed morphological classification of cross-stratification. This sets out as individual types many forms which are identical to those seen in the varyingly aligned transverse sections of festoon cross-stratification. Clearly an appreciation of the orientation of the plane of dissection of this structure in both natural sections and borehole cores is fundamental to their interpretation and effective classification.

The variety of festoon cross-stratification illustrated in Figs. 1–3 occurs when the dip of the axial line of the foresets is greater than the maximum dip on the sides of the furrows. Slightly different cross-sections may be constructed when the maximum dip on the sides of the furrows is greater than that of the axial line of the foresets. Other types differentiated by Botvinkina[7] belong to sections from this latter case.

(2) *Straight or rectilineal cross-stratification* (Fig. 4). The only required modification of Allen's explanation of how straight cross-stratification is generated is that it should be adapted to explain the parallelism of the individual sets with the regional depositional dip (Fig. 4).

It may be that differences in the depths of the advancing 'potholes' are the principal factor controlling the height of the large-scale festoon cross-stratified units. If this is the case then the systems elucidated by Simons *et al.*[3] are generally applicable, and consequently the differences in the depth of 'toe-blasting' may control the thickness of successive units of straight cross-stratification.

In conclusion, the mechanism proposed by Allen for the generation of cross-stratification is not acceptable for the exposures we have observed. However, cross-stratification with individual sets oblique to the regional depositional dip have been observed, but only on a small scale. The latter we believe are related to ripple drift, and we refer to this type as drifted cross-stratification or cross-drifting.

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DECOMPOSITION OF TRITIUM-LABELLED ORGANIC COMPOUNDS

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THE very rapid progress which is being made in the application of tritium-labelled organic compounds for many physical and biological investigations[1] has prompted the publication of this article on the decomposition of tritium-labelled organic compounds. A more general account of the self-decomposition of compounds labelled with radioactive isotopes has recently been published[2], with special reference to sugars labelled with carbon-14.

The high specific activities which can be obtained with tritium compounds, the short range[3] (0·7 mg/cm^2) of the weak β-radiation (maximum energy[4] between 17·5 and 19·4 keV and mean energy[5] 5·52 keV) and the long half-life[6] (12·3 years) of tritium, enables compounds labelled with this isotope to have a very wide variety of uses ranging, for example, from luminous paint[7] to radiotherapy[8]. The half-life of tritium, although conveniently long for most experimental uses, represents a decomposition rate of approximately 5 per cent per annum in the storage of labelled compounds, due to the natural decay of tritium to helium, that is, primary (internal) decomposition[9].

Specific Activity and Types of Molecules

In any organic compound C_nH_mX (where X is any element or group of elements other than hydrogen), the replacement of one hydrogen atom per molecule with tritium results in the compound $C_nH_{m-1}TX$ (the letter T is used to represent a tritium atom) having a specific activity of 29·12 c./m.mole. When considering the decomposition of such labelled molecules one must recognize two types: (*a*) molecules which are labelled at one position only, that is, specifically labelled; (*b*) molecules which have multiple labelling, that is, in more than one position.

As hydrogen atoms directly attached to oxygen, nitrogen and sulphur atoms are labile, that is, exchangeable in hydroxylic solvents, only compounds with tritium atoms directly attached to carbon atoms are considered. Compounds in class '*a*' are often produced with the tritium isotopic abundance at one hydrogen position of very nearly 100 per cent and the concentration of tritium in the molecule sometimes as high as 50 per cent or more of the total hydrogen atoms. Compounds at such high specific activities are required, for example, for radiotherapeutic use[8] or for luminous compound work[7]. For most tracer applications of tritium-labelled organic compounds, isotopic abundances of less than 10 per cent are normally required and in the majority of cases the tritium abundance in a molecule varies from 0·1–1 per cent only. The activity associated with one molecule of a tritium compound containing one atom of tritium is approximately 5×10^{-20} c. and the energy available from tritium corresponds to 0·88 eV/atom/day. This energy is much less than most bond dissociation energies[9]; about 4 eV are normally required to rupture a bond.

Primary Decomposition

When the tritium atom of a mono-tritiated molecule disintegrates, helium is produced leaving a non-radioactive recoil fragment. Molecules containing more than one tritium atom would give rise to radioactive recoil fragments if the tritium atoms did not disintegrate at the same time, which is unlikely to occur and ultimately only non-radioactive decomposition products will arise by primary (internal) decomposition. Consider for example the labelled amino-acid glycine-2-T ($NH_2.CHT.COOH$); primary (internal) decomposition due to natural decay will not give rise to radioactive impurities. This is shown by the figures in Table 1, glycine-2-T remaining radiochemically pure even after 21 months.

The atomic and molecular consequences of radioactive decay for tritium and one or two tritiated hydrocarbons have been examined[10]. For the simple tritiated hydrocarbon methane (CH_3T) the predominant process is one in which neutral helium-3 separates leaving a positively charged methyl group, namely, CH_3^+, and less frequently hydrogen atoms are also split off leaving CH_2^+, CH^+ and C^+. Similar results have been obtained with tritiated ethane[11] giving rise to positively charged ethyl groups, $C_2H_5^+$. These results are in direct contrast to the behaviour of tritium[10]. In molecules of TH, the pure β-emission fails to dissociate, which remain as $(He_3H)^+$. It seems likely, therefore, that in tritiated molecules of greater complexity, primary (internal) decomposition will give rise to neutral helium-3 and positively charged groups which may further loose hydrogen atoms. For the more complex molecules no information is yet available regarding the nature of these non-radioactive decomposition products, but they could possibly be conveniently studied by using doubly labelled molecules, labelled, for example, with carbon-14 and tritium. The proportion of the breakdown fragments labelled with carbon-14 could then be detected. However, unless the specific activity with respect to both isotopes is very high, the recoil fragments would have to be carefully sorted from the singly labelled molecules remaining.

Table 1. DECOMPOSITION OF TRITIATED AMINO-ACIDS

Compound	Specific activity (mc./m.mole)	Age (months)	Storage condition	Temperature (° C)	Method of analysis	Solvent systems	Radiochemical purity (per cent)
DL-Alanine-T(*G*)	1,180	4	Aqueous solution	Room	P.C.	1, 2 and 3	100
DL-Alanine-T(*G*)	1,180	12	Solid	0	P.C.	1, 2 and 3	90
DL-Alanine-T(*G*)	26	30	Solid	Room	P.C.	1, 2 and 3	100
L-Arginine-T(*G*)	27	15	Solid	Room	P.C.	1, 2, 3 and 4	100
DL-3,4-Dihydroxyphenylalanine-T(*G*)	223	7	Solid	0	P.C.	1, 2 and 3	100
Glycine-2-T	176	17	Solid	0	P.C.	1, 2 and 3	100
Glycine-2-T	176	17	Aqueous solution	0	P.C.	1, 2 and 3	100
Glycine-2-T	176	21	Aqueous solution	0	P.C.	1, 2 and 3	100
DL-5-Hydroxytryptophan-T(*G*)	927	3	Aqueous solution	0	P.C.	1, 2, 5 and 6	94
DL-Leucine-T(*G*)	105	18	Solid	Room	P.C.	1, 2 and 3	93
DL-Leucine-T(*G*)	102	7	Solid	Room	D.A.	—	100
L-Leucine-T(*G*)	197	12	Solid	0	P.C.	1, 2, 3 and 7	96
DL-Leucine-4,5-T	151	3	Aqueous solution	0	P.C.	1, 2, 3 and 7	100
DL-Leucine-4,5-T	5,400	7	Aqueous solution	0	P.C.	1, 2, 3 and 7	75
DL-Leucine-4,5-T	576	10	Aqueous solution	− 40	P.C.	1, 2, 3 and 7	90
DL-Methionine-T(*G*)	28	8	Solid	Room	D.A.	—	86
DL-Methionine-T(*G*)	28	8	Solid	−40	D.A.	—	90
DL-Methionine-T(*G*)	148	11	Solid	0	D.A.	—	83
DL-Methionine-T(*G*)	148	11	Aqueous solution	0	D.A.	—	100
DL-Methionine-T(*G*)	148	13	Solid	−40	P.C.	1, 2 and 3	97
DL-Methionine-T(*G*)	145	16	Solid	−40	P.C.	1, 2 and 3	97
DL-β-Phenylalanine-T(*G*)	126	25	Solid	Room	P.C.	1, 2 and 3	97
DL-β-Phenylalanine-T(*G*)	1,800	7	Aqueous solution	Room	P.C.	1, 2 and 3	85
DL-β-Phenylalanine-T(*G*)	1,800	16	Aqueous solution	Room	D.A.	—	73
L-Proline-T(*G*)	141	7	Solid	0	P.C.	1, 2 and 3	100
DL-Serine-T(*G*)	138	12	Solid	0	D.A.	—	100
DL-Serine-T(*G*)	138	14	Solid	0	P.C.	1, 2 and 3	96
DL-Tryptophan-T(*G*)	658	3	Solid	Room	D.A.	—	100
DL-Tryptophan-T(*G*)	658	9	Solid	Room	D.A.	—	97
DL-Tryptophan-T(*G*)	658	18	Solid	Room	D.A.	—	94
DL-Tryptophan-T(*G*)	658	24	Solid	− 40	P.C.	1, 2 and 3	95
DL-Tyrosine-T(*G*)	242	23	Solid	Room	P.C.	1, 2 and 3	100
DL-Tyrosine-T(*G*)	434	10	Aqueous solution	− 40	P.C.	1, 2 and 3	95
L-Tyrosine-T(*G*)	163	10	Solid	0	P.C.	1 2 and 3	97
DL-Tyrosine-T(*G*)	69	28	Solid	0	P.C.	1, 2 and 3	100

Table 2. DECOMPOSITION OF TRITIATED NUCLEOSIDES

Compound	Specific activity (mc./m.mole)	Age (months)	Storage condition	Temperature (° C)	Method of analysis	Solvent system	Radiochemical purity (per cent)
Adenosine-T(*G*)	199	10	Solid	− 40	D.A.	—	83
Adenosine-T(*G*)	199	24	Solid	− 40	D.A.	—	75
Adenosine-T(*G*)	680	13	Aqueous solution	− 40	P.C.	8, 9, 10, 11 and 1	97
5-Bromodeoxyuridine-6-T	552	2	Aqueous solution	0	P.C.	6, 8, 10 and 11	100
Cytidine-T(G)	159	7	Solid	0	P.C.	8, 9, 10 and 11	100
Deoxyadenosine-T(*G*)	627	14	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	100
Deoxyuridine-T(*G*)	385	14	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	85
Guanosine-T(*G*)	407	4	Solid	Room	D.A.	—	87
Guanosine-T(*G*)	407	4	Solid	− 40	D.A.	—	90
Thymidine-T(*G*)	72	9	Solid	Room	D.A.	—	83
Thymidine-T(*G*)	82	17	Solid	− 40	P.C.	8, 9, 10 and 11	98
Thymidine-6-T	2,500	15	Aqueous solution	0	P.C.	8, 9, 10 and 11	85
Thymidine-6-T	4,400	10	Aqueous solution	Room	P.C.	6, 8, 10 and 11	100
Thymidine-6-T	4,400	1	Aqueous solution	− 40	D.A.	—	95
Thymidine-6-T	4,400	5	Aqueous solution	0	D.A.	—	85
Thymidine-6-T	4,400	10	Aqueous solution	0	P.C.	8, 9, 10 and 11	92
Thymidine-6-T	14,100	1½	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	90
Thymidine-6-T	14,100	3½	Aqueous solution	Room	P.C.	8, 9, 10 and 11	100
Uridine-T(*G*)	1,080	2	Aqueous solution	0	P.C.	8, 9, 10 and 11	98
Uridine-T(*G*)	1,080	5	Aqueous solution	0	P.C.	8, 9, 10 and 11	91
Uridine-T(*G*)	3,000	2	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	85
Uridine-T(*G*)	3,000	10	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	75

Table 3. Decomposition of Tritiated Purines and Pyrimidines

Compound	Specific activity (mc./m.mole)	Age	Storage condition	Temperature (° C)	Method of analysis	Solvent system	Radiochemical purity (per cent)
Adenine-2 : 8-T	82	8	Solid	Room	D.A.	—	95
Adenine-2 : 8-T	82	36	Solid	Room	P.C.	8, 9, 10 and 11	100
Adenine-2 : 8-T	430	11	Solid	− 40	P.C.	8, 9,10 and 11	100
Caffeine-T(*G*)	1,280	9	Solid	0	P.C.	8 and 11	100
Cytosine-5 : 6-T	117	20	Solid	Room	P.C.	8, 9, 10 and 11	100
Pteroylglutamic acid-T(*G*) (Folic acid)*	166	18	Solid	Room	D.A.	—	95
Orotic acid-5-T	115	4	Solid	Room	D.A.	—	96
Orotic acid-5-T	115	4	Solid	Room	P.C.	1, 2 and 12	96
Uracil-5 : 6-T	122	8	Solid	Room	D.A.	—	99
Uracil-5 : 6-T	870	12	Aqueous solution	− 40	P.C.	8, 9, 10 and 11	95
Uracil-5 : 6-T	870	21	Aqueous solution	0	P.C.	8, 9, 10 and 11	94
Thymine-6-T	125	11	Solid	Room	P.C.	8, 9, 10 and 11	100
Thymine-6-T	224	10	Solid	Room	D.A.	—	83
Thymine-6-T	2,600	5	Solid	− 40	P.C.	8, 9, 10 and 11	96
Thymine-6-T	3,140	18	Solid	− 40	P.C.	8, 9, 10 and 11	80
Thymine-6-T	17,000	5	Solid	− 40	P.C.	8, 9, 10 and 11	86

*Pteridine.

Table 4. Decomposition of Tritiated Steroids

Compound	Specific activity (mc./m.mole)	Age (months)	Storage Condition	Temperature	Method of analysis	Solvent system	Radiochemical purity (per cent)
Δ^4-Androstenedione-T(*G*)	3,400	6	Benzene solution	Room	D.A.	—	96
Δ^4-Androstenedione-T(*G*)	3,400	20	Benzene solution	Room	D.A.	—	90
Δ^4-Androstenedione-T(*G*)	3,400	20	Solid	Room	D.A.	—	76
Cholesterol-T(*G*)	110	13	Benzene solution	Room	D.A.	—	88
Cholesterol-T(*G*)	110	16	Benzene solution	Room	P.C. (on papers impregnated with 5 per cent paraffin in ether)	13, 14 and 15	90
Cholesterol-T(*G*)	314	7	Benzene solution	Room	D.A.	—	95
Œstradiol-6 : 7-T 17β-acetate	3,200	4	Solid	0° C	D.A.	—	100
Œstrone-T(*G*)	180	21	Solid	Room	D.A.	—	100
Œstrone-T(*G*)	180	8	Benzene solution	Room	D.A.	—	98
Progesterone-T(*G*)	118	9½	Benzene solution	Room	D.A.	—	100
Progesterone-T(*G*)	118	18	Benzene solution	Room	Chromatography on paper impregnated with 5 per cent paraffin in ether, or 10 per cent ethylene glycol	25 26	100

Table 5. Decomposition of Miscellaneous Tritiated Compounds

Compound	Specific activity (mc./m mole)	Age (months)	Storage condition	Temperature (° C)	Method of analysis	Solvent system	Radiochemical purity (per cent)
Acids							
Adipic acid-T(*G*)	58,000	2½	Solid	0	P.C.	1, 2, 3 and 16	14
Benzoic acid-T(*G*)	30	14	Solid	Room	D.A.	—	100
1-Naphthylacetic acid-T(*G*)	180	9	Solid	Room	D.A.	—	100
Stearic acid-9 : 10-T	98	13	Solid	Room	D.A.	—	96
Alkaloids							
Atropine-T(*G*)	246	28	Solid	Room	P.C.	1, 17, 18 and 19	85
Nicotine-T(*G*)	41	13	Liquid	−40	P.C. (18 and 19 on paper buffered by sodium dihydrogen phosphate)	1, 17, 18 and 19	90
Amines							
Aniline-T(*G*)	40	15	Liquid	Room	D.A.	—	87
Esters							
Glyceryl tri(stearate-9 : 10-T)	397	3	Solid	Room	D.A.	—	100
Halides							
Ethyl iodide-2-T	25	34	Liquid	−40	G.L.C.	—	94
Hydrocarbons							
Benzene-1-T	30	24	Liquid	0	G.L.C.	—	95
n-Decane-1 : 2-T	210	28	Liquid	Room	G.L.C.	—	90
Sugars							
D-Glucose-6-T	468	2	Aqueous solution	0	D.A.	—	100
D-Glucose-6-T	468	7	Solid	0	P.C.	20, 21, 22 and 3	96
D-Glucose-6-T	468	11	Solid	0	P.C.	20, 21, 22 and 3	100
D-Glucose-6-T	468	15	Aqueous solution	0	P.C.	20, 21, 22 and 3	96
D-Glucose-6-T	468	7	Aqueous solution	−40	P.C.	20, 21, 22 and 3	100
Vitamins							
Pyridoxine-T(*G*) hydrochloride	331	27	Solid	−40	P.C.	1, 2, 3 and 23	85
Pyridoxine-T(*G*) hydrochloride	331	17	Solid	−40	P.C.	1, 2, 3 and 23	80
Vitamin-B_{12}-T(*G*)	2,570	7	Solid	−40	P.C.	24	99

Solvent Systems. Upper phases (where applicable) were used, ascending Whatman No. 1 paper at room temperature overnight. Papers were air-dried before elution and before scanning. Compounds were spotted on to papers at a concentration of 20–50γ in 0·005 ml. solvent. Note that acid eluents were prepared on the day of use.

1. *n*-Butanol/acetic acid/water 120 : 30 : 50. 2. Ethanol/ammonia/water 80 : 4 : 16. 3. *n*-Butanol/pyridine/water 1 : 1 : 1. 4. Phenol/ethanol ammonia 150 : 40 : 10. 5. *tert*.Butanol/formic acid/water 140 : 30 : 30. 6. *iso*Propanol/ammonia/water 100 : 5 : 10. 7. *tert*.Butanol/water/methyl ethyl ketone/diethylamine 80 : 80 : 40 : 8. 8. *n*-Butanol saturated with water. 9. *n*-Butanol/water/ammonia 172 : 18 : 10. 10. *iso*Propanol/water/hydrochloric acid 130 : 37 : 33. 11. Ethyl acetate saturated with phosphate buffer at pH 6·0. 12. *n*-Butanol/ethanol/3 N ammonia 120 : 30 : 150. 13. 90 Per cent acetic acid/paraffin 100 : 1. 14. Ethoxy-ethanol/*n*-butanol/methanol/water 35 : 10 : 30 : 25. 15. *n*-Propanol/methanol/water 15 : 82 : 3. 16. *n*-Propanol/eucalyptol/formic acid/water 50 : 50 : 20 : 25. 17. *n*-Butanol/N sodium acetate/N hydrochloric acid 7 : 120 : 60. 18. *n*-Butanol/5 per cent aq. citric acid 180 : 20. 19. *iso*Amyl alcohol/5 per cent citric acid 180 : 20. 20. *iso*Propanol/water 160 : 40. 21. Ethyl acetate/pyridine/water 120 : 50 : 40. 22. *iso*Propanol/*n*-butanol/water 140 : 20 : 40. 23. *n*-Butanol/dioxan saturated with 2 N-ammonia 4 : 1. 24. *sec*. Butanol/water/acetic acid(trace of KCN) 50 : 18 : 1. 25. Xylene/methanol 225 : 75. 26. Petroleum ether (b.p. 100°–120° C).

Table 6. SUMMARY OF THE DECOMPOSITION RATES OF TRITIATED ORGANIC COMPOUNDS

Compound class	Specific activity (mc./m mole)	Storage state	Temp. (° C)	Decomposition rate (max.) (per cent per ann.)
Acids—aliphatic	100	Solid	Room	5
	60,000	Solid	0	100
aromatic	200	Solid	Room	5
Alkaloids	250	Solid	Room	10
Amino-acids	200	Solid	0, −40	5
	200	Aqueous soln.	0, −40	5
	500	Aqueous soln.	−40	10
	2,000	Aqueous soln.	Room	30
	5,000	Aqueous soln.	0	30
Halides—alkyl or aryl	25	Liquid/solid	−40	5
Hydrocarbons—aliphatic or aromatic	200	Gas, liquid or solid	Room	5
Nucleosides	100	Solid	Room	20
	100	Solid	−40	10
	200	Solid	−40	20
	400	Solid	Room	40
	400	Solid	−40	30
	700	Aqueous soln.	−40	5
	4,000	Aqueous soln.	0	10
	4,000	Aqueous soln.	−40	10
	14,000	Aqueous soln.	−40	30
Purines	100	Solid	Room	5
	500	Solid	−40	5
Pyrimidines	100	Solid	Room	5
	1,000	Aqueous soln.	0, −40	5
	3,000	Solid	−40	15
	17,000	Solid	−40	30
Steroids	100	Benzene soln.	Room	5
	200	Solid	Room	10
	300	Benzene soln.	Room	10
	3,000	Solid	Room	15
	3,000	Benzene soln.	Room	5
Sugars	500	Solid	0	10
	500	Aqueous soln.	0	5
	500	Aqueous soln.	−40	5

Owing to the very short range of the β-particles it is reasonable to assume that all the energy of the radiation will be self-absorbed. In the solid state, therefore, decomposition due to primary (external) radiation, that is, radiation from other molecules, is likely to be a major contributing source. Interaction between radioactive and unlabelled molecules will be much smaller in solution and primary (external) decomposition will be minimized, most of the β-radiation being absorbed by the surrounding solvent, especially in aqueous solutions where the molecules may be solvated. The decomposition should be minimal at infinite dilution, but the gross effects of variation in concentration have yet to be studied in detail.

Secondary Radiation Effects

The decomposition or transformation due to the reaction of a labelled molecule with a reactive species, for example, free radicals or the excited decomposition fragments of primary decay, presents the most serious problem in the storage of tritium compounds. Such secondary radiation effects give rise to radiochemical impurities which can often present a serious disadvantage to the tracer compound user. This is especially true for experiments, for example, on the metabolism or some analytical use of tritium compounds, the results of which can only be deemed reliable if the tracer labelled compounds are of a high radiochemical purity. Fortunately for many uses the presence of a few per cent impurity is not necessarily an embarrassment, but it is obviously most desirable to know the exact radiochemical purity of the labelled compound before use.

Chemical Decomposition

All organic compounds are thermodynamically unstable, but chemical decomposition at room temperature, for many compounds. is usually very slow and can be slowed even further by storing the compound at low temperatures. In radiochemical investigations the presence of chemical non-radioactive impurities is not normally a handicap; but for work involving the absorption or reaction rates of a particular labelled compound. these chemical impurities may prove embarrassing. As chemical decomposition can give rise to radiochemical impurities, compounds are often stored at low temperatures to reduce this type of decomposition to a minimum.

Storage

The numerous tritium-labelled compounds described in this article have been kept as weighed crystalline solids, freeze-dried solids or dissolved in solvents at concentrations approximately 1–5 mc./ml. Ampoules contained air or were sealed *in vacuo*, although there is at present no evidence to suggest that the presence of a small volume of air affects the stability of compounds not normally affected by oxygen.

Methods of Analysis

Two methods of analysis were used for determining the radiochemical purity of the tritiated compounds.

(1) Dilution analysis (D.A.), which is carried out by diluting a small weight of the labelled compound with about 1,000 times its weight of carrier. The mixture is homogenized in a suitable solvent, the solvent evaporated, the sample dried and the specific activity measured. The compound is recrystallized several times until the specific activity is unchanged on two successive recrystallizations. When possible a suitable derivative is prepared and the specific activity of the pure derivative measured. If all the specific activities are identical the compound can be regarded as radiochemically pure. Care must be taken to ensure that all samples are dried to the same extent or inaccurate values will be obtained. This is especially true with compounds which contain water or solvent of crystallization. Derivatives must be selected carefully and reactions which involve the substitution of non-labile hydrogen atoms in the labelled compound should be avoided. Tritium measurements were carried out by combustion of samples to tritiated water by the Schöniger technique[12] and the activity measured in a β-liquid scintillation counter (Ekco Electronics No. 612). The overall accuracy of this method was ± 7 per cent.

(2) Liquids and gases were subjected to gas-liquid chromatographic (G.L.C.) analysis[13] and the radiochemical purity was assumed to be no better than the chemical purity (± 5 per cent). Other compounds were examined by paper chromatographic analysis (P.C.) in the solvent systems described in the Tables. The tritium activity on the developed chromatograms was determined with a 2π-gas flow (argon–10 per cent methane) proportional chromatogram scanner[14]. Identical results were obtained on scanning both sides of a paper chromatogram. The accuracy of this method was about ± 5 per cent and the limit of detectability approximately 1 μc.

Results

The Tables contain a selection of tritium-labelled organic compounds for different classes of compounds at a variety of specific activities. All the compounds studied were radiochemically pure when first prepared and their subsequent rate of decomposition under various storage conditions has been determined.

Conclusions

By an examination of the Tables it can be seen that the decomposition of tritium-labelled compounds can be minimized by storing the compounds dissolved in a suitable solvent. Chemical and secondary radiation decomposition are reduced by storing below room temperatures. For many compounds the storage in aqueous solution appears to be a most suitable method for reducing decomposition rates. In numerous cases there does not seem to be any great advantage in storing the compound at − 40° C than at 0° C. At low temperatures there is the additional problem of sublimation of the protecting solvent to other parts of the tube, if there is a temperature gradient, leaving the substance unprotected; frequent examination is then required.

Tritium compounds having specific activities less than 500 mc./m.mole, when stored under the best-known conditions suffer no serious decomposition for at least one year. For compounds with higher specific activities, the rate of decomposition will increase with increasing specific activity and will depend on the actual type of compound. Among the most stable classes of compounds are amino-acids, purines, pyrimidines and steroids. Nucleosides at high specific activity > 1 c./m.mole appear to be one of the more sensitive classes of compounds, the rate of decomposition when stored under the best conditions being 0·5 – 1 per cent per month. As more labelled compounds are prepared there will obviously be some members of a particular class or group which will be found more sensitive to radiation effects than others within that class. For example, the amino-acid methionine is slightly more sensitive to radiation effects than other amino-acids of the same specific activity and uridine-T is one of the more sensitive of the nucleosides.

There are a number of anomalies, for example thymidine-6-T at high specific activity (4 or 14 c./m.mole) appears to keep better at room temperature in aqueous solution than at –40° C. This may be due to a shorter life of reacting species (for example, free radicals) at room temperature than at – 40° C giving less time for secondary decomposition to occur[15], but at present the anomaly is unexplained.

It is hoped that this article will guide the many users of tritium-labelled compounds in selecting the best method of storage at present known. There is obviously much more information to be obtained regarding the mechanism of the decomposition and the nature of the products of primary nd secondary decomposition. Further work is in progress in our laboratories and a more detailed account of the decomposition of individual compounds will be reported on at a later date.

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EFFECT OF IONIZING RADIATION ON ADENINE IN AERATED AND DE-AERATED AQUEOUS SOLUTIONS

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THE radiation chemistry of the nucleic acids and their constituents has been investigated by various workers[1–4]. It is known that in these macromolecules the purine and pyrimidine bases are the elements most susceptible to radiation damage, and that the purines are less radiosensitive than the pyrimidines[1,2], which one would expect from the increased resonance of their structure. Pyrimidine attack is enhanced by a factor of approximately 2·5 in the presence of oxygen, and is a result of peroxidation at the 4 : 5 bond[1]. Peroxides are not found, however, when purines are irradiated in aerated solution[1,3]. This article is concerned with the estimation of the extent of radiation damage to adenine in aqueous solution, both in the presence and in the absence of oxygen, and the identification of the primary products formed.

10^{-3} M solutions of adenine in triple-distilled water were irradiated at room temperature, in the presence of oxygen, with 40 kV X-rays and under vacuum with cobalt-60 γ-rays. Total doses in the range 300,000 r. were given. The resulting solutions were subjected to ion exchange chromatography, using a 12-mm column of 'Dowex-50' and eluting with 2 N hydrochloric acid, a technique which has already been described[4]. Typical elution diagrams are shown in Figs. 1*a* and *b*.

In both cases the major product appears in section *B*, and these fractions were collected, lyophilized and the product subjected to descending paper chromatography in various solvents. Section *B* was thus shown to contain a single product of which the R_F in three solvents and absorption spectrum at different *p*H corresponded with those of 8-OH-adenine. This compound was synthesized for comparison by reacting 4,5,6-tri-NH$_2$-pyrimidine with phosgene in alkaline solution. When mixed with the synthetic compound, the irradiation product was inseparable by column or paper chromatogram.

On similar treatment, section *A* yielded traces of hypoxanthine, more in the absence than in the presence of oxygen. Other degradation products were certainly also present here, which have not been identified.

No products were detected in section *C* when the solutions had been irradiated under vacuum. In the presence of oxygen, however, this section was found to contain traces of isoguanine and a compound which shows no absorption above 220 mμ, but produces a spot on a paper chromatogram, with a strong fluorescence under ultra-violet light (2537 Å). No result was obtained by spraying the chromatogram with Fink's reagent[5] (0·5 N sodium hydroxide followed by a solution of *p*-dimethylaminobenzaldehyde) which reacts to form a coloured complex with pyrimidines saturated at the 4 : 5 bond. Michl's reagent[6] (an ethanolic solution of eosin saturated with mercuric chloride) which gives a red-violet coloration with purine derivatives, also gave a negative result with this product. Since it shows no ultra-violet absorption, the chromophoric group in the pyrimidine ring must have been changed, which, if it is a primary product, implies saturation of one of the double bonds in this ring. For stearic reasons, and considering the electronic structure of the molecule, it seems unlikely to be the 4 : 5 bond as in the case of the pyrimidines, but more probably the 1 : 6 or 2 : 3 bonds.

Nothing was detected in the fractions after the unreacted adenine, but on eluting the column afterwards with 0·25 N sodium hydroxide, 4,5,6-tri-NH$_2$-pyrimidine was found. This is presumed to arise from the formamido-pyrimidine already noted by Hems[1], which would readily lose the CHO group in 2 N hydrochloric acid. Contrary to Hems's results this compound appeared in the presence as well as in the absence of oxygen; but it may well have been that in this case the aerated solutions were not fully oxygen saturated throughout the irradiation time.

The results show the same primary reaction in both cases: attack by an OH• radical at C_8, which is the position of highest electron density when the molecule is approached by an electrophylic reagent[7,8]. The highest bond order is found in the 7 : 8 bond, thus reaction in the presence of H• and OH• radicals should occur primarily via substitution of OH at C_8 or saturation of the 7 : 8 bond. On the basis of these considerations and the experimental results obtained, the scheme as shown in Fig. 2 is suggested for the reaction in the absence of oxygen.

In the presence of oxygen, though the primary step is the same—the secondary products may differ. Reactions due to OH• radicals will be unchanged but the H• radicals should be largely converted to HO_2•. The formamido-pyrimidine should not, therefore, be formed. If hydroperoxides are formed as a result of HO_2• attack, they must immediately decompose, as they have not been detected. If the HO_2• radicals are not thus directly responsible for the augmented base attack in the presence of oxygen then this must be due to the removal of H• radicals preventing recombination with OH•, and thus leaving more OH• radicals free to attack the solute molecules.

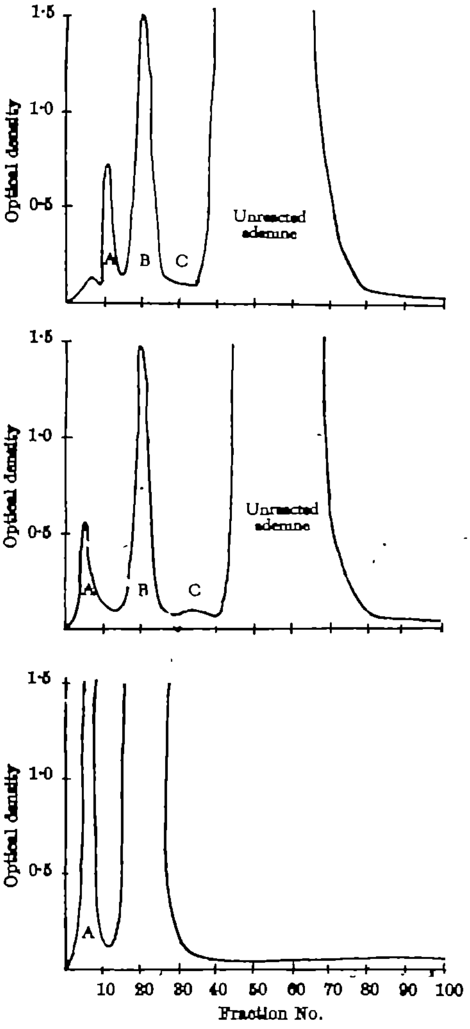

Fig. 1. Ion-exchange chromatography of irradiated adenine and 8-OH-adenine solutions. 12-mm column 'Dowex-50'. Elution with 2 N hydrochloric acid. Fraction volume 3·5 ml./10 min. optical density at 260 mμ. *a*, After irradiation of 100 ml. 10^{-4} M adenine solution, in the absence of oxygen, dose = 440,000 r.; *b*, after irradiation of 80 ml. 10^{-4} M adenine solution, in the presence of oxygen, dose = 300,000 r.; *c*, after irradiation of 40 ml. 10^{-4} M 8-OH-adenine solution in the presence of oxygen, dose = 300,000 r.

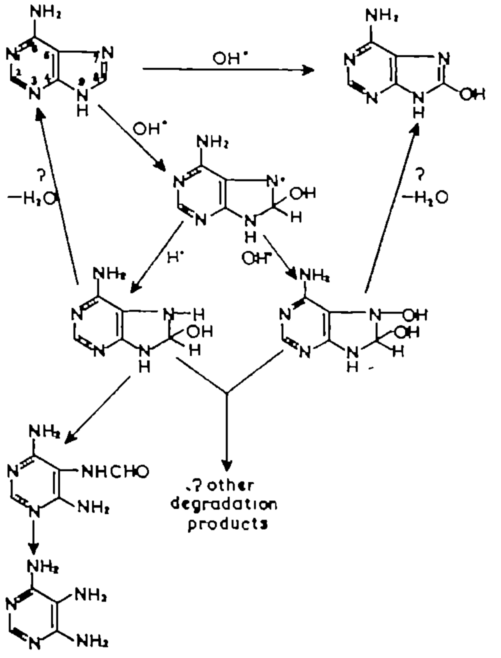

Fig. 2

It is notable that when adenine reacts with OH• radicals from Fenton's reagent, the 8-OH compound is also the product formed[9]. Other possible primary reactions include deamination at C_6, producing hypoxanthine, which is found to a slight extent, or substitution in position 2 giving isoguanine, which appears also to be present in trace quantities in section *C*, in aerated solutions. No trace of the N_1 oxide which is produced by oxidation with hydrogen peroxide[10] was found.

Table 1. R_F of Compounds on Paper Chromatograms

	n-Butanol : morpholine : diethylene glycol : water 4·5 : 1·5 : 1 : 2	*n*-Butanol : diethylene glycol : water (in atmosphere NH_3) 4 : 1 . 1	*n*-Butanol : methanol : formic acid 3 : 0·5 : 2·5 (paper impregnated with 30 per cent *cyclo*hexanol/acetone solution)
Adenine	0·55	0·61	The solvent was allowed to run off paper; therefore R_F's not calculated, only corresponding positions of products and blanks obtained
8-OH-adenine	0·48	0·32	
B	0·48	0·32	
Hypoxanthine	0·40	0·49	
A	0·39	0·40	
Iso-guanine	0·23	0·53	
4,5,6-tri-NH_2-pyrimidine	0·45	—	
Ad. N_1 oxide	0·33	—	
C	0·21	0·33	

Table 2. Ultra-violet Absorption Results

	*p*H = 1–2 max (mμ)	*p*H = 1–2 min (mμ)	*p*H = 12 max (mμ)	*p*H = 12 min (mμ)
Adenine	263	229	269	237
8-OH-adenine	272	—	278	245
B	275	—	277	245
Hypoxanthine	248	215	258	232
Iso-guanine	282	248	284	253
C	280	250		
			*p*H	Neutral
4,5,6-tri-NH_2-pyrimidine	287	240	280	240

G(-adenine) was found to be approximately 0·85 for aerated solutions and 0·35 for de-aerated ones. The ratio is thus comparable with that for the pyrimidines but the values are lower in agreement with the data already known. (*G*(8-OH-adenine) was approximately 0·15 in oxygen and 0·05 under vacuum. (These measurements were made using values for the extinction coefficients as follows: adenine λ_{max} 263 mμ, Σ_{max} 13,100 l. mole^{-1}cm^{-1}; 8-OH-adenine λ_{max} 272 mμ Σ_{max} 10,900 l. mole^{-1}cm^{-1}.) The *G* values indicate that 8-OH-adenine is more sensitive to radiation than adenine itself, if it is the major primary product formed. This was confirmed when a solution of 8-OH-adenine (10^{-4} M) was irradiated in the presence of oxygen, and under the same conditions as adenine. The resulting solution subjected to column chromatography (Fig. 1c) showed that G(-8-OH-adenine) is approximately twice the value for G(-adenine), and that the product in section A from irradiated adenine solutions is a secondary product resulting from attack on the 8-OH-adenine. The final product in section A has not been identified; but in solution it is yellow in colour and shows an absorption maximum in acid solution ($pH \sim 1$) at 335 mμ and a minimum at 298 mμ. There may be more than one product present here. It is interesting to note, in this connexion, that the N_1 oxide of adenine has also been found to be destroyed more rapidly by ultra-violet light and ionizing radiation than adenine[10], which seems to be one of the most stable purine derivatives.

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RECOVERY FROM RADIATION INJURY: A PROPOSED MECHANISM

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EXPERIMENTAL evidence has emerged over recent years which better defines the course of recuperation following injury to certain kinds of cells caused by ionizing radiation. Biological systems varying widely in complexity have been examined under somewhat different conditions, and have yielded results which appear to agree qualitatively, if not quantitatively. It is the purpose of this article to point out the common thread running through these diverse findings, and to suggest a mechanism for their explanation.

Irradiating hamster cells *in vitro* and defining survival as the ability to form macrocolonies, Elkind[1] has compared the effect on survival of single, acute radiation exposures with doses given in two fractions. Varying lengths of time were allowed to intervene between the first, or conditioning, dose and the second, or test, dose. Essentially, the net effect of a given dose delivered in two fractions was never so great as when it was delivered singly. The effect on surviving cells of the first of two fractions disappeared completely by approximately 20 h, that is, recuperation was complete. Elkind has sub-divided this 20-h period into two phases: (1) recovery, meaning changes in survival response after an initial exposure, which changes occur during the period of division delay; (2) repair, meaning changes in per cent survival that involve repopulation by viable cells. A characteristic and well-defined fine structure was described for the recuperation kinetics during the first few hours following initial irradiation, that is, the recovery period. This consisted in the curve of surviving fraction after a constant second dose as a function of time between the two fractional doses rising to a maximum some time during the first 3–6 h, then falling to a minimum shortly before 10 h, and finally rising again progressively to complete recuperation at about 20 h. This progression of sensitivities may be described qualitatively in other ways, equivalent to Elkind's description. Thus one may substitute the term population resistance for surviving fraction in certain of Elkind's fractionated dose-survival graphs (for example, Fig. 7 in ref. 1). Alternatively, the term I, defined as residual injury, would be roughly equivalent to the reciprocal of surviving fraction (Fig. 1, curve 1). (The terms, residual injury I, and conditioning dose D_C, are used throughout this article as established and defined by G. Sacher in *Radiation Biology and Medicine* (edit. by Claus, W. D., 283, Addison-Wesley, 1958).) It will be seen that the level of residual injury falls to a minimum shortly after exposure to conditioning irradiation, then rises to a maximum, and then falls until it finally reaches the level characteristic of an unexposed population. Elkind has presented an attractive hypothesis to account for this kind of behaviour, largely on the basis of changes in extrapolation number inflicted by the conditioning irradiation: recovery, he concludes, consists in the return of a radiation-altered extrapolation number to its original value, that is to say, the reappearance of the shoulder of the survival curve of the sensitive cells.

The kind of recuperation kinetics, that is, the qualitative shape of the residual injury curve in two-fraction experiments already described, is similar to that observed in at least three other biological systems. As Elkind has pointed out, two-fraction acute lethality experiments in mammals have yielded similar curves. Mole[2] originally described an initial maximum in residual injury followed by a minimum before the approximately exponential fall in two-fraction injury curves (Fig. 1, curve 4). *CBA* mice were exposed to a conditioning dose of 400 r. and were tested with second doses at intervals of 6 h–20 days later. His findings have since been amply confirmed by Storer and Sanders (Fig. 1, curve 5) (unpublished data, cited by Elkind[1]) and by myself and Silini (Fig. 1, curve 3) (unpublished). It must be noted that acute death of an animal at the LD_{50} in whole-body irradiation experiments reflects radiation depopulation of vital tissues, notably the blood-forming system, to just below a critical number of sensitive cells. The likelihood that the shape of this injury decay curve is attributable to changes in the probability of survival per unit radiation dose of bone marrow cells after conditioning irradiation is well supported by the recent findings of Till and McCulloch[3]. Thus, bone marrow cells irradiated *in vivo*, allowed to recuperate in their normal environment before exposure to a second radiation dose, and tested by their ability to form visible clones in the spleens of irradiated recipient mice exhibited a pattern of recuperation similar to that described here: an initial minimum in residual injury occurred at 5 h with a subsequent maximum at 10–15 h (Fig. 1, curve 2).

In a series of experiments on spontaneous mammary tumours of mice, we[4,5] have found indications of the same kind of recuperation pattern, though on a greatly expanded time scale (Fig. 1, curve 6). When solid tumours, *in situ*, were given a conditioning dose of 1,120 rads, there was an apparently higher degree of residual injury at the fourth day than at the second day thereafter. In these experiments, the criterion of radiation effect was 50 per cent cure, that is, the dose estimated to cure 50 per cent of tumours as determined by interpolation between the cure rates for a graded series of doses.

From a comparison of the 'smooth' curves in Fig. 1, which have been plotted freely from the available data,

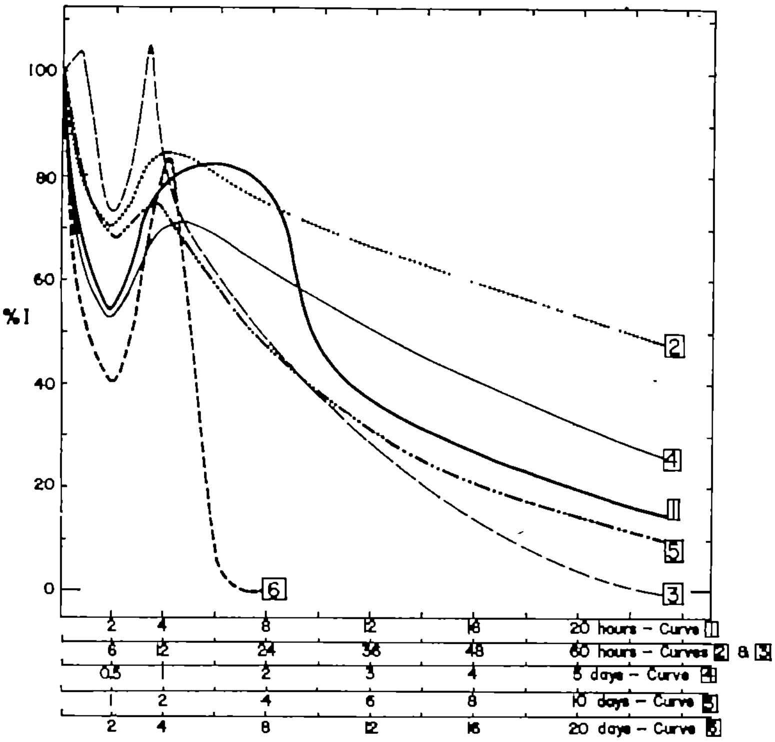

Fig. 1. Curves showing the change in effectiveness of a second dose of radiation as a function of time between first and second dose. All data have been converted to the same ordinate scale of percentage residual injury (%I). Each curve is plotted on its own arithmetic time index, as abscissa, with the different abscissal indexes arranged so that the initial minima fall at the same distance from the ordinate axis. All curves are freehand fits to experimental data. Conditioning doses, method of detecting residual effectiveness, biological system employed, and investigators were, respectively: curve 1, 433 rads, constant second dose, hamster cells *in vitro*, Elkind (ref. 1); curve 2, 400 rads, constant second dose, mouse bone marrow *in vivo*, Till and McCulloch (unpublished); curve 3, 800 r, variable second dose, *C57BL* mouse acute lethality, Kallman and Silini (unpublished); curve 4, 400 r, variable second dose, *CBA* mouse acute lethality, Hole (ref. 2); curve 5, 400 r., variable second dose, *CF*-1 mouse acute lethality, Storer and Sanders (cited by Elkind (ref. 1)); and curve 6, 1,120 rads, variable second dose, *C3H* mouse spontaneous mammary tumour cure, Kallman and Tapley (ref. 5)

the conclusion appears amply justified that, as a general phenomenon, conditioning irradiation evokes this pattern of early refractoriness succeeded by a rise in sensitivity prior to a final monotonic course of recuperation. The liberties taken in the choice of abscissal scales in Fig. 1 were deliberate—so that this conclusion might be the more apparent.

For the majority of mammalian cells that have been tested quantitatively after both *in vitro* and *in vivo* irradiation, D_0's have been reported in the range of 100–150 rads and extrapolation numbers from approximately 2 to 10. The majority of cell populations thus far studied must, until proved otherwise, be assumed to be essentially asynchronous with respect to the positions of the component cells in the generation cycle. That is to say, at any given instant an asynchronous cell population will contain cells in all stages of mitotic and interphase activity, the exact proportion of cells of any given stage being dependent on the duration of that stage. For example, if the stage of DNA synthesis lasts, say, 8 h and the cell cycle averages 20 h in duration, 40 per cent of the cells in a random population would be expected to be in DNA synthesis. The concept that cell radiosensitivity varies according to the position of a cell in its generation cycle has been bolstered by the findings of Terasima and Tolmach[6,7]. When HeLa cells were synchronized by selective means and homogeneous populations of such were irradiated during different parts of the cell cycle, it was found that, with respect to loss of reproductive integrity, radiosensitivity changed continuously and mitotic (M) cells were most sensitive (D_0 about 70 rads), early G_1 cells were minimally sensitive (D_0 baout 180 rads), cells grading from late G_1 to early S were very sensitive (D_0 about 100 rads), and G_2 cells were relatively insensitive (D_0 about 160 rads). (The notation used for distinguishing different periods in interphase is, as established in present-day usage, G_1 to denote the immediate post-mitotic, pre-DNA synthetic period, S to denote the period during which DNA is synthesized, and G_2 to denote the post-DNA synthetic, pre-mitotic period.) Quantitative estimates of the radiation sensitivities of these major stages relative to early G_1, the least-sensitive state, would therefore be: M = 2·57-fold, late G_1-early S = 1·80-fold, and G_2 = 1·13-fold. These differences in radiosensitivity were evidenced by changes in the slopes of the survival curves rather than in the extrapolation numbers which varied between 1·5 and 2·5, not correlated with phase. (In the most recent experiments the different survival curves were carried down to less than 0·01.) Undoubtedly, these findings are in themselves mean estimates of sensitivity in the various major stages identified; owing to technical difficulties in achieving and maintaining higher possible degrees of synchrony, not to mention the difficulties associated with the ability to resolve small differences in survival curve slopes or extrapolation numbers, small radiosensitivity differences between immediately adjacent stages cannot be detected with high precision or confidence.

Using a completely different technique based on pulse-labelling asynchronous populations of L cells and ascites tumour cells with tritiated thymidine, Dewey and Humphrey[8] have furnished qualitative and quantitative[9] confirmation of the preceding order of sensitivity. In contrast, however, Chinese hamster cells[10,11], subjected to the same kind of radiation treatment and analysis after pulse-labelling with thymidine, showed a different order of phase-dependent radiosensitivities. Moreover, for these cells, the phase-dependent order of sensitivities differed for ultra-violet as compared with cobalt-60 γ-radiation. Further work is necessary in order to resolve these differences between cell types as well as kind of radiation.

It is difficult conceptually to reconcile typical mammalian cell survival curves[1,12,13] which are characterized by an initial shoulder followed by a straight line down to low surviving fractions with any population that is not homogeneous. But it has been shown[7,14,15] theoretically that mixed populations may indeed exhibit net survival curves which cannot easily be distinguished from those expected for homogeneous populations; the first decade of a survival curve is the portion in which the shape will reflect mixtures of sensitivities from different sub-populations such as suggested here, and, unfortunately, it is this very region in which insufficient data are available.

Although earlier evidence indicated different periods in the cell cycle when division delay is manifested, it appears most likely that those cells the ultimate reproductive integrity of which is unimpaired by irradiation but which are delayed in their passage through the division cycle experience this delay some time during the G_2 period. As shown by Whitmore *et al.*[16], cells in stages other than G_2 will progress through the generation cycle at a rate close to the normal until they reach the point of the reversible blockage in G_2. They will then remain at that point until they have recovered from the injury responsible for division delay. The preceding is based on experiments with L cells; and it may be remarked, parenthetically, that there is now some evidence for acceleration of the passage of certain cells through the S stage during the period when they are actually being irradiated[17].

In addition to a dependence of surviving fraction on radiation dose and phase of the generation cycle, the duration of reversible division delay is also dose-dependent[16,18–21] and probably phase-dependent as well. Terasima and Tolmach[6,7] also reported phase-dependent differences in mitotic delay as a function of generation stage of irradiated cells. Thus, mitotic delay is minimal for cells irradiated in G_1 and increases as they pass through S and G_2. In confirmation of Whitmore *et al.*[16], it was also reported that the major portion of mitotic delay occurs in the G_2 period. Furthermore, the duration of division delay would appear to be correlated with the normal absolute length of the division cycle as well as with the amount of radiation absorbed, so that cells which normally experience longer generation times will not emerge from the dose-dependent division block as rapidly as will those with shorter division cycles[9].

It must be kept in mind that the populations of cells which have been used in the study of phase-dependent radiosensitivity, as well as dose-dependent division delay, are relatively homogeneous with respect to the duration of the generation cycle and, correspondingly, its several sub-divisions or phases. In marked contrast to this is the situation in a neoplastic cell population, particularly as found in a spontaneous animal tumour. We owe to Mendelsohn[22] the knowledge that cells of spontaneous mouse mammary tumours are indeed an extremely heterogeneous population with respect to generation time. Making cellular autoradiographic measurements of tumours taken from animals which were infused with tritiated thymidine for up to 10 days, Mendelsohn found that doubling times as long as this maximum infusion period of 10 days were not uncommon. Tumour doubling times were normally distributed with the majority falling between approximately 4 and 18 days. Presumably, these doubling times are reflexions of the generation times of the component tumour cells. Even though cells which do not incorporate the label over infusion periods of maximum duration might be regarded as non-dividing or incapable of division, it is unquestionable that some cells require approximately this great an interval (> 10 days) between periods of DNA synthesis. It is significant that, despite this marked heterogeneity in generation times, the durations of S, G_2 and M are relatively constant; it is the G_1 period that is capable of varying from cell to cell and ranges from hours to weeks in the growth fraction of these tumours[23].

Based on the foregoing evidence, the following hypothesis is suggested:

(1) That fraction of a cell population the future reproductive integrity of which is damaged, but not completely destroyed, by a first dose of radiation, that is, the 'surviving fraction', will nevertheless experience division delay of duration proportional to absorbed dose.

(2) The survival of cells exposed to a first dose will depend on the probability of survival per unit dose relative to each cell's generation stage; thus, immediately following initial irradiation, there will be fewer survivors in those states characterized by greater radiosensitivity or higher probability of death per unit dose, and the balance of cells in the population will be shifted toward a greater proportion in the relatively resistant states than at the beginning of irradiation.

(3) Recent evidence[7,15] supports the assumption that the duration of division delay depends not only on dose but also on state of the cell; the closer a cell is to the point of blockade in G_2, the longer the division delay per unit dose. (If the findings of Das and Alfert[17] are generally applicable to other types of cells and other radiation doses, the S compartment would be depleted faster than others.) One would then predict the following course of fluctuations in the proportions of cells in the major compartments in an exponentially growing asynchronous population: a possible initial depletion in the S compartment, with G_1 decreasing at least as rapidly, owing to the passage of its members into S; next, a progressive depletion in G_1 with S remaining essentially constant until G_1 is completely exhausted, at a time roughly that of the normal duration of G_1; and, finally, a progressive exhaustion in the S compartment at a rate consistent with the normal rate of passage through S.

Throughout the preceding succession of compartmental changes, G_2 would be expected to increase to approximately 100 per cent by virtue of the accumulation of all cells which were in earlier stages at the time of irradiation and which would be expected to be blocked during this generation stage. (The essential features of this proposed sequence of events are graphically illustrated in Fig. 2 of the paper by Painter and Robertson[24].)

(4) Assuming, on the other hand, that all cells experience division delay strictly in proportion to dose and with no dependence on generation state at the time of radiation exposure, surviving cells would progress through the division cycle until the point of blockage occurs sometime during G_2; this is contrary to the most recent evidence, but must be considered as well as the preceding, alternative, point.

(5) During the course of this compartmental shift, it is predicted that the overall radiosensitivities of the resultant mixed populations will change accordingly. As the proportion of cells increases in G_2, therefore, and particularly while it decreases in late G_1–S and M, the net or overall sensitivity of the mixed population would approach that of a pure population of G_2 cells. Since the available evidence indicates that G_2 is a relatively resistant phase, such a population would be expected to suffer less loss of reproductive integrity per unit dose than the original random population.

(6) As mentioned in (2) here, owing to the relatively greater radioresistance of early G_1 cells, conditioning doses large enough to kill all but a few cells would leave survivors predominantly in G_1. Thus, while the more sensitive late G_1–S compartment would be severely depleted promptly, this same compartment would tend to be renewed with time from the surviving G_1 pool. This kind of redistribution, leading to a temporary repopulation of late G_1–S prior to the G_2 blockade, would therefore tend to lower overall population radioresistance from its immediate post-conditioning state toward the unirradiated level.

(7) If a second radiation dose is administered at a time when more cells than normal are in G_2 and early G_1, that is, near the beginning of post-irradiation blockade, the net visible effect would be a higher probability of survival or a lower number of cells killed per unit dose than in the starting population. In other words, a state of refractoriness relative to the sensitivity of the unirradiated population would have been induced.

(8) It is attractive to assume that because cells are, in a sense, synchronized by radiation in so far as they are blocked at a common stage, G_2, they may also be then synchronized in their escape from the block. This is entirely consistent with the sequence of events outlined in (3) here: Cells irradiated close to the block point in G_2 experience longer delays than their younger counterparts and thus, in effect, 'wait' for these younger survivors to reach the same stage. Because of the tendency for the cells which reach the block latest to be delayed least, there should be a greater than normal proportion of cells entering the next mitotic division. There is good evidence[16,24–26] for a kind of compensatory overshoot in mitosis in the first post-irradiation division. If this were to follow, as a general rule, it would provide a partial explanation for the relatively rapid fall in radioresistance toward the end of the refractory state: the presence of a pulse of such mitotic cells would tend to increase the net population sensitivity and thus 'mask' the resistance of the as-yet-unrecovered blocked cells. This is supported by the recent report of Painter[25] that the mitotic overshoot in HeLa cells at 7–9 h post-irradiation is made up largely by cells which were in S during irradiation. It is likely,

however, that soon after the reversibly blocked cells emerge from G_2, they assume an asynchronous progression; and that in a relatively short time—no greater than the mean generation time, perhaps—each of the compartments of the generation cycle will contain roughly the same proportion of the total population as existed in the random population prior to initial irradiation.

It is suggested, therefore, that the finding typified by Elkind's 'recovery' is related to this shift in the proportions of cells in the different compartments which are characterized by different sensitivities. Indeed, the fact that radiosensitivity fluctuates in this way in the several systems already noted points to the universality of this phenomenon. The possibility must also be entertained that this state of relative population refractoriness may be produced by the accumulation of cells in a state other than the normal G_2—a non-normal state which simulates G_2 but differs in that it is a perturbed G_2 which is none the less characterized by a lower-than-average radiosensitivity.

(9) The foregoing considerations deal principally with the event occurring in hours to perhaps a very few days after initial, or conditioning, irradiation. The subsequent recuperative events, designated by Elkind as 'repair', may be postulated to be of a different nature and, as Elkind has suggested, concerned largely with repopulation by surviving cells which have resumed their normal course of generative activity.

If the proposed interpretation is valid, several observations take on a different aspect. Although the radiotherapeutic literature is, unfortunately, lacking in controlled quantitative data on clinical material, there are many radiotherapists[27-29] who find that fractionated treatments delivered less frequently than daily are at least as successful as a course of strict daily irradiation. While these and other regimes of radiotherapy may owe their success to different reasons (for example, altered tumour cell-stroma relationship, etc.), it may well be that daily irradiation of many tumours serves to shift an increasingly larger proportion of cells into these stages of lower radiosensitivity so that the later the fractional dose is administered in a given course of treatment, the less its tumoricidal efficiency per given number of tumour cells.

A particularly interesting extrapolation of the foregoing concerns the expected effects of dose protraction, or decreased radiation intensity, on asynchronously growing cell populations. Assuming the hypothesis to be valid, it would be expected that soon after continuous protracted irradiation is initiated, there would be some critical maximum overall time of exposure during which the maximum lethal effect could be produced. As dose rate decreases and overall exposure time increases, however, it would be expected that surviving cells would tend to accumulate at a relatively less sensitive point of reversible mitotic blockade. This would result in a net decrease in lethal effectiveness with dose rate, the population survival depending on the balance between the rate of escape or reversibility of some cells from the block with the rate of production of the same kind of block in other cells. As Thomson and Tourtellotte[30] initially demonstrated, the $LD_{50/30}$ of mice increases steeply as the dose rate falls from ~ 10 r./min to ~ 3–5 r./min—that is to say, as the irradiation time increases from ~ 1 h to ~ 3 h.

In addition, Brown *et al.*[31] and myself[32] have found that the significantly lowered lethal effectiveness of radiation delivered continuously over ~ 6 h (~ 2 r./min), as compared with single doses given in less than ~ ½ h, is quantitatively the same as the effectiveness of two or more high dose rate fractions given in the same overall 6-h time. These data, for which there are no published quantitatively comparable studies on mammalian cells *in vitro*, strongly suggest that the so-called 'dose rate effect' has a basis in the present proposal. The practical significance of the extrapolation of the proposed hypothesis to the dose rate effect is obvious and need not be discussed further at this point.

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NATURE OF THE PHOTOREACTIVABLE ULTRA-VIOLET LESION IN DEOXYRIBONUCLEIC ACID

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IT has been shown by Wulff and Rupert[1] that ultra-violet-induced thymine dimers in *Escherichia coli* deoxyribonucleic acid (DNA) can be split by treatment with a yeast extract in the presence of 'light' of longer wave-length. Such treatment can partially reactivate the biological activity of ultra-violet-inactivated transforming DNA[2]. These experiments strongly suggest, but do not prove, that some or all of the biological photoreactivation of DNA, as well as its chemical photoreactivation, arises from the elimination of thymine dimers by the yeast extract and visible light.

The answers to two questions are of interest: (1) Does the biological reactivation caused by the photoreactivating yeast extract involve thymine dimers, and, if so, (2) is the splitting of thymine dimers sufficient to account for all the increase in biological activity observed? The biological damages in transforming DNA that are caused by large doses of 2800 Å radiation are partially reversed by irradiation at 2390 Å. This reactivating effect has been quantitatively correlated with the splitting of a fraction of thymine dimers that results from a shift in the equilibrium of dimerized and undimerized thymine–thymine sequences[3]. We have attempted to answer these two questions by determining the biological effect of 2390 Å reactivation on photoreactivation with yeast extract. If the yeast extract increases biological activity by reversing thymine dimerization, DNA inactivated by 2800 Å irradiation and then treated with yeast extract should not show the reactivating effect of 2390 Å irradiation. The magnitudes of the maximum reactivation by 2390 Å irradiation and by the yeast extract after various doses of 2800 Å radiation should indicate how much of the biological effect of the yeast extract is caused by thymine dimer splitting.

Methods of ultra-violet irradiation, the transformation assay and the description of markers have been given previously[3,4]. The yeast extract was prepared according to the method of Rupert[5]. (We thank Dr. F. J. Bollum for preparing the yeast extract.) Photoreactivation was carried out at 37° on volumes of 0·4 ml. contained in the 2-cm diameter depressions of white porcelain spot plates. The illuminated solution contained final concentrations of 0·5 μgm/ml. DNA and 0·25 mg/ml. protein from the most active ammonium sulphate fraction of yeast extract. Photoreactivating radiation was supplied by two General Electric 15-W 'blacklight' bulbs, filtered so as to eliminate all wave-lengths less than 3250 Å. The incident intensity was about 7,200 ergs/mm²/min. The minimum illumination time for maximum photoreactivation was found to be less than 2 h for all the inactivating doses used. Illumination times actually used for maximum photoreactivation were 128 min. The efficiency of the yeast preparation for photoreactivation was unchanged after 2 h of illumination at 37° without DNA.

Figs. 1 and 2 show the results of an experiment in which transforming DNA inactivated with various doses of 2800 Å radiation was afterwards exposed to maximum photoreactivation, to 2390 Å radiation, to photoreactivation before 2390 Å, and to 2390 Å radiation followed by photoreactivation. It can be seen (Fig. 1) that when the 2800 Å irradiated samples have been photoreactivated, subsequent 2390 Å irradiation brings about a decrease in survival. However, when 2800 Å inactivated samples have not been photoreactivated following these large doses, 2390 Å irradiation brings about an increase in activity. Since the 2390 Å radiation apparently reverses 2800 Å damage by breaking thymine dimers[3], and because prior photoreactivation eliminates the reactivating effect of the 2390 Å, we may conclude that at least some of the biological effect of the yeast photoreactivating preparation also involves breaking thymine dimers.

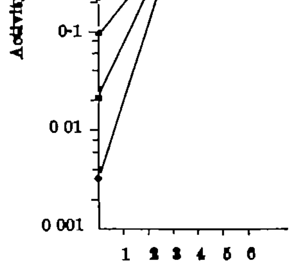

Fig. 1. 2390 Å irradiation following 2800 Å inactivation. The eight points on the ordinate represent the per cent transforming activity remaining after ●○ 2·5 × 10⁴, ▲△ 3·5 × 10⁴, ■□ 4·5 × 10⁴ and ◆◇ 6 × 10⁴ ergs/mm² at 2800 Å, with (white) and without (black) maximum photoreactivation. The increase in activity due to 2390 Å reactivation was earlier (ref. 3) shown not to increase further after a dose of 2·5 × 10⁴ ergs/mm² at 2390 Å

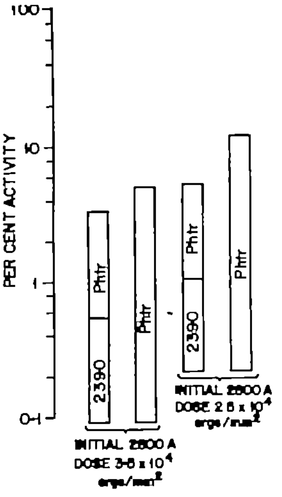

Fig. 2. Photoreactivation (phtr) with and without previous 2390 Å reactivation. Same samples as two of those shown by shaded points in Fig. 1

Fig. 2 indicates that transforming DNA exposed to 2800 Å followed by 2390 Å radiation may be further photoreactivated, but the final activity is somewhat less than that found after 2800 Å followed by photoreactivation alone. The 2390 Å irradiation does not break all the thymine dimers[3], and thus we may conclude that subsequent photoreactivation produces a further increase in activity because of further breakage of thymine dimers. The lower activity found after 2800, 2390 Å and photoreactivation indicates the production of non-photoreactivable damage by 2390 Å irradiation.

If the yeast photoreactivating preparation repairs some type of DNA damage from 2800 Å irradiation other than that repaired by the 2390 Å radiation, we would expect maximum photoreactivation following 2390 Å reactivation to result in a survival higher than that expected from splitting the remaining thymine dimers. Moreover, since we may assume that most types of damage increase with dose, there should be relatively more photoreactivation after larger initial 2800 Å doses, and thus the slope of the 2800 Å dose-curve after 2390 Å reactivation and photoreactivation should be less steep than the 2800 Å dose-curve after reactivation by 2390 Å irradiation alone. Fig. 3 shows survival-curves after 2800 Å irradiation with and without 2390 Å reactivation and photoreactivation. (Similar data were used previously[3] to calculate that about 50 per cent of the biological damage at high doses of 2800 Å is due to formation of thymine dimers. The results in Fig. 3 yield a figure closer to 70 per cent.) It is seen that the slopes of the 2390 Å reactivation and 2390 Å reactivation plus photoreactivation lines are approximately the same. This is interpreted to mean that the yeast photoreactivating preparation has little or no biological effect except that resulting from breakage of thymine dimers. Confirmation of this conclusion is provided by a comparison of the survival-level after maximum photoreactivation of 2800 Å damage with that expected if all thymine dimers were split. An estimate of this latter survival may be made graphically from a plot of reactivation of biological activity and also of relative absorbance change versus 2390 Å dose, in which the scales are adjusted so that the maximum change in survival coincides with the maximum change in absorbance[3]. The survival-level corresponding to complete restoration of the absorbance may be read from the graph. For a dose of 5×10^4 ergs/mm^2 at 2800 Å, as in a previously published graph[3], this survival-level is about 2 per cent, which is close to 2·3 per cent, the actual survival-level found after maximum photoreactivation following this dose.

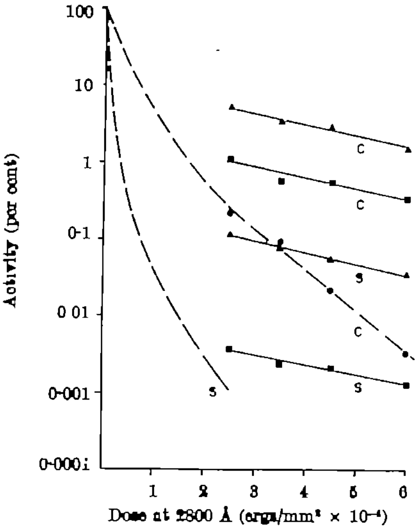

Fig. 3. Inactivation at 2800 Å alone (dashed line) and followed by $2\cdot5 \times 10^4$ ergs/mm^2 2390 Å irradiation with and without maximum photoreactivation. *S*, Streptomycin marker, *C*, cathomycin marker assayed. ▲, 2390 Å + photoreactivation; ■, 2390 Å alone

The conclusion that photoreactivation involves only splitting of thymine dimers makes it probable that the proportion of photoreactivable damage varies with different wave-lengths of inactivating radiation. Fig. 4 shows that this is indeed the case. Inactivating doses of 2800 Å and 2390 Å radiation were chosen so as to yield somewhat similar survival-levels. It can be seen that at the smaller doses there is little difference in the maximum photoreactivation obtained, but at the higher doses there is a much larger increase in activity from photoreactivation of the 2800 Å irradiated DNA. It is apparently more the initial dose than the initial survival-level which determines the point at which the photoreactivation of 2390 Å and 2800 Å radiation damage becomes different. The survival-levels of the streptomycin marker at the small doses are similar to the survival-levels of the cathomycin marker at the large doses, but there is appreciable difference in the photoreactivability of 2390 Å and 2800 Å damage only after large doses. These data may be interpreted to mean that at high doses of 2800 Å radiation there is a relatively higher proportion of damage due to reversible thymine dimerization than at high doses of 2390 Å radiation.

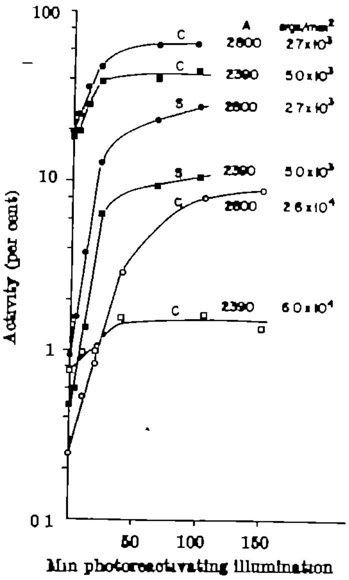

Fig. 4. Photoreactivation of transforming DNA after inactivation at two different wave-lengths. *C*, Cathomycin marker; *S*, streptomycin marker assayed

We have shown that the only photoreactivable lesion in transforming DNA is the thymine dimer, and that it is breakage of the thymine dimer which restores the biological activity. Although the thymine dimer is probably the most important photoreactivable lesion in cells, it is not the only one, for damage to RNA, which contains no thymine, is known to be photoreactivable[5].

This work was carried out under the auspices of the Union Carbide Corporation of the U.S. Atomic Energy Commission.

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LOCALIZATION OF TRITIATED 'DIGOXIN' IN THE MYOCARDIAL CELLS OF FROGS AND DOGS BY AUTORADIOGRAPHY COMBINED WITH ELECTRON MICROSCOPY

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SINCE the application of autoradiography for use with the electron microscope[1], the technique has been extensively developed and used[2-4] for the localization of isotopically labelled substances within living cells. Recently, Waser[5] utilized the technique for the localization of tritium-labelled digitoxin by light microscopy. In this article we present a method for the localization of tritium-labelled 'Digoxin' by electron microscopy in the myocardium. The method depends on the precipitation of radio-'Digoxin' within the cells in order to retain it *in situ* during the preparation of the tissue. (The 'Digoxin' was kindly supplied by Dr. Stanley Bloomfield, of Burroughs Wellcome and Co., Inc.)

The usual methods for fixing and drying tissue for electron microscopy utilize solutions of ethanol, but this procedure results in extraction of the alcohol soluble

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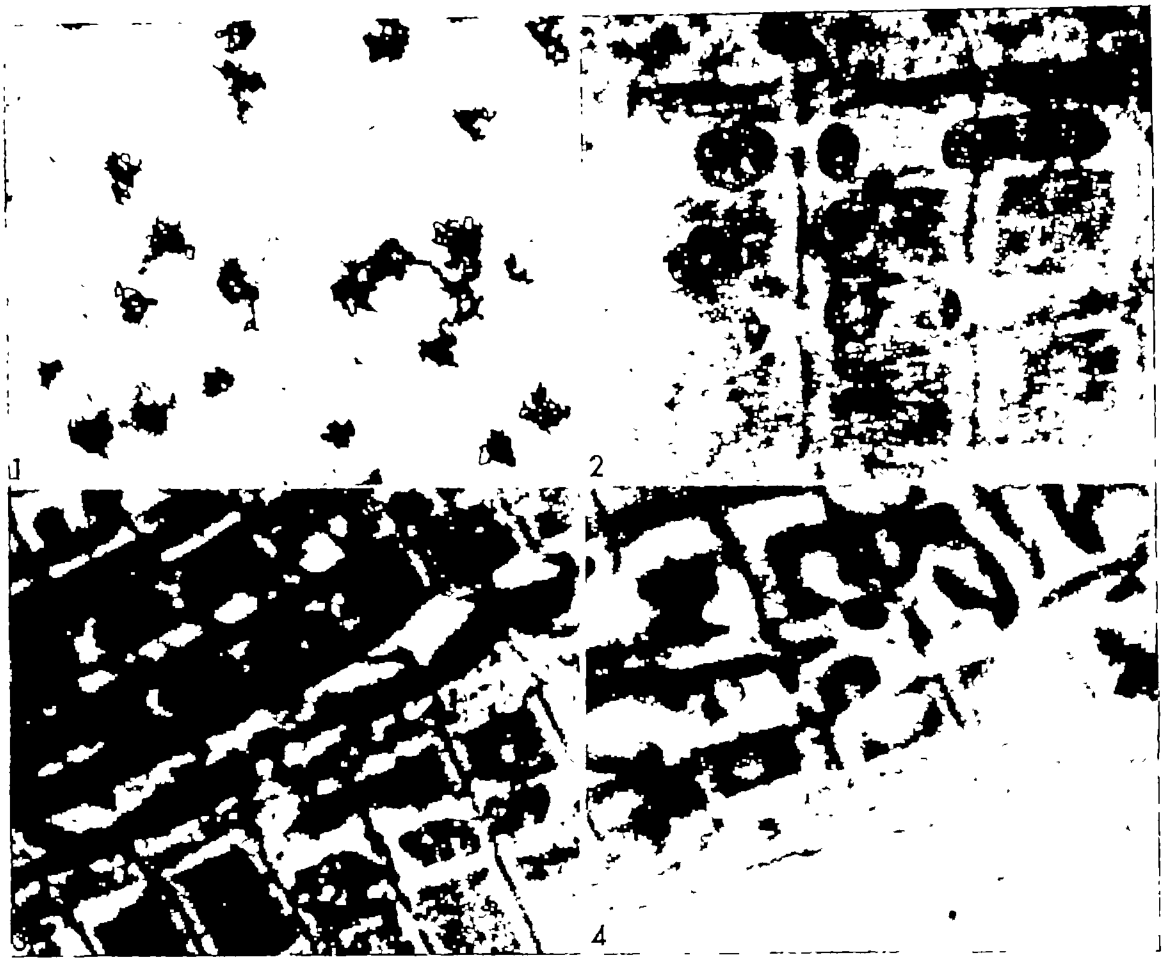

Figs. 1–4. (1) Appearance of exposed silver grains from tritiated 'Digoxin' mixed with 'NTB' liquid emulsion, exposed for 4 days and developed. Gelatin of the emulsion was not removed. (× c. 7,500) (2) Autoradiograph of a typical site of tritiated 'Digoxin' localization (at the A zone of a myofibril) (× c. 12,450) (3) Autoradiograph of tritiated 'Digoxin' localized nearer the Z bands. (× c. 7,500) (4) Autoradiograph of tritiated 'Digoxin' typically related to the A bands. Only occasionally was a silver grain at the sarcolemma.

'Digoxin' from tissue. The glycoside is, however, completely insoluble in acetone. By light microscopy it can be seen that addition of 50 per cent acetone to an alcoholic solution of 'Digoxin' results in its immediate precipitation. For this reason, acetone was used for tissue dehydration in our procedure.

Initial studies for the localization of cardiac glycosides were carried out on the hearts of bullfrogs (*Rana catesbiana*). It was considered advantageous to use this tissue because of the simplicity of preparation and the precision with which the cells may be sectioned longitudinally for study with an electron microscope. The hearts were excised and perfused with Ringer's solution (at room temperature) through a cannula introduced into the right atrium. Contractions were recorded by simple writing levers and kymograph, or by direct observation. When stability of the heart beat was assured, the tritiated 'Digoxin' (66·4 μc./mg) was added to the reservoir in 0·1-mg quantities at 10–20 min intervals until evidence of digitalis toxicity was clearly manifest (auriculo-ventricular dissociation, idioventricular rhythm and final ventricular standstill). Usually, 0·5 mg was introduced into the perfusion fluid before signs of extreme digitalis intoxication were evident. In later experiments dog hearts were studied. Starling heart–lung preparations were set up in the usual way, and tritiated 'Digoxin' was administered into the venous reservoir in 0·1-mg quantities at 15–20 min intervals until digitalis poisoning occurred. A dose of 0·9–1·2 mg of tritiated 'Digoxin' was required to produce digitalis intoxication.

When the influence of the glycoside was plainly evident in the living heart, the ventricles were cut away and trimmed for tissue blocks. The ventricles of the frog hearts were cut into 1-mm wide longitudinal strips with razor blades and fastened to applicator sticks with insect pins under slight stretch. The coronary arteries of the dog hearts were flushed with 50 per cent acetone and 10 per cent formalin solution prior to removal from the chest. Small longitudinal blocks were excised from the papillary and trabecular muscles and fixed to applicator sticks in the same manner.

The muscle specimens, thus pinned, were immersed in a mixture of 50 per cent acetone and 10 per cent formalin for 30 min for precipitation of the glycoside and partial fixation. Thereafter the specimens were transferred to a fixing solution of 1 per cent osmium tetroxide buffered in '*s*-Collidine'[7] for 1·5 h. After the fixing period, the muscle was dehydrated in successive changes of 50, 75, 85, 95 and 100 per cent acetone for 20-min periods each, and was then carried through mixtures of equal parts of acetone and toluene, pure toluene, and equal parts of toluene and methacrylate for 20 min each. The tissues were finally immersed in 70–30 butyl-methyl methacrylate (several changes) for not less than 1 h before final embedding in gelatine capsules. On subsequent sectioning, it was evident that the tissues were adequately fixed, and very thin sections were readily obtained.

Sections were cut in the usual way with a Porter–Blum microtome, and were mounted on 200-mesh steel grids coated with 'Formvar' or 'Parlodion'. When dry, the

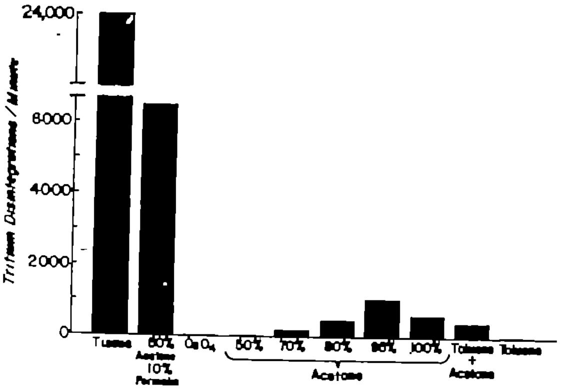

Fig. 5

sections were dipped in a 1 : 5 dilution of E. K. Co. liquid emulsion, 'NTB-3'; the excess emulsion was removed by lightly touching the edge of the grid to filter paper. The coated grids were then placed on filter paper in small Petri dishes and sealed in light-tight boxes. These boxes were kept in the refrigerator for 10–12 days. At the end of this period the specimens were developed in the dark room, washed, fixed in hypo and gently rewashed in distilled water.

Since it was difficult to obtain emulsion films that were sufficiently thin to permit adequate visualization of the underlying cells in an electron microscope, we adapted the method of Revel and Hay[5] to remove the gelatine of the emulsion coat so as to render it largely transparent to the electron beam and to enhance the discernment of cellular detail. Therefore, the grids with mounted specimens were immersed in 0·1 N sodium hydroxide for approximately 1 h. These grids were then placed in distilled water for 10–15 min to rinse away the sodium hydroxide and were allowed to dry on filter paper.

In order to measure the amount of tritiated 'Digoxin' in the tissues and the losses from the tissue during the fixing and drying processes, these fluids and the tissue were analysed using a liquid scintillation counter. With a pulse height analysis circuit, counting efficiency was about 25 per cent. The tissue and samples were prepared by either alcohol extraction or digestion of the dried specimens in 5 N sodium hydroxide. Samples were counted in a toluene solution of 2,5-diphenyloxazole and 1,4-*bis*-2-(5-phenyloxazolyl)-benzene.

The resultant autoradiographs are sufficiently clear so that the emulsion grains exposed by the tritium disintegration can be readily seen against a detailed cellular background (Figs. 2–4). From the micrographs of emulsion exposed directly to the tritiated 'Digoxin' without the presence of tissue, the resultant exposure of silver grains may be seen as fine, irregular traceries (Fig. 1). These same patterns of emulsion exposure may be readily recognized in the autoradiographs of the myocardial cells. Examination of the micrographs likewise indicates that most of the tritiated 'Digoxin' appears to be in close relation to the *A* bands of the myofibrils; rather less activity is noted in the region of the *Z* bands. Rarely, points of activity may be found over, or in the proximity of, the nuclei. On the other hand, there is a paucity of evidence of radioactivity along the sarcolemma, and only rarely does it occur in the sarcoplasm or over the mitochondria.

All attempts to utilize ethanol for dehydration of the tissue resulted in complete extraction of the radioactive 'Digoxin' from the tissue. When acetone was used the 'Digoxin' remained in the tissue in good concentration (0·16–0·60 μg 'Digoxin'/g tissue). The isotope removed during the fixing procedure (Fig. 5) was small, and little additional loss occurred during dehydration. This total amount of glycoside lost was equal to the calculated quantity that would have been present in the extracellular fluid space estimated to be 30 per cent of volume at the same concentration as in the perfusate.

Our greatest difficulty in the production of autoradiographs of intracellular 'Digoxin' has been preparation of the tissue without eluting the glycoside from the cells. It was quickly apparent that the usual procedures utilizing ethanol would not be satisfactory, for no isotope remained in the tissue after preparation. Acetone was found to fix and dehydrate the tissue satisfactorily without excessive loss of 'Digoxin'. It is unfortunate that the treatment of these tissues with acetone is partially destructive to the muscle. For this reason, passage of the muscle specimens through the acetone solutions was kept as brief as was consistent with adequate tissue preparation. Under the conditions reported, thin sections may be prepared with ease and precision without evidence of gross tissue destruction by the glass knives of the microtome.

Examination of many autoradiographs of myocardial cells treated with tritiated 'Digoxin' demonstrates that the radioactivity is usually found in relation to the *A* bands of the myofibrils. Very little activity appeared to be localized in the other sub-cellular structures. It is not certain that the location of the radioactive 'Digoxin' in the cells is actually the physiological site of action of the drug, but its specific concentration in the area of the myofibrils is strongly suggestive of this, and is at present under investigation.

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INHIBITION OF VIRUS MULTIPLICATION BY FOREIGN NUCLEIC ACID

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THE cells of many vertebrates can be induced to synthesize interferon by infecting them with many different viruses. In trying to understand what is the essential stimulus that induces cells to make interferon, an important finding is that for many different cell-virus systems, virus multiplication is not essential for the production of interferon. Thus interferon production can be induced by inactivated viruses[1–3], by live virus inoculated into cells which do not support virus multiplication[4,5], or by growth of infected cells at temperatures which do not allow virus multiplication[6]. This would imply that the stimulus to make interferon is provided by part of the virus particle that enters the cell.

There is some indirect evidence to suggest that the stimulus might be viral nucleic acid. Influenza virus

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Table 1. ANTIVIRAL ACTION OF CHICK AND MOUSE RNA ON VACCINIA VIRUS GROWN IN CHICK AND MOUSE CELLS

	Material tested	Concentration (γ/ml.)	Mouse cells Plaque counts	Mean count	Chick cells Plaque counts	Mean count
Exp. 1	Control	—	96, 111, 115, 121, 120, 122	116	332, 314, 313, 328, 323, 309	320
	Chick RNA	50	12, 21	17	78, 82	80
		10	80, 77	79	244, 274	259
		2	107, 99	103	300, 317	309
	Mouse RNA	50	127, 117	122	6, 10 (faint)	8
		10	99, 110	105	40, 50	45
		2	103, 92	97	333, 282	308
Exp. 2	Control	—	17, 21, 19	19	161, 179, 174, 185	175
	Chick RNA	50	6, 4	5	132, 119	126
		10	16, 6	11	192, 172	182
	Mouse RNA	50	12, 10	11	0, 0	0
		10	14, 14	14	35, 23	29

particles containing virus nucleic acid are able to induce virus interference whereas particles lacking nucleic acid are not[7]. The ability of influenza virus particles to induce production of interferon can be abolished by prolonged irradiation with ultra-violet light, which damages the viral nucleic acid without affecting significantly the antigenic or enzymatic (neuraminidase) activities of the particles[8]. However, if viral nucleic acid is the stimulus to make interferon this poses an awkward problem, since both DNA and RNA viruses are able to induce its production. This suggested a hypothesis—that the essential stimulus to make interferon might be nucleic acid that was 'foreign' to the cell[9]. This paper reports a test of this hypothesis which involves the use of non-viral nucleic acids. It was soon found that non-viral 'foreign' nucleic acids induced production of either very small amounts of interferon or no detectable interferon, as measured by interferon liberated from cells. In order to test therefore whether small amounts of interferon were being produced inside the cells the experimental methods were modified so that the direct antiviral action of foreign nucleic acids was measured on cells.

In preliminary experiments chick embryo fibroblasts were first treated with foreign nucleic acids for various periods of time, then infected with virus, overlaid with nutrient agar and viral inhibition measured by the reduction in plaque count. A number of different preparations of RNA and DNA showed viral inhibitory effects (Table 3), but only when very large amounts of nucleic acids (1–10 mg per plate) were used. The antiviral action of nucleic acids in animals has been previously reported[10]; but with the low degree of viral inhibition it was difficult to understand the mechanism involved. A second technique used was to suspend the nucleic acid in M sodium chloride, as is carried out when initiating infection by viral nucleic acid[11]. This technique gave preliminary indications that heterologous, but not homologous, viral nucleic acid inhibited virus growth, but the degree of viral inhibition found was small and inconstant. A third technique used was to mix the nucleic acid with protamine which was thought to increase the uptake of nucleic acid by cells[12]. A difficulty experienced in this work was that protamine itself is apparently an uncoupler of oxidative phosphorylation[13] and shows an antiviral action[14] that somewhat resembles that of interferon[15].

Recently the technique used was modified so that instead of pre-treating cells with nucleic acid, the nucleic acid was present in the medium during the whole time the virus was multiplying. With this modification regular and reproducible viral inhibition by heterologous nucleic acid has been found.

The assay method used was an adaptation[16] of the technique of Postlethwaite[17] by which vaccinia virus induces plaques in cell monolayers kept without an agar overlay. Two types of cells were utilized. Primary cultures of 10-day-old chick embryo fibroblasts were used 24 h after seeding 4-cm Petri dishes with 9 × 10⁶ cells. Secondary cultures of 18-day-old mouse embryo fibroblasts were used 24 h after seeding Petri dishes with 2 × 10⁶ cells. The nucleic acids were chick RNA, obtained from the livers of 3-week-old chickens, and mouse RNA from the livers of 4–6-week-old mice. The RNA was extracted from the ribosomes by the guanidinium chloride technique of Cox and Arnstein[18].

An assay was carried out as follows. The nutrient medium was removed from the cells and replaced by a maintenance medium consisting of 99·7 per cent Gey's buffered salt solution, 0·05 per cent yeast extract and 0·25 per cent lactalbumin hydrolysate, in which the nucleic acid under test and vaccinia virus, diluted to give about 100 plaques in chick cells, were suspended. The plates were incubated for 42–48 h at 37° in an atmosphere containing 3 per cent carbon dioxide. They were then removed, the cell sheets stained for 1 min with 0·1 per cent crystal violet, washed and drained to dryness. The plaques were counted with the help of either a hand-lens (if plaques were clearly visible) or with about ten-fold magnification in a viewing box. Chick and mouse RNA were tested at the same time with chick and mouse cells, so that essentially what were compared were the effects of heterologous and homologous RNA.

The results showed a striking viral inhibition by heterologous RNA with a smaller inhibition, or a lack of inhibition with homologous RNA. Two experiments illustrating this are shown in Table 1. In four experiments in which the chick and mouse cells were tested at the same time this type of result was found, and in a number of additional experiments in which, for technical reasons either the chick or the mouse cells did not provide good cell sheets, it was always found that heterologous RNA showed definite viral inhibition whereas homologous RNA at the same dosage showed a weaker effect or no effect. The degree of viral inhibition produced by homologous RNA was variable. Thus in Exp. 1 of Table 1, chick RNA (50 γ/ml.) showed definite antiviral activity in chick cells whereas mouse RNA at the same dosage showed no antiviral activity on mouse cells. In Exp. 2 of Table 1 there was minimal inhibitory activity of homologous RNA. The reasons for this behaviour of homologous RNA are discussed below. When very small doses of protamine were added (1γ protamine + 100γ RNA) the antiviral action of the heterologous RNA was reduced and homologous RNA plus protamine showed no viral inhibition or showed occasionally enhancement of viral growth. When experiments with nucleic acids were carried out in stoppered bottles with no added carbon dioxide the antiviral action was less than when cells were kept in parallel in open Petri dishes incubated in an atmosphere of carbon dioxide. In another experiment the time at which the nucleic acid was added to the cells was varied but with the same technique of assay[16] used throughout. Pretreatment of cells with nucleic acid for 6 h before virus was added gave a much weaker antiviral effect than when the nucleic acid was present along with the virus.

It is known that the cells of 6-day chick embryos are less sensitive to the antiviral action of interferon and produce lower yields of interferon than the cells of 10-day chick embryos[19]. If the antiviral action of heterologous

RNA were mediated through production of small amounts of interferon, it should be less evident when tested in 6-day-old than in 10-day-old chick embryo cells. Two experiments were carried out both of which clearly showed this to be the case. The results of one of the experiments is illustrated in Table 2. It is clear that the cells of 6-day chick embryos (7 days old at the time of assay) were less sensitive to the antiviral action of interferon and also to that of mouse RNA than the cells of 10-day chick embryos (11 days old at the time of assay).

Table 2. ANTIVIRAL ACTION OF MOUSE RNA AND CHICK INTERFERON ON 6-DAY AND 10-DAY CHICK CELLS

Material tested	Concentration	10-Day chick cells		6-Day chick cells	
		Plaque counts	Mean count	Plaque counts	Mean count
Control	—	161, 179, 174, 185	175	110, 105, 129, 107	113
Mouse RNA	50 γ/ml.	0 0	0	72, 65	69
	10 γ/ml.	35, 23	29	108, 104	106
Chick interferon	1/100 dilution	0, 0	0	30, 42 (faint)	36

Another point that is known about interferon is the fact that some viruses are more sensitive to its antiviral action than others[16]. This was difficult to test with the present assay since very few viruses produce plaques without an agar overlay. However, it was tested by the less-sensitive technique by which cells were treated with nucleic acid before being infected with two different viruses. The results shown in Table 3 indicate that Chikungunya virus was more sensitive than Newcastle disease virus to the antiviral action of both interferon and DNA from calf thymus.

Table 3. EFFECT OF TREATMENT WITH NON-VIRAL DNA AND INTERFERON ON THE ABILITY OF CHICK EMBRYO FIBROBLASTS TO GROW CHIKUNGUNYA AND NEWCASTLE DISEASE VIRUSES

Material tested	Concentration	No. of virus plaques with	
		Chikungunya virus	Newcastle disease virus
DNA from calf thymus	5·8 mg/ml.	1	88
	1·16 mg/ml.	6	75
Chick interferon	1/20 dilution	0	95
	1/100 dilution	3	102
Control	—	12, 16	104, 128

DNA was prepared by the technique of Kay *et al.* (ref. 21).

The findings with 6-day chick cells and with the two different challenge viruses furnish indirect evidence that the antiviral action of nucleic acids may be mediated through the production of interferon. More direct evidence for this was sought by treating chick embryo fibroblasts with 100γ of mouse RNA for 3 h at 37°, washing the cells twice, removing the RNA and replacing it with maintenance medium in which the cells were then incubated overnight at 37°. This medium was then tested for antiviral action against vaccinia virus in fresh chick cells when it showed a weak but definite antiviral action. This effect could have been due to a new substance produced by the cells or to some residual mouse RNA. However, the former explanation was favoured when it was found that chick cells pre-treated with this material and then challenged with Chikungunya virus showed definite viral inhibition. In addition, the inhibitory substance produced was found to be non-dialysable, stable at pH 2, inactivated by incubation at 37° and pH 2 for 1 h with 10 γ/ml. of pepsin, stable on heating at 60° for 30 min but largely inactivated by heating at 100° for 5 min. In all these properties it resembled interferon.

The weak antiviral action of some preparations of homologous RNA (Table 1) might be accounted for by the fact that neither the mice nor the chickens used in these experiments were inbred. It is possible, therefore, that small differences would be present between the extracted RNA and the RNA of the homologous animal cells. This point is now under investigation using isologous and homologous RNA with cells from two inbred strains of mice. However, so far as they go, the present experiments favour the hypothesis that interferon production occurs as a reaction of cells to foreign nucleic acids.

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VIRUSES, GENES AND CISTRONS

ORIGIN of viruses. My suggestion[1] that the pathogenesis of viruses involves primarily an interference inflicted on the metabolism of the host by the expropriation of nucleotides by the virus from the host had been expressed earlier by Pirie[2], who has recently suggested further[3] that viruses "(1) . . . can be the extreme form of degeneration of micro-organisms so that, from merely being able to multiply in a host, they have become totally parasitic and dependent on the host's synthetic systems. (2) They can arise in a cell when, as a result of an aberration in metabolism, it produces a structure robust enough to withstand normal scavenging processes, and able to act as a model for further synthesis. (3) They can be structures with qualities similar to those needed in possibility (2) but coming into the cell from outside—from another species, for example.

"Of these three possibilities (1) and (3) are probably the most generally accepted. The possibility that anything similar to the viruses we now study was a stage in the evolution of more elaborate organisms, though often touted, need not be taken seriously. Present-day viruses demand so much from an environment before they can multiply in it that they are improbable components of the pre-biotic world."

Pirie's pronouncement that substances like viruses need not be considered seriously by those interested in the origin of life must have been made with his tongue in his cheek, because he is only too well aware of the fact that the basic hypothesis which directs the scientific activities of most of the foremost geneticists and biochemists of the present time is founded on precisely that assumption.

Receptor-hypothesis of gene-action and gene-structure. I[4,5] have proposed the receptor-hypothesis of gene-structure according to which the gene comprises: (1) a side-chain of DNA embedded in the histone backbone of the chromosome; (2) a segment of RNA, corresponding to the exposed DNA segment which is transcribed *in situ* and bound to the DNA segment by hydrogen bonds; (3) the receptor, which is an adjacent side-chain on the histone comprising a folded protein which resembles the enzyme. The application of the inducer to the outer surface of the receptor produces an excitation which passes from the receptor to the DNA side-chain and causes the release of messenger RNA. Mutations in the receptor are heritable changes in the coiling of the receptor which determine which one of one or more inducers may act as initiators of adaptive enzyme synthesis. The nucleic acid components of the chromosome replicate according to the Watson–Crick[6] theory of nucleotide-pairing; but the chromosome, as a whole, is a coacervate[7] and each new chromosome is produced by coacervation of all the respective components from the nuclear vacuole. The specific folding of the protein is heritable and the protein in each parent receptor directs the folding of the protein which is coacervated on its lateral surface to produce the 'new' receptor. The structural DNA holds the RNA messenger in a relatively stable complex, but the union of inductor with the receptor makes the complex unstable and leads to the release of messenger RNA. Induction and cessation of enzyme synthesis is an automatic homeostatic process beginning when the inductor or inductors make contact with the receptor, and ceasing when all the inductor molecules have been altered by the induced enzyme. According to this hypothesis, gene-systems are exclusively adaptive.

Viruses as fragments of gene-systems. On the basis of the recent biochemical advances which have specified the relation between genes and proteins, one may assume that vegetatively transmitted, non-infective RNA and DNA (prophage) are segments of nucleic acid which are somehow resistant to the ribonucleases and the deoxyribonucleases which must abound in the cytoplasm to prevent the replication of either RNA or DNA in the cytoplasm. Under normal conditions, the replication of DNA and the transcription of RNA from DNA must be confined to the nucleus in a region free from nucleases[8]. The controlling devices which lead to the emergence from the nucleus into the cytoplasm of a limited number of specific RNA templates must be capable of being reversed almost completely from instant to instant. An enzyme needed for a few seconds or minutes in the life-cycle of a micro-organism may be exceedingly detrimental a few moments later. The activity of the nucleases makes the cytoplasm particularly rich in nucleotides, and any nucleic acid which persisted in the cytoplasm would be presented with an abundance of the materials essential to its replication. If a segment of RNA, which was relatively resistant to the cytoplasmic ribonuclease, appeared in the cytoplasm, it might (after replication) become attached to a ribosome and effect the transcription of a protein which could envelop and protect the replicated RNA segment as soon as it was detached from the ribosome just as the TMV–RNA (ref. 9) virus is protected by a protein particularly resistant to proteases.

One might expect, as Pirie suggests, that the RNA templates which develop into viruses generally originate in another species. Parallel considerations suggest that DNA viruses are segments of 'structural' DNA capable of surviving in the cytoplasm to transcribe RNA templates which, when attached to ribosomes, could control the transcription of these proteins, which comprise the envelope essential to an infective virus particle. Neither the RNA nor the DNA viruses would be expected to have an independent life outside the cytoplasm because only the cytoplasm can supply the ribosomes, the enormous pool of nucleotides, the amino-acids and the energy sources necessary for their replication. The growth of an infective virus would be expected to come to a final end when the stored reserves present in the host were exhausted. On this hypothesis, viruses may not be compared with the functional genes of higher organisms, but only to parts of gene-systems. Instead of genes which have taken up a cytoplasmic existence, viruses represent nucleic acid fragments of gene-systems which are normally destroyed in the cytoplasm by the natural scavenging processes. They are related only to the structural DNA and the template or messenger RNA of the gene-system.

Vegetatively propagated viruses (like prophage or kappa) which persist in the host from generation to generation without producing particles capable of initiating infection and which replicate at a rate more or less in synchrony with the replication of the cell may be looked on as persistently transmitted hereditary 'garbage', outside the normal metabolic requirements and, hence, dispensable, and only slightly, if at all, detrimental to the host.

Bacteriophage as an end-to-end sequence of structural elements. Although a single gene carries only a single structural element capable of specifying the amino-acid sequence of a single protein, a bacteriophage requires a variety of different proteins to produce an infective particle, and must be capable of producing many different RNA templates which specify a variety of different kinds of proteins. Thus, the bacteriophage virus must comprise a linear array corresponding to the end-to-end attachment of a number of different structural elements arising from many different and probably rearranged nucleotide sequences derived from structural elements of pre-existing genes. Since each single gene-system controls the synthesis of only a single enzyme (or a single protein) the amount of nucleic acid present in a gene-system would be expected to be much less than that in a bacteriophage, which must specify the synthesis of a great variety of different proteins.

Mapping and crossing-over in chromosomes and in viruses. The receptor-hypothesis (which is consistent with the Taylor–Freese[10] model of the chromosome) implies that there are, at least, two different kinds of cross-overs; (1) those which occur in the histone backbone between genes; (2) those between nucleotides in the structural DNA within a gene. The theory suggests that the former are much more frequent than the latter, and it also implies that synapsis at meiosis is controlled by the mutual attraction of two exposed complementary single-stranded DNA side-chains. Thus, the phenomenon of two-by-two pairing in triploid synapsis, described by Darlington[11], in which one of three chromatids is excluded at any given level, is finally comprehensible.

Mapping and crossing-over occurring in viruses has no direct relation to the mapping of genes on the chromosome because, in mapping genes, the cross-overs which are counted are only the relatively numerous breakages and reunions in the histone backbone of the chromosome occurring at the four-strand stage between the genes, while in the mapping of bacteriophages (and structural elements of genes) the much rarer cross-overs which are counted are those which occur between two nucleotides within a nucleotide sequence to produce a change in the nucleotide sequence. (Gene-conversion, which results in non-reciprocal recombination, involves loss, alteration or transfer of receptors.)

The cistron. The cistron[12] of a bacteriophage comprises that particular portion of the nucleotide sequence which specifies the amino-acid sequence in a single one of the proteins essential either to protection from the cytoplasmic nucleases or the development of a mature infective particle. A cistron becomes non-functional when a deletion occurs in a region of the segment essential to the structure of its specific protein. A cistron is identified as a limited region within which respectively overlapping deletions produce the same defective phenotype. If the deletions present in two different defective bacteriophage mutants overlap in a given cistron, no amount of recombination between two mutant bacteriophages can produce a functional infective particle. The test for the identification of a cistron, therefore, resembles the test for allelomorphism of genes, but this operational similarity does not equate genes and cistrons.

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LINDEGREN'S suggestion that I might have made a comment without wholly serious intent is not likely to commend itself to anyone who knows me. Nevertheless, the comment that he finds surprising may be worthy of a little amplification. Even supposing Lindegren is right in saying that the idea that viruses resemble an early stage in biopoesis, is basic to the activities of the "foremost geneticists and biochemists of the present time", it is not necessary that we should all accept the idea. To argue the contrary seems to be carrying the principles of democracy, and the validity of anything agreed by majority vote, too far. Heretics may not be often right, but the history of science suggests that they sometimes are.

One essential quality in any system proposed as an early stage in biopoesis is that it should have been able to carry out its characteristic activity, whatever that was, in the absence of more highly integrated vital forms. The absence of this capacity is the characteristic unifying quality of the viruses. It may be that there are undiscovered systems (in the sea, for example) with some virus-like qualities but able to engineer their own synthesis without the help of a host. It will be time enough to discuss the relationship between such a system and the accepted viruses when it has been discovered. The discovery may be difficult for so far it is not we who have found viruses, it is viruses that, by modifying the behaviour of hosts in which we are interested, have found us. As I have remarked, reviewing a discussion on the origins of life[1]: "It is unfortunate therefore that so much attention was devoted at the Symposium to viruses and autotrophic bacteria. The former are irrelevant because they do too little, and the latter because they do too much. Viruses only exist by courtesy of the synthetic mechanisms of a more elaborate host cell, which has a normal metabolism that is concerned with making a wide range of substances such as polysaccharides, steroids, peptides and also nucleoproteins. The viruses about which we have any chemical information seem to be predominantly nucleoprotein. There is no reason to think that when a virus invades a cell it does more than upset the normal cell metabolism, so that the cell then makes copies of the invader instead of making the things it was accustomed to. The manner in which this switch over is brought about is not yet understood. It is the central problem of virus research, but it has no close connexion with biopoesis. Autotrophs, on the other hand, contain mechanisms for making every type of molecule used in biochemistry and they do this without help from any other organism. This implies a biochemical complexity and competence greater than that of man or of the other organisms that have to get vitamins, amino-acids, and so on, pre-formed by other organisms. The apparent morphological simplicity of an autotroph should not mislead us into looking on it as a primitive form. All these capacities could not have developed simultaneously, but are more likely to have been slowly accreted in response to the competitive stresses of one or two thousand million years of evolution."

Two further comments may be made on Lindegren's communication. The three ways in which viruses could reasonably be expected to originate were all discussed by the virus workers in the 1930's and, in my opinion, some viruses have arisen in each way. Lindegren's picture of gene structure and action is plausible but may well be too precise. Scientists rarely discover a phenomenon outside the domain of their interest. This is the era of the nucleic acids; phenomena involving them are therefore very often discovered and very readily credited. When general interest moves, perhaps to initiating the intensive study of fats or the renewed study of specific polysaccharides, other mechanisms parallel to those dependent on nucleic acids will probably be found: and these mechanisms are just as likely to be primitive as mechanisms based on nucleic acids.

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QUANTITATIVE HISTOCHEMISTRY OF SUCCINATE DEHYDROGENASE IN TISSUE SECTIONS

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ALTHOUGH methods of estimating succinate dehydrogenase quantitatively in tissue sections have been described[1–3], such techniques do not appear to have been widely adopted. In these communications tetrazolium salts have been used as electron acceptors, and enzymatic activity has been estimated by relating formazan production to the area of the section at constant thickness. The main disadvantage of these methods is that the thickness of sections is not easy to control accurately or to measure directly; thus determinations of activity as related to area of section will inevitably reflect variations in thickness. Greater accuracy can be gained by the use of more sections per tissue determination, but the method can thereby become cumbersome and tedious. In the present investigation a simple and more accurate method is described in which formazan production is related to the total nitrogen content of the section itself.

Portions of liver taken from male August rats fed on a diet low in protein and supplemented with methionine[4] were frozen and sectioned at 8μ by the controlled-temperature freezing sectioning technique[5]. The sections were mounted on slides and dried at room temperature for 10–15 min prior to incubation, which was carried out in

Table 1. SUCCINATE DEHYDROGENASE AS ESTIMATED IN TISSUE SECTIONS

1 h incubation at 37° C; formazan extracted with 0·5 ml. 10 per cent *n*-heptanol in tetrachloroethane

Section	Formazan production log I_0/I at 550 mμ	Amount of formazan (μg)	Nitrogen determination, log I_0/I at 450 mμ	Amount of nitrogen (μg)	Formazan + nitrogen (μg formazan per μg nitrogen)	Area (cm²)	Formazan per unit area (μg per cm²)	Nitrogen + area (μg nitrogen per cm²)
1	0·974	10·15	0·524	6·08	1·67	0·378	26·9	16·1
2	0·652	6·80	0·341	3·96	1·72	0·347	19·6	11·4
3	0·641	6·69	0·340	3·95	1·69	0·349	19·2	11·3
4	0·857	8·94	0·462	5·36	1·67	0·332	26·9	16·2
5	0·792	8·26	0·473	5·49	1·51	0·388	21·3	14·2
6	0·567	5·91	0·284	3·30	1·79	0·341	17·3	9·7
7	0·799	8·33	0·509	5·90	1·41	0·351	23·7	16·8
8	0·494	5·15	0·275	3·19	1·61	0·282	18·3	11·3
9	0·610	6·36	0·306	3·55	1·79	0·336	18·9	10·6
Average					1·65		21·3	13·1
S.D.					0·044		1·28	0·97
Standard deviation (%)					2·7		6·0	7·4

25 ml. of solution in Coplin jars. The jars were maintained at 37° C in a water bath in the dark. The solutions contained sodium succinate (0·05 M), sodium phosphate buffer (0·05 M) at various *p*H values, neotetrazolium chloride (0·75–0·80 mg/ml.) and ethylenediamine tetraacetic acid (0·001 M). The time of incubation was usually 1 h. The reaction was stopped by placing the slides in distilled water; after a further rinse in distilled water the slides were dried and used immediately or stored unmounted in the dark for not longer than two weeks.

Small squares of glass bearing the intact sections were cut from the slides. Each square was placed in a 16 × 150 mm 'Pyrex' test tube. The formazan was extracted by adding 0·5 ml. of 10 per cent *n*-heptanol in chlorine-free *sym*-tetrachloroethane. Extraction was usually rapid, but if the section was folded it was sometimes necessary to wait 15–20 min to obtain total extraction. The extinction of the formazan solutions was measured at 550 mμ in a Hilger spectrophotometer with a microcell attachment (volume of microcells, 0·25 ml., path-length, 10 mm). Solutions of the formazan obeyed the Beer–Lambert law up to extinction values of 1·2. The colour of the solutions was fairly stable, but faded by 0·5–1 per cent in 30 min. Excess solvent was removed with a Pasteur pipette; the sections were washed with a few ml. of ether, and dried at 80°–90° C for at least 2 h.

The micro-method of Long and Staples[6] has been made more sensitive in order to estimate the total nitrogen of each section. To each tube was added 0·1 ml. 72 per cent perchloric acid. A 'Teflon' chip was added to prevent bumping and loss of acid during digestion[7]. The acid was refluxed gently at 195°–200° C for 90 min and cooled. 0·5 ml. water was added and the tubes were cooled in a refrigerator. 1·0 ml. chilled Nessler's reagent was added, and the absorption of the solution was read at 450 mμ. Under these conditions 0·5 μmoles of nitrogen gave a reading of approximately 0·60 in the spectrophotometer. Nessler's reagent was prepared according to the method of King[8] except that an equimolar proportion of sodium iodide was substituted for potassium iodide[9]. Areas of sections were obtained by tracing the projected outline of the stained section on paper at a ten-fold magnification, and by measuring the area of the tracing with a planimeter.

A typical set of results is given in Table 1. The spectrophotometric readings for both the formazan and nitrogen values are sufficiently high to be determined accurately. When enzymatic activity is related to the total nitrogen of the section, the percentage deviation is ± 2·7, but when the activity is related to the area of the section the percentage deviation becomes ± 6·0, that is, more than twice as great. It can also be seen from the table that there is an approximate correlation between formazan production per unit area and section thickness as given by total nitrogen per unit area; in other words, thick and thin sections show high and low activity respectively. Sections incubated in a medium from which succinate had been omitted gave a very weak reaction, which was about 0·5 per cent of that obtained in the presence of substrate. Formazan deposition was obtained only on the section.

The *p*H activity curve of succinate dehydrogenase with neotetrazolium as electron acceptor is shown in Fig. 1. Five sections were used for each point on the graph. When succinate was omitted from the medium a negligible reaction was recorded. It has been found that the inclusion of sodium azide at a concentration of 0·01 M enhances the production of formazan under anaerobic conditions. Several workers[10] have directed attention to the qualitative histochemical finding that cyanide produces an identical effect when substituted for azide. The enhancement of anaerobic activity was attributed to the blockage of the cytochrome system which was otherwise possibly activating traces of oxygen still present despite anaerobiosis, thereby permitting competition for electrons at some site on the transport chain.

From Fig. 1 the *p*H optimum of the succinate dehydrogenase–neotetrazolium reductase system is 8·0. A visual examination of the unmounted sections showed that it was evident that the intensity of the stain at *p*H 7·4 was very much stronger than that obtained at *p*H 6·8, but the staining reaction between *p*H 7·4 and 8·6 was so similar that it was impossible to make any assessment of where the *p*H optimum lay. It can, however, be seen from the

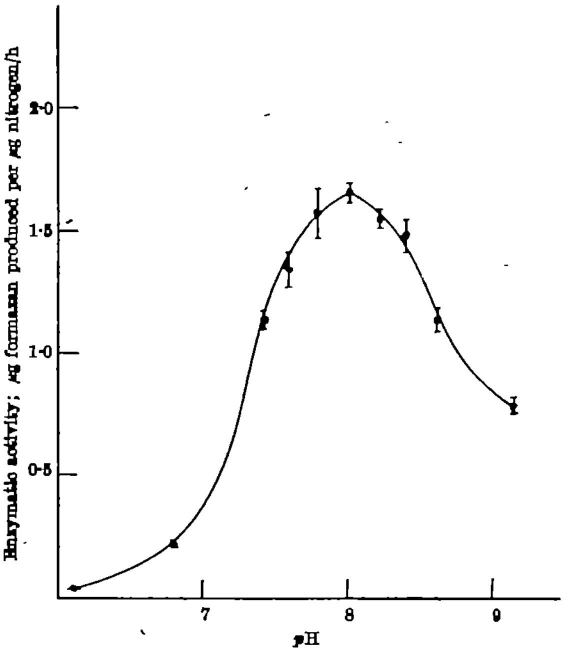

Fig. 1. Variation of succinate dehydrogenase activity with *p*H; sections incubated in neotetrazolium–succinate mixture for 45 min

graph that the activity at pH 8·0 is 50 per cent higher than the activity at pH 7·4, and that the differences in activity of adjacent points is very slight. It is often difficult to assess microscopically the intensity of a dehydrogenase reaction. Granular deposition is generally taken as being indicative of enzymatic activity, but under given conditions with the same material the size and number of formazan granules can vary. Qualitative comparisons may not be necessarily valid even when differences between sections are apparently clear. Moreover, sections of different thickness which have been treated in the same way can show considerable differences in intensity of staining both microscopically and to the naked eye. When such sections are subjected to the procedure described and formazan production related to total nitrogen the agreement between the values obtained is very close.

No comparison of enzymatic activity at this, the cellular level, has as yet been made with succinate dehydrogenase activity as determined with neotetrazolium in an homogenate preparation, but a comparison with the results of Slater[11] shows that the activities reported here are of the order of two-thirds of those obtained at the homogenate-level. Jardetzky and Glick reported a value of 0·27 μg formazan produced per μg nitrogen per hour, but their nitrogen determinations were performed indirectly on untreated sections.

The amount of tissue needed for each sample is very small, and is but a fraction of the amounts required for assay by manometric methods. It is, however, of the same order as that required for methods involving a direct measurement of rate of fumarate formation[12]. The accuracy obtainable here is probably no better than ± 7 per cent. It is likely that the error arises largely in the nitrogen determination; further work on this is in progress. The outstanding advantage possessed by this technique is that it is possible to examine microscopically the section itself before analysis and, if necessary, to examine a serial section stained with hæmatoxylin and eosin. With standard biochemical procedures the sample is homogenized, and the only histological appraisal of the material which can be made must rest on pieces removed from the material prior to a treatment which often involves disruption. The danger then exists that the histological section can give information which is no more than an approximation to the real composition of the sample. At present this quantitative method is probably not sufficiently sensitive for different areas of the section to be analysed separately, but this should be possible when the nitrogen estimation method is made more sensitive and reliable.

The potential value of this quantitative microtechnique is considerable, even if it is used only as a means of microsampling for enzymatic assay. It is stressed, furthermore, that this technique enables dehydrogenases to be studied at the level of the intact cell instead of at the level of individual cellular components at various stages of isolation after the cell has been disrupted. The simultaneous combination of a quantitative enzymatic determination with direct microscopical examination of the material studied should reduce experimental ambiguity of tissue sampling when heterogeneity is a problem, especially in the study of tumours.

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BETA-GALACTOSIDASE OF *SHIGELLA SONNEI*

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β-GALACTOSIDASE is an inducible enzyme in many organisms[1–4]. Lederberg[5] developed an assay method for β-galactosidase which was based on the hydrolysis of a chromogenic substrate, *o*-nitrophenyl-β-*d*-galactoside (ONPG), to *o*-nitrophenol (ONP). Electrophoretic analysis has shown that the hydrolysis of lactose and ONPG was catalysed by the same enzyme[6]. According to Monod *et al.*[7] the induction of β-galactosidase in *Escherichia coli* was limited to substances possessing an intact galactosidic radical. β-Galactosidase production was increased by sugars which were not its specific substrate[8]. Certain inducers such as melibiose, which is a β-galactoside, are not hydrolysed by β-galactosidase[7]. Galactose was found to be a good inducer of β-galactosidase in *Bacillus megaterium*[8], *Staphylococcus aureus*[9] and *E. coli* B[10], whereas it was a poor inducer in a wild type of *E. coli*[11]. Methyl-β-*d*-galactoside and methyl-β-*d*-thiogalactoside (TMG) were good inducers of β-galactosidase but phenyl-β-*d*-thiogalactose was not[12]. Fluoro-β-*d*-galactoside was reported to be a good inducer but not as efficient as TMG (ref. 11). This was because the fluorogalactoside was hydrolysed by the enzyme whereas TMG was not. It is considered advantageous to use 'gratuitous' inducers which do not influence general cellular metabolism since their effects would not mask the true induction process[1]. Lactose was found to be the only inducer of β-galactosidase in *Paracolobactrum aerogenoides*[13]. Attempts have been made to induce the enzyme in *Shigella sonnei* with lactose, melibiose, and TMG (ref. 14). TMG was the most efficient inducer particularly in toluene-treated cells.

The purpose of the present investigation was to study the properties of the β-galactosidase in *S. sonnei* and to determine the factors which affected this induction process. Concurrently, we studied the lactose fermenting patterns of *S. sonnei*[15].

Four strains of *S. sonnei* were used. In addition, *S. boydii*, *S. flexneri*, *S. dysenteriae*, *alkalescens-dispar* group, and *E. coli* were studied. The cells were collected, washed and suspended to yield an optical density reading of 1·0 (420 mμ). The enzyme assay was based on the colorimetric determination of ONP released from ONPG[5]. Intact cells and toluene-treated cells were assayed simultaneously; toluene-treatment procedures were those utilized by Koppel *et al.*[10]. The amount of ONP liberated in the reaction tubes was determined from a standard calibration curve which was prepared by measuring the spectral changes for varying concentrations of

ONP. 1 ml. of the original cell suspension was dried in the hot-air oven at 85° C to determine the enzyme activity on a per weight basis. The activity of β-galactosidase was recorded as the number of μmoles of ONP liberated from ONPG in 20 min at 37° C per mg dry weight of cells. Cell-free extracts were obtained by shaking the cell suspensions for 60 min at 4° C with glass beads in the Mickle tissue disintegrator. The disrupted cells were then assayed for enzyme activity. Extracts were also prepared by alternate freezing and thawing for 5 times at – 20° C.

Attempts to increase the production of β-galactosidase were made by growing the organisms in the presence of various carbohydrates (lactose, galactose, glucose, melibiose, raffinose and TMG) for 3 days prior to the assay. The enzyme was also induced in washed cell suspensions with either lactose or TMG. The inducers were added to the cell suspensions 2–18 h before the assay. The induction was carried out at 37° C, which was found to be the optimum temperature for enzyme induction. Starved cells were also induced. The cells were starved by placing washed cells at 37° C for 24 h prior to the addition of the inducer. Starved cells were also induced in the presence of an exogenous carbon source (0·5 per cent maltose, glucose, or lactose). The organisms were grown at 15°, 25°, 37° and 40° C for 7 days prior to the assay to determine the effects of growth temperature on enzyme production. We also grew the organisms at pH values of 6, 7, 8 and 9 for 3 days to determine the effects of pH on enzyme production. We also examined the stability of the enzyme by assaying cell suspensions which were stored at 4°–40° C for periods up to 7 days.

Conventional manometric methods[16] were utilized to measure oxygen uptake of cells using lactose as the substrate. Both induced and uninduced cells were examined. In some experiments the inducing agents were placed directly into the Warburg flasks to measure induction during the observation period. The concentration of the substrate was varied in other experiments. Attempts were made to isolate mutants by ultra-violet irradiation. Cells were assayed immediately after irradiation and also following growth of the irradiated cells in nutrient broth for 7 days. Three different irradiation doses were used.

In order to determine the role of the composition of the culture media on β-galactosidase production the organisms were grown in nutrient broth and in a defined medium[17]. The enzyme was induced during the growth of the organisms in these two media and in other experiments casamino-acids and polypeptides were added during enzyme induction to learn if additional nitrogen sources increased the enzyme formation.

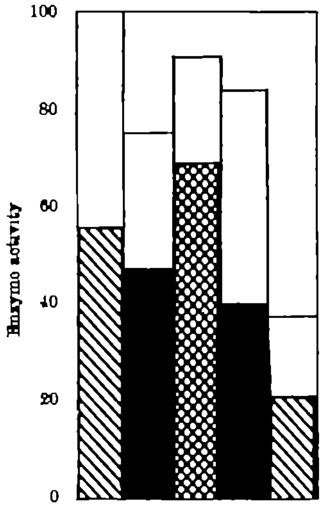

Fig. 1. Effect of toluene treatment on β-galactosidase activity of *S. sonnei* and *E. coli*. Lower portion of bars indicate activity of intact cells; upper (unfilled) portion of bars represent the enzyme activity of cells treated with toluene
The strains are listed by numbers from left to right: *B*–2506–3, *F*-6, *F*-141, *B*–2509–2, *E. coli*

S. sonnei exhibited β-galactosidase activity as reported previously by many workers. There was, however, considerable strain difference in the level of enzyme production. The other species of *Shigella* possessed no β-galactosidase. The enzyme-level in uninduced *E. coli* was approximately half that in *S. sonnei*. Toluene treatment for 10 min increased the enzyme activity; additional treatment resulted in diminished enzyme activity. Toluene treatment for 10 min did not lyse the cells; however, the cells appeared larger and elongated under microscopic examination. Fig. 1 shows the results of enzyme assays with intact and toluene-treated cells in four strains of *S. sonnei* and one of *E. coli*. The enzyme activity of cells prepared by standard methods is compared with cell-free extracts in Table 1. Cells disrupted by the tissue disintegrator possessed more enzyme than intact or toluene-treated cells. Freezing and thawing reduced the enzyme-level.

Table 1. VARIATION IN β-GALACTOSIDASE ACTIVITY OF DIFFERENT CELL PREPARATIONS

Cell preparation	Enzyme activity *E. coli*	 *S sonnei*, *F*–6
Intact cells	21	48
Toluene-treated cells (10 min)	38	77
Cell-free extract (disrupted in Mickle)	82	115
Cell extract prepared by freezing and thawing	0	25

The β-galactosidase activity of cells grown in the presence of several inducers for 3 days is recorded in Table 2. The inducing sugars were used at a concentration of 0·5 per cent and TMG was used at a concentration of 5 × 10^{-4} M. Neither of the monosaccharides found in lactose, namely, glucose and galactose, induced enzyme formation in *S. sonnei* and *E. coli*. Lactose was an effective inducer of β-galactosidase in *E. coli*, but had only a slight stimulatory effect on *S. sonnei*. Specifically, lactose increased the enzyme activity of *E. coli* 17 times the basal level and only 1·3 times the basal level in *S. sonnei*. Melibiose, which also consists of glucose and galactose (α-linkage), induced the β-galactosidase in *E. coli* but not in *S. sonnei*. Raffinose, a trisaccharide containing galactose in an α-linkage with sucrose, failed to induce the enzyme. Only TMG was an effective inducer of β-galactosidase in *S. sonnei*. Enzyme induction was not observed in the other species of *Shigella*.

Table 2 EFFECT OF INDUCERS ON THE β-GALACTOSIDASE ACTIVITY OF *E. coli* AND *S. sonnei*, STRAIN *B*–2506–3

Inducer	Enzyme activity *E. coli*	 *S. sonnei*
None	38	101
Glucose	0	7
Galactose	20	30
Lactose	638	131
Melibiose	568	100
Raffinose	145	100
TMG	682	482

Maximum enzyme induction occurred at 4 h in *E. coli* when 0·5 per cent lactose was used as the inducer; on the other hand, in *S. sonnei* maximum induction occurred after 18 h. When TMG was used as the inducer maximum enzyme induction occurred after 18 h in both *E. coli* and *S. sonnei* (Fig. 2). A comparison was made of the inducibility of the enzyme in distilled water and in nutrient broth; the induced enzyme-level was approximately 30 per cent higher in nutrient broth. Attempts to induce β-galactosidase in cell-free extracts of *S. sonnei* with TMG met with variable results but was generally unsuccessful. Washed cell suspensions of *S. sonnei* starved for 24 h were induced with TMG. Starved cells still retained their capacity for β-galactosidase production at a level comparable to unstarved cells. However, cells starved for 48 h produced less enzyme under TMG induction. Cells starved for 96 h exhibited no detectable enzyme.

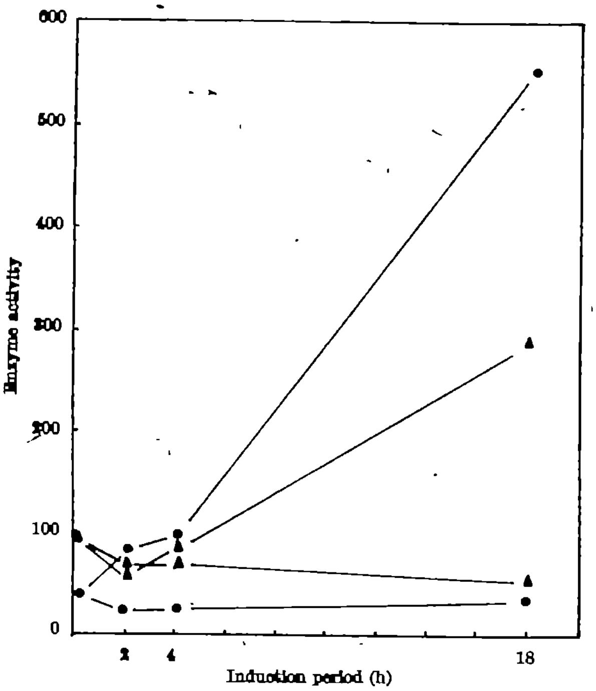

Fig. 2. Induction of β-galactosidase with TMG at 37° C for 0–18 h. The cells were treated with toluene
●, *E. coli*; ▲, *S. sonnei*. The two lower lines represent uninduced controls

Fig. 3 illustrates the optimum temperature for the induction of β-galactosidase. In Fig. 4 is shown the effect of the temperature of growth of *S. sonnei* on the production of β-galactosidase. Cells grown at various *p*H values were examined for their enzyme activity; between values of 6 and 9 there was little difference in the enzyme-levels of *S. sonnei*. The stability of the β-galactosidase of *E. coli* and *S. sonnei* was studied next. Washed cells were stored at 4° C and enzyme assays were performed from aliquots after 1, 3, 5 and 7 days of storage. There was no appreciable loss in the enzyme activity within the time that we studied the cells. As a matter of fact, there was only a slight decrease in enzyme activity even when the cells were stored for 7 days at 15°, 37° or 40° C. Induced cells of *S. sonnei* respired (took up oxygen) at a more rapid rate and to a greater extent than uninduced cells. When the inducing agent was added directly to the Warburg flasks during the observation period, TMG produced an increase in respiratory rate. The increase was slight at the beginning and gradually became accelerated after several hours. TMG was the only effective inducer among the several tested.

Washed cells were irradiated with ultra-violet light and assayed immediately for β-galactosidase activity. The results are illustrated in Fig. 5. Irradiation, in general, produced a slight decrease in enzyme activity which stabilized at a minimal level. It is possible that increased doses would have completely eliminated enzyme activity. Repeated attempts to obtain a mutant of *S. sonnei* by irradiation that produced more or less enzyme failed. When enzyme induction was attempted in the presence of casamino-acids, polypeptides and peptone preparations, there was a considerable increase in the enzyme-level. However, when the organisms were grown for several days in media containing these nitrogen sources the enzyme-levels remained unchanged.

In our investigations *S. sonnei* possessed β-galactosidase activity and the other *Shigellae* were devoid of the enzyme. Rickenberg[14], on the other hand, was able to detect the enzyme in several other *Shigella*. Lowe[15] was unable to observe β-galactosidase activity in *S. alkalescens*, which agrees with our findings. *S. sonnei* produced 2–3 times more basal (uninduced) enzyme than *E. coli*. Others have reported that *Paracolobactrum aerogenoides* possessed 10 times more basal enzyme than *E. coli*[13]. We have found that mechanically disrupted cells revealed more detectable enzyme than intact cells. It is possible that this procedure released the enzymes into solution thus increasing total enzyme activity. On the other hand, cells treated by alternate freezing and thawing were devoid of β-galactosidase activity. Possibly the procedure was sufficiently damaging to inactivate the enzyme. We cannot fully explain the fact that melibiose, which was reported as an excellent inducer in several organisms[2,7,20–22], failed to induce the enzyme in *S. sonnei*. It is possible that *S. sonnei* is only poorly induced, as was suggested by Anderson and Rickenberg[16] for the low level of induction in *P. aerogenoides*. From our work it appeared that the presence of nutrients favour enzyme induction. However, it has been reported that the addition of glucose, ribose, xylose and glycerol as exogenous energy sources to nitrogen-deficient media blocked enzyme formation[23]. We have found that additional nitrogen

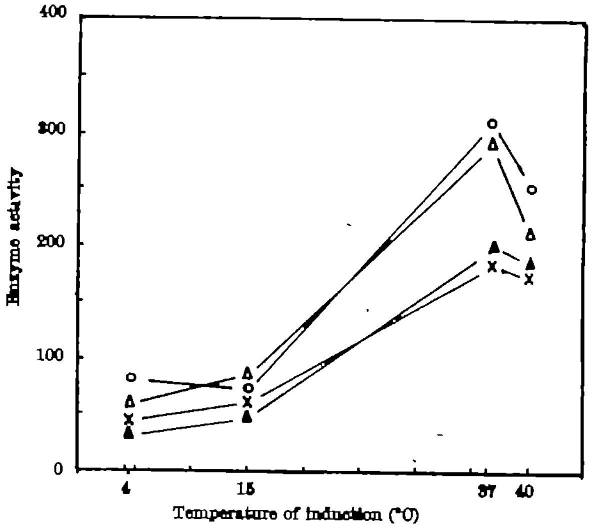

Fig. 3. Induction of β-galactosidase of *S. sonnei* at various temperatures, using TMG in distilled water as the inducer for 18 h
▲, Strain *F*-141; ×, strain *B*-2509-2; Δ, strain *B*-2506-3; ○, strain *F*-6. Cells were treated with toluene

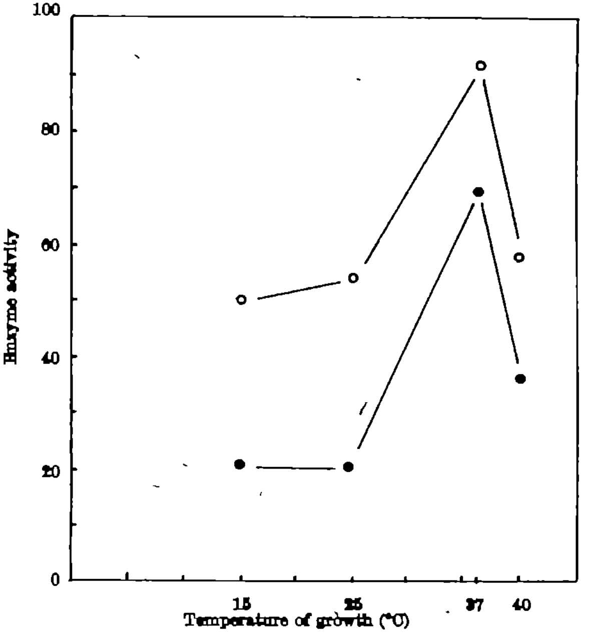

Fig. 4. β-Galactosidase activity of *S. sonnei*, strain *F*-141, grown at various temperatures
●, Intact cells; ○, cells treated with toluene

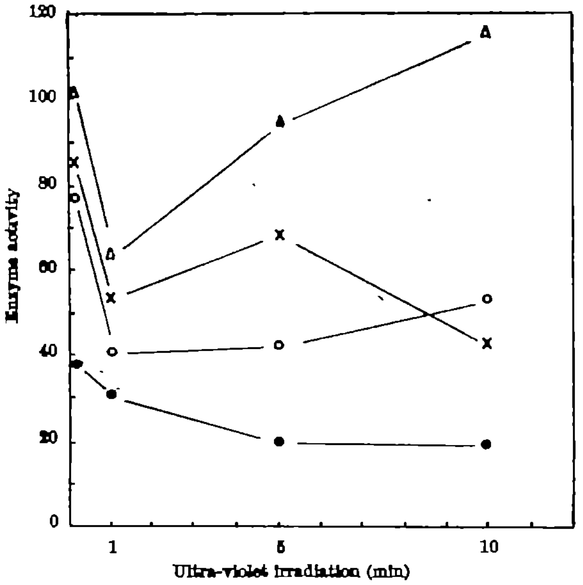

Fig. 5. Effect of ultra-violet light on β-galactosidase activity of *E. coli* and *S. sonnei*. The washed cell suspensions were irradiated, treated with toluene, and assayed for the enzyme
●, *E. coli*; ○, *S. sonnei*, strain *F*–6; ×, *S. sonnei*, strain *B*–2500–2; *Δ*, *S. sonnei*, strain *B*–2500–3

sources were necessary for good enzyme induction and therefore suggest that the endogenous nitrogenous supply is inadequate in *S. sonnei* for optimal enzyme induction. Washed cells irradiated with ultra-violet light possessed less enzyme than unirradiated cells. The irradiation procedure may have destroyed pre-existing enzyme and also inhibited the synthesis of new enzyme. Kameyama and Novelli[24] reported that β-galactosidase induction in *E. coli* was inhibited by ultra-violet irradiation.

In summary, the β-galactosidase of *S. sonnei* behaves quite differently from that of *E. coli*. We have compared the enzymes in these two organisms, studied various factors which affect their activity, and examined the stability of the enzyme.

This investigation was supported by a research grant (*E*–3734–*C*1) from the National Institutes of Health, U.S. Public Health Service. One of us (C. R. C.) was the recipient of a Summer Fellowship for Graduate Teaching Assistants.

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DIFFERENTIATION OF PHASE I AND PHASE II *COXIELLA BURNETII* BY EQUILIBRIUM DENSITY GRADIENT SEDIMENTATION

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FRESHLY isolated *Coxiella burnetii* cells from animals and ticks are characteristically in Phase I and when serially passed in embryonated chicken eggs are converted to Phase II after a variable number of passages[1–3]; conversion of cells from Phase I to Phase II is determined by the serological reactivity of Phase II cells with early convalescent sera from animals infected with *C. burnetii* cells of either phase[3]; Phase I cells do not react with early convalescent sera. Phase I cells can also be converted to Phase II serological reactivity by oxidation with periodate or by physical treatment such as ultrasonication[4].

Ormsbee[5] has noted qualitative physical differences in Phase I and Phase II cells during purification, and this article describes the difference between these two phases as determined by equilibrium density gradient sedimentation in cæsium chloride solution as described by Meselson *et al.*[6].

Yolk-sac suspensions of *C. burnetii* cells, strains Ohio and Nine Mile, were purified by various techniques[5,7], including differential sedimentation in M potassium chloride or M sodium chloride, treatment with ethyl ether, and the use of linear gradients of sucrose or glycerol, and various combinations of these procedures. In most instances the starting material was treated initially with 0·2 per cent formaldehyde. Some purified preparations were lyophilized prior to use in cæsium chloride gradients.

About 1 mg of Nine Mile strain cells, fifth egg passage (Phase I) and seventeenth egg passage (Phase II) were uniformly suspended in 4 ml. cæsium chloride solution, relative density 1·3, and centrifuged in a Spinco *SW*-39 rotor for 16 h at 35,000 r.p.m. (about 100,000*g*). The Phase I cells formed a granular band 22 mm from the meniscus and a homogeneous, well-dispersed band 20 mm from the meniscus. The relative density of the cæsium chloride solution in the zone between these two bands was about 1·33. The Phase II cells formed a dense pellicle at the meniscus (relative density 1·25) which had to be slightly stirred to obtain satisfactory photographs. A mixture of the two preparations gave bands characteristic of the Phase I and II preparations examined individually. The bands are shown in Fig. 1. In addition, Nine Mile cells in Phase I were treated with periodate, shown to have Phase II serological activity, and centrifuged as above in 1·3 relative density cæsium chloride solution. Bands at the 20- and 22-mm levels characteristic of the original Phase I suspension were observed and no detectable material was present at the meniscus.

Cells of Ohio strain, fifth egg passage (Phase I), and twenty-second egg passage (with Phase II serological

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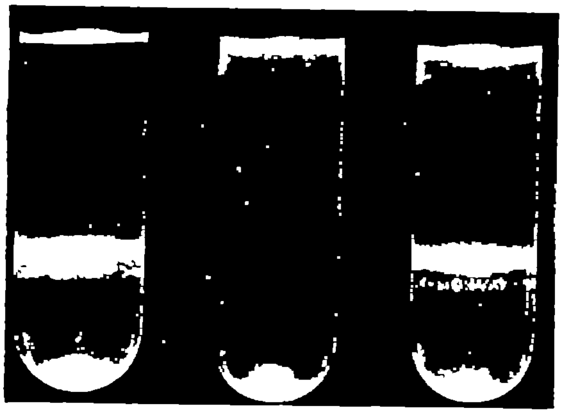

Fig. 1. Bands obtained by equilibrium density gradient sedimentation (16 h, 100,000g) of Phase I and II *Coxiella burnetii* cells in caesium chloride solution, initial relative density, 1·3. I, Nine Mile (Phase I), II, Nine Mile (Phase II), II–I, mixture of Phase I and Phase II cells

reactivity) were also centrifuged as previously described in a 1·3 relative-density caesium chloride solution. The Phase I Ohio cells formed bands at 20 and 22 mm as did the Phase I Nine Mile cells, and in addition some preparations formed a band with weak turbidity at 17·5 mm. There was no material at the meniscus. Ohio cells with Phase II reactivity, unlike the Phase II Nine Mile cells, formed principal bands at 20 and 22 mm from the meniscus and in addition a pellicle at the meniscus indicating that the cell population consisted of a mixture of Phase I and Phase II cells. Mixtures of fifth and twenty-second egg passage cells yielded principal bands at 20 and 22 mm and a pellicle at the meniscus. To determine the density of the material at the meniscus, twenty-second egg passage *C. burnetii* cells of Ohio strain were centrifuged as above in caesium chloride solutions with initial relative density of 1·2. Material collected at the meniscus of this gradient was amorphous and a band which appeared at 22 mm from the meniscus (1·21 relative density region) was composed of rickettsial cells. A pellet at the bottom of the tube was composed of the denser Phase I cells.

Thus, it appeared that natural and artificial mixtures of Phase I and Phase II rickettsiae could be separated by reason of their different densities. To confirm this observation, material collected from appropriate bands was examined by darkfield, phase and electron microscopy as well as complement fixation and slide agglutination tests.

Microscopy confirmed that the material in the 1·33 relative density region consisted of rickettsial cells which, when washed free of salts, were not observably contaminated when viewed by the electron microscope. Material from the meniscus of the same gradient consisted of rickettsial cells and amorphous material. However, the 1·21 relative density region of a different gradient formed from caesium chloride solution with 1·2 initial relative density contained only rickettsial cells as determined by phase microscopy and preparations stained with Machiavello stain.

Complement fixation tests were done with early convalescent guinea pig sera (Phase II antibody only) which reacted with material collected from the meniscus of the density gradient (initial relative density 1·3) and in some instances with material collected from the zone 20 and 22 mm below the meniscus. If cells were not treated with formaldehyde early in the purification process, the cells from the zone characteristic of Phase I often showed Phase II reactivity. This is most probably explained by an unmasking of Phase II reactive sites by treatment of the cells prior to equilibrium density gradient sedimentation; it was necessary to suspend thoroughly preparations prior to sedimentation, and this was often done by vigorous shaking with glass beads or by ultrasonication. Presumably these treatments disturbed the surface of unfixed cells sufficiently to uncover Phase II reactive sites; formaldehyde treatment 'fixed' the surface sufficiently to prevent this uncovering. These minor changes in cell surface were not reflected in observable density differences. Cells from both density regions reacted with hyperimmune (Phase I, II) guinea pig antisera.

Cells were also examined for serological reactivity in a slide agglutination test read by darkfield or phase microscopy. A few drops of early convalescent (Phase II) or hyperimmune (Phase I–II) guinea pig sera were mixed with suspended, washed cells from the appropriate density region sufficient to give several thousand cells per oil immersion field. A cover slip was sealed over the cell-antibody mixture with clear nail polish and the preparation observed with an oil immersion objective. Cells from the less-dense (Phase II) zones were characteristically slightly clumped, but this clumping increased dramatically as incubation with either antiserum proceeded. Cells from the denser (Phase I) zones were characteristically uniformly suspended and clumped dramatically as incubation in Phase I–II antiserum proceeded, but either did not clump at all (formaldehyde-treated cells) or formed scattered small clumps in Phase II antiserum.

With two strains of *C. burnetii*, morphological as well as serological evidence, therefore, supports the observation that Phase II rickettsiae (relative density about 1·21) can be separated from Phase I rickettsiae (relative density 1·33) by equilibrium density gradient sedimentation in caesium chloride. Cells with Phase I density characteristics, however, can exhibit some serological reactivity with Phase II antisera if treated by physical or chemical means; fixation of the surface of Phase I cells early in the purification procedure prevents this unmasking of Phase II reactive sites.

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IN VIVO STUDY OF CELL KINETICS IN ACUTE LEUKÆMIA

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THERE have been several reports of *in vitro* studies designed to assess the proliferative capacity of leukæmic cells, from patients with acute leukæmia, with tritiated thymidine as a cell label[1–4]. A preliminary report of an *in vivo* study in a patient with acute myeloblastic leukæmia has been made[5]. In this communication we present the results of an *in vivo* study with tritiated thymidine in a child with untreated acute lymphoblastic leukæmia.

The patient, a five-year-old white girl, was admitted to the hospital because of pallor. The initial laboratory values were: hæmoglobin, 7·3 g per cent; white blood

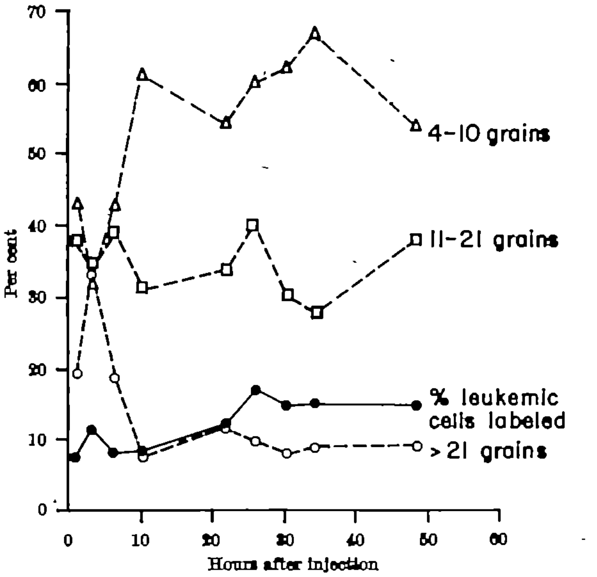

Fig. 1. Time-course of label in the leukaemic cells of the bone marrow. The closed figures represent the percentage of leukaemic cells having greater than four grains over the nucleus and the open figures represent the percentage of labelled leukaemic cells in the three groups of label concentration

cell count, 7,650/mm^3 with 31 per cent lymphoblasts; and platelet count, 86,000/mm^3. On the second hospital day at 11.45 a.m. she was given intravenously 200 μc. of tritiated thymidine (specific activity 360 mc./m.mole) per kg body-weight. One h after the injection and at intervals afterwards blood and bone marrow samples were obtained for the preparation of autoradiographs. A transfusion of 500 ml. of stored bank blood was given 50 h after the injection and treatment with prednisone and 6-mercaptopurine was begun 76 h after the start of the study. During the course of the study, the white blood count varied between 5,800 and 8,000/mm^3 with 25–35 per cent lymphoblasts.

The autoradiographs prepared from the blood and bone marrow smears were exposed for 90 days. After developing and staining the smears, 2,000–2,500 nucleated cells were observed from each sample and the number of labelled cells and the grain count above each cell were determined. All differential cell counts were compared with those obtained from examination of directly stained smears of the samples.

The time-course of the label in the leukaemic cells in the bone marrow is shown in Fig. 1. In the 1-h sample 7·5 per cent of the leukaemic cells contained label, with 20 per cent of the cells having greater than 21 grains over the nucleus, 38 per cent of the cells having 11–21 grains and 42 per cent of the cells having 4–10 grains. Any cell having less than 4 grains was not considered to be different from the background grain count. In the 3-h samples the percentage of heavily labelled cells had increased at the expense of the other two groups. At 6 h, the percentage of heavily labelled cells decreased as the other two groups increased. By 10 h, both the groups containing heavy and intermediate amounts of label had decreased as the lightly labelled cells increased further in percentage. A similar cycle of change in the percentage of labelled cells in each compartment was found starting with the samples obtained 22 h after the injection of the label.

One possible explanation for this time-course is that early division of cells incorporating thymidine more rapidly near the end of the deoxyribonucleic acid (DNA) synthesis phase of the mitotic cycle at the time of injection of the label resulted in the observed increase of heavily labelled cells in the 3-h sample. Afterwards, division of cells in earlier stages of DNA synthesis at the time of the injection and incorporating label at a slower rate, resulted in the increase of cells with less label. The repetition of these changes, beginning with the 22-h sample, suggests redivision of the labelled cells indicative of a generation time for these cells.

Changes in the percentage of leukaemic cells containing label occurred with the changes in concentration of label described here. An increase in labelled cells was found at 3 h, but a decrease at 6–10 h. In part this decrease might have resulted from the division of lightly labelled cells producing daughter cells containing insufficient label to be detected above the background grain count. Another increase in the percentage of labelled cells was found on the second day, with the maximal concentration occurring at 26 h.

Analysis of these results leads to the suggestion that a considerable part of the marrow leukaemic cell population either divided very slowly or not at all. At the time of labelling, about 7·5 per cent of the cells incorporated the thymidine or were in the DNA synthesis phase of the mitotic cycle. Thereafter, progressive changes in the concentration of label were observed during an 8–10-h period, which would likely reflect the time required by these cells for DNA synthesis. Repetition of those changes in label concentration and an increase in labelled cells occurred 22 h after the injection, which would indicate a generation time of about 20–22 h for the labelled cells. Thus, if the actively proliferating segments of the leukaemic cell population acted in a uniform manner, the 7·5 per cent of the leukaemic cells found to be labelled were in a phase of the mitotic cycle requiring about one-third of the generation time. Therefore, the size of the actively proliferating pool of cells represented only about 22·5 per cent of the total population of leukaemic cells in the marrow.

Further evidence that the labelled cells were dividing in a mixed population was the increase in the percentage of labelled cells which occurred during the second day. Only if the labelled cells increased by division in comparison with a static or slowly dividing group of leukaemic cells could this observation have been made.

A comparison of the labelling of leukaemic cells in the blood and bone marrow is shown in Fig. 2. Once again the difference in the initial labelling and subsequent time-course of the label in the two compartments is in favour of a concept of non-uniform leukaemic cell populations in acute leukaemia[7]. The appearance of labelled leukaemic cells in the blood is most reasonably explained by immigration of labelled cells from the bone marrow or other tissues rather than proliferation of labelled cells within the blood. The effect of the blood transfusion on the time course of the label after 50 h cannot be evaluated, although no change in total white blood count or percentage of lymphoblasts was found.

The appearance of label in normal blood cells is shown in Fig. 3. The rate of maturation of the myeloid cells

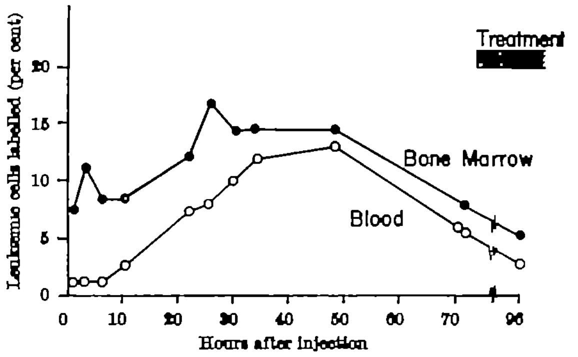

Fig. 2. A comparison of the percentage of leukaemic cells in the blood and bone marrow having greater than four grains over the nucleus. Treatment with prednisone and 6-mercaptopurine was begun 76 h after the injection of label

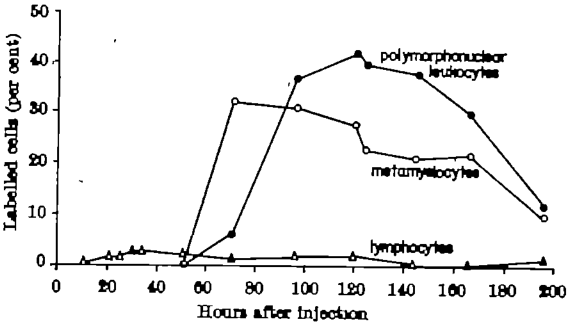

Fig. 3. The percentage of normal blood lymphocytes and granulocytes containing label

was similar to that described by Bond *et al.* in hæmatologically normal subjects[8]. The number of erythroid and myeloid cells in the marrow was too small for an adequate evaluation of the kinetics of proliferation in these cells.

Considering the variability of the clinical and laboratory findings in patients with acute lymphoblastic leukæmia, the findings presented here cannot be assumed to be characteristic of the kinetic pattern of cell proliferation in all patients with this disease. From this investigation, however, there is further evidence that the leukæmic cell population cannot be considered to be homogeneous for the purpose of the calculation of various kinetic measurements. It is of interest that Mendelsohn[9,10] has recently reported *in vivo* studies of the proliferative capacity of mouse autochthonous breast tumour cells with tritiated thymidine and similarly found a non-uniform population of cells in these tumours.

This investigation was supported by a Public Health Service research grant, *CY*–4826, Cancer Institute, U.S. Public Health Service.

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HAPTOGLOBIN TYPES

Haptoglobin Sub-types in Three Racial Groups

SMITHIES, Connell and Dixon[1-3] have shown that purified haptoglobin, after reductive cleavage by urea and mercaptoethanol, is electrophoretically separable into two polypeptide chains, α and β. The β-chain is apparently unaffected by the genes at the Hp locus, but the α-chains, differentiated by migration rate and staining intensity, represent the expression of a series of alternative Hp genes. Four distinctly different α-polypeptides, called hp 1F, hp 1S, hp 2 and hp 2J have been demonstrated. A fifth polypeptide, hp 2M, found in individuals having the phenotype Hp 2–1(mod), is characteristically reduced in quantity, but its mobility is very similar, if not identical, with that of hp 2.

Investigations of inheritance studies[1] indicate that these five polypeptides represent the alleles Hp^{1F}, Hp^{1S}, Hp^2, Hp^{2J} and Hp^{2M}, although further subdivisions, particularly of hp 2, are likely to exist[4]. Of the 15 possible genotypes, the phenotypic expressions of nine have been differentiated (Fig. 1). Three of the remaining six, Hp^{2J}/Hp^{2J}, Hp^{2J}/Hp^{2M} and Hp^{2J}/Hp^{1F}, are very rare, and no appropriate subjects have yet been studied. The genotypes Hp^{2M}/Hp^{2M} and Hp^{2J}/Hp^2 appear to be associated with greatly reduced haptoglobin synthesis[5,6]; while the two polypeptides associated with Hp^2 and Hp^{2M} are so similar that the Hp^2/Hp^{2M} genotype is at present indistinguishable from Hp^2/Hp^2 (ref. 5).

Smithies *et al.*[1] sub-typed 93 random blood donors in the United States and Canada, estimating the frequencies of Hp^{1F} and Hp^{1S} to be 0·16 and 0·24 respectively, assuming a value of 0·60 for Hp^2. The work recorded here was undertaken in order to obtain further information on the racial distribution of the various Hp genes, particularly Hp^{1F} and Hp^{1S}.

Random donors at the King County Central Blood Bank were classified into three groups: Caucasoid, Negro and Oriental on the basis of general appearance. The surnames of the Oriental group were considered as a rough index of geographic origin; nearly all were Japanese. Starch-gel electrophoresis was carried out in the usual manner[7] to determine the haptoglobin phenotype, and from these data the frequency of the Hp^1, Hp^2 and Hp^{2M} genes was calculated.

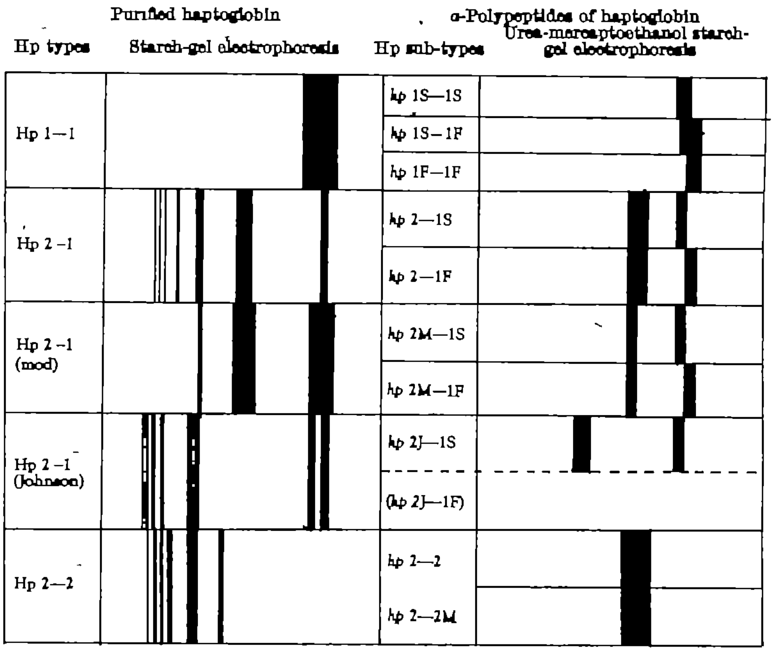

Fig. 1. Diagrammatic representation of the starch-gel electrophoretic patterns of the haptoglobin phenotypes and their respective sub-types. Those examples of Hp 2–1 (Johnson) which have so far been sub-typed have had the α-polypeptides hp 2J and 1S. The starch-gel pattern associated with hp 2J and hp 1F may vary from Hp 2–1(Johnson)

Table 1. HAPTOGLOBIN TYPES AND SUB-TYPES IN BLOOD DONORS OF THREE RACIAL GROUPS

The gene frequencies of the Negro subjects were calculated by subtracting those with Hp 0 and obtaining the approximate incidence of Hp^{2M} from the ratio of Hp 2–1 to Hp 2–1(mod). Hp^{1F} and Hp^{1S} frequencies were obtained by applying the sub-type proportions to the respective Hp^1 frequencies. All results fit Hardy-Weinberg equilibrium with a probability of $>0·05$ for the Negroes and $>0·2$ for the Caucasoids and Orientals

Ethnic group	No. tested		Haptoglobin types					Gene frequencies		
			1–1	2–2	2–1	2–1(mod)	0	Hp^1	Hp^2	Hp^{2M}
Caucasoid	409	No.	54	149	206	—	—	0·384	0·616	—
		%	13·2	36·4	50·3	—	—			
Negro	1,657	No.	472	307	641	181	56	0·552	0·349	0·099
		%	28·5	18·5	38·7	10·9	3·4			
Oriental	494	No.	34	270	190	—	—	0·261	0·739	—
		%	6·9	54·7	38·4	—	—			

		Haptoglobin sub-types of selected donors (see text)							Gene frequencies	
		1S–1S	1F–1F	1S–1F	2–1S	2–1F	2M–1S	2M–1F	Hp^{1F}	Hp^{1S}
Caucasoid	66	4	0	5	36	21	0	0	0·133	0·251
Negro	222	26	20	49	61	55	6	5	0·259	0·293
Oriental	80	14	0	1	64	1	0	0	0·005	0·256

Serum of Hp types 1–1, 2–1 and 2–1M, containing adequate amounts of haptoglobin and no visible hæmoglobin, were selected for sub-typing, since the haptoglobin–hæmoglobin complex is not suitable for haptoglobin polypeptide identification. Partial purification by DEAE cellulose chromatography, reductive cleavage, and electrophoresis in starch gels containing urea, mercaptoethanol and formate buffer were performed as described by Smithies *et al.*[1]. In most instances, a duplicate specimen was afterwards tested, and the results recorded prior to comparison with the previous test. The two readings agreed in all but a few instances, when the haptoglobin-level was not sufficiently high to produce an adequate yield of polypeptide.

Table 1 indicates the haptoglobin types of 409 Caucasoid, 1,657 Negro and 494 Oriental donors, as determined by conventional starch gel electrophoresis, as well as the calculated Hp^1, Hp^2 and Hp^{2M} gene frequencies. The lower portion of the table shows the results of sub-typing 66 Caucasoids, 222 Negroes and 80 Orientals, and the frequencies of Hp^{1F} and Hp^{1S} in those populations. The Caucasoid figures are in good agreement with those of Smithies *et al.*[1] and the calculated probability figures for all three groups are consistent with the hypothesis that Hp^{1F} and Hp^{1S}, like Hp^2 and Hp^{2M}, are alleles at the Hp locus.

Although only 11 specimens of type 2–1(mod) were sub-typed, the nearly equal distribution of Hp^{1F} and Hp^{1S} confirms the fact that the peculiarity in the pattern representing this phenotype is not dependent on a variant of the Hp^1 gene[5].

Among American Negroes, the incidence of Hp^1 is higher than that of Caucasoids and much higher than in Orientals. Similarly, the percentage of Hp^1 genes which are Hp^{1F} is highest in the Negroes, intermediate in the Caucasians and extremely low in the Orientals. The latter group, whose Hp^1 genes are nearly all Hp^{1S}, are of particular interest in view of the possibility that they might provide some information about the frequency of intragenic cross-over. Smithies *et al.*[4] suggest that the Hp^2 gene may have originated from the very rare phenomenon of non-homologous cross-over occurring in a Hp^{1S}/Hp^{1F} heterozygote, and that subsequent cross-overs involving the Hp^2 gene and either Hp^{1S} or Hp^{1F} may have produced two or even three Hp^2 variants. Most, but not all, of the hp 2 α-polypeptides which they have analysed contained those peptides characteristic of both hp 1F and hp 1S. It is conceivable that in a population such as the Japanese, the very low frequency of Hp^{1F} might be associated with an increased incidence of hp 2 α-chains lacking the peptides characteristic of hp 1F, as the result of unequal cross-over in Hp^2/Hp^{1S} individuals. Lack of information concerning cross-over frequencies and selective factors makes such a correlation entirely speculative at present. However, it is possible that further data on the fine structure and distribution of the hp α-polypeptides among various populations may provide useful clues about the evolution of their respective genes under different environmental circumstances.

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Haptoglobin Types of the Nez Perce Indians

THE genetic determination of the three common haptoglobin types, Hp 1–1, Hp 2–1, Hp 2–2, was first discussed by Smithies and Walker[1,2]. Subsequent population surveys have shown marked variations in the frequencies of the Hp^1 and Hp^2 alleles in different races. In general, the focus for the lowest Hp^1 frequency appears in India, where Kirk *et al.*[3] reported a Hp^1 frequency of 0·09. An easterly increase is also noted in comparing the Japanese Hp^1 frequency of 0·24 (ref. 4) with the frequency of 0·33 in the Alaskan Eskimoes[5]. The highest Hp^1 frequency so far encountered, 0·93, is exhibited by the Lacondons of southern Mexico[6].

Recently, Connell *et al.*[7] found that after purification and reductive cleavage of haptoglobin in the presence of 8 M urea two types of polypeptides could be distinguished corresponding to the gene Hp^1. They therefore suggested that the gene Hp^1 is not a unique entity but can have one of two closely similar forms, Hp^{1F} or Hp^{1S}. The Hp 2–1 heterozygote can consequently have two forms distinguishable after reductive cleavage, Hp 2–1F or Hp 2–1S, and Hp 1–1 can be either Hp 1F–1F, Hp 1F–1S, or Hp 1S–1S. Subsequent sub-typing of families[8] confirmed their hypothesis that there are three common alleles at the Hp locus, Hp^2, Hp^{1F} and Hp^{1S}.

Table 1. ESTIMATION OF *Hp* GENE FREQUENCY ACCORDING TO THE WEIGHTING METHOD OF COTTERMAN[10]

No. of parents tested	No. of children tested	1–1 a	2–1 b	2–2 c	$b+2a$ x	$2(a+b+c)$ y	Weight w	wx	wy
unrelated		21	34	18	76	146	1·00000	76·0000	146·0000
1	1	3	8	7	14	36	0·66667	9·3334	24·0012
1	2	2	7	0	11	18	0·50000	5·5000	9·0000
1	3	1	7	0	9	16	0·40000	3·6000	6·4000
1	4	0	0	5	0	10	0·33333	0·0000	3·3333
0	2	5	23	12	33	80	0·66667	22·0001	53·3336
0	3	1	3	2	5	12	0·50000	2·5000	6·0000
0	5	2	0	3	4	10	0·33333	1·3333	3·3333
$r^*=1/8$		1	0	3	2	8	0·88888	1·7778	7·1112
$r=1/4$		4	5	3	13	24	0·80000	10·4000	19·2000
Σ						360		132·4446	277·7116

* r refers to coefficient of relationship:

$q=\frac{\Sigma wx}{\Sigma wy}=0·477=Hp^1$ Variance $=V(q)=\frac{q\times(1-q)}{wy}=0·0008983$

This communication concerns the *Hp* gene frequencies found among the Nez Perce Indians residing in Idaho and Wyoming. The specimens were collected in acid citrate dextrose and flown to Madison, where the blood typing, haptoglobin and transferrin determinations were done. (Serum samples were provided in the course of blood typing by Dr. R. Littlewood of the Department of Sociology and Anthropology, University of Washington, Pullman, Washington.) Determination of the Hp 1–1, Hp 2–1 and Hp 2–2 types was carried out according to the vertical electrophoretic method of Smithies[8], and the sub-types determined according to the method of Smithies *et al.*[8]. The results obtained are summarized in Table 1. No Hp 0 or Hp 2–1M phenotypes were encountered. One exception to the hypothesis of three common alleles, Hp^2, Hp^{1F}, Hp^{1S}, was encountered in this sample. An offspring of type Hp 2–2 was noted among four offspring of a type Hp 1S–1S male. No sample was obtained from the mother. The other three siblings were Hp 2–1S. Probably this exception is due to illegitimacy, but this could not be confirmed by the blood groups of the father and child. Since some of the specimens were obtained from related individuals, the gene frequencies were calculated by the weighting method of Cotterman[10]. The Hp^1 frequency obtained is 0·477. The population studied shows evidence of Caucasian admixture since it contains the Rh factor, *cde*; the extent of this admixture was estimated by using the per cent admixture $=\frac{q_h-q}{Q-q}$, where q_h is the gene frequency in the hybrid population, q is the expected gene frequency in the absence of admixture, and Q is the Caucasian gene frequency. For the Rh factor, *cde*, in the Nez Perce population, q_h was calculated as 0·129. The Caucasian frequency (Q) of *cde* used was 0·40, and the assumed aboriginal Nez Perce frequency (q) used was 0·00 (ref. 11). The percentage of Caucasian admixture calculated was 32·3. Using this calculated admixture, the aboriginal Hp^1 frequency in this population is estimated to be 0·50, using the Caucasian Hp^1 frequency of 0·43 (ref. 12). This frequency agrees with those reported for other North American Indian tribes: Anaktuvuk, 0·52 (ref. 13); North Athabascan, 0·42 (ref. 13); Tlingit, 0·44 (ref. 13); Apaches, 0·59 (ref. 12); Navajo Indians, 0·45 (ref. 14).

Recently, Smithies *et al.*[8] reported a gene frequency for Hp^{1F} and Hp^{1S} of 0·16 and 0·24 in 26 Caucasians, and Giblett and Brooks reported a corresponding frequency of 0·133 and 0·251 in 66 Caucasians and 0·259 and 0·293 in 222 Negroes (preceding communication). In the Nez Perce sample, only one Hp^{1F} gene was encountered in a Hp 1–1 individual; all other Hp 1–1 and Hp 2–1 samples were Hp 1S–1S and Hp 2–1S. This is of considerable interest, since Giblett reported a Hp^{1F} frequency of 0·005 in 80 Hp 2–1 and Hp 1–1 Orientals tested in her laboratory. The Oriental descent was determined by general appearance; by the surnames she concluded that most of them were Japanese. Possibly the Hp^{1F} gene is absent in the Mongoloid race.

By using the Caucasian Hp^{1F} frequency of 0·133 obtained by Giblett, the expected Nez Perce hybrid Hp^{1F} frequency is 0·043 on the bases of 32·3 per cent admixture. Due to hæmolysis in some cases and inadequate amounts in others, not all Hp 2–1 and Hp 1–1 samples were sub-typed. A separate *wx* value was therefore calculated based only on the Hp 2–1 and Hp 1–1 samples sub-typed; this value is 89·7, which represents the effective number of independent Hp^1 genes sub-typed. Of these 89·7 independent Hp^1 genes, one or 1·115 per cent were observed to be Hp^{1F}. Similarly, of the 75 or 38·4 per cent Hp^1 genes reported by Giblett for Caucasians, 26 or 34·6 per cent of them are Hp^{1F}. Assuming an aboriginal Hp^{1F} frequency of 0·00, the percentage Caucasian admixture based on 1·115 per cent Nez Perce Hp^{1F} genes and 34·6 per cent Caucasian Hp^{1F} genes, is 3·2 per cent. A test of significance of the difference between these two values (that is, 32·3 and 3·2 per cent) gives a t value of 2·82, corresponding to a probability of $< 0·01$ The discrepancy could be accounted for by postulating an early nineteenth-century introduction of one or several *cde* genes that became established by genetic drift in the small Nez Perce population. This would cause the calculated percentage admixture to be spuriously high when calculated from the *cde* frequency. The other possible explanation for the discrepancy would be a selective force acting against the Hp^{1F} gene. As of yet, no selective force has been found which influences the haptoglobin polymorphism.

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LETTERS TO THE EDITOR

SPACE SCIENCE

Measurement of Ionospheric Electron Content Regardless of Approximations

THE forthcoming advent of the Polar Beacon Ionosphere Satellite[1] has again focused scientific interest on the use of simultaneous Faraday rate and Doppler measurements for obtaining further new data about the integrated electron density along paths in the ionosphere. It might be useful, therefore, to describe a method by means of which the electron content can be obtained without approximation assumptions other than the following: isotropic and static medium, validity of reciprocity theorem, and equal propagation paths (for the various frequencies used simultaneously).

The well-known equations for the Faraday fading rate $\mathrm{d}a/\mathrm{d}t$, and for the Doppler shift Δf in a static ionosphere are:

$$\frac{\mathrm{d}a}{\mathrm{d}t} = \frac{K}{f^2}\,\overline{B\cos\varphi}\,\frac{\mathrm{d}}{\mathrm{d}t}\int_0^{S(p)} N_t\,\mathrm{d}s \tag{1}$$

$$\Delta f = \frac{f}{c}\,\frac{\mathrm{d}S}{\mathrm{d}t} - \frac{k}{cf}\,\frac{\mathrm{d}}{\mathrm{d}t}\int_0^{S(p)} N_t\,\mathrm{d}s \tag{2}$$

where the constants $K = 2\cdot36 \times 10^4$ and $k = 4\cdot03 \times 10^7$ are in C.G.S. units; f is the transmitted frequency; the line integral from the observer O to the satellite's position p has the geometrical length S; the integral is extended over the local electron density N_t along the radio path; c is the velocity of light in free space; and $\overline{B\cos\varphi}$ is the component of the geomagnetic field in the direction of the ray path (it is assumed that this field component remains constant during each single observation of one or a few seconds' duration, and can be replaced by an effective value of $\overline{B\cos\varphi}$). We solve equation (2) for the differential of the integral and write for equation (1):

$$\frac{\mathrm{d}a}{\mathrm{d}t} = \frac{K}{k}\,\overline{B\cos\varphi}\left[\frac{\mathrm{d}S}{\mathrm{d}t} - \frac{c\Delta f}{f}\right] \tag{1a}$$

The fading rate is, other things being equal, proportional to the difference between the change of the geometric path length and the change of the optical path length. At the moment of Doppler inflexion the sign of the rate $\mathrm{d}a/\mathrm{d}t$ changes, but not the sense of rotation. This effect is seldom observed because the temporal changes of $\overline{B\cos\varphi}$ are dominant for most passages. However, such passages for which $\overline{B\cos\varphi}$ is nearly constant (low orbital inclination, small changes in altitude, close to overhead passage) reveal the influence of the foregoing difference (see Fig. 1).

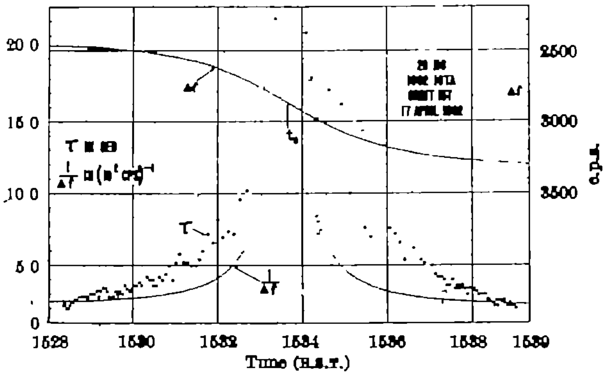

Fig. 1. Simultaneous measurement of Doppler shift and Faraday fading rate. Note the parallel trend of the centre part of the curves for $1/\Delta f$ and for τ; τ is the time-interval between two successive fade-outs; the incoming and outgoing 2-min section of the τ data is not true Faraday fading but is disturbed by simultaneous antenna fading from ground reflexions at very low elevations

With two simultaneous Doppler shift observations Δf_1 and Δf_2, we obtain from equation (2) the following expression for $\mathrm{d}S/\mathrm{d}t$:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = c\,\frac{\Delta f_2 f_2 - \Delta f_1 f_1}{f_2^2 - f_1^2} \tag{3}$$

At the same moment, we measure Δf_1, Δf_2 and at the frequency f_1 the fading rate $\mathrm{d}a_1/\mathrm{d}t$. These values, together with the result from equation (3), are substituted in equation (1a), which is solved for the magnetic field component $\overline{B\cos\varphi}$ as follows:

$$\overline{B\cos\varphi} = \frac{\mathrm{d}a_1}{\mathrm{d}t}\,\frac{k}{cK}\,\frac{f_2 - f_1\dfrac{f_1}{f_2}}{\Delta f_2 - \Delta f_1\dfrac{f_2}{f_1}} \tag{4}$$

The numerator of the fraction in equation (4) is close to f_2 for $f_2 > f_1$; the denominator is the so-called 'differential Doppler'; for absolute values of Δf_1 and Δf_2 it is always positive, it changes sign together with $\mathrm{d}a_1/\mathrm{d}t$. (At the Doppler inflexion point equation (4) is no longer applicable.)

A precise measurement of the differential Doppler has to be performed with great care. First, the equality of the radio paths has to be checked, for example, by a comparison of the time moments of the occurrence of Doppler inflexion at the two frequencies. Experience shows that this condition does not apply for a good number of passages (mainly because of frequency scintillations at the lower frequency). Secondly, the application of equation (4) has to be restricted to observations at small zenith angles (this condition is synonymous to the path equality). Thirdly, the values of the Δf have to be measured against the observed value of f at inflexion moment.

Having obtained the value for $\overline{B\cos\varphi}$ in equation (4) we recall the origin of equation (1), which is :

$$a = \frac{K}{f^2}\,\overline{B\cos\varphi}\int_0^{S} N_t\,\mathrm{d}s \tag{5}$$

and which with the help of equation (4) is converted to:

$$\int_0^{S(p)} N_t\,\mathrm{d}s = \frac{c}{k}\;a_1\left(\frac{\mathrm{d}a_1}{\mathrm{d}t}\right)^{-1} f_1^2\;\frac{\Delta f_2 - \Delta f_1\left(\dfrac{f_2}{f_1}\right)}{f_2 - f_1\;\left(\dfrac{f_1}{f_2}\right)} \tag{6}$$

The total phase shift a_1 of the frequency f_1 at the time moment of observation of $\mathrm{d}a_1/\mathrm{d}t$, Δf_1, and Δf_2 is gained[2] by counting the total number of phase rotations (one-half of the number of fading periods) from the moment when the Faraday effect disappears, that is, when φ becomes close to $\pi/2$ (larger than 80°). With amplitude recording systems it is often difficult to observe this moment exactly. Further, it is important to consider the change of sign of $\mathrm{d}a/\mathrm{d}t$ when counting a. Reliable results are obtained when the resultant phase is continuously measured. G. Vogt of our laboratory has designed an analogue phase-follower system which measures the phase shift during the entire passage of the satellite. In case the foregoing method cannot be applied (for example, when as in the case of Fig. 1 the angle φ never comes close to $\pi/2$). other methods for the final calculation have to be considered. Then the above values of $\overline{B\cos\varphi}$ can be used in the differential rotation method or in other methods described in the literature[3]. However, with the polar orbit of the planned S-66 Satellite such unfavourable geometry will not apply.

The method described is free from additional assumptions about path length, path curvature, altitude, ionospheric stratification, geomagnetic field. The method needs experimental care, but gives results without ambiguity.

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RADIOPHYSICS

38-Mc/s Radiation from Jupiter

Soon after the discovery by Burke and Franklin[1] in 1954 of 22-Mc/s sporadic-type radiation from Jupiter, an attempt was made by F. G. Smith[2] to detect similar radiation at higher frequencies from old records taken with the Cambridge 38- and 81-Mc/s interferometers. Suitable records were available for 16 days in 1952 and 1953 at 38 Mc/s and for 10 days in 1955 at 81 Mc/s. In spite of the fact that both aerial systems were considerably more sensitive than anything normally utilized in the study of Jupiter at frequencies close to 20 Mc/s no emission was detected. For the next few years it was tacitly assumed by most workers, with the notable exception of Douglas[3], that some frequency below 38 Mc/s represented an upper limit to the Jupiter radiation.

As other investigations progressed, however, it became clear that, not only did the radiation become much less intense at higher frequencies (see, for example, accounts of 27-Mc/s observations by Franklin and Burke[4], Gardner and Shain[5], and Carr *et al.*[6]), it also became much less prolific. I have pointed out[7] that the emission mechanism might be such that at higher frequencies, on rare occasions, an event might occur which would contain bursts of a sufficient strength to be detectable on the Earth. It is possible that if F. G. Smith had had records of 38 Mc/s observations available for a longer period he might have found a Jupiter event recorded.

Warwick[8] has recently reported that a few events extending to frequencies in the 38 Mc/s region have been recorded by the High Altitude Observatory dynamic spectrograph. Radiation at 38 Mc/s has also been detected at Florida State University during 1962 using a phase-switched interferometer consisting of two square corner reflectors, each containing a single half-wave dipole and separated by six wave-lengths. A more sensitive system was not desirable for the purpose of the experiment, which was to demonstrate low day-by-day frequency of occurrence as the dominant feature rather than low radiated power. The records examined by F. G. Smith should already have revealed some emission were it simply a matter of using higher sensitivity.

Observations were made nightly during April 25–August 25, 1962. All observations were monitored by an observer. During this period two events from Jupiter were recorded. The first, recorded on June 18, lasted for about 1 min only. The second, recorded on August 18, lasted for about 30 min and contained a number of weak bursts of estimated intensity about 5×10^{-22} W m^{-2} $(c/s)^{-1}$. On both occasions strong bursts of noise were observed simultaneously on lower frequencies between 16 and 22 Mc/s and receiving conditions were good. The second event was recorded while the most active region of the planet was facing the Earth. This is shown in Fig. 1. A histogram of 18 Mc/s observations for the corresponding period is shown for comparison. The histograms have been made on the basis of a rotation period of 9 h 55 m 29·37 s, the value which has been provisionally adopted by the International Astronomical Union for Compilation of a System III Ephemeris[9]. The first event was of such short duration that it cannot be represented on the histogram, which is computed for 5° intervals of central meridian longitude. This event occurred when the System III central meridian longitude was 325°. The experiment is to be repeated at a slightly higher frequency next year.

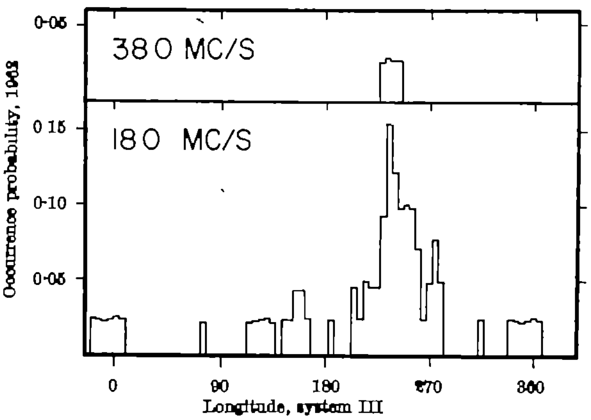

Fig. 1. Histograms comparing occurrence probability of Jupiter radiation at 38 and 18 Mc/s for 1962 as a function of System III central meridian longitude

It is worth noting that there are indications[10,11] that the increase in day-by-day frequency of occurrence continues to the lowest frequencies of observation, close to 5 Mc/s rather than reaching a maximum around 18 Mc/s as was originally suspected. Some disagreement exists, however, concerning average intensities at the lowest frequencies. Those reported by Ellis[10] are rather less than typical values for 18 Mc/s, whereas those reported by Carr *et al.*[11] are higher.

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Abrupt Phase Shifts on *MSF** (60 kc/s)

Watt-Carter and Corke[1] have reported phase variations on the *GBR* (16 kc/s) transmissions from Rugby as a result of aerial detuning by changes in weather conditions and aerial sway. We report here man-made phase jumps on the *MSF* (60 kc/s) transmissions.

Daily phase measurements of *MSF* (60 kc/s) against local frequency sources are made at the Signals Research and Development Establishment and our chart records show occasions when abrupt phase changes of about 12° occurred. Between April 25 and July 11, 1962, there were 20 days on which one abrupt phase change occurred. It was always in the same sense and was completed in less than a few seconds. The changes were always found within −3 to +10 min of the nominal start of the *MSF* transmission at 1430 G.M.T.

Investigation revealed that the phase jumps resulted from earthing the *GBR* aerial system during its maintenance period—which coincided with the start of the *MSF* transmission. The resultant detuning of the nearby *MSF* aerial system was responsible for the abrupt phase shifts observed.

It remains necessary to earth the *GBR* aerial system from time to time, but so far as possible this will be done only when *MSF* (60 kc/s) is not transmitting. The corrections for *MSF* (60 kc/s), published by the National Physical Laboratory, are based on the average rate of phase accumulation with time over the daily one hour transmission period. They are intended to be representative of the average frequency over this period. For this reason, on the days when the foregoing phase jumps occurred, the jump of 12° was not included as part of the total accumulated phase. If the jump had been included the *MSF* correction would have been in error by 1·5 parts in 10^{11}*.

These results emphasize the care necessary in operating and using the standard frequency transmissions when high-precision measurements are required in a limited time-interval.

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* *MSF* is the call sign of the standard frequency transmissions from Rugby Radio, England.

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GEOPHYSICS

Motion of Current Systems in the Ionosphere

THEORETICAL studies of the behaviour of electric current systems in the lower ionosphere, treated as a rigid anisotropic shell, indicate that the Hall-current conductivity produces a rotation of these systems from west to east about the geomagnetic axis. When a given current system is expressed in terms of spherical harmonics, it is found that the components of the system corresponding to harmonics of different order n rotate with different speeds, because the angular velocity contains a factor $(2n + 1)/(n^2 + n)$ (refs. 1 and 2). It follows that the pattern of the current system is distorted as it rotates, but the rate of distortion may be fairly slow if only a few close values of n are involved. Using the conductivity tensor adopted by Ferris and Price, the corresponding velocities of the current pattern at latitude λ range from $8{\cdot}3 \times 10^4 \sin \lambda$ cm/sec for $n = 1$ to $1{\cdot}06 \times 10^4 \sin \lambda$ cm/sec for $n = 10$.

In the course of an investigation of the spatial distribution of rapid geomagnetic fluctuations during a typical magnetic storm (November 9–13, 1956) evidence has been found of a general motion eastwards of the pattern of disturbance, and, since the velocity found is of the same order as that given here, it is tempting to interpret this motion as due to the afore-mentioned effect of the Hall current. If this interpretation is correct further studies of geomagnetic fluctuations may assist in evaluating the conducting properties of the ionosphere more precisely.

Various comparative investigations of magnetograms from Thule, Godhavn, Lerwick, Lovo, Rude Skov, Wingst, Abinger, Chambon-la-Foret and Tamanrasset were made. In particular, simultaneous polar diagrams for these stations showing the projections of the disturbance vectors in the *HD* plane, and also when possible in the *DZ* or *HZ* plane, were constructed from measurements made at 20-sec intervals for the period 10*d* 1125 to 1135 G.M.T. These diagrams showed many similarities at neighbouring stations. Taking in turn the *HD* diagram for each station, time-intervals were selected corresponding to distinct magnetic field changes, the beginning and end of each interval being estimated to the nearest 10 sec. Many of these bounding times were found to correspond at different stations, particularly the European ones, though the times were not exactly the same at all stations. The stations lie roughly on two lines, broadly north–south and east–west, and an attempt was made to detect phase differences in each direction. In Fig. 1 the times are plotted against geomagnetic latitude, and in Fig. 2 against geomagnetic longitude. The lines running from top to bottom in the figures are the best fitting straight lines, and their slopes to the vertical are a measure of the apparent phase gradients. The averages of the two sets of phase gradients were tested for significance. With respect to longitude (Fig. 2), the *t*-test shows that there is less than 5 per cent probability of the calculated average having arisen from pure chance. This average is approximately 10^{-7} sec/cm, events being later towards the east. Likely estimates of timing errors or instrumental lags are rather too small to account for this phase gradient. The average with respect to latitude (Fig. 1) is, however, not significantly different from zero.

The longitudinal phase gradient implies some motion of the disturbance field pattern from west to east. Assuming the form and amplitude of this pattern does not change too rapidly, the mean velocity is approximately the reciprocal of the phase gradient, that is, 10^7 cm/sec.

A different method of studying the field also indicated a general motion eastwards of the field pattern. Vector diagrams of the horizontal field deviations at specified epochs were plotted on charts showing the geographic positions of the stations. It was found that the vectors for the European stations tended either to converge to or to diverge from a small area, and this area progressed towards the east with a speed of order 10^4 cm/sec, suggesting the existence of a pulsating current circulation travelling eastwards.

It will be noted that there is no evidence of any north–south motion, while the velocity derived for the west–east motion is approximately that suggested by the above theory. The detailed analysis of the observations is being

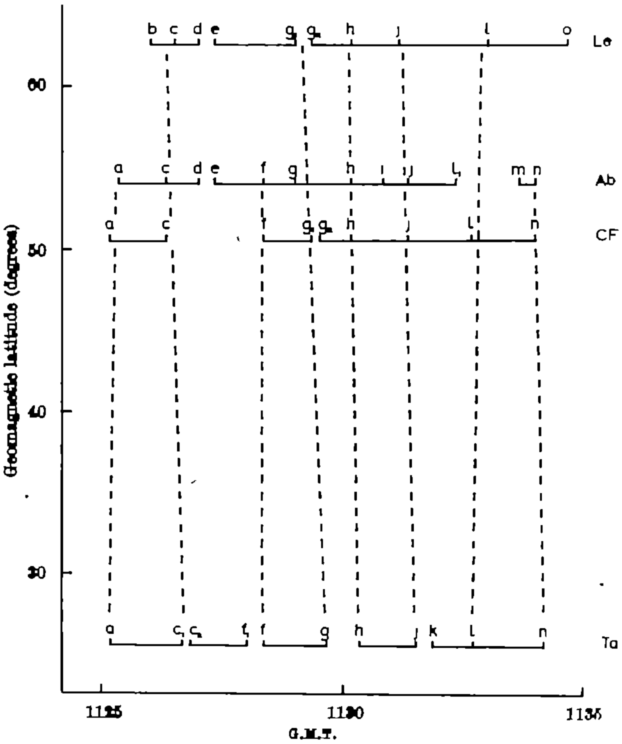

Fig. 1. Bounding times at various stations, indicated by the letters *a*, *b*, . . . *o*, plotted against geomagnetic latitude. Dotted lines are best fitting straight lines through corresponding points

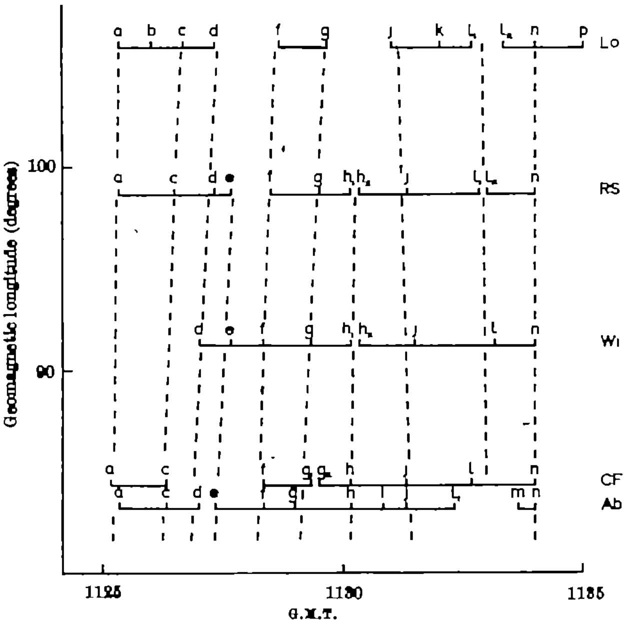

Fig. 2. Bounding times at various stations indicated by the letters *a*, *b*, . . . *o*, plotted against geomagnetic longitude. Dotted lines are best fitting straight lines through corresponding points

published by one of us (J. L.) elsewhere. It is clearly desirable to make more extensive analyses of a similar character, but if ordinary magnetograms are used the work involved would be very considerable. If a sufficient number of automatic observatories were established giving the field deviations in digital form[3], it would be possible to programme such an analysis for an automatic computer.

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A New Mechanism for Convection in the Mantle and Continental Accretion

THE study of palæomagnetism has necessitated the contemplation of continental drift and the most plausible explanation appears to be convection in the mantle[1,2]. It has been assumed that the convection is thermal, being maintained by heat from the core. The purpose of this communication is to point out that there is another, quite natural cause for convective-type motion, which avoids certain difficulties encountered in the purely thermal explanation.

Ringwood[3] has summarized the evidence for the existence in solution in the core of 10–20 per cent silicon and pointed out that this metallic silicon would react with iron oxide in the bottom layers of the mantle. Further, there is seismic evidence[4] for a light layer approximately 200 km thick at the bottom of the mantle, and it is natural to interpret this as a product of iron–silicon exchange between the core and mantle. Metallic silicon from the core reduces iron oxide in the mantle, so that liquid iron falls into the core leaving silica in the bottom of the mantle.

It must therefore be supposed that any convective overturn of the mantle will bring material from the silica-rich layer to the surface, which accounts for the accretion of the continents. Since this material is substantially less dense than the bulk of the mantle the so-called convection is really a geological differentiation, with thermal gradients having only a secondary role.

The mantle must be presumed to yield slowly under a steady stress of 10^8–10^9 dynes/cm^2 (ref. 5); for the present purpose it is assumed that the necessary stress is 3×10^8 dynes/cm^2. A density difference of 6×10^{-4} g/cm^3 between up- and down-going convective columns with radii of 3,000 km would produce a stress of this magnitude. Such a density difference would result from a relative enrichment of the up-going columns with 0·2 per cent of the silica-rich, continental or granitic material, the density of which differs from that of the bulk of the mantle by about 0·3 g/cm^3.

The average rate of convective motion must be close to 1 cm/year, since this is the minimum consistent with palæomagnetic data and probably the maximum consistent with the geothermal flux[5]. It gives a rate of accumulation of continental material equivalent to the addition of a layer of thickness 4×10^{-3} cm/year to continents occupying a quarter of the Earth's surface. At this rate, continents of average thickness 30 km could accumulate in $0{\cdot}75 \times 10^9$ years little more than one-tenth of the age of the Earth. The explanation must depend on the 200 km reservoir of silica-rich material at the bottom of the mantle. Assuming that continental accretion equivalent to 4×10^{-3} cm/year is in equilibrium with the rate of production of silica at the bottom of the mantle, then, allowing for its compression, the 200 km layer would have taken about 10^{10} years to accumulate. However, the initial silicon–iron reaction through a thin layer of silica must have been more rapid, so that these figures are mutually consistent.

The proposed model for the history of the mantle is therefore a gradual accumulation of silica at the bottom of the mantle by reaction with the core, until a layer about 200 km thick was built up. When irregularities arising from minor convective motions accompanying the core–mantle interaction became sufficiently large to cause the instability of the layer as a whole to exceed the limit which the mantle would support, they initiated convective-type movements by which the silica layer was maintained at an average thickness of about 200 km and the surplus was carried to the surface to form continents.

It may be noted that in the foregoing model there is no need to maintain the conventional concept of convection with up-going and down-going currents of similar dimensions. Rather the motion might be expected to have relatively narrow silica-rich up-currents depositing the surplus silica and causing a general lowering of the rest of the mantle without recognizable down-currents. We must even allow the possibility of irregular, spontaneous overturn, involving rising 'blobs' of silica.

The convective instability of a 200 km layer of density lower than that of the material above it by as much as 0·3 gm/cm^3 is enormously greater than the instability which could result from any temperature gradient. Indeed, the presence of such a layer makes the thermal gradient of no consequence to the convection mechanism. It now becomes reasonable to suppose that convective-type motion can begin in the crystalline solid mantle, the creep law of which (on any time scale) cannot be that of a pseudo-Newtonian liquid[5], as it appears necessary to assume in the hypothesis of thermal convection[6].

[The settling into the core of any iron not already there will also contribute to the chemical differentiation of the mantle, but we cannot suppose, as Runcorn[7] has done, that the whole core has accumulated in this way over the past 3×10^9 years, because such a settling process would release an enormous amount of gravitational energy which would have to escape as geothermal flux. Neglecting any change in size of the Earth, this energy is about 6×10^{38} ergs, equivalent to about 30 μcal. cm^{-2} sec^{-1} for the whole Earth for 3×10^9 years, that is, nearly 30 times the present average geothermal flux. Lyttleton[8] pointed out that the greater compressibility of core material would lead to an overall contraction of the Earth during settling of the core and hence an even greater release of gravitational energy. It is therefore necessary to assume that the core was substantially complete at an early stage in the Earth's history and that its subsequent growth has been slight.]*

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* Note added in proof.

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DR. STACEY's suggested mechanism is a specific one by which convective overturn can be brought about by the chemical separation which must occur if the Earth has been formed by accretion and the iron core has grown gradually in radius. It seems doubtful, however, to me that the rate of release of gravitational energy by chemical separation can be as great as that released by the decay of radioactive elements, although in any event it appears that the form of convection should not depend on the cause of the density differences which give rise to convective instability.

However, Dr. Stacey's mechanism of chemical segregation makes the specific proposal that there is a silicon-rich layer 200 km thick at the bottom of the mantle which is as much as 0·3 g/cm^3 less dense than the material above it. It can be shown that for any reasonable value of viscosity such a layer would be highly unstable. This can perhaps best be seen from calculations made by me[1] which show that for a viscosity as high as 10^{21} poise convection velocities of 1 m/sec are to be expected with a temperature difference between the up-going and down-going streams of as little as 1/3°, which of course means density differences between the up-going and down-going streams of one part in ten thousand.

Further, I think there is now evidence that there may be a thin layer of solid iron at the base of the mantle of variable thickness not exceeding about 10 km. It has long been known that the geomagnetic secular variation and the non-dipole field are systematically small over a large area of the Earth roughly co-extensive with the Pacific[2].

A convective mantle essentially means one with very nearly uniform physical properties and it is hard to suppose that the turbulence in the Earth's core which gives rise to the secular variation of the Earth's magnetic field can for long be systematically different in its properties below one area of the Earth, especially as the core is slowly rotating relative to the mantle. The cause has long been a puzzle to geomagneticians.

New interest in the problem has been raised by palæomagnetic observations on recent lavas from Hawaii by Doell and Cox[3], who find that the low secular variation in the Pacific seems to have been a property of the Earth over the past few tens of thousands of years. In a full account of this topic elsewhere, I am suggesting that the cause is electromagnetic screening by a layer of iron which has collected at the base of the mantle by separation from the descending convection currents, but which has not freed itself completely from lighter silicates and may, in fact, be similar to the Palliasite meteorites. As this is a thin layer it is reasonable to suppose that its distribution may be highly irregular, although any one distribution would be expected to persist for the order of a million years.

While this does not exclude the specific mechanism of chemical separation suggested by Dr. Stacey it seems more likely to me that it results from convection currents of a fairly regular kind existing throughout the whole of the mantle.

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METEOROLOGY

Meteorological Conditions Dependence of Radon Concentration in the Air above the Atlantic Ocean

CONCENTRATION of radon in the air above the Atlantic Ocean at the *K* ('kilo') point was measured during May 28–June 28, 1962, and now we are attempting to relate the values of natural radioactivity to meteorological conditions.

In previous papers[1,2], an apparatus for measuring the natural radioactivity of the air was described. We have examined the meteorological conditions by referring to the daily weather information of the Meteorologie Nationale, and the local data obtained at the *K* point, especially the wind direction (front-crossings).

We have systematically examined the way followed by the air, at ground-level, getting the *K* point. We have also investigated whether the *K* point was concerned with cold descending air, although such a phenomenon is not very likely to happen in June, and, finally, we have connected the concentration of radon and of its descendants to the origin of the air masses, for the air reaching the *K* point. As a first approximation, taking into account the mean speed of movement of the air masses, it may be assumed that the air takes one day to move from Brittany and Ireland, and half-a-day from the Cantabric coast.

The following results were obtained:

(1) If the air comes from the north-west of France or from Great Britain, its radon concentration is always between 10 and 27 pc./m^3; the mean speed wind blowing from east to north-east which has brought this air during the observed period was generally weak ($\leqslant$10 m/sec), mostly when leaving the coast, but a stronger wind could give higher values. We noticed, during the morning of June 1, a decrease in radon activity, and we are inclined to impute this to a descending draught of descending cold air, poorer in radon.

(2) When the air comes from the north of Spain, its radon concentration is greater than 27 pc./m^3. The maximum activity obtained was 40 pc./m^3. The arrival of this air at the *K* point is always conditioned by an isobaric constriction prevailing for 12 h at least above the north-western side of the Iberian Peninsula, and on the neighbouring oceanic zone. This constriction is bound sometimes to a centre of depression located above western Spain or Portugal. If, in this centre, the minimum pressure comes down enough (1,005 millibars) the air supply feeding the *K* point will be deviated towards the south before it reaches that point. Such a phenomenon occurred, for example, on June 27.

(3) During the recording time, there was no crossing of warm fronts, but there were four crossings of cold fronts with one quasi-stationary front: we observed in three of these five cases that maximum radioactivity was always very low ($<$ 6 pc./m^3) when the front was running. Then, the cold air located behind the front comes from the northern to the north-western direction, but it seems risky to take into account its previous behaviour. As we noticed before, there was only one cold front subsidence, and the

supply of air from the Continent for the *K* point was disturbed at its origin, along the seashore, by a slight north-western flow. Thus, so far as this is concerned, we are not able to draw any conclusions.

This work is supported by a contract of the Commissariat à l'Energie Atomique (service of Physical Electronics). We thank Mr. Lambert for helpful discussions on this matter.

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PHYSICS

Electrical Breakdown in High-temperature Gases

Relatively few experimental results are available for the breakdown of high-temperature gases at atmospheric pressure and above. Alston[1] has confirmed Paschen's law for air up to 1,100° C and obtained flashover voltages which were independent of electrode material. On the other hand, it is well known from investigations of the decaying properties of freely recovering arcs[2] that 'thermal' breakdown can occur at considerably reduced voltages while the charge concentration remains high and the electrodes retain their heat. Similar low voltages of breakdown have been observed in shock tube investigations[3]. Forman[4] has recently described experiments with rare gases up to 300 mm pressure and for refractory cathodes at temperatures up to 3,000° K. The cathode was a filament of 0·01-in. diameter and the concentric anode had a diameter of 1 cm. Breakdown occurred at anode voltages considerably lower than the ionization potential of the gas for argon and xenon and cathode temperatures greater than 2,400° K, but was not observed for helium. Although the gas in the immediate vicinity of the cathode was assumed to be in thermal equilibrium at the cathode temperature, the bulk of the gas and certainly the anode were considerably cooler.

The experiments recorded here were carried out in high-temperature (1,500–2,500° K) argon and helium between concentric graphite electrodes with an arrangement leading to a more uniform temperature distribution. In this case the outer $\frac{1}{2}$-in. diameter electrode was made the heating element and the inner $\frac{1}{4}$-in. diameter electrode is heated by radiation, conduction and convection in the gas. Measurements with an optical pyrometer through a small hole in the outer electrode indicated a temperature difference between the electrodes of not greater than 25° C at 2,500° K. Results were obtained both with d.c. voltages between the electrodes and also with 400-c/s a.c. voltages to permit display of the current/voltage characteristics on an oscilloscope. The latter frequency was chosen so that a complete characteristic could be displayed over a time during which the 50-c/s a.c. heating current in the outer tube is relatively small, thus reducing any effect of the electrical field of the heating element. Fig. 1 shows typical current/voltage characteristics for argon at 2,200° K and 1,200 mm pressure (with the right-hand side representing the outer electrode as cathode) and for two different values of circuit resistance. Four regions are in evidence:

(1) For low applied voltages where an excess of electrons is available at the cathode, the characteristic rises approximately linearly. An effective 'conductivity' for the gas can be measured in this region, by enlarging this region of the trace.

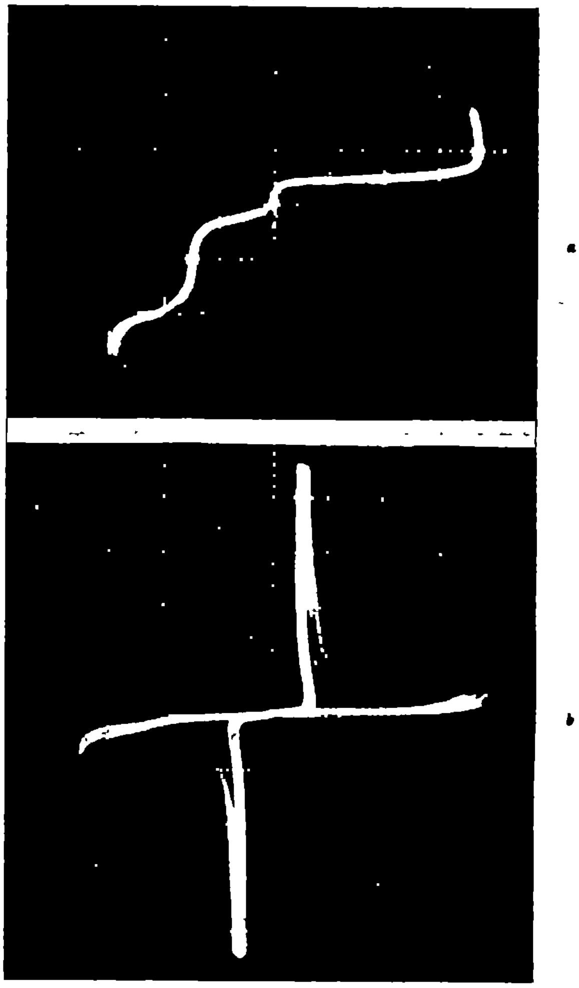

Fig. 1. Current/voltage characteristic for argon at 2,200° K and 1,200 mm pressure. Vertical axis is current and horizontal axis voltage. *a*, Series resistance, 700 Ω; voltage scale, 50 V per division current scale, 50 m.amp per division *b*, Series resistance, 80 Ω, voltage scale, 50 V per division; current scale, 0 5 amp per division

(2) At intermediate voltage, a constant current is obtained corresponding to the saturated thermionic emission current of the cathode. No more electrons are available and a voltage gradient is built up at the cathode as the applied voltage increases.

(3) At a particular voltage, characteristic of the temperature, a very rapid increase in current occurs for no further increase in applied voltage. The maximum current reached is restricted by the circuit resistance, but if permitted to rise to a high enough value (also characteristic of the temperature) will result in a transition to a fourth region.

(4) This region (Fig. 1*b*) is the normal current/voltage characteristic of the arc for this geometry and has been confirmed with 'cold' tests. Once established, the discharge remains in this mode until the voltage reduces to zero.

Hysteresis effects are in evidence if the transition (4) is prevented, but these have been darkened out in Fig. 1*a* for clarity. Also it is seen that the lower saturation current for the inner electrode as cathode (corresponding to the smaller surface area) is followed by a transition to region (3) at a much lower voltage than for the outer electrode as cathode. A further saturation of current occurs before the final breakdown voltage is reached. Not all these regions are observed throughout the temperature-range 1,500° K–2,500° K; but in general the effect of increasing temperature is: (*a*) an increase in the effective 'conductivity' in region (1); (*b*) a higher saturation emission

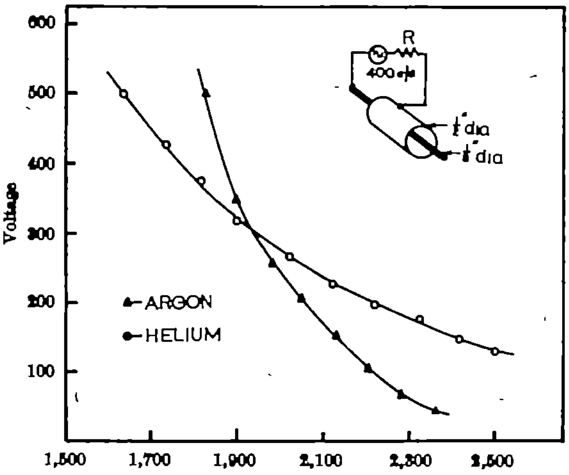

Fig. 2. Transition (or breakdown) voltage as a function of temperature for outer electrode as cathode

current in region (2); (c) a steady reduction in the transition voltage (3), but an increase in the maximum stable current at that voltage; (d) a transition to the same arc characteristic irrespective of initial electrode temperature.

In Fig. 2 the transition (or breakdown) voltage is shown as a function of temperature for both argon and helium. Similar forms of breakdown have been obtained with commercial and reactor-grade graphites and recently with a tantalum outer electrode and tungsten inner electrode.

Smooth transitions from glow discharges into arcs have previously been observed for refractory cathodes both at low pressures[5] and more recently at atmospheric pressures[6]. In such experiments the only source of heat is the discharge current itself, and the transition is associated with a steady increase in the thermionic component of current as the cathode temperature rises. In our experiments the thermionic component of current is established prior to breakdown occurring and is an independent variable.

This work, to be described more fully elsewhere, was supported financially by the Australian Institute of Nuclear Science and Engineering.

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Representation of a Group in Relativistic Quantum Mechanics

ESSENTIALLY all infinite unitary representations of semi-simple Lie groups are known[1], they are of Murray–von Neumann[2] type I. Further, while it has been known for a long time that non-relativistic quantum mechanics is such a representation for the rotation group of space[3], the same does not carry over to relativistic quantum mechanics. Infinite representations of the Lorentz group[4] do not correspond to anything physically identifiable. This, among other things, suggests that the underlying group of relativistic quantum theory is not the Lorentz group but rather one for which non-unitary representations have a special significance.

Previously[5] a physical argument was given for the exceptional Lie group F_4 as candidate for the above. This turned on the interpretation of the wave equation as not being (Hermitian) self-adjoint, thus implying the existence of a non-unitary representation. An attempt[6] was made to give a path integral form to the latter, in which paths oppositely oriented in 'time' belonged to different measure spaces A, Ω say. It was conjectured that the uniqueness of A, Ω depends on a special relation which exists between the co-homology structure of F_4 and certain algebraic number fields. Here I give some preliminary details to substantiate this idea.

The structure of a semi-simple Lie group G of rank L and dimension R is determined[7] by its so-called Weyl group $W(G)$, which is a group generated by reflexions $S_1 \ldots . S_L$ and is at the same time irreducible and 'crystallographic' (in L dimensions). The period h of $W(G)$, given by $S_0^h = (S_1 \ldots . S_L)^h = 1$, is $(R - L)/L$ and the co-homology structure of G is determined by the 'form problem' for $W(G)$ which was shown by Coxeter[8] to be equivalent to finding the characteristic roots of S_0 as g_i/h^{th} roots of unity. For $W(F_4)$, $h = 12$ and $g_i = 1, 5, 7, 11$ and the latter, less $g_0 = 1$, are the same (in the representation given by Klein) as for the octahedral group $G_{24} \sim W(C_3)$, which explains the appearance of these numbers in the many results of algebraic field theory which depend on elliptic functions.

The invariant forms $F_i(z)$ of degrees $(g_i + 1)$ for G_{24} were given by Klein[9], who observed that the parametrizations $(z_1, z_2) = \theta_{10}(\tau,0), \theta_{00}(\tau,0)$ in terms of Jacobian elliptic functions gives an infinite representation of G_{24}, for $p = 4$, under the action of the factor group Γ_∞/Γ_p, where Γ_p is the modular congruence group, $T\tau = -1/\tau$ and:

$$S_p = \frac{a\tau + b}{c\tau + d}$$

$$\left.\begin{matrix} a, d \equiv 1 \\ b, c \equiv 0 \end{matrix}\right\} \text{mod. } p, |S| = 1$$

However, one notes that G_{24} is contained in Γ_p for all primes $p \equiv \pm 1$ (mod. 8) and therefore the possibility exists of constructing a measure in the closure of all such p The details require considerably more; namely, first, for $p_i \equiv \pm 1$ (mod. 24) the least primitive roots a_i have maximum density for just the values g_i; secondly, if $\tau = \omega_1/\omega_2$ is restricted to the quadratic number field $K(\sqrt{g_i})$ then we have complex multiplication[10] by $\gamma_1 = \sqrt{g_i}$ for elliptic functions, the effect of which is to change the normalization $|S| = 1$, to $|S_\gamma| = g$. Then the operations $S_g = S_\gamma S_p S_\gamma^{-1}$ form the so-called transformation group which leaves invariant a modular form of degree $g + 1$.

It was shown by Hasse[11] that something like the converse of the foregoing is possible. The field $K(\theta, p)$ of elliptic functions mod. p admits a non-commutative (quaternion) multiplication ring depending on the residue class $p_i \equiv g_i$ (mod. 12). It thus appears that there does exist a quasidual approach for the construction of the measure spaces A, Ω.

The underlying phenomenon here is the following. First, the elementary fact[12] that 24 is the highest resolution modulus (erklärungsmodul) with all real characters, which therefore can be represented by a quadratic residue character. Secondly, the fact noted here about the least primitive roots, which has to be completed by $a_j = 2, 3$ for $p_j \equiv \pm g_j$ (mod. 24). Consequently the fields $K(e^{2\pi i/24})$ and $K(\sqrt{g_i})$ 'contain each other' in the approximate sense of ignoring those primes p_j for which $a_j > 11$.

Lastly, the group $W(F_4)$ leaves invariant a 24-cell in 4-space or, with an involution, a system of 3 desmic tetrahedra[13] in 3-space. It therefore involves two independent representations of G_{24} which, if associated with A, Ω, must include an operation interchanging them. As pointed out previously[6], this should introduce a very large number N into a theory with physical interpretation. A rough guess is $N = \exp 2\pi(\sqrt{D} - \sqrt{D'})$, where $D = 5.7.11$ and $D' = 24$ (so that $N \approx 10^{44}$) since this is the factor that might be expected to appear in the functional equation for elliptic functions over these fields.

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ENGINEERING

An Elastohydrodynamic Lubrication Experiment

THE thickness and shape of the lubricant film between highly loaded rollers is the feature of elasto-hydrodynamic lubrication which has received most attention in recent years. The efficacy of hydrodynamic lubrication in such contacts is directly related to the thickness of the lubricant film compared with the surface irregularities on the solids, and the recent activity reflects the significance of this quantity to the designer of machine components.

Another aspect of elasto-hydrodynamic lubrication which is of interest to the designer is the stress field within the solids. In this respect the distribution of pressure within the lubricant is of paramount importance, and it is the purpose of this communication to report a preliminary investigation of the pressure and temperature distributions in a highly loaded sliding contact.

One of the difficulties associated with the measurement of pressure and temperature profiles in contacts representative of gear teeth is the very small width of the contact zone in relation to the size of conventional sensing elements. Higginson[1] overcame this difficulty by using a low modulus elastic solid for the stationary component in a sliding contact. In order to examine the pressure and temperature distributions in a highly loaded metal contact a novel disk machine has been constructed which opens out the contact zone and yet retains the essential features of elastohydrodynamic conditions. A stationary phosphor bronze shoe having a 6·5-in. concave radius is loaded against the periphery of a rotating phosphor bronze disk of 6-in. radius in the manner of a brake shoe as shown in Fig. 1. A mineral oil supplied to the disk surface ensures the existence of a coherent oil film between the shoe and the disk. The shoe is provided with a small central oil pressure tapping and four thermocouples buried at various depths below the working surface. The pressure tapping hole communicates with a cylindrical, wire wound pressure transducer. The position of the shoe is varied circumferentially with respect to the line of action of the applied load, thus traversing the pressure tapping and thermocouples through the pressure zone.

Results have been obtained for various speeds and loads. A typical pressure traverse is shown in Fig. 2 for a load of 1·35 tons per in. of face width and a disk peripheral speed of 64 in./sec. Also shown in Fig. 2 are contours of rise in temperature above ambient for the bulk metal of the shoe. The lower curves show the distribution of shoe surface temperature and the lubricant viscosity related to the local pressure and the shoe surface temperature.

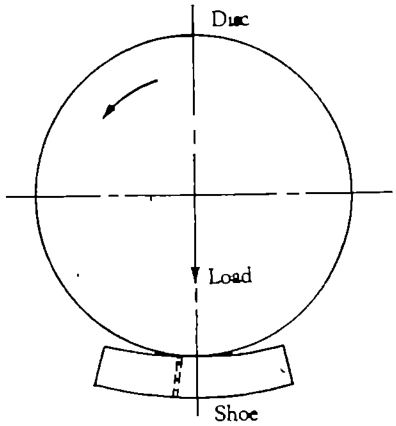

Fig. 1. Disk machine

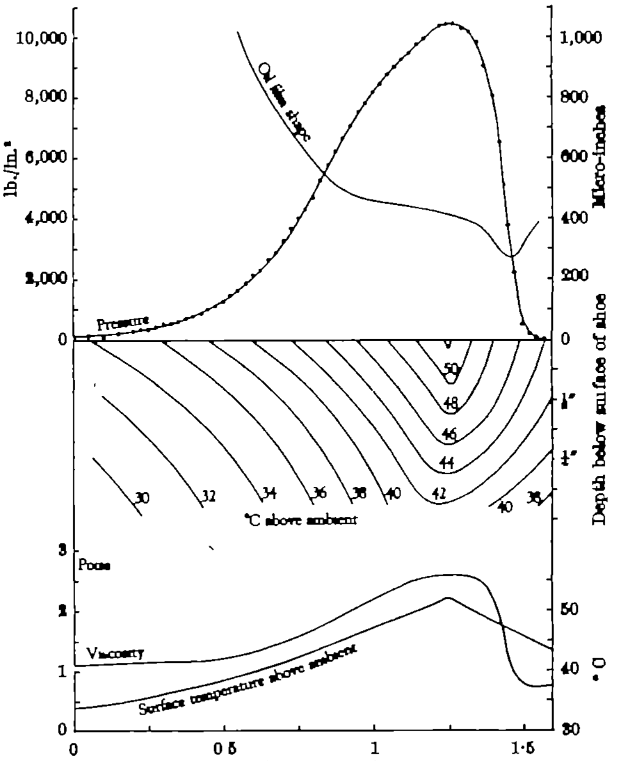

Fig. 2. Pressure and temperature profiles

The shape of the pressure curve is consistent with the predictions of elasto-hydrodynamic theory[2], the most notable feature being the very steep pressure gradient at the outlet end of the zone. If side leakage is neglected, the shape of the oil film corresponding to the measured pressure distribution can be determined according to a method described in ref. 2. The shape of the oil film shown in Fig. 2 exhibits the well-known characteristics of elastohydrodynamic films. The similarity between this shape and the picture presented by Crook[3] based on a direct measurement is striking. This result demonstrates that the local restriction in film thickness at the outlet end of the zone does not necessarily imply the existence of the pressure spike exhibited by some theoretical solutions. It should be recalled that theoretical solutions demonstrate the dependence of the pressure spike on material properties[4]. An interesting feature of the surface temperature profile is that the maximum temperature is attained before the end of the pressure zone is reached; a result attributable to the rise in lubricant viscosity in the vicinity of the peak pressure.

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METALLURGY

G Phases containing Scandium

We have noted the occurrence of a *G* phase (structure type *D8a*) in the Sc-Ni-Si, Sc-Co-Si and Sc-Ni-Ge systems. Alloys were made by arc melting in a water-cooled copper crucible under argon. Diffraction patterns were taken with copper radiation on powder annealed at 900° under vacuum. The composition and lattice parameters of the newly found *G* phases are given in Table 1.

Table 1. LATTICE PARAMETERS OF *G* PHASES

Composition	a_0 (Å)
$Sc_6Ni_{16}Si_7$	11·429
$Sc_6Co_{16}Si_7$	11·438
$Sc_6Ni_{16}Ge_7$	11·663

We failed to detect a *G* phase in the following alloys: $U_6N_{16}Si_7$, $Y_6Ni_{16}Si_7$ and $Th_6Ni_{16}Si_7$, but we confirm the existence of a *G* phase in $Nb_6Ni_{16}Si_7$ (ref. 1).

Gladyshevskii *et al.*[1] have reported data for *G* phases of the generalized formula $A_6Ni_{16}Si_7$ in which *A* may be Ti, Zr, Hf, V, Nb, Ta, Cr (with 1 *a*/0 Ta) or Mn. Our data extend this list to include Sc but not Y. It is noted that the *A* element may come from groups IIIb to VIIb of the Periodic Table, but is restricted to atoms having small to intermediate size, that is, Mn to Sc.

This work was performed at the Argonne National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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Aluminium Nitride Whiskers

In the course of an investigation into the stability of certain nitrides, whisker growth has been observed on aluminium nitride. Aluminium nitride powder was prepared from the elements, cold pressed at 15 tons/in.² and sintered for 4 h at 1,750° C in high purity argon. The sintered compacts measured approximately 8 mm in diameter by 4 mm high. X-ray powder photographs of filings of the sintered nitride showed the structure to be hexagonal, close-packed (a near-wurtzite structure according to Jefferey and Parry[1]) with $a = 3{\cdot}11$ Å and $c = 4{\cdot}98$ Å. The sintered density was 68 per cent of theoretical and the Vickers micro-hardness 1,010 ± 15 kg mm^{-2}.

A stack of three compacts was mounted under a bell jar in an arc image furnace[2] and the uppermost one heated, in an atmosphere of high-purity nitrogen. The initial nitrogen pressure was 745 mm and the pressure at the end of the heating period was 750 mm. The following sequence of events was observed on the heated surface as the temperature was increased: (*a*) short 'woolly' filaments formed; (*b*) longer filaments grew normal to the surface; (*c*) nodes formed on these filaments and growth continued; (*d*) 'fir-tree' growth occurred from these nodes. This latter growth appeared to act as centres of sublimation and the needles decreased in height. There was no sign of melting. Stage (*d*) was accompanied by the formation of a thin film (presumably aluminium nitride) on the inside of the bell jar.

When heating was stopped after stage (*b*) it was found that the needles were hexagonal prisms about 1,600μ long by 20μ thick and almost transparent. They could be bent through an angle of about 15° before fracture occurred.

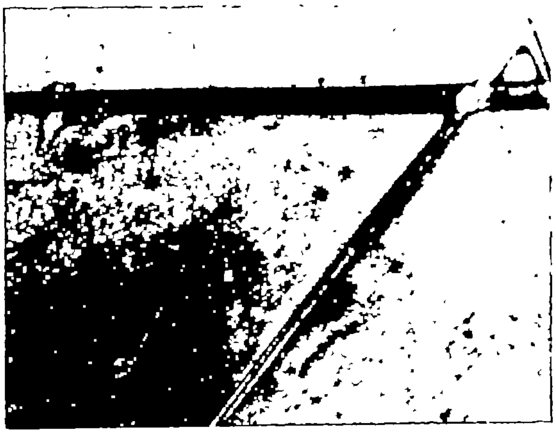

Fig. 1. Aluminium nitride needles, reflected light. (× c. 375)

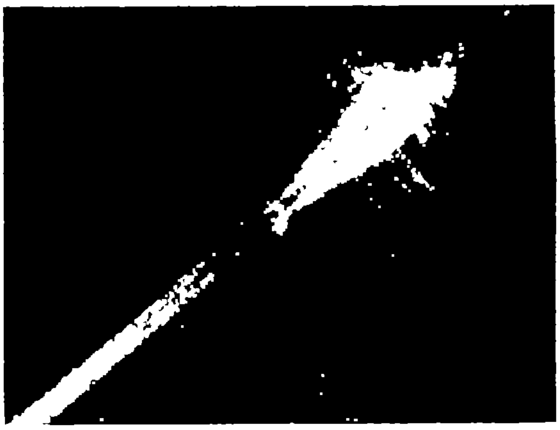

Fig. 2. Sublimation stage ('fir-tree' growth) during heating of aluminium nitride whiskers; transmitted light. (× c. 560)

Specimens examined after stage (*c*) showed well-defined nodes (Fig. 1.) separating transparent hexagonal prismatic crystals. The fir-tree growth (stage (*d*)) is shown in Fig. 2.

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CHEMISTRY

Oxidation of Plutonium in Moist Air and Argon

The only published works on the oxidation of plutonium are by Waber[1], Poole[2] and Sackman[3]. Waber and Sackman's work was carried out only in air at temperatures < 100° C, whereas Poole's work was at 320°–480° C in air. In order to understand the mechanism of oxidation, we have found it necessary to compare the oxidation-rate of plutonium in air and in argon under both dry and humid conditions at a temperature sufficiently low that changes in oxidation behaviour are readily detected. Results presented here indicate that oxidation is anodically controlled.

Abraded specimens of plutonium (both cast disks and rolled sheet) were oxidized for 1,800 h in air and in argon at room temperature and 95 per cent relative humidity. Despite some irreproducibility of results caused by varia-

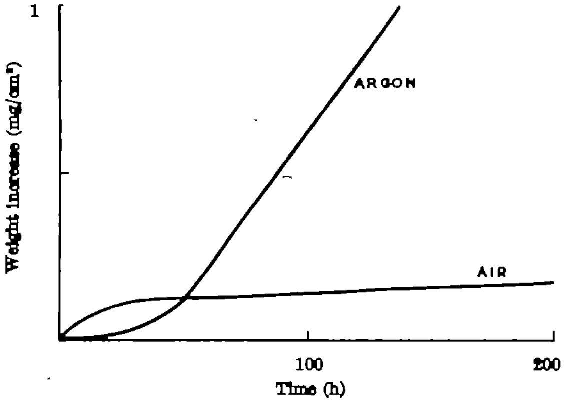

Fig. 1. Uptake of oxygen by plutonium in moist air and in moist argon at 25° C

tions in microstructure and purity of different casts, oxidation proceeds many times faster in moist argon than in moist air and extremely slowly under dry conditions (Table 1). This suggests that oxidation proceeds primarily by reaction with moisture and is under anodic control. The only other example of a metal oxidizing in this way is uranium[4]. At this temperature, oxidation of plutonium is paralinear after the initial deviations from linearity caused by the protective oxide film on the metal in air (Fig. 1). This protective oxide film continues to be built up to its maximum thickness in moist air; thereafter, the oxidation-rate is reduced as the oxygen seals the oxide surface, thus reducing the anodic surface area and the rate of reaction with moisture. In moist argon, the protective film takes some time to be broken down; thereafter, oxidation proceeds quickly because there is no oxygen to inhibit the reaction with moisture.

Table 1. OXIDATION-RATE OF PLUTONIUM AT 25° C

Cast	Rate of oxidation in* Argon k_{Ar}†	Air k_{Air}†	$\frac{k_{Ar}}{k_{Air}}$	Humidity (%)
92	0·0071	0·00028	25·4	95
106	0·0040	0·00096	4·2	95
108	0·0062	0·00029	21·4	95
119	0·0043	0·00079	5·5	95
119 A ‡	0·0087	0·00066	13·4	95
All	0§	0§	–	0

* Derived from slope of line after initial deviations from linearity.
† Mg/cm²/h.
‡ As 119, but electropolished surface.
§ Not detectable.

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Electrochemical Generation and Visible Spectrum of Nitrobenzene Free Radical Anion in Dimethylformamide

POLAROGRAPHIC behaviour and electrolytic reduction of nitrobenzene in a solution of 0·2 N sodium nitrate in dimethylformamide show that free radical anions of nitrobenzene are formed[1,2]. In this solution nitrobenzene is reduced in two polarographic waves, the first of which corresponds to the formation of the free radical anion $C_6H_5{-}NO_2^-$. These anions can be generated, in a special electrolytic vessel containing the solution, by electrolysis at a mercury pool cathode at a potential corresponding to the diffusion current of the first polarographic wave of nitrobenzene in dimethylformamide. The nitrobenzene anions generated were relatively stable in the absence of oxygen and were of a yellow-brown colour in solution. The anions formed gave a polarographic oxidation current (Fig. 1) corresponding to the formation of nitrobenzene: $C_6H_5{-}NO_2^- - e \rightarrow C_6H_5{-}NO_2$.

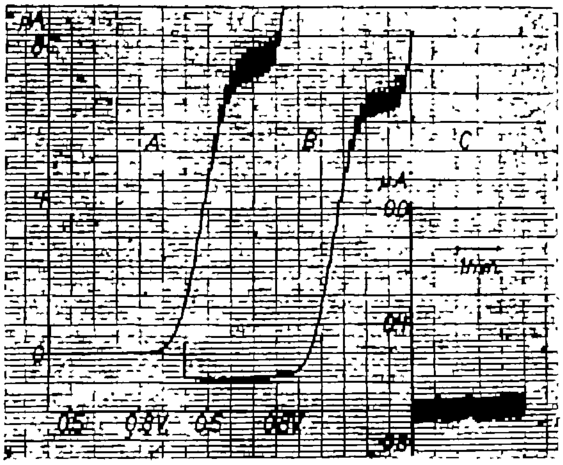

Fig. 1. Polarograms of solution of 2·7 × 10⁻⁴ M nitrobenzene in 0·2 N sodium nitrate in dimethylformamide. *A*, first one-electron wave before electrolysis; *B*, the same after electrolysis; *C*, registration in time of anodic current at potential –0·65 V (for standard calomel electrode) of nitrobenzene free radical anion. Height of anodic current corresponds to about 2·5 × 10⁻⁴ M concentration of nitrobenzene anions

On the assumption of the equality of diffusion coefficients of nitrobenzene and its free radical anion, it was possible to determine from the height of the anodic oxidation curve the concentration of these anions in solution[2].

In the present communication we are concerned with the visible spectrum of the free radical anion of nitrobenzene formed in the solution.

Weissman *et al.*[3] and Hoijtink *et al.*[4] investigated the optical spectra of negative ions produced by the action of alkali metals on aromatic hydrocarbons in aprotic solvents. Pointeau and Mme. Favede[5] investigated spectra of free radical anions of aromatic hydrocarbons after their generation by electrolysis in dimethylformamide. The latter workers, however, did not give in their method the concentrations of generated free radical anions and hence the extinction coefficients.

We performed our experiments using a special cylindrical, electrolytic cell made of quartz. Electrolysis was carried out with 2·5 × 10⁻³ M solution of nitrobenzene in 0·2 N $NaNO_3$ in dimethylformamide at a mercury pool cathode against a standard calomel electrode at a potential corresponding to the first polarographic reduction wave of nitrobenzene. The solution was mixed during electrolysis by passing through a stream of hydrogen which had been very carefully purified to exclude oxygen and water. After the electrolysis mixing was continued for a few minutes in order to achieve a uniform concentration of the anions formed. A stream of hydrogen was passed above the solution to prevent the entry into the cell of small amounts of air through the joints. Spectrophotometric measurements were made with a single-beam Optica-Milano '*CF-4*' spectrophotometer. Simultaneously, the spectrophotometric measurements of extinction and the polarographic anodic oxidation current (Fig. 1c) of the nitrobenzene anions present in solution at potential –0·65 V for the standard calomel electrode were recorded. From the height of this oxidation current it was possible to determine the actual concentration of the nitrobenzene free

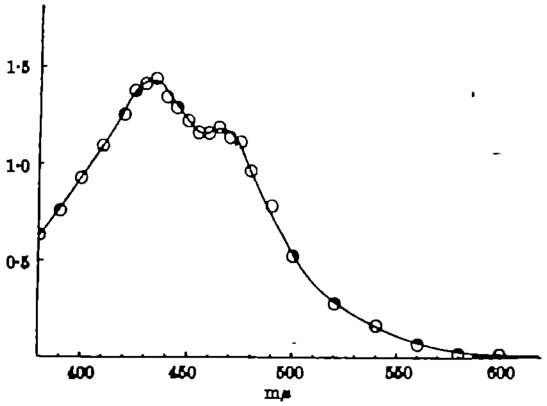

Fig. 2. Visible spectrum of nitrobenzene free radical anion in solution of 0·2 N sodium nitrate in dimethylformamide. Spectrum was made for concentrations of the free radical anion of about $1·3 \times 10^{-4}$ M and for a cell length of 35 mm

radical anion. By combining this with measurements of extinction for different wave-lengths caused by the presence of the free radical anion, the extinction coefficients of the whole spectrum were calculated.

The visible spectrum of nitrobenzene free radical anion exhibits two distinct maxima: the first is at 435 mμ region and the second at 465 mμ (Fig. 2).

The method applied by us for simultaneous measurements of the optical properties and oxidation current of the nitrobenzene free radical anion can be used for determining the extinction coefficients and spectra of different free radical anions which are stable, or which only slowly decay with time. This method also enables the measurement of the kinetics of the decay of radical anions to be made.

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Electron-donating Properties of Central Sympathetic Suppressants

ELECTRON-TRANSFER reactions between organic substances have often been implicated in various biological processes[1-3]. Recently, Karreman, Isenberg and Szent-Györgi[4] have suggested that certain central sympathetic suppressants act by donating electrons on the inside of the electric double layer of the central nervous system. A consequence of this hypothesis would be that pharmacologically active substances should possess relatively low ionization potentials.

Although it has been inferred both from molecular orbital calculations[4-6] and charge-transfer spectra[7] that these substances should be good donors, as yet there have been no reports of ionization potentials of suppressants. Though the direct measurement of ionization potential is quite difficult, it is nevertheless possible to derive values of this quantity from photoelectric measurements on the solid.

The photoelectric threshold, I_C, of a molecular crystal has been shown[8] to be related simply to the molecular ionization potential, I_G, by:

$$I_C = I_G + P \qquad (1)$$

where P is the polarization energy of the crystal. P can be calculated for crystals of known structure.

We have measured I_C for several central sympathetic suppressants and threshold energies are given in Table 1.

Table 1. I_C AND I_G FOR SOME PHARMACOLOGICALLY ACTIVE SUBSTANCES

	I_C (eV)	I_G (eV)
Phenothiazine*	5·14 ± 0·05	6·7
Promazine	5·16 ± 0·05	6·8
Promazine hydrochloride	5·46 ± 0·05	7·1
Chlorpromazine	5·06 ± 0·05	6·7
Chlorpromazine hydrochloride	5·22 ± 0·05	6·8
Trifluoperazine dihydrochloride	5·68 ± 0·10	7·3
Prochlorperazine	5·45 ± 0·10†	7·1
Reserpine	5·40 ± 0·05	7·0

* Inactive pharmacologically.
† As viscous oil.

I_G is estimated from equation (1) by assuming an average value for P of 1·6 eV (ref. 8). We expect the error in P to be no greater than 0·3 eV.

The measurements indicate that these substances have striking electron-donating properties. Chlorpromazine is an even better donor than the readily oxidizable naphthacene for which I_C = 5·25 eV (ref. 9) and I_G = 6·88 eV (ref. 10). Low values of I_C for phenothiazine derivatives are seen to be associated with the parent nucleus.

Though it is appreciated that alternative mechanisms of suppression of the central nervous functions are feasible, the present work is consistent with the electron-transfer theory. It must be emphasized, however, that the possession of a low ionization potential does not in itself render a substance pharmacologically active. Phenothiazine, for example, is not a central sympathetic suppressant.

Since such biochemically important molecules as the chlorophylls and β-carotene also have low values of I_C (ref. 11) it is still possible to maintain that electron-transfer reactions may be of importance in many biological processes.

We thank Prof. S. E. Wright, Mrs. R. Temple and Dr. W. J. O'Reilly for useful discussions.

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Photoconductivity of Manganous Phthalocyanine

WE have investigated the dark- and photo-conductivity of single crystals of metal-free and some transition metal phthalocyanines. Some of our results for metal-free, zinc, copper, nickel and cobalt phthalocyanines have been published elsewhere[1]. Meanwhile we have extended our investigations to include ferrous and manganous phthalocyanines. Here we wish to report briefly that we have found manganous phthalocyanine to differ in its behaviour from the other members of the series in a striking manner. The measurements were made on the needle-shaped crystals using two electrodes. No guard ring was used, and the arrangement is therefore equivalent to a surface conductivity cell.

First, we find the dark resistivity of the manganous compound is much lower than that of the other phthalocyanines. The dark resistivities are presented in Table 1, from which it can be seen that while manganous phthalocyanine has a specific resistivity as low as 10^6 ohms/c.c.,

Table 1

Phthalocyanine	Specific resistance (ohm/c.c.)
Metal-free	c. $10^{[illegible]}$
Manganous	4×10^4
Ferrous	4×10^9
Cobalt	9×10^9
Nickel	6×10^{10}
Copper	2×10^{11}
Zinc	3×10^9

the other members of the series have specific resistivities lying between 10^9 and 10^{13} ohms/c.c.

Secondly, while the photoconductive action spectra of compounds other than manganous phthalocyanine were generally similar, having two or more peaks in the region 5000 Å–8500 Å, and another, usually weaker, peak in the region 10000 Å–12000 Å, manganous phthalocyanine showed its spectral response over the range 7000 Å–20000 Å (the limit of our monochromator) with broad maxima at 13500 Å and 16000 Å. The photocurrent in the infra-red was stronger in this compound than in any of the others.

Thirdly, the photocurrent is depressed in a remarkable way by gases. We have used dry electrolytic oxygen, dry oxygen-free nitrogen and water-vapour, and find all three, but particularly water-vapour, to have a marked effect on the photocurrent. A pressure of 1 cm mercury of any of these gases was sufficient to depress the photocurrent to the value in air, which is less by a factor of forty than its value at 10^{-5} mm mercury. The effect of evacuation or the introduction of gases was, as far as could be measured, instantaneous, and was repeatable, apparently indefinitely.

We could find no evidence of absorption bands corresponding to the spectral response of manganous phthalocyanine in its diffuse reflexion spectrum. However, a series of three peaks was found in the solution spectrum in *o*-dichlorobenzene, at 8500 Å, 10900 Å, and 13500 Å, the last being the most intense and corresponding to one of the maxima of the action spectrum. The extinction coefficients of these absorption bands are less by a factor of at least one hundred than the extinction coefficient in the very intense first singlet absorption band of the phthalocyanines. We are, as yet, unable to assign these bands definitely; but one possibility is that they are due to triplet transitions. Low optical absorption can give inordinately large photocurrents if they lead to an efficient photoconductive mechanism.

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Differential Isotope Effects in the *Ortho*- and *Para*-Bromination of Dimethylaniline

AN account of the kinetic isotope effect observed in the bromination of dimethylaniline in aqueous acid containing added bromide ions has been given previously[1]. We now report that the observed isotope effect varies with the bromide ion concentration, but not with the acid nor with the amine concentration. The relative rates of bromination of dimethylaniline and its 2,4,6-trideutero derivative (0·005–0·0125 M) in sulphuric acid (6·4 M), measured by Bell and Ramsden's method[2], are:

$[Br^-]$ (M)	0·0125	0·025	0·050	0·100	0·250
k_H/k_D	1·9	$1{\cdot}7_5$	1·5	1·3	1·1

Under comparable conditions, similar isotope effects are found for 2,6-dideuterodimethylaniline, whereas 3- and 4-deuterodimethylaniline give no isotope effect at any bromide concentration in the foregoing range. These results, together with the observation[3] that the *ortho*/*para* ratio for the bromination of dimethylaniline in aqueous acid is 2·3 and 0·15 at bromide concentrations of 0·025 and 0·5 M respectively, indicate that the isotope effect is 2·6 for the *ortho*-bromination and unity for the *para*-bromination of dimethylaniline under the conditions described.

The isotope effects obtained imply either that the *ortho*- and the *para*-substitutions proceed by different mechanisms, the rate-determining step involving the fission or the lengthening of the *C–H* bond in the *ortho*-, but not in the *para*-bromination, or that steric effects supervene in the *ortho*-deutero compounds, owing to the smaller amplitudes of *C–D* vibrations compared with the corresponding *C–H* vibrations. The red complexes formed by dimethylaniline and bromine, with molar ratios of 1 : 1 and 1 : 2, may be intermediates in the reaction, since the colour of aqueous bromine deepens on the addition of dimethylaniline, giving a band with an absorption maximum at 4650 Å. *Ortho*-deuterium atoms may stabilize such complexes by allowing fuller conjugation between the dimethylamino group and the benzene ring, resulting in a slower subsequent reaction between the complex and additional bromine. Using an arsenite titration method, which avoids the uncertainties connected with the particular electrode processes involved in the potentiometric method[3], the reaction order is found to be 1·3 in titratable bromine, suggesting that the transition state of the reaction contains more than one molecule of bromine with each dimethylaniline molecule.

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BIOCHEMISTRY

Chromatography of Infective Ribonucleic Acid from Foot-and-mouth Disease Virus on Calcium Phosphate

ALTHOUGH it is fairly generally accepted that the molecular weight of the infective unit of several ribonucleic acid-containing plant and animal viruses is approximately 2×10^6, there is still some objection to this conclusion. For example, Fraenkel-Conrat and his colleagues[1] consider that the infective ribonucleic acid of tobacco mosaic virus may be an aggregate of smaller polynucleotide sub-units. Furthermore, Vizoso and Burness[2], using the technique of chromatography on calcium phosphate, have shown that the infective material obtained by phenol extraction of Krebs-2 ascites tumour cells infected with mouse encephalomyocarditis virus is eluted at phosphate molarities ranging from 0·01 to 0·21 M. As this method separates ribonucleic acids according to their molecular weights, the smaller molecules being eluted first[3], it appears that the infectivity is associated with components of several molecular sizes, some with molecular weights considerably smaller than 10^6. This finding is of considerable importance. It seemed of interest, therefore, to apply the same method to the examination of the ribonucleic acid of another virus. Consequently, the chromatographic behaviour of the infective ribonucleic acid obtained by phenol extraction of both pig kidney tissue culture cells and the vesicular fluid from guinea pigs infected with the virus of foot-and-mouth disease has been investigated. Two strains of the virus have been used for the preparation of the infective ribonucleic acid: (*a*) strain 1 (type *O*) from the fluid from vesicular lesions of guinea pigs infected with the virus, and (*b*) strain 997 (type *C*) passaged more than

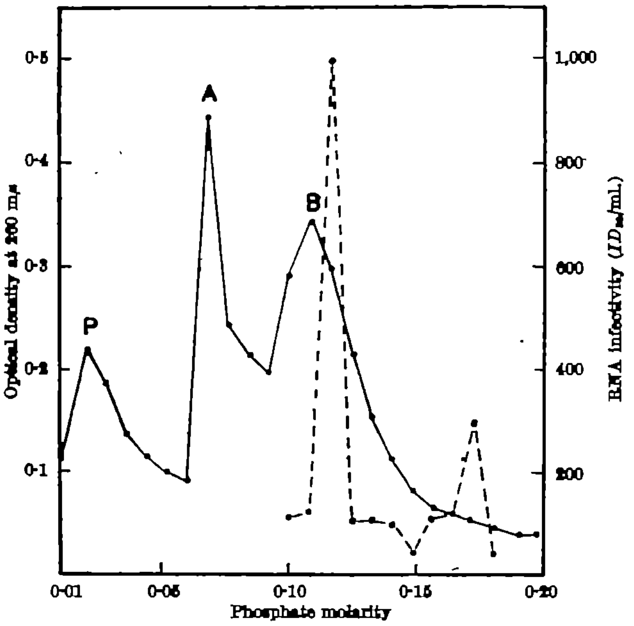

Fig. 1. Chromatography of ribonucleic acid from virus-infected pig kidney cells on calcium phosphate. Optical density at 260 mμ is shown by the unbroken line and the infectivity of the ribonucleic acid by the broken line. Peak *P* is due to residual phenol in the preparation

100 times in pig kidney cell monolayer cultures. Suspensions of each virus strain were extracted with phenol at 2° C in the usual manner and the infective extracts introduced on to 5 × 1 cm calcium phosphate columns (prepared according to the method described by Main and Cole[4]) in equilibrium with 0·01 M phosphate buffer, *p*H 7·2. Gradient elution at 0° C was achieved by adding 0·5 M phosphate to a reservoir containing 0·01 M phosphate. Fractions (5 ml.) were collected and titrated in mice (for strain 1) or pig kidney cell monolayers (for strain 997). In each case the viral ribonucleic acid was mixed with cell ribonucleic acid (prepared by phenol extraction of uninfected pig kidney tissue culture cells) before addition to the column so that the behaviour of the viral and cell ribonucleic acids on the same column could be determined. Ribonucleic acid was estimated in each fraction by the orcinol method or by measuring the ultra-violet absorption at 260 mμ.

A typical elution diagram is shown in Fig. 1. This shows that the two main peaks (*A* and *B*) of ribonucleic acid from pig kidney cells were eluted at the same molarities as the ribonucleic acids from Krebs-2 ascites tumour cells[2]. Recovery of both chemical and infective ribonucleic acid was almost quantitative. Infectivity could not be detected in the first ribonucleic acid peak (*A*) eluted from the column but was found mainly in conjunction with the second peak (*B*). The peak of infectivity did not coincide, however, with the peak of the cellular ribonucleic acid but was eluted at a slightly higher molarity. In addition to the main peak of infectivity a smaller peak was eluted with 0·17 M phosphate (Fig. 1). This second peak represented about 20 per cent of the total infectivity and has been obtained in all the fractionations so far carried out.

A similar elution pattern was obtained with the phenol extracts of pig kidney tissue culture cells infected with the virus and collected 4 h after infection. At this stage of the multiplication cycle, more than 50 per cent of the infective ribonucleic acid present in the cells is in a form which is susceptible to ribonuclease and is probably a virus precursor[5]. This evidence suggests that this ribonucleic acid has the same molecular weight as the viral ribonucleic acid.

The results of Burness and Vizoso[3], obtained with Krebs-2 ascites cells, show that the molecular weight of peak *B*, eluted at 0·11 M phosphate, is of the order of 10^6. Consequently, the molecular weight of the infective unit or units obtained by phenol extraction of foot-and-mouth disease virus is greater than this value. These results thus provide supporting evidence for the figure of $3·1 \times 10^6$ obtained by Strohmaier and Mussgay[6] by ultracentrifugation of the infective ribonucleic acid of the same virus. They differ from those of Vizoso and Burness[2], obtained with the ribonucleic acid of mouse encephalomyocarditis virus, in that no low molecular weight infective units have been detected.

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Dextran-gel Filtration in the Purification of a Crustacean α-Amylase

DURING studies[1] on the α-amylase from the hepatopancreas of *Carcinus maenas*, the common shore crab, the need arose for a purified enzyme preparation. Highly purified preparations of α-amylases have been prepared previously using starch as an adsorbent[2,3], but partition systems consisting of starch in water have serious drawbacks for chromatographic purposes since commercial starches are of poorly defined composition[4]. However, by using dextran-gel columns a purification stage has been developed which is reproducible and which gives a good enrichment.

In an early purification scheme a partially purified extract of hepatopancreas tissue was applied to a dextran-gel column, 25 × 3 cm, which had been prepared from 15 g of dry 'Sephadex *G*-75' (AB Pharmacia, Uppsala, Sweden). The internal volume, V_i, of the column was calculated from the mass and the water regain of the dry 'Sephadex.' The void volume, V_0, had been previously determined with hæmoglobin in the normal manner[5]. The enzyme was eluted with a solution containing calcium acetate, 0·02μ, and sodium chloride, 0·03μ, *p*H 7, at a rate of 10 ml./h. The enzymatic activity was assayed in the eluted fractions and the elution volume, V_e, of the enzyme was determined as the volume of eluant which gave the fraction containing the maximum activity.

The values recorded for V_i, V_0 and V_e were substituted in equation 1 to give a value of 0·29 for K_D, the distribution coefficient[5] of the α-amylase:

$$K_D = (V_e - V_0)/V_i \qquad (1)$$

Having established the value of the distribution coefficient it was unnecessary to redetermine the response of the enzyme to gel filtration in subsequent purifications using gel columns of different sizes. This was because the elution volume of the enzyme could be calculated by substitution of the values of K_D, V_0 and V_i in equation 1 and solving for V_e.

The course of a typical gel filtration experiment is illustrated in Fig. 1. A high proportion of the protein content of the partially purified α-amylase preparation applied to the column was found to be totally excluded from the gel phase. This extraneous material could be separated from the enzyme protein provided that the

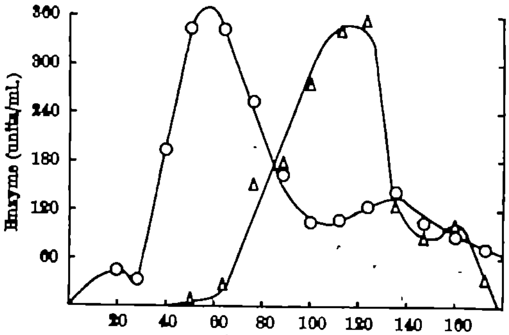

Fig. 1. Gel filtration of a crab α-amylase on a column of 'Sephadex *G*-75' at room temperature. V_0 = 60 ml., V_t = 120 ml. The partially purified enzyme, sample volumes 28 ml., specific activity 1·3, was applied to the column and eluted with a solution of calcium acetate, 0·02*M* and sodium chloride, 0·03*M*, *p*H 7. Approximately 10 ml. fractions were collected and assayed for enzymatic activity (ref. 7) and protein concentration (ref. 8). Total enzymatic activity applied to the column, 2,840 units. Yield, 1,810 units (64 per cent) contained in 75 ml. of eluate with a specific activity of 4·4, enrichment 3·5. ○, Enzymatic activity (units/ml.); △, protein concentration (mg/ml.)

volume of the sample applied to the column did not exceed 0·29 V_t (ref. 6). The enzymatic activity is partially retained on the column enabling the isolation of an α-amylase enzyme preparation with a specific activity 3–5 times greater than that of the partially purified preparation applied to the column and in a 60–80 per cent yield. This preparation was obtained by pooling the two or three fractions obtained on each side of the elution volume. In all cases, any material which appeared after collection of the enzyme was ignored.

The experiments were simple to perform and the purification stage could be carried out within 12–15 h using a freshly packed column. The results were reproducible and each column could be used repeatedly after washing with buffer following each purification.

Details of additional purification and properties of the purified crab α-amylase will be published elsewhere.

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Fractionation of a Substance containing Hexosamine, isolated by Precipitation with Calcium Chloride from Normal Urine

In urine there are many dialysable as well as many non-dialysable substances containing hexosamine. Recently, considerable attention has been directed to their examination[1–4]. By a number of methods various substances have been isolated; for the most part they represent complicated mixtures the composition of which depends on the manner of preparation. From the alcoholic supernatant after the precipitation of mucoproteins by precipitation with calcium chloride, Marcotte-Boy *et al.*[5] prepared another, non-dialysable, fraction of unknown nature, containing hexosamine. This communication reports further fractionation of this substance.

The soluble part of alcohol and ether-dried precipitate was extracted with distilled water; the extract was frozen and then thawed. In this procedure a part of the substances not containing hexosamine, giving positive murexide reaction as well as a characteristic ultra-violet spectrum for uric acid, remains unsolved. The supernatant was freeze-dried and used for further work.

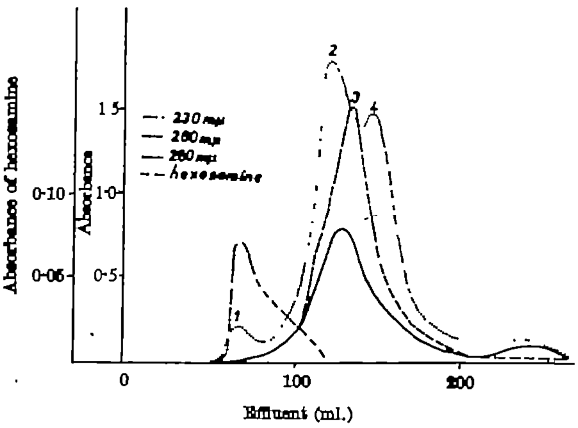

Fig. 1. Gel filtration on the column 'Sephadex *G*-25' medium

By the gel filtration of the solution of this substance on 'Sephadex *G*–25' medium four fractions were obtained among which the first two contained hexosamine (Fig. 1).

By refiltration of the column 'Sephadex *G*–50' as well as '*G*–75' a simple peak is obtained which, however, in the latter case is shifted to higher elution volume. Subsequent fractionation was performed on a DEAE-cellulose (phosphate buffer *p*H 6·0, ionic strength 0·01μ) using linear gradient up to the ionic strength 0·6μ, and after this using linear gradient up to the concentration of 0·2 N sodium hydroxide. By means of chromatography of the first fraction following the gel filtration eight sub-fractions were obtained, five of which contained hexosamine and hexoses (Fig. 2).

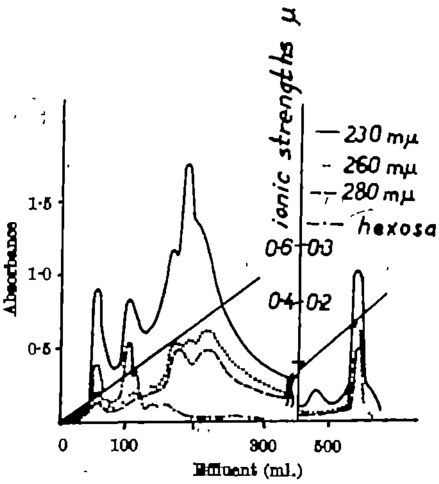

Fig. 2. Chromatogram of fraction I on the DEAE-cellulose column

By means of chromatography also the second fraction could be separated into eight sub-fractions, only the first of them, however, containing both hexosamine and hexoses (Fig. 3). By means of repeated chromatography of this fraction containing hexosamine on the DEAE-cellulose at *p*H 7·0 three fractions could be obtained.

The material isolated using the Marcotte-Boy technique is thus a varied mixture of different substances. Approximately two-thirds of the hexosamine is contained in non-dialysable substances (molecular weight 8,000–50,000) and can be divided into five fractions. The remaining one-third of hexosamine is in the form of dialysable substances, probably having a molecular weight lower than 5,000.

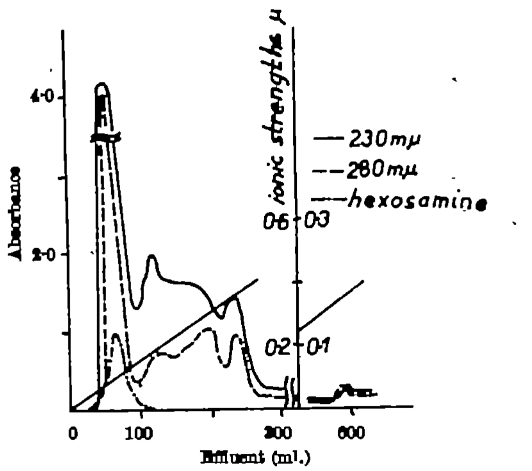

Fig. 3. Chromatogram of fraction II on the DEAE-cellulose column

Detailed chemical composition of these fractions is now being investigated.

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Changes in Hydroxyproline-containing Fractions in the Developing Chick Embryo

DURING recent years increasing attention has been paid to free and peptide-bound hydroxyproline in order to explain their role in the metabolism of collagen[1-10]. Since chick embryos contain relatively large amounts of free hydroxyproline at least[1,11-13], it seemed worth while to follow the contents of various hydroxyproline containing fractions during the development of the chick embryo.

The following four fractions were analysed: (1) free hydroxyproline; (2) peptide-bound hydroxyproline soluble in 80 per cent ethanol; (3) hydroxyproline soluble in 1 M sodium chloride, insoluble in ethanol, designated as soluble collagen hydroxyproline; (4) total hydroxyproline. In addition, the amount of insoluble hydroxyproline could be calculated from these four values. The values for cortisone-treated embryos in the same fractions are given in another preliminary report[14].

The amount of peptide-bound hydroxyproline was calculated from the difference of analyses of the ethanol-soluble fraction before and after hydrolysis. Hydroxyproline was determined by the method previously described[15], with some modifications, except for the soluble-collagen fraction, for which the method of Prockop and Udenfriend[16] was used, since the amount of hydroxyproline in this unpurified fraction is very low as compared with that of other substances. The latter method[16] was also used for all fractions from embryos up to the 8th day of development. The error of the methods of determination was about ± 3 per cent. Up to the 10th day of development each symbol in the figures is a mean value of three pooled samples, thereafter a mean value of 6–18 embryos, analysed separately.

The amount of hydroxyproline in various fractions expressed per unit of dry tissue is presented in Fig. 1 (upper and lower). The amounts of free and soluble collagen hydroxyproline increase to maximum values on the 14th day of development. Thereafter the content of free hydroxyproline decreases rapidly, but the content of soluble-collagen hydroxyproline only slightly. In contrast to the free hydroxyproline, peptide-bound hydroxyproline was found to be present in very low concentrations. A tendency to increase is to be seen during embryonic development, and by the 20th day peptide-bound hydroxyproline forms a noteworthy part of the total ethanol-soluble hydroxyproline. The values are in agreement with those presented for acid-extractable peptides in 10- and 17-day-old chick embryos[13].

Since substances other than collagen compose the bulk of the dry weight used as basis for Fig. 1, the values are given as a percentage of the total hydroxyproline in Fig. 2 (upper and lower). The amount of insoluble hydroxy-

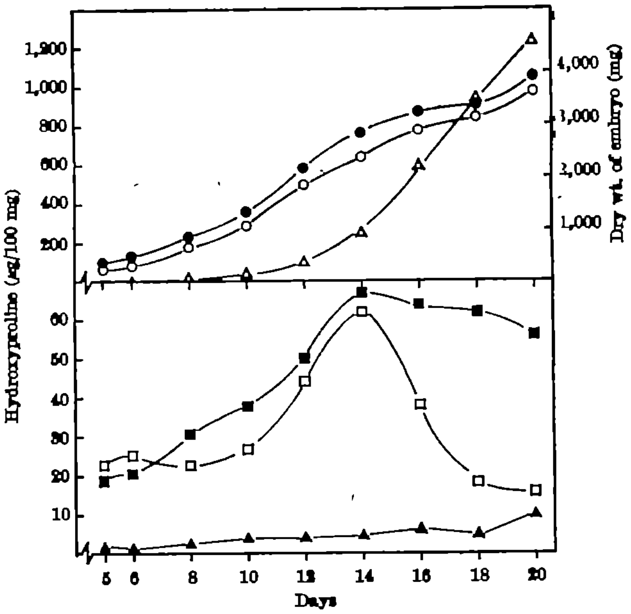

Fig. 1. Hydroxyproline in various fractions expressed per unit of dry matter of the embryo. ●, Total; ○, insoluble; ■, soluble collagen; □, free; ▲, peptide-bound. In addition, in the upper graph, the absolute amount of dry matter per embryo is shown on the ordinate on the right, △

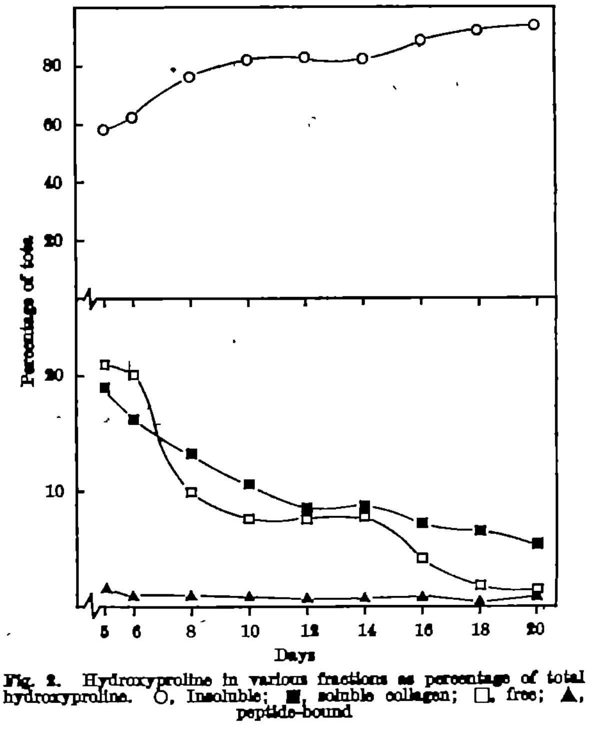

Fig. 2. Hydroxyproline in various fractions as percentage of total hydroxyproline. ○, Insoluble; ■, soluble collagen; □, free; ▲, peptide-bound

proline increases continuously during development. Nevertheless, even on the 5th day of development, 58 per cent of the hydroxyproline is in insoluble form. It is of interest that in young embryos not only soluble collagen but also free hydroxyproline constitute a noteworthy part of the total hydroxyproline. Similar values for free hydroxyproline have also been reported recently during the early development of carrageenin granuloma[4,6] and fibroblast cultures[10]. It has been generally accepted that administered hydroxyproline is not utilized for collagen synthesis and that the collagen hydroxyproline is derived from proline that is hydroxylated in bound form[17]. Therefore the part played by free and peptide-bound hydroxyproline in the metabolism of collagen is of interest. Recent work suggests that the ultra-filtrable hydroxyproline present in tissues during the early development of collagen represents a precursor of collagen[4,8,10]. It is evident, too, that part of the urinary hydroxyproline is derived from collagenic precursors[5,6,7]. The present work, likewise, suggests that in young chick embryos free hydroxyproline may be derived from precursors of insoluble collagen.

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Oxidation of β-Carotene: Formation of Vitamin A Acid

Although Moore[1] had demonstrated that β-carotene was transformed into vitamin A in the animal body, the actual process of this transformation has not as yet been fully understood. There are two views: one postulating oxidation of β-carotene at the central double bond, with the formation of, at first, two molecules of retinene[2,3] (vitamin A aldehyde), which are immediately reduced to give two molecules of vitamin A alcohol; the other claiming that if an excentric bond be attacked it would give only one molecule of vitamin A, resulting from the further degradation of the larger fragment. Moore[4] has given an outline of the evidence for and against these views.

The oxidation products of β-carotene therefore are of interest as they can help in giving an insight into the problem of provitamin - vitamin A transformation. A number of investigations in the oxidation of β-carotene, has been reported. When β-carotene was oxidized with chromium trioxide, semi-β-carotenone and β-carotenone were produced[5]. When, however, alkaline permanganate was used β-apocarotenals were formed[6]. Hunter and Williams[7] oxidized β-carotene with hydrogen peroxide in chloroform–acetic acid medium and found retinene as one of the oxidation products which gave vitamin A alcohol on reduction, the yield being only 0·4–0·5 per cent. With osmium tetroxide as catalyst a moderate yield of 30 per cent was reported[8,9]. Meunier[10] reported formation of vitamin A aldehyde, retinene hydrate, and vitamin A alcohol from carotenoids, especially from β-carotene by oxidation with various metallic oxides, namely, TiO_2, V_2O_5, Fe_2O_3. His reported yield of about 95 per cent was, however, disputed by Sebrell and Harris[11], who were unable to reproduce Meunier's results.

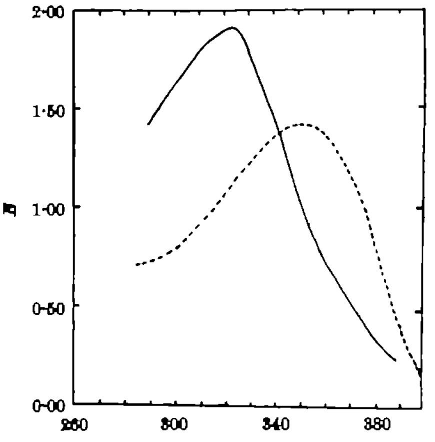

Fig. 1. Broken line, absorption spectrum of vitamin A acid obtained by oxidation of β-carotene and subsequent chromatography; solid line, absorption spectrum of vitamin A alcohol obtained after reduction of vitamin A acid followed by chromatography

In none of the foregoing oxidation investigations, however, was the formation of vitamin A acid reported from β-carotene. In an investigation carried on in this laboratory, it has been possible to obtain vitamin A acid as one of the products of oxidation of β-carotene. When β-carotene in petroleum ether or ether solution was kept over a mixture of V_2O_5/MnO_2 (5 : 2) for about 80–90 h the solution changes to a greenish yellow colour. This was then chromatographed on a column of deactivated alumina[12] (8 per cent water), when three zones separated at the top. The first, second and the third zones gave on extrusion and elution with ethanol, spectra with $\lambda\lambda_{max}$ at 335 mμ, 285–287 mμ, and 350–351 mμ, respectively (solvent–ethanol). The third zone has spectral characteristics corresponding to vitamin A acid (λ_{max} at 347 mμ in petroleum ether, 350–351 mμ in ethanol). With $SbCl_3$, it gave a purple colour having λ_{max} at about 570 mμ. The acid was then selectively reduced and chromatographed on deactivated alumina, when it gave a product spectroscopically identical with vitamin A alcohol (λ_{max} at 325 mμ in ethanol, and with $SbCl_3$ gave a blue colour with λ_{max} at 618 mμ). Fig. 1 shows the absorption spectra in ethanol of vitamin A acid (shown with broken line) and that of vitamin A alcohol obtained after reduction and chromatography (shown with unbroken line).

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A Significant Variable in the Rat Hemidiaphragm Assay of Insulin

While using the rat hemidiaphragm method for assay of insulin[1,2], it was noticed that the right hemidiaphragm, when incubated in the presence of insulin (2 milliunits/ml.), takes up significantly more glucose per unit weight, per unit time than the left hemidiaphragm. The basal uptake (without insulin) of the right hemidiaphragm was slightly more than the left, but the difference was not significant. The combined results of seven separate experiments are shown in Table 1.

Table 1

	Basal			Insulin 2 m.units/ml.		
Hemidiaphragm	No. of hemidiaphragm	Weight of hemidiaphragm (mg) ± *S.E.*	Glucose uptake/ g wet diaphragm/ 90 min ± *S.E.*	No. of hemidiaphragm	Weight of hemidiaphragm (mg) ± *S.E.*	Glucose uptake/ g wet diaphragm/ 90 min ± *S.E.*
Right	24	80 ± 1·7	2·56 ± 0·15	24	80·4 ± 1·7	5·22 ± 0·23
Left	17	72 ± 2·3	2·32 ± 0·12	31	72·5 ± 1·5	4·04 ± 0·25
Difference and significance		R > L $P < 0·01$	None $P = 0·3$		R > L $P < 0·01$	R > L $P < 0·001$

Table 2

Comparison	No. of hemidiaphragm	Correlation coefficients (*r*) and significance		Regression coefficients (*b*)		
		Right hemidiaphragm	Left hemidiaphragm	Right hemidiaphragm	Left hemidiaphragm	Difference and significance
Wet weight versus dry weight	30	0·97 $P < 0·001$	0·98 $P < 0·001$	4·00	3·64	None $0·2 > P > 0·1$
Wet weight versus non-collagen N_2	30	0·87 $P < 0·001$	0·93 $P < 0·001$	5·46	4·70	None $0·2 > P > 0·1$

Table 3

Experiment	Hemidiaphragm	No. of hemidiaphragm	Weight of diaphragm ± *S.E.*	Glucose uptake of hemidiaphragm (2 m.units insulin/ml.)/100 mg dry diaphragm/90 min ± *S.E.*	Difference in uptake and significance
Left hemidiaphragm removed before right	R	8	17·1 ± 0·4	2·04 ± 0·08	R > L $P < 0·02$
	L	8	15·2 ± 0·3	1·75 ± 0·07	
Left phrenic nerve cut first	R	8	17·1 ± 0·75	3·03 ± 0·15	R > L $P < 0·02$
	L	8	15·4 ± 0·8	2·45 ± 0·13	

The diaphragms were all dissected out in a standard manner from the ventral aspect. The right hemidiaphragm was always removed before the left and cuts were always made through comparable areas of muscle. The left phrenic nerve was always sectioned after removal of the left hemidiaphragm.

The following factors which might contribute to the difference in uptake of glucose between right and left hemidiaphragms have so far been investigated:

(1) The left hemidiaphragm might hold more water or contain less muscle than the right hemidiaphragm. It has been shown that (Table 2): (*a*) The wet weight of the diaphragm correlates very significantly both with the dry weight (dried at 110° C for 2 h) and with the non-collagen nitrogen content of the diaphragm. The latter[3] has been shown to correlate with the muscle content of a tissue such as the rat diaphragm. (*b*) The regression coefficient for the correlations of: (i) wet weight versus dry weight; (ii) wet weight versus non-collagen nitrogen of right and left hemidiaphragms do not differ significantly from each other.

Thus there is no evidence that right and left hemidiaphragms differ in their water or muscle content.

(2) In the foregoing experiments, the right hemidiaphragm and right phrenic nerve were always cut before the left hemidiaphragm and the left phrenic nerve. Metabolites such as corticosteroids or adrenaline might accumulate in the left hemidiaphragm more than the right and lead to antagonism of the effect of insulin. Buse and Buse[4] have shown that section of the phrenic nerve hours or days before use, can lead to decreased sensitivity of the diaphragm to insulin. It is conceivable that discharges from the sectioned spinal cord down the phrenic nerves might affect the diaphragm longer in contact with its nerve supply. Such effects should be altered by: (*a*) removal of the left diaphragm before the right—any metabolites will now affect the right diaphragm more than the left; (*b*) section of the left phrenic nerve before cutting the right phrenic nerve and hemidiaphragm.

Table 3 shows the results of these two experiments. These procedures do not affect the original pattern of the right hemidiaphragm taking up more glucose, in the presence of insulin, than the left. The difference does not therefore depend on the technique of removing the diaphragm. The possibility that the right and left hemidiaphragms have a different surface area to weight ratio is being explored.

A similar difference between right and left hemidiaphragms was described by Mann[5]. The difference was noted only with smaller concentrations of insulin and under basal conditions. With higher concentrations of insulin, in the range of the experiments recorded here, the difference between the two sides of the diaphragms were minimal or absent. The existence of this difference when concentrations of about 2 m.units insulin/ml. are used is important, as such concentrations are commonly used experimentally. The difference will increase the variability of any insulin assay and may bias results if unequal numbers of right and left hemidiaphragms are used in any one assay.

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Œstrogens and Progesterone in Ovaries of the Marine Teleost *Conger conger*

RESULTS of biological assays have shown *Conger conger* ovary[1] as well as other teleost ovaries[2-4] to contain œstrogenic material. No indication of the chemical structure of the steroids present in the ovaries of these fishes has so far been ascertained. Only recently œstradiol-17β has been identified in the ovarian extracts of *Salmo irideus* and œstradiol-17β, œstriol and 16-epi-œstriol in the ovarian extracts of *Cyprinus carpio*[7].

In order to clarify this point, ovaries of the marine teleost *Conger conger* were extracted according to the method of Dean and Chester Jones[8]. From 740 mg of fresh ovarian tissue, obtained from one sexually mature female, 30 mg of neutral material and 75 mg of phenolic material were isolated.

The neutral fraction was resolved by paper chromatography in systems developed by Bush[9] and Zaffaroni[10]. The first chromatography by the Bush *C* system showed the presence of a spot absorbing in the ultra-violet region, the R_F of which was the same as pure progesterone. This spot was eluted and chromatographed successively by two systems (Zaffaroni and Bush *A*); the R_F of this component corresponded in both systems to that of progesterone. The steroid material was eluted and at this stage of purification showed an absorption maximum at 240 mμ. It was again chromatographed by the Bush *A* system, and, likewise pure progesterone, showed a positive Zimmermann reaction on the paper.

According to these findings, therefore, this component is progesterone.

On the basis of the ultra-violet absorption curve, progesterone was calculated to be present in the amount of approximately 15 μg/kg of fresh tissue.

The phenolic fraction was resolved by paper chromatography in 1 : 1·75 per cent methanol–toluene system. In order to detect the position of the œstrogens, 0·5 cm of the paper strip (total width, 1·5 cm) was cut and sprayed with a solution of 1 per cent ferric chloride and 1 per cent $K_3(Fe(CN)_6)$ (ref. 11). The colour reaction which followed this treatment showed the presence of œstriol and œstrone in amounts, as it appeared from semi-quantitative estimations, of about 50 and 80 μg/kg respectively. Traces of œstradiol-17β were also present.

The remaining part of the strip was eluted; the eluates of the zones corresponding to the three œstrogens were acetylated for 3 h by means of acetic anhydride and pyridine. The acetylated derivatives were chromatographed in 1 : 1 benzene – 80 per cent methanol system. After accomplishing hydrolysis *in situ* with ammonia the spots were detected by the colour reaction already described. They showed the same R_F as the authentic pure œstrogen derivatives.

These results show a similarity between the œstrogens secreted by the teleost *Conger conger* ovary and those observed in the case of both mammalian and non-mammalian vertebrates. So far as fishes are concerned, œstrone (not quantitatively), œstradiol-17β (120 μg/kg) and progesterone (although not conclusively) have been identified in the ovaries of the elasmobranch *Squalus suckleyi*[12]; only œstradiol-17β (not quantitatively) has been identified in the ovaries of *Salmo irideus*[7] and œstriol, 16-epi-œstriol and œstradiol-17β (not quantitatively) in the ovaries of *Cyprinus carpio*[7]; while œstriol (700 μg/kg), œstradiol-17β (traces), œstrone (70 μg/kg) and progesterone (60 μg/kg) have been detected in the ovaries of the lungfish *Protopterus annectens*[6].

The only differences existing between these species of fishes appear to be the absence of œstriol in the ovarian extracts of *Squalus suckleyi* and *Salmo irideus* and of œstrone in those of *Salmo irideus*, and the discrepancies in relative concentrations of the various œstrogens. The latter could be due to different concentration of the enzymes involved in the metabolism of the œstrogens.

The presence of progesterone in a teleost, as well as in other fishes, raises the question of its role in the reproductive behaviour of lower vertebrates.

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Species Difference in Regard to the Distribution of Glucose-6-phosphatase

THE enzyme glucose-6-phosphatase cannot be classified as either acid phosphatase or alkaline phosphatase. It is highly specific and is differentiated from non-specific acid and alkaline phosphatases on the basis of location and behaviour. So far, no systematic study has been made regarding the distribution of this enzyme in different species of animals. Hers and de Duve[1] first reported that in rats it is abundant in the liver, but only present in small amounts in the kidney; it is, however, absent in the intestine and not present in detectable amount in other organs and blood. But afterwards they contradicted their own previous finding and showed that glucose-6-phosphatase was also present in the intestine. The present communication concerns an investigation of the distribution of this enzyme in different species of animals.

The results presented in Table 1 show that in most of the species the glucose-6-phosphatase activity is concentrated mainly in the liver and to some extent in the kidney. The brain and testes have little, if any, glucose-6-phosphatase activity. In mammals like mouse, rat and goat both the liver and the kidney have more or less similar activity. It is interesting to note that in lower forms of animals, namely, toad, tortoise and fish, the kidney tissues possess a very little glucose-6-phosphatase activity. It also

Table 1. DISTRIBUTION OF GLUCOSE-6-PHOSPHATASE IN TISSUE HOMOGENATES OF DIFFERENT SPECIES OF ANIMALS

The test system contains: 0·2 ml. of 0·1 M glucose-6-phosphate, 0·55 ml. of maleate buffer (0·1 M, *p*H 6·5) and 0·25 ml. of enzyme dispersion (homogenate or microsomes as the case may be) in a total volume of 1 ml. At the end of the incubation period (30 min at 37°), 2 ml. of 10 per cent trichloroacetic acid was added. After keeping for a few min in ice, the samples were diluted to 10 ml. and centrifuged. An aliquot (0·5 ml.) was used for the determination of phosphate by the method of Chen *et al.* (ref. 5)

Natural order	Name of the species	Activity of glucose-6-phosphatase in units*				
		Liver	Kidney	Intestine	Brain	Testis
Amphibia	Tortoise	35·0	1·3	1·1	1·8	—
	Toad	36·2	8·6	3·8	1·1	—
Pisces	Mrigal (*Cirrhina mrigala*)	19·0	3·1	1·4	2·8	—
Aves	Chick	38·1	38·8	2·3	2·4	2·2
	Pigeon	34·1	34·2	2·1	1·8	3·2
Mammalia	Mouse	47·5	36·6	1·6	1·8	2·2
	Rat	46·1	48·3	8·5	4·5	5·0
	Goat	41·8	51·8	—	2·9	—
	Guinea pig	23·6	23·4	13·2	2·8	4·2
	Rabbit	18·6	19·1	10·5	1·8	3·6

In one invertebrate species studied, namely, crab, the hepatopancrease homogenate contains only a trace of glucose-6-phosphatase activity, but the microsomes obtained after sedimentation at 88,700*g* for 60 min have been found to possess a fair activity of the enzyme.

* 1 unit = μg of P_i/mg protein/30 min.

appears from the results given in Table 1 that in amphibians and in fish, the liver tissue possesses the maximum glucose-6-phosphatase activity whereas in the avian species and in mammals both the liver and the kidney were almost equally active. It has been observed that intestines of rat, guinea pig and rabbit possess a small activity of glucose-6-phosphatase.

In one invertebrate species examined, namely, crab, it has been found that though the hepatopancreas homogenate possesses a trace amount of activity, the microsomes obtained from the hepatopancreas homogenate contain a fair amount of activity. The reason why the hepatopancreas homogenate of the crab possesses such a low activity is not clear.

In studying the activity of the microsomal fractions sedimented under different centrifugal forces it has been observed that the fraction sedimented at 30,000*g* for 60 min, which was mainly composed of the heavier microsomes, possesses the highest activity in the case of the livers of the rat and goat. A similar finding has also been observed in the case of rat kidney; de Duve *et al.*[4] also reported previously that glucose-6-phosphatase activity resides mainly in the microsomal fraction of the rat liver separated by centrifugation at 20,000*g* for 60–90 min. Regarding the different enzymatic activities of the heavier and the lighter microsomes it has very recently been shown by Kar *et al.*[3] that the heavier microsomes sedimenting at 26,390*g* for 60 min are more active than the lighter microsomal fractions sedimenting at 59,380*g*, 88,700*g* and 109,500*g*, 60 min each time, respectively, in regard to the synthesis of L-ascorbic acid in rat liver.

The microsomal glucose-6-phosphatase can be rendered soluble by treatment with a variety of surface-active agents like sodium deoxycholate, 'Triton *X*–100', sodium dodecyl sulphate and 'Duponol'.

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Accumulation of Ribonucleic Acid in *Escherichia coli* due to the Action of 5-Methyl Tryptophan

In bacteria, the syntheses of protein and ribonucleic acid (RNA) are normally closely linked but, under certain conditions, protein synthesis may be slowed down or stopped relative to the synthesis of RNA, so that RNA accumulates within the cells. With *Escherichia coli* this may be achieved by incubation of cells with chloramphenicol[1] or puromycin[2] and also when a certain methionine-requiring mutant strain is starved of this amino-acid[3]. It has been shown that in each of these cases[4–6] at least a part of the accumulated RNA appears in the cells in the form of ribonucleoprotein (RNP) particles which sediment more slowly than ribosomes, at about 14–18*S*. It has been suggested[6] that although these particles have some of the properties claimed for 'messenger' RNA[7], they are more likely to be related to precursors of the ribosomes.

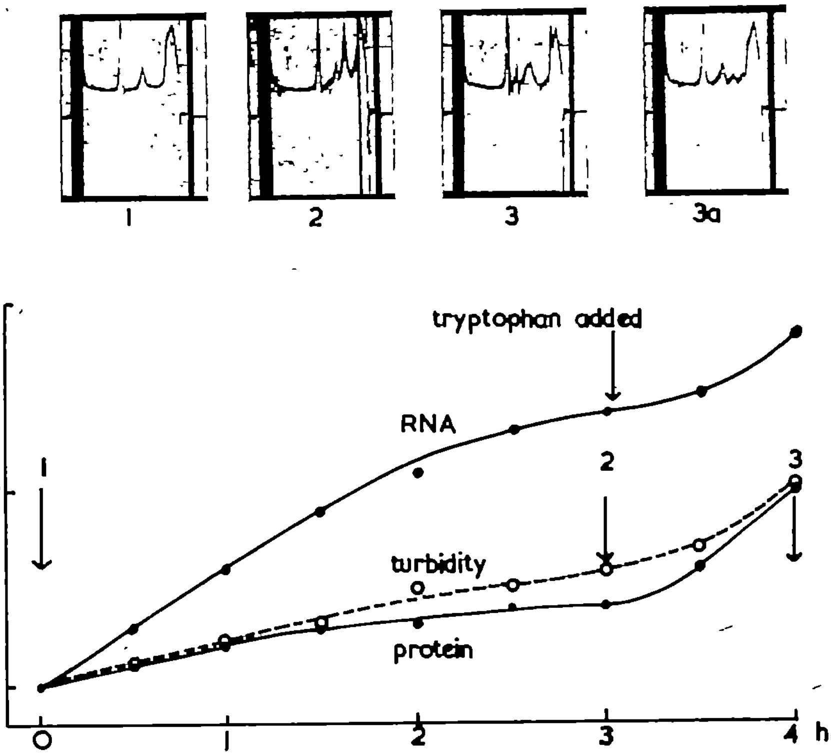

Fig. 1. Ribonucleic acid content of a methionine-requiring mutant of *E. coli*. At zero time, 5-methyl tryptophan (50 μg/ml.) was added to a culture of the bacteria growing exponentially in an aerated glucose and mineral salts medium containing methionine. Curves show the increases in turbidity, RNA and protein of the culture expressed as multiples of initial values. L-Tryptophan (100 μg/ml.) was added after 3 h. Cell-free extracts were prepared at times indicated by arrows and the corresponding Schlieren diagrams are shown. Photograph (3a) shows the extract in photograph (3) after treatment with 1 μg/ml. RNase. Sedimentation at 187,000*g* is to the left and the photographs were taken 16 min after reaching speed. The leading peaks in every photograph are those caused by the 50*S* and 30*S* ribosomes

The mutant strain in which the control of RNA synthesis is relaxed has also been investigated by Stent and Brenner[8]: they found that the ability of the cells to synthesize RNA in the absence of protein synthesis is genetically determined and that, by suitable conjugation experiments, mutants can be obtained which require amino-acids other than methionine and which synthesize RNA in the absence of this 'new' amino-acid requirement. We have now found that if the tryptophan analogue, 5-methyl tryptophan, is added to growing cultures of the 'relaxed' methionine-requiring mutant, growth and protein synthesis are inhibited but considerable synthesis of RNA (and DNA) occurs. In the experiment in Fig. 1, after 3-h incubation with 5-methyl tryptophan, the protein content of the cells had increased by 35 per cent, the RNA content by 140 per cent. If extracts are prepared from inhibited cells and examined in the analytical ultracentrifuge, then additional boundaries sedimenting at 14–18*S* are once again seen (Fig. 1).

In similar experiments with a second methionine auxotroph of *E. coli* which does not synthesize RNA when starved of methionine, inhibition of growth can also be brought about by 5-methyl tryptophan, but there is comparatively little 'extra' RNA synthesis and no new boundaries can be seen in ultracentrifuge diagrams. It therefore seems likely that the production of 14–18*S* material by the action of 5-methyl tryptophan may be confined to the 'relaxed' strains.

When tryptophan is added to a culture of the 'relaxed' mutant which has been inhibited by 5-methyl tryptophan, the cells retain their accumulated RNA as growth and protein synthesis slowly recommence and interesting changes can be observed in ultracentrifuge diagrams (Fig. 1). The 14–18*S* components now sediment very close to the 30*S* ribosomes and an additional small boundary which sediments at about 40*S* appears. Like the 14–18*S* material, these new components are extremely sensitive to RNase. In normal *E. coli* cells, particles sedimenting at about 30 and 40*S* have been implicated in the synthesis of ribosomes[9]. We may therefore be observing in these experiments further stages in ribosome synthesis from 14–18*S* precursors.

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PHYSIOLOGY

Transmission of Noradrenaline across the Human Placenta

Fœtal bradycardia during labour is usually taken to be a sign of 'fœtal distress', its occurrence being interpreted as a consequence of fœtal hypoxia. Beard[1] has recently considered the possibility of an endogenous humoral agent such as noradrenaline in the maternal circulation contributing to, or causing, this type of fœtal response. After injecting noradrenaline into the pregnant human subject, he noted a transitory fœtal bradycardia but was not able to decide from his results whether this effect stemmed from uterine vasoconstriction with subsequent fœtal hypoxia or from a direct action of noradrenaline on the fœtal cardiovascular system.

It seemed important to try to elucidate this point. Although the administration of radioisotopically labelled noradrenaline to the mother followed by direct detection of activity in the fœtal blood appeared to offer the best chance of obtaining this information, it was not considered justifiable to perform such an experiment in the presence of a normal fœtus. Procedures have thus been confined to clinical circumstances incompatible with fœtal survival; choice and availability of clinical material have of necessity been limited.

Following intravenous injection of ^{14}C-DL-noradrenaline into the mother, the labelled noradrenaline content of serum deriving from maternal venous, umbilical venous (afferent) and fœtal heart blood was measured (Table 1). In all cases, the placenta appeared to be normal for the stage of pregnancy.

Although there was wide variation, appreciable amounts of labelled noradrenaline crossed the placenta to appear in blood flowing from the placenta to the fœtus. During the passage of this blood through the fœtus, almost all labelled noradrenaline had disappeared from the circulation, presumably by a combination of tissue binding and metabolic change.

In certain circumstances, intravenous noradrenaline will give rise to bradycardia in the human new-born infant[3]. It would be reasonable therefore, in the light of our results, to interpret Beard's findings[1] as indicating that fœtal bradycardia following noradrenaline injection into the mother may be due, in part at least, to a direct action of noradrenaline on the fœtal circulatory system. One proviso, however, must be mentioned; the ^{14}C-noradrenaline used in these experiments was a racemic DL mixture. Even though it is likely that the inactive D- and the physiologically active L-isomers are metabolized similarly[4], we have no information as to whether there is any preferential transfer of either across biological membranes. Final verification of this point must await the availability of radioisotopically labelled L-noradrenaline.

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Table 1

Clinical features	Duration of pregnancy (weeks)	Maternal serum	Cord serum		Fœtal heart serum	
		C.p.m./1 ml.	C.p.m./1 ml.	Percentage of maternal count	C.p.m./1 ml.	Percentage of maternal count
Hydrocephalic fœtus	37	1,925	200	10	Nil	—
Anencephalic fœtus	38	314	Specimen lost		4	1·3
Termination of pregnancy by hysterotomy (rubella in pregnancy)	24	417	76	18	9	2·2
Termination of pregnancy by hysterotomy (psychiatric reason)	15	640	13	2	4	0·6

β-[^{14}C]-DL-Noradrenaline (5 μc.) was injected intravenously into the mother over a period of 2–5 min immediately before delivery. Immediately after delivery specimens of blood were collected as quickly as possible from the venous circulation of the mother, from the placental end of the umbilical cord and from the fœtal heart, either simultaneously or within a few minutes of each other. In every case collection of blood was completed within 10 minutes of the end of the injection. Serum was separated without delay.

Noradrenaline was assayed by a modification of the methods of Weil-Malherbe (ref. 2). The *p*H of the serum was adjusted to 8·4 and, without protein precipitation, was passed through a small column of alumina on to which the noradrenaline was absorbed. After washing with *p*H 8·4, 0·2 M acetate buffer, the column was eluted with 0·2 N hydrochloric acid. The eluate was evaporated to dryness, and dissolved in 0·1 ml. water, to which 4 ml. methanol and 16 ml. liquid scintillator (1 l. toluene + 0·8 g 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene + 5 g 2,5-diphenyloxazole, were added. Radioactivity was determined in a 'Tri-Carb' liquid scintillation spectrometer. Each sample was counted for a length of time sufficient to give a standard error of ± 1 per cent.

Presence of a Phosphatido-peptide Fraction in Liver Cell Membrane and its Possible Role in the Active Transport of Amino-acids

Previous work in this laboratory has shown that a phosphatido-peptide fraction becomes promptly labelled during the perfusion of the isolated rat liver with rat blood containing labelled amino-acids, and reaches a maximum in its radioactivity a few minutes after the start of the experiment[1–3]. The reaction is strongly inhibited by metabolic inhibitors, such as ethionine, potassium cyanide and 2,4-dinitrophenol. It was also shown that the phosphatido-peptide contained in the 1,000 × g fraction of liver homogenates, which contains at least a large part of cell membranes[4], incorporates the radioactivity more rapidly than that contained in the mitochondrial or in the microsomal fraction and in the 105,000 × g supernatant. The radioactivity bound to the phosphatido-peptide fraction was released after incubation with a liver cathepsin preparation; other proteolytic enzymes so far tested were devoid of such a property.

The results have been interpreted assuming that the phosphatido-peptide fraction may play a part in the active transport of labelled amino-acids and, as a working hypothesis, such a transport was depicted as follows:

$$\text{extracellular amino-acids} \xrightarrow{\text{ATP}} \text{phosphatido-peptide} \xrightarrow{\text{cathepsin}} \text{intracellular amino-acids}$$

This scheme recalls that described by Hokin and Hokin[5], assuming a role of phosphatidic acid in the active transport of cations and hydrophylic secretory materials.

As a first attempt to confirm our hypothesis, the chemical composition of rat liver cell membranes and the incorporation of labelled amino-acids were investigated. Liver cell membranes were isolated by Neville's method[4]. Electron microscope examination, kindly performed by Prof. Pernis, Department of Industrial Medicine, University of Milan, showed that our preparation consisted essentially of cell membranes with the exception of very few dense granules. For each preparation at least two rat livers (16–24 g) were used, and the yield was 10–16 mg dry weight of cell membranes.

The membranes were extracted twice with 5 ml. of cold acetone and twice with 5 ml. of ethanol/diethylether (3 : 1) for 10 min at 50° C. The insoluble residue was next subjected to two extractions with 5 ml. of the mixture chloroform/methanol/hydrochloric acid (200 : 100 : 1, v) and the protein residue washed with diethylether and dried at 50° C. All the operations were carried out in conical centrifuge tubes.

Table 1. COMPOSITION OF RAT LIVER CELL MEMBRANE

Constituent	Percentage of dry weight
Proteins	59·6
Acetone and ethanol-diethylether soluble lipids	22·1
Phosphatido-peptide fraction	18·3

Each value is the average of the analyses performed on five different preparations.

The results are reported in Table 1. It should be noted that the composition of liver cell membrane is very similar to that of blood red cell membrane[6]. The extract obtained with the acidified mixture of chloroform–methanol was taken as the phosphatido-peptide fraction[3]; the presence of a bound peptide was indicated by the increase of amino-nitrogen from 0·89 to 2·38 per cent after hydrolysis for 24 h with 6 N hydrochloric acid. A chromatographic examination showed that acid hydrolysates contain most of the common amino-acids, with the apparent exception of cysteine, tyrosine and tryptophan. All the analyses of the phosphatido-peptide fraction were performed after drying the extract obtained from the membranes at 50° C under a nitrogen stream and re-extracting the residue with chloroform/methanol (2 : 1). Usually none, or a negligible amount, remained undissolved.

The isolated rat liver was perfused as already described[2] with heparinized rat blood containing 20 μc. of algal protein-^{14}C hydrolysate; at intervals of 3–12 min the experiment was stopped and the liver rapidly perfused with cold physiological saline. The liver was then homogenized with cold bicarbonate solution (0·001 M) brought to pH 7·4 and centrifuged 10 min in the cold at 1,500 × g. The membranes were then separated from the sediment[4]. Thereafter, 10 per cent trichloroacetic acid (TCA) was added to both the membranes and the 1,500 × g supernatant and the precipitates centrifuged and treated as previously described[3] for the separation of lipid fractions and proteins and the determination of their radioactivity.

It was thus observed that the radioactivity of the membrane phosphatido-peptide rose very rapidly, reaching a maximum within 3 min, but thereafter diminished; however, the proteins both of the 1,500 × g supernatant and of the membrane gradually took up the labelled amino-acids (Fig. 1). The total radioactivity taken up by the liver rose sharply at the beginning of the experiment and thereafter rose at a reduced rate. The values obtained after 3, 6 and 12 min were respectively 82,000, 119,000 and 143,000 c.p.m. for the sum of radioactivity of the TCA-soluble and TCA-insoluble fraction.

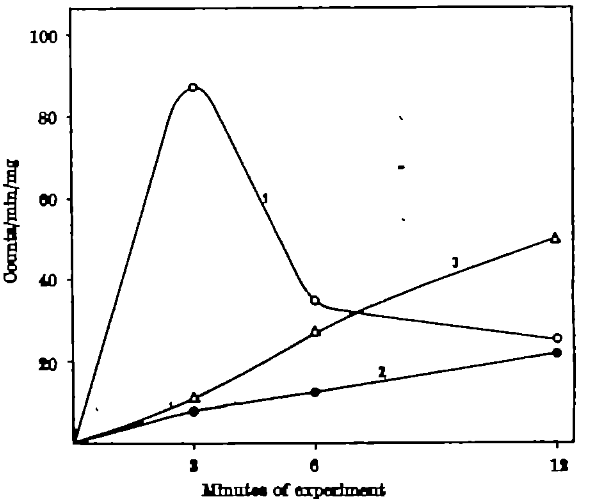

Fig. 1. Time-course of the incorporation of labelled amino-acids (algal protein-^{14}C hydrolysate) into the phosphatido-peptide fraction of cell membrane (curve 1), into the proteins of cell membrane (curve 2) and into the proteins of 1,500 × g supernatant (curve 3)

The curve in Fig. 1 relative to the membrane phosphatido-peptide is closely similar to that obtained by Hunter and Goodsall[7], when studying the incorporation of labelled amino-acids into the lipids of protoplasts of *B. megatherium*.

Such a behaviour of the membrane phosphatido-peptide seems to support its possible role in the active transport of labelled amino-acids in liver cells. The second assumption of our hypothesis, the role of cathepsin, is under investigation; the presence of these enzymes in cell membrane has been known since 1953[8]. We believe at present that the phosphatido-peptide fraction fulfils at least many of the criteria listed by Heald[9] for a substance which takes part in the active transport of a molecule.

Very recently Hendler[10] suggested that lipid–amino-acid complexes are involved both in the transport of amino-acids and in the synthesis of proteins. Although our results are not sufficient to support the last proposal, we have found that the radioactivity bound to the phosphatido-peptide fraction is easily incorporated into the proteins of isolated rat liver mitochondria. Obviously, further experimentation is necessary to demonstrate a direct utilization of the peptide moiety of our lipid fraction for the synthesis of proteins.

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Effect of Adrenalectomy on Histamine Metabolism in the Rat

THE effect of adrenalectomy on the metabolism of histamine in the rat has been investigated by several workers, but with conflicting results. Though it has been established that after adrenalectomy the histamine contents of some rat tissues are raised[1–4], there is considerable discrepancy regarding how the rise is brought about and the conditions under which it occurs. For example, increased levels of tissue histamine in adrenalectomized rats have been attributed by different workers to: (1) increased uptake of histamine from the gut[2]; (2) increased rate of tissue histamine formation[3]; (3) increased ability of tissues to bind histamine[4]; (4) decreased activity of histaminase[1]. Some workers have reported that the increases in histamine-levels after adrenalectomy are greater if the animals are given water to drink than if they are given saline[1], while others have found that in animals given saline increases in the tissue levels of this amine do not occur[3].

The present experiments were performed in the hope of clarifying these discrepancies. Groups of 6 female rats of Wistar strain (120–180 g) were bilaterally adrenalectomized under ether anæsthesia. The animals received their normal food and either 0·9 per cent saline or drinking water, and were killed 4, 14 or 49 days after the operation. The histamine contents of various tissues were determined by the method of Parratt and West[6]. The results, each of which is the mean of the values from at least 3 experiments, are presented in Table 1.

Table 1. EFFECT OF ADRENALECTOMY ON THE HISTAMINE CONTENT OF RAT TISSUES

Tissue	Water 4 days	Saline 4 days	Saline 14 days	Saline 49 days
Abdominal skin	256	158	238	147
Skin of the feet	281	173	205	128
Ears	278	139	251	114
Fundic stomach	249	180	200	144
Pyloric stomach	333	229	320	120
Jejunum	220	156	201	121
Ileum	267	136	202	127
Colon	275	182	229	98
Liver	340	260	240	94
Kidney	406	271	325	113
Lung	285	162	300	100

The animals received their normal food and either saline or drinking water, and were killed 4, 14 or 49 days after the operation. All values are expressed as percentages of the control values obtained using unoperated or mock-adrenalectomized rats.

The results demonstrate two points. First, the increases in tissue histamine after adrenalectomy are as great in the skin and some other tissues as in the alimentary canal; the findings of some authors that major increases in histamine-levels are confined to the gastro-intestinal tract could not be confirmed[1,2,4]. Secondly, the increases are of similar magnitude in rats maintained on water and in rats maintained on saline. In rats maintained on water, the histamine contents of the tissues reach their highest values 4 days after the operation; in rats maintained on saline the values are highest 10–15 days after the operation and do not return to pre-adrenalectomy levels until 5–6 weeks later.

In a further series of experiments, the daily urinary excretion of histamine (μg free histamine/100 g body-weight/18 h) was followed before and after removal of the adrenals. No significant changes were noted, either in animals drinking water or in animals drinking saline. This result is in contrast with that of Bjurö and Westling, who have reported a two-fold increase in the excretion of histamine in adrenalectomized rats maintained on saline[5]. In the present work, however, the rats did not receive aminoguanidine to inhibit histaminase. The excretion of histamine was also measured in non-operated rats receiving cortisone, prednisolone or deoxycortone acetate (10 mg/kg daily for 9 days), and again no changes were noted.

Injections of glucocorticoids reduce the histamine content of the skin and small intestine, but increase the content of this amine in the pyloric stomach[7]. As is shown in Table 1, adrenalectomy likewise increases the histamine content of the pyloric stomach. While the changes in histamine-levels after glucocorticoids are probably due to changes in the rate of histamine formation[8], little or no alterations occur in the histamine-forming capacities of rat tissues after adrenalectomy. Thus, the histidine decarboxylase activities of the liver and pyloric stomach, the two major sources of this enzyme in the adult rat[9], are unaffected by adrenalectomy[9,10]. Schayer *et al.* have reported increases in the histidine decarboxylase activity of the skin and lung of adrenalectomized rats[11], but such increases are unlikely to account for the major and rapid changes in histamine-levels since these tissues contain only minute amounts of the enzyme, and are capable of forming only very small quantities of histamine.

It is more probable that the levels of tissue histamine are raised in the absence of the adrenals because of increased firmness in the binding of the amine to tissue components, coupled with a reduction in histaminase activity. It is known that after adrenalectomy the activity of this enzyme in the rat is reduced[9,12]. A final series of experiments showed that inhibition of histaminase activity is greater when the rats are maintained on water than when they are maintained on saline; it is possible that this latter finding accounts for the increases in the levels of tissue histamine occurring more rapidly in rats maintained on water.

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Influence of Pregnancy and an Oxidized Lipid Diet on the Fatty Acid Composition of Blood and Tissues

RECENTLY it has been shown that a diet which is low in vitamin E and which contains 5 per cent of oxidized lipid is capable of producing the generalized Shwartzman reaction in pregnant rats[1,2]. This reaction is characterized by an episode of disseminated intravascular coagulation and, in the past, has only been produced experimentally by intravenous injection of bacterial endotoxin. The fact that the only variant in the diet which was responsible for its lethal effect was the oxidized lipid plus the fact that an increase in stainable lipid could be demonstrated in the kidney, liver, and placentas of some animals led to an investigation of the distribution of fatty acids in the blood and tissues of these animals. It seemed appropriate to compare the fatty acid compositions of tissue lipids of pregnant and non-pregnant female rats fed the oxidized lipid diet (20 per cent casein, 61 per cent corn starch, 10 per cent brewers' yeast, 4 per cent salt mixture, and 5 per cent of the ethyl esters of a molecularly distilled fraction of oxidized cod liver oil) with those of similar rats fed a pellet stock diet containing approximately 5 per cent fat.

The rats were killed on the last day of pregnancy (or 21st day of experiment for the non-pregnant controls), and lipids were extracted from serum, perirenal fat, liver,

Table 1. PERCENTAGES OF PALMITATE AND LINOLEATE IN ORGAN LIPIDS OF NON-PREGNANT AND PREGNANT RATS

The rats were fed a stock ration (Rockland rat diet) or a vitamin E-low diet containing the esters of a fraction of oxidized cod liver oil. 'Pregnant, sick' denotes rats with the generalized Shwartzman reaction. Standard deviations are given for averages of at least three values; individual values are given when there were only two samples

Group	No. of samples	C 16 : 0	σ	C 18 : 2	σ
	Perirenal fat				
Stock diet:					
Non-pregnant	4	23·6	1·77	26·9	1·96
Pregnant	4	24·9	1·44	26·4	1·35
Oxidized cod liver oil esters:					
Non-pregnant	2	31·6	30·7 32·6	10 6	10·9 10·4
Pregnant, normal	2	25·2	25·1 25·4	14·7	14·0 15·4
Pregnant, sick	3	28·1	1·65	16·1	3·20
	Serum				
Stock diet:					
Non-pregnant	4	27·6	2·63	25·2	4·62
Pregnant	4	31·9	6·76	30·4	1·93
Oxidized cod liver oil esters:					
Non-pregnant	8	22·5	5·01	13·1	3·24
Pregnant, normal	7	36·9	4·76	13·1	3·19
Pregnant, sick	5	36·5	4·60	15·2	3·46
	Liver				
Stock diet:					
Non-pregnant	4	19 4	2·76	17·7	1·93
Pregnant	4	25 6	2·19	16·2	2·07
Oxidized cod liver oil esters:					
Non-pregnant	2	25·2	27·3 23·2	5·4	3·5 7·4
Pregnant, normal	2	34·5	34·5 34·5	11·6	12·2 11·0
Pregnant, sick	3	30·6	4·61	12 7	1·79
	Kidney				
Stock diet:					
Non-pregnant	4	25·6	0·41	20·9	1·15
Pregnant	4	24·2	1·89	18·7	1·40
Oxidized cod liver oil esters:					
Non-pregnant	2	25·0	24·1 26·0	7·3	3·7 11·0
Pregnant, normal	2	29·2	29·6 28·9	11·4	13·9 8·9
Pregnant, sick	3	32·6	2·98	10·5	2·74
	Placentas				
Stock diet	4	28·3	2·46	15·9	1·97
Oxidized cod liver oil esters.					
Normal	6	28·7	2·44	9 5	1·58
Sick	4	35·0	1·55	13·1	3·78

kidneys, and placentas with a mixture of 3 : 1 ethanol/ethyl ether. Fatty acid analyses were carried out on the methyl esters of these extracts on a Perkin-Elmer model 154C vapour fractometer having a 2 meter polydiethylene glycol succinate column at 220° C.

Palmitate- and linoleate-levels in these lipids are given in Table 1; the remaining data have been omitted because of limitations of space and because there were few significant differences. The linoleate-levels of all rats fed the fraction of oxidized cod liver oil were significantly lower than those of the rats fed the stock diet. In one series in which only the serum was examined, feeding of α-tocopherol to rats fed this diet did not raise the linoleate-level. It seems likely that the presence of oxidized fat, *per se*, is responsible for the lower levels.

Pregnancy brought about significantly higher levels of palmitate in serum and livers and also in the kidneys and placentas of the rats having the generalized Shwartzman reaction. This elevation in tissue palmitate-levels was more marked in the rats fed the oxidized cod liver oil diet.

Further control experiments were carried out on 3 non-pregnant and 2 pregnant rabbits fed a pelleted rabbit ration. The average palmitate values were: in the serum, 28·2 per cent in non-pregnant and 38·5 per cent in pregnant; in the fat, 27·5 per cent in non-pregnant and 36·7 per cent in pregnant; in the liver, 27·4 per cent in non-pregnant and 43·3 per cent in pregnant; kidneys, 24·3 per cent in non-pregnant and 27·2 per cent in pregnant. There was no overlapping of values except for one kidney value. The average palmitate-level in the placentas of these normal rabbits was 34·3 per cent. A rabbit given the generalized Shwartzman reaction by means of endotoxin had a serum palmitate-level of 45·7 per cent.

The variability of individual fatty acid-levels in rats can be seen in Table 2, which represents a compilation from many experiments of different kinds. The range of such levels are given for each sex without regard to tissue source and diet of the animals. The palmitate-levels of males and females are similar, and the coefficients of variation are the lowest of all acids.

Palmitate synthesis and tissue-levels would seem to be controlled within narrow limits but would appear to be mobilized under the stimulation of pregnancy, perhaps by hormones. The fact that, in most naturally occurring fats, palmitate in non-randomly distributed in the more labile 1,3 positions of the triglyceride molecule would suggest one reason why palmitate-levels increase as lipids are mobilized. That this may be a more generalized effect is suggested by studies in which X-irradiated rats had higher liver palmitate-levels than their non-irradiated controls.

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HÆMATOLOGY

In vitro Abnormality of the Blood in Von Willebrand's Disease correctable by Normal Plasma

VON WILLEBRAND'S disease is a familial hæmorrhagic disorder affecting both sexes and characterized by a prolonged bleeding time and a low level of antihæmophilic factor (AHF, factor VIII). The prolonged bleeding time is associated with abnormal *in vivo* platelet adhesiveness[1]. However, no *in vitro* abnormality has been detected in the factors believed to be essential for hæmostasis, such as platelet viscous metamorphosis[2] and aggregation of platelets by adenosine diphosphate (unpublished observation) and by collagen[3]. The platelet-aggregating ability of the patient's connective tissue is also unimpaired[3]. Recent reports have indicated that the abnormal bleeding time can be corrected by transfusion of fresh plasma or a plasma fraction[4–6]. This effect can only be tested in patients. Identification of an *in vitro* abnormality in von Willebrand's disease would facilitate purification of this plasma factor; it would also help to elucidate both the

Table 2. VARIABILITY OF FATTY ACID COMPOSITION OF TISSUE LIPIDS OF RATS

Data include values from liver, serum, kidney, depot fat, and placentas of rats maintained under widely differing conditions

	C 14 and below	C 16 : 0	C 16 : 1·2	C 18 : 0	C 18 : 1	C 18 : 2	C 20 : 4
			Females (23 values)				
Range	0·2–3·8	19·4–36·9	1·3–18·0	1·8–20·2	15·5–38·4	5·4–30·4	0–16·2
Average	1·6	28·6	6·8	11·9	29·0	16·2	4·7
Coefficient of variation (%)	56	17	63	72	23	39	90
			Males (25 values)				
Range	0·3–40·4	15·9–38·2	0·6–25·0	0–22·2	15.5–46·5	0–21·1	0–16·1
Average	9·9	27·8	9·7	8·6	29·2	9·4	4·8
Coefficient of variation (%)	147	21	69	85	30	64	112

pathological physiology of the disease and the normal physiology of hæmostasis.

During an examination of platelet adhesiveness by Hellem's method[7], it was found that the platelets of a subject with von Willebrand's disease did not adhere when fresh citrated blood was passed through a column of glass beads. The beads (Propper Co., Long Island City, New York), were soaked in 2 per cent sodium carbonate and then in sodium chromate–sulphuric acid cleaning solution before use. Columns were made of vinyl tubing (Becton, Dickinson and Co., Rutherford, New Jersey). After glueing with vinyl cement (Schwartz Chemical Co., Long Island City), air was drawn through the columns to remove any traces of cement solvent. In mixing experiments, 1 ml. citrated plasma, separated after 10 min centrifugation at 15,000*g*, was added to 3 ml. citrated blood.

Initial results were striking (Table 1). In three subjects with von Willebrand's disease, platelets did not adhere to the beads. Fresh normal plasma restored adhesiveness, whereas the patient's plasma was ineffective. By the end of October, all this batch of beads had been used once. A second batch was obtained from the same company but had almost certainly been manufactured at a different time from the first. The new batch, as well as beads obtained from Hellem's source, demonstrated only minor and inconstant differences between subjects *A* and *B* and normal individuals. Consequently, investigations were resumed on December 4 with the original beads which had been used and then washed again as before. The number of washings is not known, but increased as the beads were used again and again. On December 18 a trip was made to another city to examine the mother and sister of subject *A*, and an unrelated family (subjects *G*, *H*, *I*) in whom three generations could be tested. Only the mother of subject *A* demonstrated abnormal platelet adhesiveness. Afterwards, the beads no longer distinguished patients *A*, *B* and *D* from normal individuals. This is in accord with the results of O'Brien[8] and Cornu and Larrieu (unpublished observations).

Recent use of columns made of unwashed soft glass beads (Minnesota Mining Co., St. Paul, Minnesota) on subjects *A*, *B*, *E* and four additional patients with von Willebrand's disease gave an average platelet adhesiveness of 26·9 per cent ± *S.D.* 11·0, whereas in 11 normal subjects, adhesiveness averaged 39·2 per cent ± *S.D.* 7·5. The probability that this difference would occur by chance is 0·02. Data in Hellem's original work actually suggest that the adhesiveness of platelets is subnormal in von Willebrand's disease since adhesiveness was below 35 per cent in 11 of 15 patients with this disease, but it was below 35 per cent in only 22 of 104 observations on normal subjects. Hellem failed to comment on this difference, and attributed some of the adhesiveness values below the lower level of normal to subnormal hæmatocrits. Further study, I hope, will eliminate the unpredictability of the method and permit consistent demonstration of an *in vitro* abnormality in von Willebrand's disease correctable by normal plasma.

Table 1. PERCENTAGE OF PLATELETS ADHERING TO GLASS BEAD COLUMN IN PATIENTS WITH VON WILLEBRAND'S DISEASE AND IN NORMAL SUBJECTS. (CONSECUTIVE STUDIES WITH ONE BATCH OF GLASS BEADS)

Subjects with von Willebrand's disease*					Normal subjects				
Date	Subject	Pt. Bl.	Pt. Bl. Pt. Pl.	Pt. Bl. N. Pl.	Date	Subject	N. Bl.	N. Bl. Pt. Pl.	N. Bl N. Pl
					14/7	*K*	62		
					17/7	*L*	28		
2/10	*A*	2			2/10	*M*	55		
		−2					46		
9/10	*B*	9	8	38‡	9/10	*M*	47	50‡	62
12/10	*A*	8	2	16‡	12/10	*M*		30‡	35
17/10	*C*†	25	33	37	17/10	*M*	22	41	23
					25/10	*L*	48		39
27/10	*D*	−4	−3	35	27/10	*L*	33	34	54
4/12	*A*	0			17/11	*K*	45		
12/12	*A*	7	—10	41	12/12	*L*	63	40	54
14/12	*A*	11							
18/12	*E*	3	6						
18/12	*F*	66	59						
18/12	*G*	77	72						
18/12	*H*	46	47						
18/12	*I*	51	36						
20/12	*J*§	32	17		22/12	*M*	25		32
15/1	*B*	25	10	27	23/12	*N*	24		
16/1	*D*	38			2/1	*O*	38		
12/3	*B*	9	15		14/3	*L*	62		
20/3	*A*	36			15/3	*M*	54		
17/5	*A*	41	27	10					

* All subjects had histories of excessive bleeding and normal platelet counts and hæmatocrits. All patients except *C* and *J* had prolonged Ivy bleeding times and AHF levels below 60 per cent of average normal. Normal range of AHF was 60–200 per cent.

† This patient had a long bleeding time but normal AHF and thromboplastin generation test on several occasions.

‡ Patient *A* is blood type O; patient *B*, type AB; normal *M*, type A. In subsequent mixing tests, both patient and normal subject were of same blood type.

§ This patient had true hæmophilia. His AHF level was about 4 per cent of normal, which is lower than any of our subjects with von Willebrand's disease. He was bleeding and probably had a low hæmatocrit. This may account for the low adhesiveness when the hæmatocrit was lowered still further by adding his plasma to his blood.

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Immunoelectrophoretic Studies of the β_1-Hæm-binding Globulin (Hæmopexin) in Hereditary Hæmolytic Disorders

NEALE *et al.*[1] have demonstrated by paper electrophoresis a hæm-binding globulin in the β-globulin fraction of human serum. Since then, several investigators examined this protein[2-6], which was identified by immunoelectrophoresis with β_1-*B*-globulin and called hæmopexin[7]. On starch-gel electrophoresis the hæm-binding protein was found to migrate with the fast α_2-globulins[8,9]. Hæmopexin is present in the perchloric acid-soluble fraction of serum[7,10] from which Schultze *et al.*[11] obtained a highly purified preparation by combined ammonium sulphate precipitation and zone electrophoresis. Hæmopexin is a glycoprotein containing approximately 20 per cent carbohydrate and has a sedimentation rate of 4·8*S* corresponding to a molecular weight of approximately 80,000. The purified protein has a binding capacity of 15–20 mols hæmin per mol hæmopexin; evidence for binding of hæmoglobin or cytochrome *c* could not be obtained. The hæmin binding capacity of normal human serum is approximately 10 mg/100 ml. (refs. 2 and 9).

Information on the biological significance of this protein is limited. In the sera of several patients with hæmolytic disorders, ahaptoglobinæmia was associated with deficiency or absence of hæmopexin[1,9].

In the work reported here the sera of 38 children with hereditary hæmolytic disorders were examined. The material comprised 36 samples from 11 patients with hereditary spherocytosis, 35 samples from 19 patients with thalassæmia major, 11 samples from 8 patients with sickle-cell anæmia, and included 4 samples from 4 individuals with thalassæmia minor. Sera were obtained during periods of active hæmolysis; in 13 patients sera were studied before and at various times after splenectomy. In patients with spherocytosis, splenectomy results in almost complete cessation of hæmolysis, while in thalassæmia major the effect is incomplete and transient.

Vertical starch-gel electrophoresis was performed at *p*H 8·6 in borate buffer with a voltage gradient of 5 V/cm

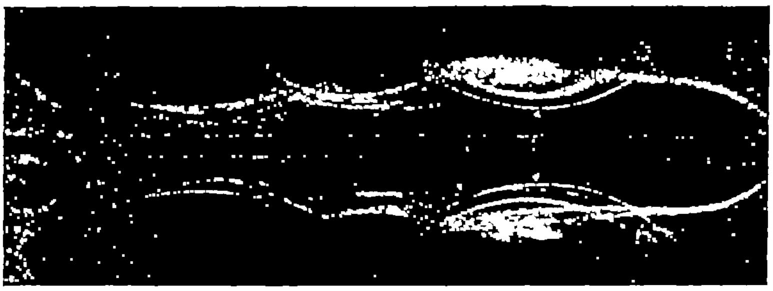

Fig. 1 Deficiency of hæmopexin ($\beta_1 - B$) in serum from a patient with thalassæmia major (upper part) is demonstrated by immunoelectrophoretic analysis. Normal serum is illustrated for comparison (lower part). The precipitation lines of hæmopexin ($\beta_1 - B$) and transferrin (Tf) are designated by arrows

for 16–18 h at room temperature[12]. Prior to electrophoresis, hæmolysate from normal individuals was added to the sera in amounts sufficient to saturate haptoglobin. The gels were sliced into two layers and stained for protein with amido black and for haptoglobin by the benzidine reagent. Sera which demonstrated a complete absence of peroxidase-positive haptoglobin bands were classified as ahaptoglobinæmic. Immunoelectrophoresis on agar gel was carried out on microscopic slides according to Scheidegger[13] in *p*H 8·2 barbital buffer and a voltage gradient of approximately 7 V/cm was applied for 80–100 min. Horse anti-serum against normal human serum No. 13411 from the Institut Pasteur, Paris, was used for the development of the precipitation lines. Samples were tested before and after addition of hæmolysates prepared from individuals with hæmoglobin A; they were stained for protein with azocarmine and examined for peroxidase activity with the benzidine reagent.

Fig. 1 illustrates the comparative immunoelectrophoretic analysis of sera from a patient with thalassæmia major and from a normal individual. In the patient's serum the precipitation line of hæmopexin ($\beta_1 B$) is absent. Experiments in which varying concentrations of serum and antiserum were used demonstrated that the absence of the precipitation line was due to a decrease of hæmopexin below the sensitivity of the method.

Table 1. HÆMOGLOBIN- AND HÆM-BINDING GLOBULINS IN HÆMOLYTIC ANÆMIAS. EFFECT OF SPLENECTOMY (SPL.)

Diagnosis	No. of cases	Ahaptoglobinæmia		Deficiency of β_1 hæm-binding globulin	
		Before spl.	After spl.	Before spl.	After spl.
Spherocytic anæmias					
Studied before and after spl.	8	8/8	4/8	4/8	0/8
Studied only after spl.	3	—	2/3	—	0/3
Thalassæmia major					
Cases without spl.	5	3/5	—	5/5	—
Studied before and after spl.	5	2/5	0/5	5/5	4/5
Studied only after spl.	9	—	2/9	—	9/9
Thalassæmia minor					
Cases without spl.	4	0/4	—	0/4	—
Sickle-cell anæmia					
Cases without spl.	7	7/7	—	6/7	—
Studied only after spl.	1	—	1/1	—	0/1

The results obtained from 42 patients are summarized in Table 1. In all patients with spherocytic anæmia active hæmolysis was associated with ahaptoglobinæmia; a deficiency of hæmopexin was found in 4 of 8 patients studied before splenectomy. Decreased hæmolysis following splenectomy was associated with the reappearance of haptoglobin in some patients, and of hæmopexin in all patients studied. In thalassæmia major ahaptoglobinæmia was found only in 5 of 10 patients and hæmopexin deficiency in all patients examined. Following splenectomy haptoglobin reappeared in all cases. In contrast, the deficiency of hæmopexin persisted in 4 out of 5 patients studied, although in one individual a faint β_1-B-precipitate indicated the presence of small amounts of hæmopexin. A deficiency of hæmopexin was observed in 9 additional cases studied at various time-intervals after splenectomy, and was usually not associated with ahaptoglobinæmia. No abnormalities of the hæmoglobin and hæm-binding globulins were disclosed in thalassæmia minor. In sickle-cell anæmia all sera studied were ahaptoglobinæmic and 6 out of 8 cases were also deficient in hæmopexin.

Deficiency of β_1-hæm-binding globulin in hæmolytic disorders associated with ahaptoglobinæmia has previously led to the conclusion that β_1-hæm-binding globulin might represent an additional mechanism capable of eliminating hæmoglobin from the circulation. Although the possibility of hæmoglobin-binding to hæmopexin after complete saturation of haptoglobin has not been excluded, and indeed may explain the findings in spherocytosis and sickle-cell anæmia, binding of hæm appears to be the major function of hæmopexin[3,6,11]. The absence of detectable amounts of hæmopexin in the presence of haptoglobin as observed in thalassæmia major indicates that these two proteins have distinctive functions. Although it cannot be excluded that impaired synthesis of hæmopexin is responsible for the deficiency of this protein in thalassæmia, the observation that in one case of thalassæmia major the protein was present in decreased amounts suggests that this explanation is unlikely. Moreover, heterozygous individuals revealed no detectable abnormalities of hæmopexin. It is more probable that the deficiency of hæmopexin in hæmolytic disorders can be ascribed to the formation of a complex of hæmopexin with hæm or related protoporphyrins which is rapidly eliminated from the circulation. The deficiency of β_1-hæm-binding globulin in patients with thalassæmia major suggests that increased concentrations of a hæm compound may be present in this disorder. Further investigations are required to determine whether this compound is a normal or abnormal product of porphyrin metabolism.

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Determination of Haptoglobin-groups Frequencies by Starch-gel and Agar-gel Electrophoresis : Application to Belgian and Barundi Populations

IT has been frequently stated that the determination of the haptoglobin groups of some subjects may be difficult, even by the Galatius-Jensen[1] method for detecting hæmoglobin–haptoglobin complexes with benzidine. Some 2–2 subjects with a low haptoglobin blood concentration may erroneously be considered as ahaptoglobinæmic[2]. It may also be hazardous to distinguish a subject lacking haptoglobin from one belonging to the 1–1 group, when Smithies's standard technique of starch-gel electrophoresis with borate buffer at pH 8·4 is used[3], since with this method the mobility of the hæmoglobin is nearly the same as that of the hæmoglobin–haptoglobin complex of the 1–1 pattern[4–6].

We have found that these difficulties can be overcome by using high-voltage electrophoresis in agar-gel according to Wieme's method[7,8].

The method was as follows: 1-mm thick agar-gels prepared with a barbital buffer (μ 0·05; pH 8·4), poured on 0·8 mm thick microscopic slides; identical buffer in tanks; 20 V/cm for 25 min; gel immersed in petroleum ether as sealer and refrigerant; detection of the hæmoglobin-containing fractions with a benzidine solution. This method is very different from Hirschfeld's method[9], who demonstrated the possibility of determining the haptoglobin groups by agar-gel electrophoresis; our technique will be described in detail elsewhere[10].

Fig. 1 shows typical results obtained in each of the haptoglobin groups.

Two fractions migrating towards the anode and one towards the cathode are detected: first, a fast-moving fraction of low concentration, likely to be hæmoglobin bound with methæmalbumin[11,12], which shows an albumin mobility by naphthalene black staining; secondly, a fraction corresponding to the hæmoglobin–haptoglobin complex; its mobility is different in each group, being the fastest in the 1–1 and the slowest in the 2–2 group; this fraction is lacking in ahaptoglobinæmic subjects; thirdly, migrating towards the cathode, a fraction with the same mobility in all the groups and representing free hæmoglobin.

Therefore this technique makes it possible: (1) to recognize with certainty the difference between 1–1 and ahaptoglobinæmic subjects, since free hæmoglobin is not superposed on the 1–1 haptoglobin fraction; (2) to distinguish 2–1 and 2–2 phenotypes of subjects with a low blood haptoglobin concentration because the hæmoglobin–haptoglobin complex is not spread out over several bands as it is on a starch-gel medium; (3) to recognize the haptoglobin type in 1–1, 2–1 and 2–2 subjects. Yet, for routine analysis, starch-gel electrophoresis seems the procedure of choice, since the electrophoretic mobilities of 2–1 and 2–2 hæmoglobin–haptoglobin complexes are very similar in agar-gel (Fig. 1).

With the combined techniques of starch-gel and agar-gel electrophoresis we have determined the haptoglobin groups frequencies in several populations:

(1) *Belgian population*. Blood specimens of 416 healthy Belgian adults, 141 of them living in Antwerp, were investigated. Table 1 gives the results and compares them with some data from the literature.

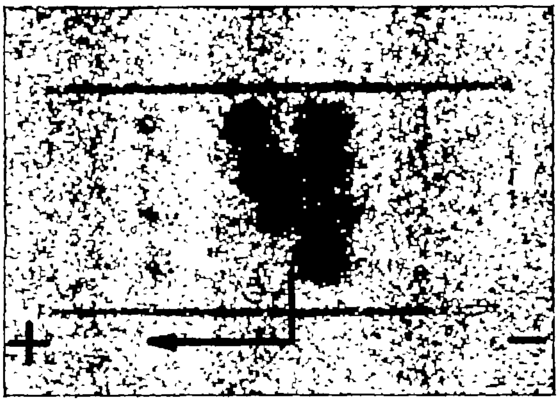

Fig. 1. Haptoglobin group patterns by agar-gel electrophoresis on microscopic slide (benzidine staining reaction). From above to below: 1–1, 2–1, 2–2 and 0–0 groups

Table 1

Populations	No. of subjects	Phenotypes (%) 1–1	2–1	2–2	0–0	Hp^1 gene frequencies[5]
Antwerp	141	17·0	47·5	35·5	—	0·407
Belgium	416	19·0	47·6	33·4	—	0·428
Sweden (ref. 11)	275	15 0	47·0	38·0	—	0·385
France (Paris) (ref. 13)	406	15 27	49·75	34·98	—	0·4015
Austria (Vienna) (ref. 14)	621	16·1	48·2	35·7	—	0·402
England (Oxford) (ref. 15)	218	10·1	55·5	31·7	2·7	0·406
Germany (Westphalia) (ref. 14)	201	19·9	46·8	33·3	—	0·433
Germany (Bavaria) (ref. 14)	273	19·4	54·2	26·4	—	0·465

The haptoglobin gene frequencies in the Belgian population are similar to those in other populations of Western Europe and more especially to the English and German ones, the German population showing the highest Hp^1 frequency ascertained in Europe[14]. No ahaptoglobinæmic subject was found among our samples of Belgian origin and the χ^2 test shows that the theory of two autosomal allelic genes[16] applies to these populations: Antwerp inhabitants: $\chi^2 = 0{\cdot}0420$, or $0{\cdot}90 > P > 0{\cdot}80$; Belgians: $\chi^2 = 0{\cdot}3241$ or $0{\cdot}70 > P > 0{\cdot}50$ for one degree of freedom.

(2) *Negro population of Burundi*. A sample of 182 healthy Burundi soldiers was examined; among them 86 had two Tutsi parents and 96 had two Hutu parents. Bahutu and Batutsi are the two most important ethnic groups of Burundi. Table 2 summarizes the results.

Table 2

	No. of subjects	Phenotypes (%) 1–1	2–1	2–2	0–0	2–1 *M*	Hp^1 gene frequencies[5]
Burundi	182	28·0	47·8	20·9	3 3	—	0·52
Tutsi	86	27·9	47·7	22·1	2·3	—	0·52
Hutu	96	28·2	47·9	19·8	4·1	—	0·52

From these results the following conclusions may be drawn: (1) The Hp^1 gene frequencies are not so high in Burundi populations as they are in Bantus living in West Africa ($Hp^1 = 0{\cdot}70$)[17] and in Western Congo ($Hp^1 = 0{\cdot}77$)[18]. (2) There is a low percentage of ahaptoglobinæmic subjects (0–0 group) in Burundi, with a frequency similar to that found in Senegalese (4·0 per cent)[1] and in American Negroes (4·2 per cent)[19]. The frequency is yet much inferior to that found in two Nigerian populations (Fulani: 50·0 per cent[20] – Noluba: 32·2 per cent[15]). (3) There is no significant difference in haptoglobin groups frequencies between Tutsi, who are of Hamitic origin, and Hutu, who are Bantus. (4) No 2–1 *M* pattern was found in our Burundi samples.

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A Simple Screening Test for Waldenström's Macroglobulinæmia

THE most important characteristic which the pathological proteins in Waldenström's macroglobulinæmia have in common is the molecular weight defined by a sedimentation ratio within the range S 18–20.

The final diagnosis of Waldenström's macroglobulinæmia is therefore based on the demonstration of such a paraprotein by ultracentrifugation of the serum.

Immuno-electrophoresis with demonstration of a β 2M bow, hypo-β-2A- and hypo-γ-globulinæmia is a useful diagnostic aid. However, in routine laboratory work a screening test is needed. The Sia test is commonly used as such. In this test a drop of serum is added to a large amount of demineralized water. If cloudy precipitation occurs, the test is positive. However, the Sia test often gives a positive reaction in cases in which subsequent ultracentrifugation fails to reveal macroglobulins of the type characteristic of Waldenström's macroglobulinæmia. In rare cases, no precipitate is obtained although the serum does contain macroglobulins.

By the simple test described here it seems to be possible with greater probability to decide whether a given serum contains the proteins characteristic of Waldenström's macroglobulinæmia.

A slip of filter paper (Schleicher and Schull, 'Selecta' No. 2043, 39 mm × 310 mm) is moistened by dipping it into demineralized water. Excess water is allowed to drip off. The paper is placed on a plastic ladder (Fig. 1). The ladder with the paper is placed in the horizontal position and 10 µl. serum is applied as a streak across the paper, 8 cm from one end of the paper. That part of the paper to which the serum is applied is placed between two rungs of the ladder. After 1 min, the paper is removed to a glass plate, so that 5 cm of the paper at the end to which the serum was applied lies on the glass plate, while the rest is freely suspended. The glass plate is then placed in a jar containing demineralized water, so that the paper dips about 2 cm into the water (Fig. 2). The free end of the paper hangs down vertically into another jar with demineralized water, so that about 2 cm of it is immersed in the water. Water will then migrate (from the upper jar) through the paper past the serum streak. For this reason I have coined the term 'hydrophoresis' for the method. After 75 min, the paper is removed. It is stained with bromophenol blue in absolute alcohol for 20 min, after which excessive dye is removed by 2 per cent acetic acid. An intense blue stain is obtained by exposing the paper to strong ammonia.

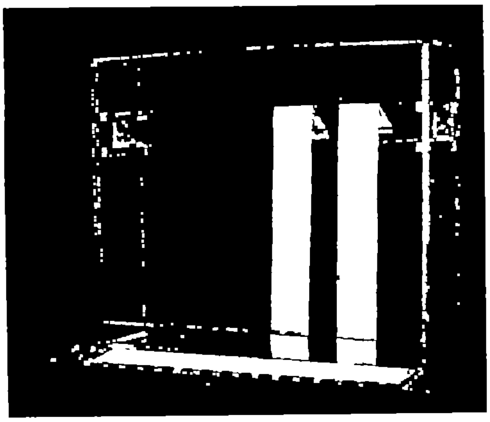

Fig. 1

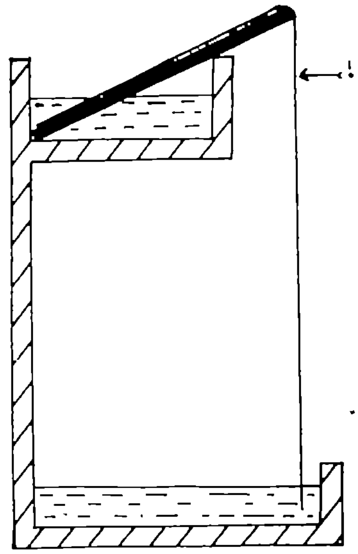

Fig. 2. Schematic drawing of the apparatus. The arrow indicates the side of the serum streak

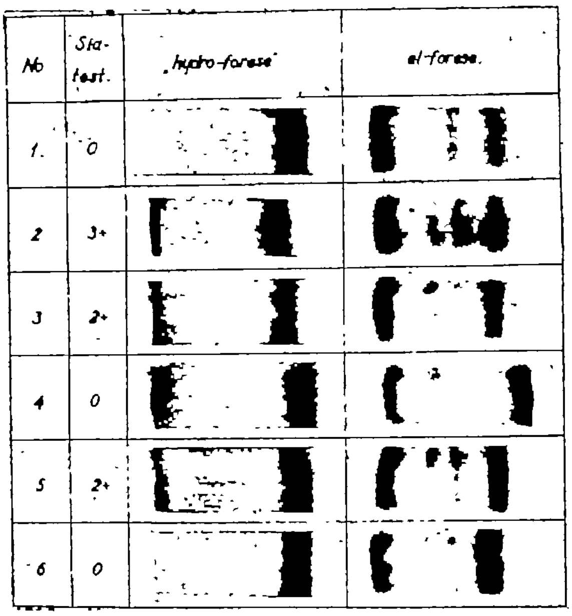

Fig. 3. The results of the Sia test are indicated by 0, 2+ and 3+

Sera from a number of healthy individuals were examined by this method. Uniform 'hydrophoretic' patterns with only slight variations were obtained. A pattern typical of a normal individual is shown in Fig. 3. No. 1. As a control measure, electrophoresis was also performed.

Two patients with Waldenström's macroglobulinæmia were studied. The diagnosis has been made after repeated demonstration of characteristic paraproteins by ultracentrifugation. In both cases, electrophoresis revealed an intensely stained band in the γ-fraction. The Sia test was strongly positive for both sera.

'Hydrophoresis' yielded identical results in these two patients (Fig. 3, Nos. 2 and 3), namely, a well-defined, densely stained, narrow band at the site of application, followed by a weakly stained area and, finally, a wide, diffusely defined band at the front.

If the paper is moistened with a 0·2 per cent aqueous solution of cysteine hydrochloride instead of demineralized water, the sharply defined band fails to appear at the site of application of serum. The Waldenstrom's macroglobulinæmia paraprotein is depolymerized by certain sulphydryl-rich compounds, for example, cysteine, to more low-molecular proteins, which are not precipitated in water at low ionic strength. The absence of precipitation after addition of cysteine is thus in support of the assumption that the proteins precipitated in the sharply defined band are 'Waldenström's macroglobulinæmia proteins'.

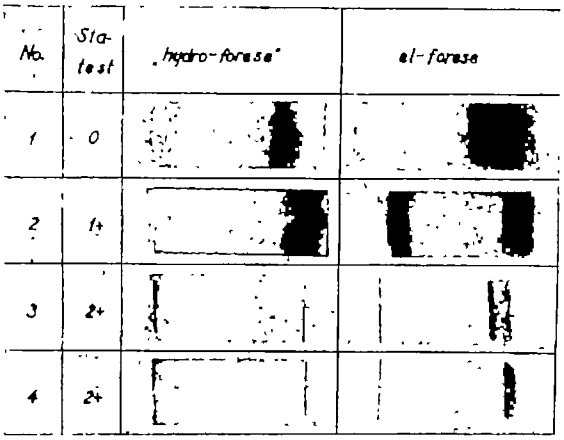

Fig. 4. The results of the Sia test are indicated by 0, 1^+ and 2^+

The sharply defined band which seems to be typical of Waldenström's macroglobulinæmia is not seen in myelomatosis. There is often relatively intense staining at the site of application, but it decreases over a wider area. In addition, distinct longitudinal stripes are often seen on the paper, and the wide band at the front may split up into two narrower bands (Fig. 3, Nos. 4, 5 and 6).

In hydrophoresis of a 10 per cent aqueous solution of human γ-globulin, moderate staining at the site of application occurs. The intensity of the staining decreases gradually along the paper, and a wide, diffusely defined band is seen at the front (Fig. 4, No. 1). In a patient with a large content of normal immune γ-globulin in the serum, a similar, but less pronounced, pattern was obtained (Fig. 4, No. 2). The serum from this patient was examined by ultracentrifugation, which failed to reveal an increase in the amount of macroglobulin. On the other hand, the Sia test was strongly positive.

Hydrophoresis was performed on sera from 50 patients with neoplastic disease. In none of these cases could the results obtained be mistaken for those typical of Waldenstrom's macroglobulinæmia.

In order to throw light on the characteristics of the precipitate obtained by the Sia test the following experiment was performed. To each of four centrifuge tubes with tapered bottoms containing about 10 ml. of demineralized water were added three drops of serum. After centrifugation and decanting of the supernatant the precipitate was re-dissolved in a small amount of physiological saline. Electrophoresis and hydrophoresis were then performed on this solution. In a patient with Waldenstrom's macroglobulinæmia (Fig. 3, No. 2), hydrophoresis now again revealed a sharply defined narrow band at the site of application, but otherwise no staining of the paper (Fig. 4, No. 3). By similar treatment of serum from a patient with myelomatosis (Fig. 3, No. 5), on which the Sia test was positive, hydrophoresis yielded only moderate staining at the site of application and an even decrease in the intensity of the staining of the paper (Fig. 4, No. 4).

The test, which may be reproduced with fairly good accuracy, seems to permit a semi-quantitative measure of the amount of macroglobulin in the serum.

Moreover, we find that it is of value that the result can be filed for comparison with subsequent tests in the same patient.

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PATHOLOGY

Growth of Transplantable Cancer in Rats previously injected with Placental Tissue

An antigen in malignant and embryonic tissues capable of retarding the growth of a transplantable human tumour (HS_1) in rats has recently been described by Buttle *et al.*[1]. Seven days after injecting a variety of human tumours and normal adult and fœtal human tissues into weanling rats, they challenged the animals with HS_1 and cortisone. Significant inhibition of tumour growth was produced by prior injection of homogenates of HS_1, sarcomata and a variety of normal fœtal tissues, especially spleen, whereas normal adult plasma and muscle had little if any effect. Buttle *et al.* attributed these findings to a labile antigen, destroyed by freezing and thawing, present in high concentration in embryonic and malignant tissue, and related to a high rate of cellular division in those tissues.

On the basis of its normally rapid growth, placental tissue was tested for its ability to inhibit tumour growth in rats. Interspecific differences and modifying effects of adrenal corticosteroids were eliminated by challenging the rats with a suspension of tumour obtained from other animals of the same strain, without administration of cortisone. In all cases the tumour used was the so-called chorionepithelioma induced by Stein in 1960, and still highly malignant, though considerably different histologically from the original[2].

Wistar albino rats weighing 90–150 g received injections of 0·2–0·3 ml. of a 40 per cent suspension of placental extract in saline intraperitoneally. Placental tissues from the same strain of rat were obtained from dated pregnancies of each day of gestation from the seventh to the twenty-first (term). The tissue was removed from the uterus at hysterectomy and immediately minced and passed through a gauze strainer, avoiding contamination with other fœtal tissues, blood and decidua. Two weeks after placental injection, the animals and an 'unimmunized' control received 0·2 ml. of a 40 per cent tumour suspension in saline intraperitoneally. A group of five animals and a control were used to test the effect of placental tissue of each day of gestational age.

Table 1. Effect of intraperitoneal injection of rat placenta on the growth of transplanted 'Chorionepithelioma' in the rat. Criterion for inhibition is absence of tumour masses 2 cm. or more in diameter and of metastases 14 days after transplantation

Series Albino Wistar	Immunizing injection of placental homogenate	Experimental group inhibition (per cent)	Control group inhibition (per cent)
I	40 per cent suspension, age 7–21 days, warm	4/70 = 5·7	1/15 = 6·7
II	Full-strength, age 7–21 days, warm	13/68 = 19	2/15 = 13
III	Full-strength, age 10–20 days, warm and cold (4° C)	1/80 = 1·2	1/20 = 5·0
IV	Full-strength, age 16 days, warm and cold	1/8 = 12*	1/8 = 12*
II–IV	Summary of groups receiving full-strength	15/156 = 9·6	4/43 = 9·3
I–V	Summary of entire series	19/226 = 8·4	5/58 = 8·6

* These animals were born of tumour-bearing mothers from the first series

The chorionepithelioma ordinarily causes death of the rat in 12–14 days after transplantation, autopsy revealing masses 4 cm in diameter and extensive metastases. Our criterion for inhibition of growth was survival of the host for 14 days, without evidence of a mass 2 cm or more in diameter or of metastases at the time of necropsy or laparotomy. In the initial series, only 4 of the 70 test animals and 1 of the 15 controls met the criterion of inhibition. The experiment was repeated, changing the placental preparation from a 40 per cent suspension to a full-strength homogenate. In the second series, 13 of the 68 test animals (those receiving placenta of 10, 12 and 17 days of age) showed inhibition of tumour growth. The control animals in the 12 and 17 day groups, however, also showed inhibition, implicating the tumour itself as the variable in these cases. A third series of rats received full-strength placental injections of tissues from 10 to 20 days of gestational age. Half the animals and appropriate controls were treated exactly as in the second series; the other half of the test animals received injections of placental tissue that had been stored at 4° C for 24 h after removal from the uterus. Again, except for one animal in the 17-day-old group (cold) and the control from the same group, there was no inhibition of tumour growth in the third series. A final experimental group consisted of 16 weanlings delivered by Caesarean section of mothers which were alive but bearing large tumours on the 14th day after transplantation in the initial experimental series. Four animals received 16-day placenta warm and four received the same type of preparation cold; equal numbers of test and control animals were used in this group. Here too only one test animal and one control (cold group) met the criterion of inhibition. The combined experimental results are presented in Table 1.

It appeared that prior intraperitoneal injection of placental extract into rats, whether in 40 per cent or full-strength suspension, and whether warm or cold, had no effect on the growth of transplantable rat chorion epithelioma, nor did animals born of the tumour-bearing mothers manifest resistance to that tumour. Finally, there was no critical developmental age of the placenta at which differences in its protective function against tumour growth could be demonstrated. No prediction is made, however, regarding the possible effect of human or other heterologous placental tissue on the growth of transplantable tumours in the rat.

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Normal Mitotic Activity and Karyotype of Leucocytes from Pernicious Anæmia Patients cultured in Vitamin B_{12}-deficient Medium

IN vitamin B_{12}-deficiency there are profound alterations in the metabolism of the cell nuclei, as shown by the megaloblast phenomenon, the increase in the nuclear size in various tissues, and the hypersegmentation of the peripheral granulocytes. The basic cause of these phenomena appears to be blocked replication of deoxyribonucleic acid and cell division[1,2].

It occurred to us that owing to deficiency in vitamin B_{12} the peripheral leucocytes of patients with manifest pernicious anæmia might behave abnormally, for example, fail to divide, in the cell culture system used in our laboratory for cytogenetic studies, especially if the medium were made deficient in 'anti-pernicious anæmia' factors.

Another reason for performing this work was that recently doubt has been cast on the finding of Court Brown *et al.*[3] that the chromosomes of patients with untreated megaloblastic anæmia are normal. In an investigation of 100 mitotic plates from patients with pernicious anæmia, Astaldi *et al.*[4,5] found frequent deviations from the normal, both regarding chromosome number and morphology.

Four female patients with untreated pernicious anæmia were used. Peripheral blood was withdrawn into heparin and the leucocytes were separated from the erythrocytes with the aid of phytohæmagglutinin[6]. The suspension of viable leucocytes in the patient's own plasma was then used for preparing leucocyte cultures[7]. The final medium in which the leucocytes were grown consisted of: (1) 20 per cent of the patient's own plasma; (2) 80 per cent culture medium; (3) a small amount of phytohæmagglutinin; (4) some heparin. Four different culture media were used, namely: (1) Parker's solution containing minerals, amino-acids, vitamins and antibiotics; (2) a modified Parker's solution without vitamins and antibiotics; (3) Hanks's solution containing minerals and sugar; (4) Dulbecco's solution containing inorganic salts only.

The microbiological vitamin B_{12} activity was assayed with *Euglena gracilis* and found to be 12 and below 1 μμg per ml., respectively, in cases 3 and 4. Using the same method, the components other than the serum in the culture fluid were found to contain no detectable amount of vitamin B_{12} activity if media 2–4 were used. Thus, the vitamin B_{12} content of the culture fluid was about 2·4 μμg and below 0·2 μμg per ml., respectively, in cases 3 and 4 in those flasks where media 2–4 were used.

Leucocytes from case 2 were taken and cultured twice, and in the other cases cultures were made on one occasion only. Incubation at 37° C for 72 h gave excellent growth of the leucocytes and after colchicine treatment good yields of mitoses were obtained in all the cases and with all the media. The incidence of mitoses was perhaps even higher than in normal subjects. In no case was the growth less abundant in those flasks which contained minimal amounts of vitamin B_{12} activity.

We conclude that leucocytes from the peripheral blood of patients with pernicious anæmia are capable of growth and mitotic division in the presence of extremely low amounts of vitamin B_{12}. It is also remarkable that the very simple inorganic salt solutions are sufficient as culture media in short-term cultures of leucocytes. Thus it is quite unnecessary to use the very rich and more expensive media usually recommended.

Table 1 presents the incidence of different chromosome counts in the four patients. The frequency of aneuploidy is low (14 aneuploid cells among 351 examined) and tallies with the results of a similar study on 20 normal females in this laboratory[8]. Detailed analysis of altogether 28 mitotic cells of excellent quality with the modal number 46 from the four different subjects failed to display anything abnormal in their chromosome morphology. Thus, in contrast to the results of Astaldi *et al.*[4,5], the karyotypes of our four patients with pernicious anæmia were entirely normal.

Table 1. DISTRIBUTION OF CHROMOSOME COUNTS FROM FOUR PATIENTS WITH UNTREATED PERNICIOUS ANÆMIA

Case No.	No. of chromosomes							
	< 44	44	45	46	47	48	> 48	Total
1	—	1	1	98	—	—	—	100
2	—	1	2	74	—	—		77
3	—	—	2	66	—	1	—	69
4	1	1	3	99	1	—	—	105

A few erythrocytes are always present in the leucocyte suspensions separated with phytohæmagglutinin. However, erythrocytes from patients with pernicious anæmia contain less vitamin B_{12} than those of normal subjects[9]. The number of erythrocytes was very small in our cultures (macroscopically mostly not detectable), and the red cells are not destroyed during treatment and culture. Therefore, their content of vitamin B_{12} can be neglected as a source of it. Thus, the leucocytes grow and divide in a medium containing exceedingly small amounts of vitamin B_{12} as determined by microbiological assay. It has been shown that the majority of the mitoses seen after 72 h are probably primary, that is, mitoses of blood cells originally present[10]. It may be anticipated, however, that some of the mitoses at 72 h have started in daughter cells of those originally present. Long-term cultures may indicate whether these vitamin B_{12}-deficient daughter cells are then able to subdivide further.

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Transfer of Stimulation of Deoxyribonucleic Acid Synthesis in Antigen-stimulated Rabbit Spleen Cell Cultures

LITTLE is known about the nature of the antigenic stimulation to antibody production or the manner in which antigen reaches the necessary site of stimulation. A recent report[1] implicates the sequential action of at least two different types of cells. One type is a mononucleated cell with phagocytic activity, and the other a non-phagocytic cell belonging to the lymphocytic series. Fishman suggests that an RNase sensitive material is transferred from one to the other, and it has been suggested[2] that complexes between antigenic fragments and RNA may play an important part in antibody formation. A physical means whereby such a transfer might occur[3,4] has been reported. This communication reports some preliminary experiments which were designed to demonstrate a transfer of antigenic stimulation from one cell culture to another.

It has been shown[5] that DNA synthesis in spleen cell preparations from previously immunized rabbits is stimulated *in vitro* by the addition of antigen. Furthermore, only brief contact with the antigen is required for stimulation[6]. After washing, less than 0·1 per cent of the added antigen remains. In contrast, 1 per cent of the original amount (or 10 times the amount remaining after washing) of the antigen added to fresh cells is too little to produce stimulation.

This system has been used here to test whether cells 'primed' by brief contact with antigen are able to transfer stimulation to fresh cells from the same spleen. In these experiments, the response of the 'primed' cells was inhibited by irradiation or freezing and thawing. The design of the experiments and results are presented in Table 1.

Table 1. TREATMENT OF CELLS

Spleen cell suspension from immunized rabbits

C	618	
C + *C*	1,144	(1,236)
CR	71	
C + *CR*	579	(689)
P	5,168	
P + *P*	10,600	(10,336)
PR	253	
C + *PR*	3,200	(871)
PF	1,503	
C + *PF*	6,143	(2,121)
CR + *PR*	621	(324)
CR + *P*	5,087	(5,239)
C + *P*	7,737	(5,786)

(*P*) 'Primed' cells. (*C*) 'Control' cells. (*PF*) 'Primed' frozen cells. (*PR*) 'Primed' irradiated cells. (*CR*) 'Control' irradiated cells.

Part of the cell suspension (3×10^8 cells) was incubated with 3 mg/ml. of human serum albumin labelled with iodine-131 for 15 min at 37° C. The cells were washed three times in 5 ml. of medium/wash. Determination of residual radioactivity showed that less than 0·2 μg labelled human serum albumin remained/cell culture. Such cells are designated 'primed' cells (*P*). 'Control' cells (*C*) were treated identically, except they were not exposed to antigen. One portion of the 'primed' cells received a dose of 300 rads, and these were called 'primed irradiated' cells (*PR*). 'Control irradiated' cells (*CR*) were similarly prepared. In addition, another portion of 'primed' cells were frozen and thawed once to give 'primed frozen' cells (*PF*).

Cultures of such cells were incubated alone or in various combinations, and the rate of DNA synthesis was measured in the second 24-h period, as previously described[5]. The figures represent the total c.p.m. incorporated/culture during 24-h incubation with ^{14}C-thymidine. The figures in parentheses in Table 1 indicate the expected results of combinations calculated from the figures for single cultures. The results which show evidence of transfer have been underlined.

A definite enhancement of DNA synthesis was observed when 'primed' cells were incubated with control cells. This was clearly shown when DNA synthesis had been inhibited in the 'primed' cells. There was no enhancement of DNA synthesis when 'control' irradiated cells were added to 'control' cells or to 'primed' cells. The enhancement of DNA synthesis obtained when 'primed' cells, which had been frozen and thawed, were added to 'control' cells demonstrated that it is the 'control' recipient cells which respond.

The results of a single experiment are presented, but identical findings were obtained in all of three other experiments using other antigens. The enhancement of DNA synthesis was only observed when the animal had been previously immunized to the antigen. The system is therefore antigen specific.

Although the results are preliminary, two positive statements can be made.

(1) The 'primed' irradiated cells contain some factor capable of stimulating DNA synthesis in control recipient cells.

(2) The presence of this factor is dependent on contact of the 'primed' cells with specific antigen.

The nature of this transfer factor, and the cells concerned, need further investigation before definite conclusions can be drawn. At least three possibilities exist: (*A*) The minute quantity of antigen taken up represents an extremely potent fraction of the original antigen preparation. (*B*) The antigen taken up has had its antigenicity enormously enhanced. (*C*) The cells taking up the antigen hand on some material, other than antigen, which is capable of stimulating cell division. Such a material need not necessarily retain the antigen specificity.

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Malaria and Glucose-6-phosphate Dehydrogenase Deficiency

DRS. C. Kidson and J. G. Gorman[1] report observations on frequencies of glucose-6-phosphate dehydrogenase (G6PD) deficiency in different parts of New Guinea and New Britain, which make a welcome addition to our knowledge of the population genetics of this area. They note that low frequencies of G6PD deficiency occur in some populations living in malarious parts of New Guinea, New Britain and elsewhere. On the basis of this fact, and a report[2] suggesting that "no significant difference in *Pl. falciparum* parasite densities occurs between affected and normal children under 3 years of age in Thailand and that affected children in this age-group may have higher densities of the non-lethal *Pl. vivax*", Kidson and Gorman issue "a challenge to the concept of selection by malaria in glucose-6-phosphate dehydrogenase deficiency".

In assessing these arguments it must be recalled that there is substantial evidence that the G6PD-deficiency gene carries with it disadvantages owing to hæmolysis in the neonatal period and later life[3]. Theoretical considerations establish unequivocally that the G6PD-deficiency gene could not have become common without some advantage[4]. This conclusion is not, in fact, disputed by Kidson and Gorman: the question at issue is the nature of the advantage. As pointed out previously[3], the only convincing argument from gene distribution against the malaria protection hypothesis would be the finding of high frequencies of abnormal hæmoglobin or G6PD-deficiency genes among populations known to have lived for long periods in malaria-free environments. No such populations have yet been found to exist. The converse argument carries very little weight, since there are many reasons why the G6PD-deficiency gene might not have attained high frequencies in populations exposed to malaria, including relatively recent introduction of the gene and unusually severe selection against it. The approach to genetic equilibrium may have progressed to different stages in different populations living in New Guinea and New Britain. The remarkable thing about the distribution of G6PD deficiency is not that it should occasionally be rare among populations living in malarious regions but rather that it should so often be common in such populations. Its distribution is much more widespread, for example, than that of hæmoglobins S, C or E.

Kidson and Gorman also report figures for G6PD-deficiency in Eastern New Guinea showing an inverse relationship with altitude among all populations tested, from low frequencies in the malaria-free highlands to high frequencies at the coast. Although conceding that these observations could be explained by malarial selection, they suggest that it is "equally possible that this pattern may in fact be the result of spread from a large gene pool on the coast near the river mouth, with dilution and diminution of the gene frequency towards the highlands". However, precisely analogous clines are seen in Sardinia[5] and in several different parts of Africa[6,7], where single foci of origin are very improbable, and in every case high frequencies of the enzyme-deficiency gene are confined to the malarious regions. In both New Guinea and Africa the cline involves several different linguistic groups which have probably, as Kidson and Gorman point out, maintained their separate identity for long periods of time.

The evidence of Kruatrachue and Harinasuta from Thailand[2] is available only in abstract form. Although it does not support the malaria protection hypothesis, neither does it provide critical evidence against the concept of protection against *P. falciparum*. The numbers involved are small (it can be calculated that one group of young children with enzyme deficiency and *P. falciparum* malaria would have included about 10 subjects and the other about 7 subjects; and the numbers with *P. vivax* malaria would have been slightly lower). It is remarkable that even in such small numbers a statistically significant excess of *P. vivax* should have been found in enzyme-deficient subjects (although there was no difference in parasite counts). This parasite preferentially attacks young erythrocytes with high enzyme activities, and no protection by G6PD deficiency would be expected. Hæmolysis as a result of *P. vivax* infection might well reduce the mean erythrocyte age, and increase susceptibility to subsequent infection with *P. falciparum*, so obscuring the difference between normal and enzyme-deficient subjects which is more obvious where *P. vivax* is less frequent.

Both our own observations in East Africa[8] and those of Gilles *et al.* in West Africa[9,10] have shown a significantly lower incidence and count of *P. falciparum* parasites in young children with enzyme deficiency than in children with normal enzymes. Moreover, in Gilles's investigations the incidence of cerebral and other complicated forms of malaria was significantly lower in enzyme-deficient subjects than in those with normal enzymes, providing direct evidence that the former are protected against the potentially lethal effects of falciparum malaria.

Finally, Kidson and Gorman argue that other parasitic diseases, in addition to variable degrees of nutritional inadequacy, are associated with climatic differences and might influence G6PD-deficiency rates. The situation is certainly complex, as already emphasized[3], and many factors might produce selection against the enzyme-deficiency gene. But in searching for the compensatory advantages required by genetic theory attention is directed first to erythrocytes, where the difference in G6PD activity between normal and affected subjects is much greater than in other tissues, and where there are good reasons for supposing that G6PD deficiency would limit multiplication of malaria parasites[3,4]. That protection against *falciparum* malaria exists has been shown by carefully conducted field investigations, the results of which cannot be seriously challenged by evidence of the type presented by Kidson and Gorman. Malaria may perhaps not be the only factor favouring the enzyme-deficiency gene, but it is an important factor, and no one has yet been able to offer any specific alternative.

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Skin Reactions in Neurological Patients after Intracutaneous Administration of Proteolipid A extracted from Human Brain

EXPERIMENTAL allergic encephalomyelitis as produced in monkeys, dogs, rabbits, guinea pigs and mice serves as an experimental model for the demyelinating diseases occurring in man. Such a form of encephalomyelitis can be reliably produced using either whole brain, whole white matter or diverse extracts derived from white matter. Among the latter, an especially potent effect has been ascribed to proteolipid A, which may be extracted from white matter following the procedure of Folch and Lees[1]. Kies and Alvord found this compound highly antigenic although not quite so potent as whole brain when they produced the experimental disease[2]. Åström and Waksman[3] and Waksman[4] demonstrated that experimental allergic encephalomyelitis follows the pathogenic pattern of a delayed-type hypersensitivity reaction. These authors observed a delayed reaction against myelin antigen in the diseased guinea pig, and transmitted the disease through lymphocytes from a diseased donor rabbit to a healthy recipient. So far, however, it has proved impossible to elicit a positive skin reaction against whole brain or myelin antigen in two series of human patients who were suffering from demyelinating disease of the central nervous system[5,6].

In the present investigation two different preparations of proteolipid A derived from two patients without organic brain disease were tested. The proteolipid was administered in three concentrations: (1) 0·05 mg/c.c., (2) 0·5 mg/c.c., and (3) 1 mg/c.c. The antigen was suspended in normal sterile saline and injected intracutaneously into the skin of the forearm in a final volume of 0·2 c.c. A control injection of 0·2 c.c. normal sterile saline was given simultaneously into the skin of the opposite forearm. The patients were observed up to four days after injection. The ensuing reaction was recorded as to strength and size and biopsies were taken at the height of the reaction. Twenty-four patients were tested. Twenty-one of these suffered from multiple sclerosis, one from syringomyelia and two neurotic patients without demonstrable neurological findings served as controls. The diagnosis was based in each case on careful clinical study and the result of spinal fluid electrophoresis.

As can be seen from Table 1, fifteen out of twenty-one patients with multiple sclerosis, one patient with syringomyelia and two neurotic patients gave a positive skin test. The observable reaction never fulfilled the criteria of a delayed hypersensitivity reaction. In all instances it consisted of a diffuse, poorly demarcated area of erythema without induration (Fig. 1) which appeared 3–4 h after injection and vanished again after various time intervals (54 h in two extreme cases, 36–48 h in two others). Histologically, the reaction was characterized by oedema of the sub-epidermal connective tissue and dense perivascular cellular infiltrates composed of lymphocytes interspersed with neutrophils (Fig. 2). The proteolipid preparation extracted from the brain of the second patient proved more potent than the first since it was effective in all cases tested. The fact that two patients without demyelinating disease of the central nervous system developed a positive reaction casts considerable doubt on the specific character of the phenomenon. In these control cases, however, the erythema disappeared much faster than in the cases of multiple sclerosis and the case of syringomyelia.

Table 1

Group *A*. · Patients with multiple sclerosis

No.		Conc. (mg/ml.)	Diameter of reaction (cm)
	First proteolipid preparation		
1	Multiple sclerosis	1	1·0
2	,,	0·5	0·5
3	,,	0·5	—
4	,,	1·0	0·5
5	,,	1·0	—
6	,,	1·0	0·4
7	,,	0·05	—
8	,,	0·05	—
9	,,	0·5	—
10	,,	0·5	—
	Second proteolipid preparation		
11	Multiple sclerosis	1·0	1·0
12	,,	0·5	1·2
13	,,	0·5	1·2
14	,,	0·5	1·0
15	,,	0·5	1 0
16	,,	0·5	1·3
17	,,	0·5	1·0
18	,,	0·5	1·3
19	,,	0·5	0·6
20	,,	0·5	1·0
21	,,	0·5	1·3

Group *B*. Controls. All tested with second proteolipid preparation

No.		Conc. (mg/ml.)	Diameter of reaction (cm)
22	Neurosis	0·5	0·8
23	,,	0·5	1·0
24	Syringomyelia	0·5	1·0

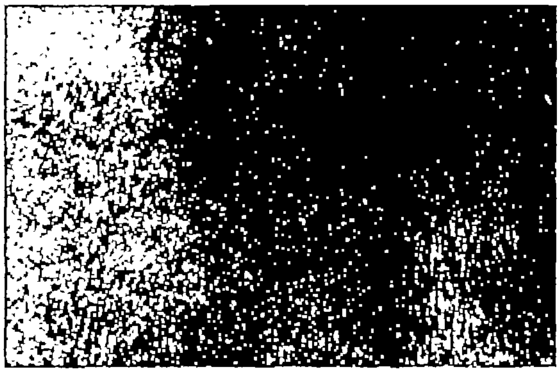

Fig. 1

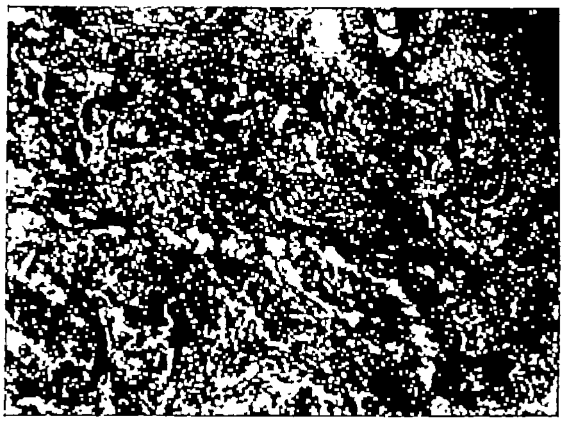

Fig. 2

While these results do not favour the view which considers multiple sclerosis a delayed-type hypersensitivity reaction against myelin antigen, some immunological process may very well be involved. Further investigations with various antigen preparations using larger numbers of patients appear to be justified.

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HISTOCHEMISTRY

Serotonin in Human Mast Cells

It has been demonstrated that, besides heparin and histamine, mast cells contain serotonin (5-hydroxytryptamine, 5-HT)[1–3]. However, serotonin-containing mast cells, having been found exclusively in mice and rats, it has been held that human mast cells contain no serotonin[4]. Evidence that the mast cells in these two species contain serotonin has been obtained by parallel histological and biochemical investigations and also by serotonin assay in isolated mast cells. Yet hitherto available techniques have failed to disclose direct histochemical proof of the presence of serotonin in mast cells, mast cells in mice and rats lacking the so-called enterochromaffin reactions.

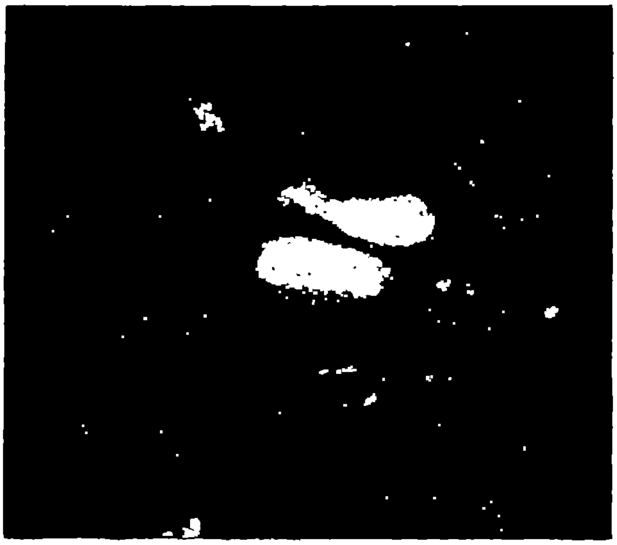

Fig. 1. (× 1,800)

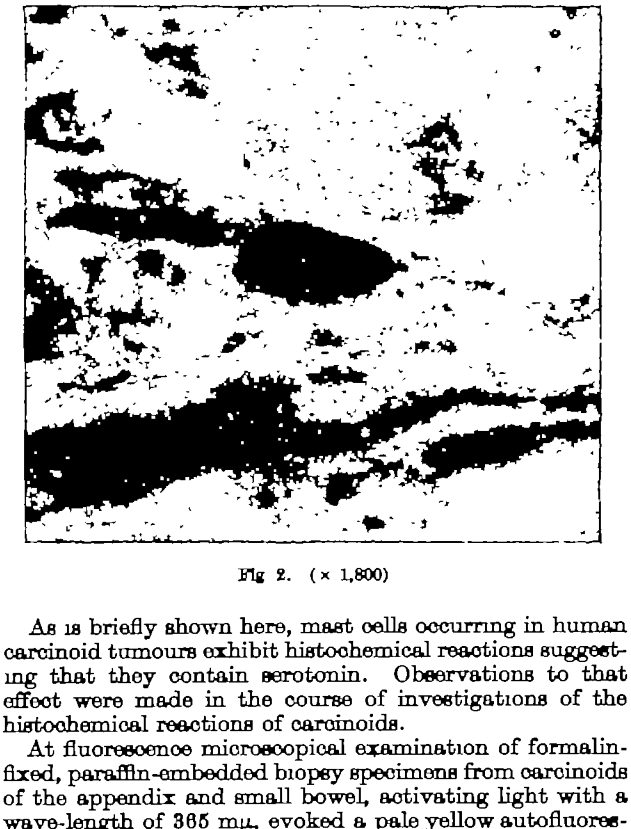

Fig 2. (× 1,800)

As is briefly shown here, mast cells occurring in human carcinoid tumours exhibit histochemical reactions suggesting that they contain serotonin. Observations to that effect were made in the course of investigations of the histochemical reactions of carcinoids.

At fluorescence microscopical examination of formalin-fixed, paraffin-embedded biopsy specimens from carcinoids of the appendix and small bowel, activating light with a wave-length of 365 mμ, evoked a pale yellow autofluorescence from the clumps of tumour cells as well as from cells situated in the interstitial connective tissue. Enterochromaffin cells in normal intestinal mucosa showed autofluorescence of similar colour. Some of the biopsy specimens included interstitial cells exhibiting positive argentaffin (hot methenamine method), alkaline diazo (fast red *B* and fast garnet *GBC*) and Schmorl's reactions. The fluorescent interstitial cells could be identified as mast cells by photographing fields containing such cells in the fluorescence microscope and then, after staining the sections with toluidine blue and May-Grünwald-Giemsa, re-photographing the same fields in regular transmitted light. In a representative pair of such photomicrographs Fig. 1 depicts a fluorescence photomicrograph displaying two fluorescent cells, one located peripherally in a cluster of tumour cells, the other in the interstitial tissue. Fig. 2 represents the same field as it appears in transmitted light after May–Grünwald–Giemsa staining. It will be seen that the cell situated just outside the tumour mass contains methachromatic granules and, accordingly, must be a mast cell. In mast cells with a few widely scattered granules, it could be perceived that the fluorescent as well as the diazo-positive substances were localized to these granules.

One of the specimens examined is especially interesting because it reveals that large numbers of mast cells with positive enterochromaffin reactions may occur even in so-called anargentaffin carcinoids. This specimen was from an appendiceal carcinoid in which the tumour cells showed a negative argentaffine reaction and a weakly positive diazo reaction. Conversely, the interstitial tissue was the site of a large number of fluorescent mast cells with positive diazo, argentaffin and Schmorl's reactions.

The so-called enterochromaffin reactions shown by enterochromaffin cells and carcinoid cells after fixation in formalin have been proved to be caused by a phenolic hydroxyl group, probably a form of tetrahydro-4-carboline[5,6]. Considerable evidence suggests that the substance that reacts with formalin is serotonin[7]. It has been assumed that mast cells in mice and rats have a serotonin content lower than the sensitivity threshold of the enterochromaffin reactions[8]. My results suggest that, under conditions of enhanced serotonin secretion, human mast cells are capable of storing histochemically demonstrable amounts of serotonin. Apparently the serotonin is localized to the granules of such mast cells rather than to the intergranular substance, as postulated in the case of mice and rats[9]. It remains to be seen whether carcinoids containing mast cells with positive enterochromaffin reactions constitute a special type of tumour or if all carcinoids normally contain such mast cells. Another interesting problem is whether the mast cells elaborate their own serotonin or merely store serotonin that has been elaborated by the tumour cells.

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RADIOBIOLOGY

Quantitative Determination of Oxygen-18 by Activation Analysis

RADIOACTIVE isotopes of carbon and hydrogen are available for exchange and mechanism studies investigation in biology, but work on oxygen must use the stable isotopes oxygen-16 and -18. The ratio of these stable isotopes can be determined with mass spectrometer or density methods.

Activation analysis is a frequently used technique for the determination of trace elements in biological materials[1]. The reaction $^{16}O(T:n)^{18}F$ has been used for the analytical determination of oxygen[2]. Similar results with the $^{16}O(n:2n)^{15}O$ and the $^{16}O(n:p)^{16}N$ reactions have been reported by Meinke *et al.*[3,4], and the $^{16}O(\gamma, n)^{15}O$ reaction has been utilized by Beard *et al.*[5]. These methods, however, are only useful for the determination of gross oxygen content. Activation analysis of oxygen-18 by the reaction

$^{18}O(p,n)^{18}F$ has been reported by Fogelstrom-Fineman *et al.*[6] who used oxygen-18 as a tracer for studying photosynthesis in algae. In general, little use has been made of thermal neutrons in the reaction $^{18}O(n,\gamma)^{19}O$ for activation analysis because of the small cross-section (0·00021 barns) of oxygen-18 and the short half-life (29·5 sec) of the resulting nuclide.

Recent measurements show that the determination of ^{18}O and $^{18}O/^{16}O$ ratios by activation analysis with reactor neutrons is practical. Samples of water with oxygen-18 concentrations ranging from 0·20 atom per cent (natural abundance) to 10·79 atom per cent were irradiated for 4 min (0·9964 of saturation activity) in a thermal neutron flux of 7·2 × 10^{10} neutrons/cm^2 sec. The pneumatic 'rabbit' system in the University of Florida Training Reactor was used for the irradiations. Irradiated samples were transferred to fresh sample containers in order to avoid spurious radioactivity, and counting was started within 30–40 sec after removal from the reactor. Preliminary results, obtained with a multichannel analyser, showed only the requisite 0·20 MeV and 1·35 MeV photopeaks characteristic of oxygen-19. Actual analytical results were obtained by a different procedure. Photons were detected with a 2 × 2 in. cylindrical well-type sodium iodide (Tl) crystal, amplified by the usual photomultiplier, and fed into a single channel analyser which was set to bracket either the 0·20 MeV or the 1·35 MeV photopeak. Output data were handled in two different ways.

In the first method, the pulses were fed into an amplifier, Brush model *RD*–5621–00, followed by an oscillograph, Brush model *RD*–2321–00. The recorded results for each sample were corrected for background, fitted with an exponential decay curve by the method of least squares and extrapolated to give the activity at the time of removal from the reactor. This standard activity plotted as a function of the mass of oxygen-18 present in the sample is shown in Fig. 1. The upper detection limit was established by the size of sample which overloaded the amplifier, and the lower limit was established by insufficient activity. The mean deviation from the linear plot is 7·9 per cent.

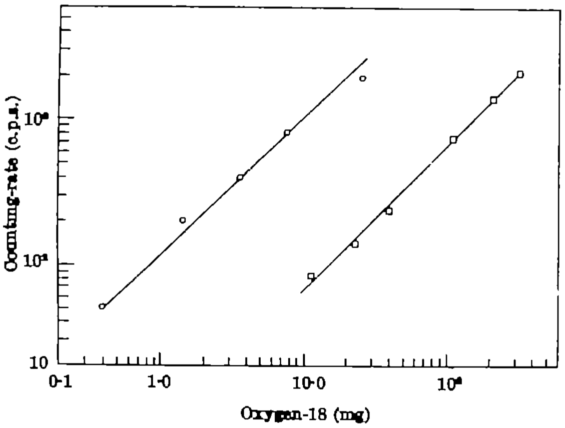

Fig. 1. Maximum activity of samples of oxygen-18. ○, 0·197 MeV photopeak; □, 1·35 MeV photopeak

In the second method, a scaler was used for obtaining the integral number of counts observed from 40 to 160 sec after the sample was removed from the reactor. Results obtained by this method are shown in Fig. 2. The mean deviation is 13·9 per cent.

The sensitivity of the method can be improved by the use of higher neutron fluxes. The minimum amount of oxygen-18 detectable with our equipment was approximately 350 µg, but a flux of 10^{14} neutrons/cm^2 sec would decrease this limit to 0·2 µg.

The thermal neutron activation of oxygen-18 can definitely be used as an analytical method for $^{18}O/^{16}O$ isotope ratio studies. This method is particularly useful for oxygen metabolism studies in living systems, and, since radioactivity is introduced after both the experiment and subsequent separations are completed, results are not obscured by possible effects of ionizing radiation on the system under investigation.

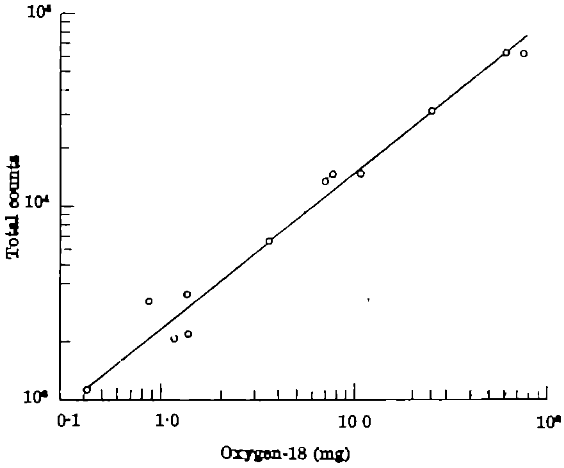

Fig. 2. Counts observed between 40 and 160 sec after removal from reactor

In collaboration with G. J. Fritz, we have obtained results on the uptake of oxygen-18 in corn seedlings exposed for varying intervals to an atmosphere containing 10 atom per cent oxygen-18. Preliminary results confirm the usefulness of the method for oxygen metabolism investigations.

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Accumulation of Radium-226 by the Freshwater Gastropod *Lymnaea stagnalis* L.

DIRECT absorption of ions in solution in the environment occurs frequently in freshwater invertebrates[1-3] and could be partly responsible for radiocontamination of the freshwater fauna[4,5] by radioisotopes in solution.

If the absorbed radioactivity is firmly fixed in the animals, and eventually concentrated at a level far above that found in the surroundings, these animals could be used as indicators for the radiocontamination of their environment. The possible use of freshwater molluscs as such indicators has already been emphasized[6-8].

Exposure of the snail *Lymnaea stagnalis* L. in solutions containing 0·04 µc. radium-226 per ml. revealed, as for calcium and strontium[8], the accumulation of the radium mainly in the shell-border. Since tap-water constitutes the optimal conditions for life of *L. stagnalis* in the laboratory and approaches fairly well the natural medium, the solutions of radium-226 were made in tap-water (containing in p.p.m.: 40 Ca$^{\cdot\cdot}$, 8 Na$^{\cdot}$, 4 K$^{\cdot}$, 4 Mg$^{\cdot\cdot}$, 25 Cl$'$, 120 HCO$_3'$). The content of radium-226 of the different samples was estimated by measuring the ^{214}Bi (= Ra *C*) formed after an equilibration period of 3 weeks in hermetically closed flasks. This ^{214}Bi radioactivity was measured in a well-crystal with a single channel pulse-height analyser on the γ-peak of 609 keV. Pools of 35 mature animals in 350 ml. solution were used in five parallel experiments.

When the animals are placed for 24 h in the radium-226 solution, 70 ± 8 per cent (at 95 per cent fiducial limits) of the radium was absorbed by the animals, as appeared by measuring an aliquot of the solutions. After 7 days, 10 animals were killed, dissected and measured for their radium-226 content. At this time, more than 90 per cent of the radium originally present in the solution was absorbed by the snails.

Table 1 presents the concentration factors observed. They are expressed as:

$$\text{Concentration factor} = \frac{\text{Ra-content in the animal per unit fresh weight}}{\text{Ra-content of the solution per unit of weight}}$$

Table 1. RADIUM-226 CONCENTRATION FACTORS IN DIFFERENT PARTS OF *L. stagnalis* AFTER 7 DAYS EXPOSURE IN A SOLUTION CONTAINING ORIGINALLY 0·04 μC./ML.

Parts of the snails	Mean	*S.D.*
Newly formed (2 mm) border of shell	1,277 ± 375	135
Shell without border	376 ± 100	36
Soft parts	140 ± 26	9
Whole animals	108 ± 18	7
Blood	21 ± 4	1

Error = fiducial limit with 95 per cent probability.

To verify that the absorbed radium-226 was firmly fixed, some of the animals were returned to uncontaminated water. During the following 24 h the animals lost about 1 per cent of their radium; this quantity fell to 0·5 per cent during the third day. Further measurements were disturbed by the low level of radioactivity and problems in relation to adsorption of radium-226. The death of an animal caused a sudden increase of radioactivity lost by the soft parts in the surrounding water, contrary to the shell, which firmly retains its radioactivity even when submerged in water after removing the soft parts of the animal. This fact and the high concentration factor in the shells confirm the possibility of using this animal as an indicator for contamination by radium-226.

I observed similar results with the accumulation of calcium-45 and strontium-85 in *L. stagnalis*. The net inward movement of the radium-226, notwithstanding the fact that the concentration of radium is 20 times higher in the blood than in the surrounding medium, seems to indicate the intervention of some active transport mechanism for this accumulation of radium-226, as has already been suggested by me for calcium absorption[9].

I thank R. Kirchmann for his advice.

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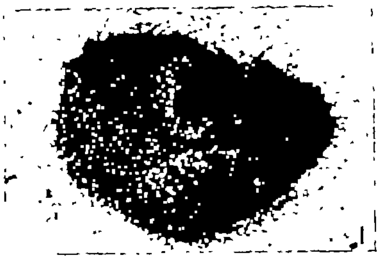
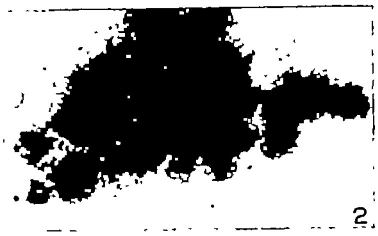
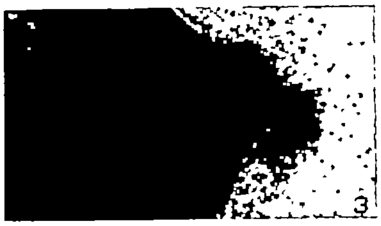
Incorporation of Iron-59 into the Cells of Different Tissues of *Chironomus plumosus*

ALTHOUGH iron plays an important part in the physiology of the living cell little is known about its localization in different parts of a cell or concerning its variation from tissue to tissue. Mammals have mainly been the object of investigations relating to the incorporation of labelled iron into the cells where the use of iron is known, such as the blood-forming cells, the liver cells, etc.[1–3]. We are not aware of the incorporation of labelled iron into the cells of other kinds of tissues and especially into the cells of invertebrates, except *Amoeba*[4].







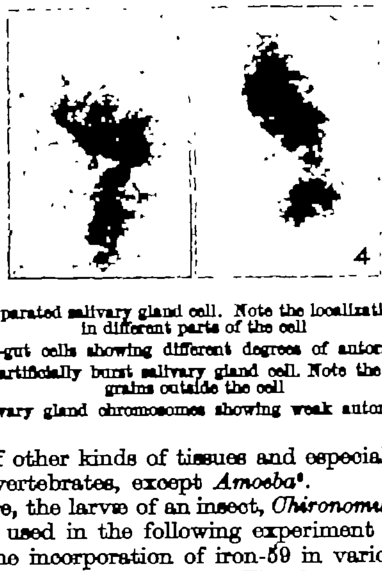


Fig. 1. A separated salivary gland cell. Note the localization of iron-59 in different parts of the cell

Fig. 2. Mid-gut cells showing different degrees of autoradiography

Fig. 3. An artificially burst salivary gland cell. Note the existence of grains outside the cell

Fig. 4. Salivary gland chromosomes showing weak autoradiography

Therefore, the larvæ of an insect, *Chironomus plumosus*, have been used in the following experiment in order to examine the incorporation of iron-59 in various parts of the cells of different tissues. The larvæ were transferred in tap-water containing $^{59}\text{FeCl}_3$ solution (sp. act. 100 mc. ^{59}Fe/g Fe). The experiment was repeated in four series, each of which involved 5 ml. of tap-water containing the following parts of $^{59}\text{FeCl}_3$: (1), 1; (2) 10; (3) 88; (4) 20. The larvæ were kept in the medium in the first and second series for 72 h, in the third for 21 h and in the fourth for 68 h. Preparations were made from salivary glands, mid-gut, Malpighian tubules and rectum according to the squash preparation technique, stained with aceto-orcein, covered with Kodak '*AR* 10' stripping films and exposed for 7–90 days.

No difference was observed resulting from the concentration of $^{59}\text{FeCl}_3$ and immersion period. In almost all the preparations cytoplasm showed autoradiography (Figs. 1 and 2). But the number of grains per mm^2 varied from larvæ to larvæ, from tissue to tissue and even from cell to cell in the same tissue (Fig. 2). Such a variation could be observed even in different parts of the same cell, but preferential localization of the label could not be detected. In some preparations the grains were also found outside the cells. This was especially the case where the cell had been burst artificially during the preparation (Fig. 3). This shows that at least some of the molecules containing iron-59 were distributed in the enchylemma of the cytoplasm and were not bound to the morphological structures of the cytoplasm.

An accumulation of grains around and on the nucleus could be seen in some autoradiographs. However, the number of the grains per mm^2 on the cytoplasm was clearly greater than those on the nucleus. The chromosomes were either unlabelled or showed very weak autoradiography. In squash preparations where the cytoplasm and the nucleus were burst artificially one could observe grains on the chromosomes, too (Fig. 4). But the number of grains seems to be less than those on the intact nucleus and varies from chromosome to chromosome. Therefore, it can be suggested that some iron-59 molecules are not bound to the chromosome, but are localized in the nucleoplasm as observed by Feldherr. No relation could be found between labelling of cytoplasm and chromosomes. For example, in some cells the cytoplasm showed a light radioactivity, whereas the chromosomes were not labelled, and vice versa. In some cases the nucleus showed stronger autoradiography than the cytoplasm.

Similar results were obtained in another experiment, when *Drosophila melanogaster* was used instead of *Chironomus plumosus*.

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BIOLOGY

Glucose Utilization in Pregnant Sheep by a Continuous Infusion Isotope Dilution Method

THE importance of carbohydrate as a metabolic substrate in the ruminant has given rise to much discussion[1]. The question of the source(s) of glucose is of special interest because there is no evidence that hexose is absorbed from the ruminant alimentary tract and because acetate is the major end product of ruminal fermentation. The foetal lamb, however, has considerable hexose requirements[2], and, as a consequence, it is thought that the pregnant female sheep is normally in a precarious state of carbohydrate balance[3]. It is known that even a slight fall in food intake in the pregnant sheep may cause the accumulation of ketone bodies in the tissues and blood stream followed by clinical signs of pregnancy toxaemia.

The continuous infusion isotope dilution technique[4] has been used to study glucose utilization rates in normal pregnant sheep and those with induced pregnancy toxaemia. Four determinations on two normal Clun Forest ewes, pregnant for 130 and 142 days respectively, gave a mean utilization rate of 1·35 ± 0·18 mg/min/kg, and two ketotic ewes, pregnant for 135 and 140 days, gave a mean rate of 1·22 ± 0·13 mg/min/kg. This difference is not significant ($P > 0·5$). The opportunity was also taken to use 1-^{14}C-glucose and 6-^{14}C-glucose in the same animal on successive days and the specific activities of the expired carbon dioxide were compared. It is generally accepted that the ratio of the specific activity of expired carbon dioxide, produced when 1-^{14}C-glucose is injected, to the specific activity of expired carbon dioxide, produced when 6-^{14}C-glucose is injected, that is, the C1/C6 ratio, should be unity if the main catabolic route for glucose is the Embden–Meyerhof pathway to pyruvate, followed by conversion to carbon dioxide in the tricarboxylic acid cycle. Deviation from unity is considered to indicate the existence of another pathway which incorporates C1 of the glucose into carbon dioxide at a greater rate than C6, for example, the pentose cycle.

The C1/C6 ratios for the two normal pregnant sheep were 1·24 and 1·38, respectively, and for the two ketotic animals 1·74 and 2·05. The mean value for the ketotic sheep did not differ significantly from the mean for the normal sheep ($0·1 > P > 0·05$). The situation in ketotic pregnant sheep therefore differs from that in ketotic lactating cattle in which lower C1/C6 ratios than in normal lactating cattle were found[5].

The glucose utilization rates in the pregnant sheep are of the same order as those in non-pregnant sheep after 24 h starvation, but only about half the rates in fed non-pregnant sheep[6]. It would seem therefore that tissue glucose supply is indeed marginal in pregnant sheep, but is not significantly reduced when the animals are showing clinical signs of ketosis. The C1/C6 ratio is of special interest. The decreased ratio in ketotic cattle has been interpreted[5] as indicating a reduction of glucose metabolism by the hexose monophosphate pathway. In a detailed discussion on the use of glucose labelled with carbon-14 for evaluating glucose metabolism[7] it has been pointed out that the C1/C6 ratio may be influenced by at least four variables, that is, the activity of the pentose and of the glycolytic pathways, the use of glucose in synthetic pathways and the proportion of triose phosphate oxidized to carbon dioxide. It is therefore not possible to interpret the C1/C6 ratio in pregnant sheep in terms of percentage pentose cycle activity, but it can be concluded that 1C of glucose is incorporated into CO_2 more readily than 6C in both normal and ketotic pregnant sheep.

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Effects of Lysergic Acid Diethylamide on the Nesting Behaviour of Male Pigeons

PRESENT-DAY advances in psychotherapy have added impetus to investigations into the actions of drugs which affect the central nervous system and thus cause changes in behaviour patterns. One such psychomimetic agent, lysergic acid diethylamide (LSD-25), exerts both peripheral and central actions[1]. Some of these effects in various animals and humans are nausea, vertigo, hyperhydrosis, hypersalivation, mydriasis, vasodilation or vasospasm, hypotension or hypertension, bradycardia or tachycardia, and changes in the white blood cell count[2].

The vasomotor changes are partially related to disturbances in the hypothalamic-hypophyseal-adrenal axis[2]. Rinkel *et al.*[3], studying the effects of LSD-25 on human beings, suggested that this drug stimulated the pituitary-adrenal axis yet rendered the adrenal glands unresponsive to adrenocorticotrophic hormone. It is reasonable to assume that this pituitary response may also cause a change in the blood or pituitary levels of prolactin, which has been shown to be responsible for broodiness or nesting behaviour as well as the development of the crop in pigeons[4–6].

Thus, we considered that an investigation into the effect of LSD-25 on the nesting cycle of pigeons would shed further light on the above concepts.

Eight mated male homing pigeons were removed from their respective nests in a loft between 11 a.m. and noon 3 days after their mates had laid eggs. Observations have shown that at this time the nesting behaviour becomes stabilized into a distinct cyclic pattern. The males sit on the eggs from about 10 a.m. until about 3 p.m. The females will then sit from 3 p.m. until 10 a.m. on the following day. Four of the male birds received LSD-25 in a single dose of 0·2 mg/kg (intraperitoneal). The other 4 males served as controls and received an equivalent volume of distilled water as a placebo. 0·5 h after treatment, the females were removed from the loft and all 8 males were returned. The time required for each bird to return to its own nest, after being placed back in the loft, was recorded and the data were statistically analysed. This programme was repeated until each of the 8 pigeons had received 5 doses of the drug and 5 doses of water with at least a 48-h interval between repeated administrations.

Table 1 INHIBITION OF NESTING BEHAVIOUR OF PIGEONS WITH LSD-25

Bird No.	Drug-treated*	Control*
1	62·7	0·6
2	13·0	7·4
3	73·8	0 0
4	48·4	5·5
5	87·2	0·8
6	55·0	8·6
7	18·4	13·6
8	76·2	11·4
Grand mean ± *S.D.*	57·4 ± 25·8	6·0 ± 12·2

* Average time (min) for return to nests.

The results obtained are summarized in Table 1. They represent the average time (min) required by the pigeons to return to their nests. Each value shown is based on 5 injections of the drug or distilled water. The grand mean was derived from the individual responses of each of the 8 birds. The difference between the experimental and control grand means is highly significant as determined by the standard error of the difference between the means ($P < 0.01$).

It is interesting to note that 2 pigeons (2 and 7) showed very little change in behaviour. These birds responded to the drug only during the first 3 exposures, after which they did not appear to show any reaction.

This temporary inhibition of the nesting response is not due to a peripheral motor inhibition. In view of the rapid metabolic rate of the pigeon and the fact that the peripheral symptoms wear off within 30–40 min (ref. 7) leads us to believe that the effect is due to changes within the central nervous system. It is possible that the drug, by acting on the hypothalamic centre in the brain, causes an inhibition of prolactin release from the pituitary. This lowering of the prolactin-level would decrease or inhibit the 'desire' to nest. It is also possible that the drug causes a change in the bodies' response to prolactin as it does with adrenocorticotrophic hormone. In order to substantiate these mechanisms, biochemical or bioassay studies are required.

We thank Sandoz Pharmaceuticals, New Jersey, for providing the *LSD*-25.

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Effect of Reserpine on Rate of Mortality and Growth of Duck Embryos

ADAMS *et al.*[1], who injected varying doses of reserpine into the yolks of eggs of White Plymouth Rock hens during the first week of incubation, reported that in the embryos treated the mortality rate increased with increasing dosage, and that the average body-weight of the surviving embryos was lower than that of the controls. On the other hand, van Limborgh *et al.*[2] were unable to demonstrate a similar influence on the mortality rate in one-day Khaki Campbell duck embryos. Uchida[3] observed a rise of the mortality-rate with increasing dosage but no significant changes in the body-weight in eighteen-day-old White Leghorn egg embryos.

The discrepancies in these results seemed to justify a re-examination of the influence of reserpine. For this work eggs of Khaki Campbell ducks were used. Three experimental series were drafted: in series I reserpine was injected after four days of incubation, in series II after eleven days, and in series III after eighteen days. Each series consisted of 96 eggs with living embryos, and these were subdivided at random into four series of 24 eggs (sub-series *a*, *b*, *c* and *d*). Each time doses of 10, 50 and 250γ of reserpine (0·20 ml.) in the form of 'Serpasil' were injected into the yolks of the eggs of the sub-series *b*, *c* and *d*; only the solvent was injected into the eggs of sub-series *a*. Four days after injection the eggs were opened and the number of deaths determined. The surviving embryos were all fixed and weighed under standardized conditions. Statistical analysis of the results included determination of the significance of differences between percentages, Student's *t* tests on the body-weights and Terpstra's tests against progression in samples.

The results of the experiments are summarized in Table 1.

Table 1. SUMMARY OF THE RESULTS OF THE EXPERIMENTS

Series	No. of embryos treated	No. of embryos died	Mortality-rate (%)	Surviving embryos No.	Surviving embryos Mean body-weight (g)
I*a*	24	2	8·3	22	0·271
I*b*	24	3	12·5	21	0·232
I*c*	24	4	16·7	20	0·222
I*d*	24	10	41·7	14	0·214
II*a*	24	4	16·7	20	3·61
II*b*	24	6	25·0	18	3·90
II*c*	24	9	37·5	15	3·56
II*d*	24	10	41·7	14	3·62
III*a*	24	4	16·7	20	22·0
III*b*	24	8	33·3	16	21·9
III*c*	24	14	58·3	10	18·7
III*d*	24	23	95·8	1	(17·1)

With regard to the mortality-rate, Table 1 shows that, within each series, this rate rises with increasing dosage. The differences between the individual mortality percentages of the various sub-series are significant in only part of the cases, but the rise noted is highly significant in each series (series I: $P < 0.002$; series II: $P < 0.02$; series III: $P < 0.0001$). Comparison of the mortality-rates found in sub-series I*a*, II*a* and III*a*, shows that in the controls mortality does not rise with increasing age ($P > 0.20$). However, following the administration of any dose of reserpine the mortality rate rises significantly with the age of the embryos (10γ, $P < 0.05$; 50γ, $P < 0.02$; 250γ, $P < 0.002$).

With regard to the body-weights of the surviving embryos, Table 1 shows that the mean body-weight of the controls of series I is much higher than that of the reserpine-treated embryos of this series. The differences are significant ($P < 0.05$, $P < 0.001$ and $P < 0.001$ respectively). The smaller differences between the sub-series I*b*, I*c* and I*d* are not significant. Nevertheless, there is a significant correlation between increase of the dose and decrease of the body-weight ($P < 0.0003$). In series II there are neither significant differences nor a systematic rise or fall of body-weight with the dose; however, in this series the individual body-weights varied extremely. In series III the only significant difference is that between the sub-series IIIb and IIIc ($P < 0.05$), but again a significant correlation exists between increase of the dose and decrease of the body-weight ($P < 0.01$).

These findings show that reserpine has a noxious action on duck embryos, and it is of particular interest to note that older embryos are more sensitive to this action than younger ones. This indicates that reserpine acts on a factor which, with increasing age, becomes of increasing importance to life. Since the drug is known to bring about changes in metabolic processes[4,5], it might be that it acts on some enzyme system. However, further work is needed to elucidate this problem.

We thank Ciba N.V., Arnhem, for the generous supply of the solvent of 'Serpasil'.

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Pogonophora from British Coastal Waters

A SUB-LITTORAL faunistic survey of the Scottish west coast has recently produced specimens of the genus *Siboglinum* from 55–62 fathoms in the North Minch about 8 miles east of Lewis. *Siboglinum* has been found in deep water in the north-east Atlantic and an undescribed species has been recorded from 80–90 fathoms off the west coast of Ireland by Southward[1], but Pogonophora were not previously known from inside British coastal waters.

The samples were collected with a 1/10 m² spring-loaded grab and the grounds surveyed included the Minches from Dubh Artach north to the Butt of Lewis, as well as all navigable mainland sea lochs and most of the Hebrides lochs. Of several hundred samples covering this area, Pogonophora were found in only four, all from the extreme north-west corner of the Minch. Details of these collections are given in Table 1.

Table 1. DISTRIBUTION OF SPECIMENS

Position	Depth (fath.)	No. of specimens per 1/10 m²
58° 24′ N., 05° 55′ W.	60	3 specimens plus several tubes
58° 27′ N., 05° 55′ W.	58	Several empty tubes
58° 31′ 48″ N., 05° 55′ W.	55	1 specimen
58° 33′ 30″ N., 05° 55′ W.	62	21 specimens plus many tubes

The bottom on which they occurred consisted of fine grey sand, and an analysis of the associated fauna is given in Table 2. At stations farther south and east where Pogonophora were not found the bottom was generally softer and more muddy and the fauna consisted either of a typical *Ditrupa* community, or of an association, common in the lochs, dominated by the polychaete *Rhodine*.

Hydrographically the distribution of the Pogonophora seems to be closely associated with a tongue of comparatively saline water from west of the Hebrides which flows round the Butt of Lewis and influences the north-west Minch. This gives bottom temperatures ranging annually from 7°–8° C in February to 11°–12° C in late September, and salinities of about 35·10 parts per thousand.

These animals thus seem to be restricted to a small part of the Minch with faunistic and hydrographic characteristics which differentiate it clearly from the rest of the area.

I thank Dr. Eve C. Southward for examining the specimens. She considers them to be identical with the species referred to from off Ireland, and is preparing a description.

Table 2. ANALYSIS OF ASSOCIATED FAUNA (NO. PER 1/10 M²)

Astrorhiza limicola Sandahl	18	*Spiophanes bombyx* (Claparède)	4
Dentalium entale L.	1	*Prionospio malmgreni* Claparède	10
Abra prismatica (Montagui)	14	*P. cirrifera* Wiren	4
Spisula elliptica (Brown)	1	*Polydora* sp.	72
Opisthobranchia	5	*Chaetozone setosa* Malmgren	2
Ophiura affinis Lutken	5	Cirratulidae	5
Spatangidae juvs.	30	*Pectinaria auricoma* (Muller)	12
Echinocyamus pusillus (O. F. Muller)	1	*Diplocirrus glaucus* Haase	5
Amphiura filiformis (O. F. Muller)	1	*Ammotrypane aulogaster* Rathke	1
Thyone sp.	2	Maldanidae	5
Leptosynapta sp.	5	Ampharetidae	4
Sthenelais limicola Ehlers	2	Terebellidae	14
Pholoe minuta (Fabricius)	1	*Owenia fusiformis* Delle Chiaje	12
Eulalia sp.	1	*Myriochele heeri* Malmgren	65
Nephtys hombergi Lamarck	5	Nemertini	2
Glycera sp.	2	Amphipoda	18
Goniada maculata Oersted	2		

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Oysters killed by Trematode Parasites

DURING laboratory breeding experiments with the New Zealand mud oyster, *Ostrea lutaria* Hutton, sectioned material showed specimens with gonads containing sporocysts of a digenetic trematode parasite. The sporocysts were tubular and branched as in the family Bucephalidae, and contained rediæ, but as cercariæ were not found the trematode has not yet been identified.

Twenty-two of the oysters were fixed on arrival from Foveaux Strait, New Zealand, and showed 13·6 per cent parasitized. The remainder were kept in aquaria for 6 months and those that died were fixed and sectioned. Samples of living animals were also fixed and sectioned at intervals during the same period. The parasitization in the two groups was as follows.

	No. parasitized	No. not parasitized	Parasitization (%)
Oysters that died	35	17	67·3
Oysters that remained alive	3	17	15·0

The highly significant difference indicates that the parasites were an important cause of death.

Heavy infection causes almost complete destruction of the gonad, and later, death of the oyster. The importance of this parasite in Nature is unknown, but under laboratory conditions all the animals initially parasitized may die. Until the species is identified and the hosts are known, the probability of accidentally spreading the trematode to oysters in other countries cannot be assessed, but the danger should be recognized by anyone introducing oysters from New Zealand.

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Extension and Retraction of the Tube-Feet of Ophiuroids

THE greater part of the basic anatomy of ophiuroids was worked out in the latter half of the last century and the results summarized in Bronn[1]. In the water-vascular system, the absence of tube-foot ampullæ comparable with those of asteroids or echinoids is striking. It has been generally recognized that the feet are less important in locomotion than the arms, and they are frequently attributed only sensory and adhesive functions. In several common genera the tube-feet are small and show only slight changes of shape, although protraction has been recorded[2,3]. Perhaps for these reasons there has been little discussion of possible mechanisms of protraction and it seems often to have been tacitly assumed that none is present, and that retracted feet resume the extended condition only on relaxation of their musculature (which is wholly longitudinal), by the general pressure of the coelomic fluid in the water-vascular canals or by the elasticity of the podial wall. Ludwig[4] was the first to describe the valve at the head of the podium, the lips of which prevent water from flowing back into the radial canal. The presence of this valve has been remarked upon by all subsequent reviewers of the group; but its significance, namely, that pressure is developed within the foot, has been overlooked. Comparisons between active and anaesthetized specimens of various species suggest that in a number of cases podial extension is far in excess of that which could be achieved by relaxation alone.

A number of genera have been examined histologically (*Acrocnida*, *Amphipholis*, *Amphiura*, *Ophiactis*, *Ophiocomina*, *Ophiopholis*, *Ophiopsila*, *Ophiothrix* and *Ophiura*) and in all cases the podial musculature is differentiated into two systems, one in the long stem and the other in the proximal internal end or 'head' of the foot, which is generally embedded in a cup-like expansion of the vertebral ossicle. We contend that these systems are generally antagonistic and that the head of the ophiuroid podium is the functional equivalent of the ampulla of other classes.

This arrangement is clearest in the burrowing genus *Amphiura*. The head of the podium is distinctly bulbous, and its muscle fibres are deeper and more numerous than those of the stem and quite distinct from them. In *Amphipholis squamata*, on the other hand, the two muscle systems are less differentiated; some fibres are common to both systems, but there are many more in the slightly inflated head of the podium.

When the foot retracts, the ampulla-like head of the foot cannot expand so much as the ampulla of an asteroid, because it is enclosed by ossicles and because there are no large body spaces in the arm. If the foot is to be fully retracted, a limit is set to the volume of hydraulic fluid within the podium and thus to the amount by which the foot can be protracted. The full retraction of longer feet is obtained in many species by forcing excess fluid into the radial canal. This situation can often be recognized by two structural characteristics.

First, the valve between the head of the podium and the branch from the radial canal is equipped with muscle fibres which can force the lips apart. Secondly, the radial canal is partitioned by muscular sphincters situated between each vertebra, so that each pair of tube-feet has its own elastic expansion chamber and can operate independently of its neighbours. These sphincters have been described in *Ophiactis virens* by Simroth[2] who thought they were responsible for the slight protraction of tube-feet which he observed. We have found them in *Acrocnida brachiata*, *Amphiura chiajei*, *A. filiformis*, *Ophiactis balli*, *Ophiocomina nigra*, *Ophiopholis aculeata* and *Ophiothrix fragilis*. In addition to the circular muscle fibres forming the sphincters, longitudinal fibres generally run along the floor of the radial canal and are more strongly developed in the region of the sphincters, to which they may be antagonistic.

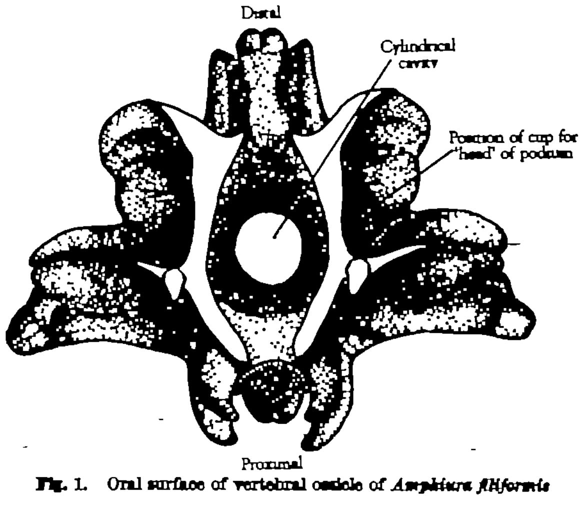

Fig. 1. Oral surface of vertebral ossicle of *Amphiura filiformis*

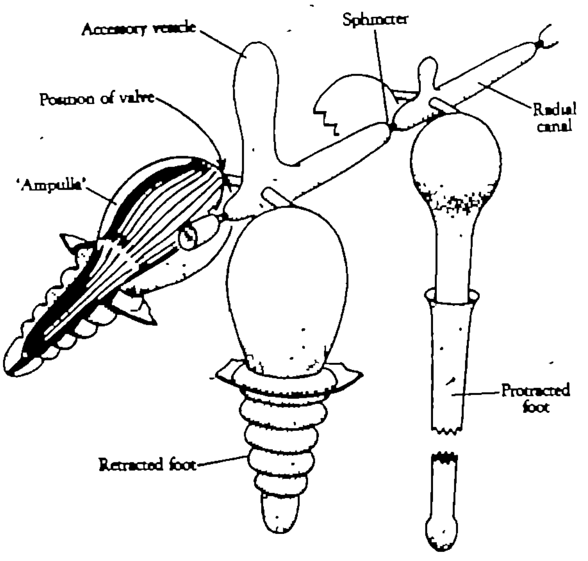

Fig. 2. Diagram of part of radial water-vascular system of *Amphiura filiformis*

Amphiura filiformis is capable of very considerable extension of the tube-feet. It possesses a series of accessory vesicles which emerge from the radial canal in each segment at the point where the lateral branches are given off to the podia. The vesicles are non-muscular, but apparently elastic, and are sometimes fixed in an expanded and sometimes in a relaxed condition. Each is suspended by connective tissue strands in a cylindrical cavity which passes completely through the middle of the vertebral ossicle (Fig. 1). This hole through the ossicle occurs in several members of the Amphiuridae[3], but not in *A. chiajei*.

Fig. 2 represents the water-vascular system of two arm segments of *A. filiformis*, and summarizes our ideas of how the system works. The right-hand pair of podia are protracted; the accessory vesicle and radial canal are relaxed, the valve is shut, the foot-head muscles are contracted and the stem of the foot is elongated. The left-hand pair are retracted; the 'ampullae' are dilated, the valves have been opened, and the accessory vesicle is expanded.

It seems possible that active protraction is general throughout the Ophiurae, because evidence for it has been found in all species examined, drawn from five families. Modification of the radial canal to permit greater extension is also widespread, but the details vary between species and much further work remains to be done. The configuration of the arm water-vascular system seems to have been similar in fossil somasteroids[6]; it will be interesting to have histological reports on *Platasterias*, the living somasteroid reported by Fell[7].

One of us (J. D. W.) is grateful for a studentship from the Department of Scientific and Industrial Research. Both of us thank Dr. D. Nichols for his useful criticism.

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'Campaniform-type' Organs in Nematodes

CAMPANIFORM organs are common in insects[1], where they have been demonstrated to act as proprioceptors[2,3]. Morphologically they show considerable variation but "In vertical section the cuticular parts of the organs generally have the form of a small dome into which the sense cell process is inserted like the clapper of a bell"[1] and the structure of various types is illustrated by Snodgrass[1].

In the Nematoda lateral gland cells and pores have been known to occur in many groups for some time and are considered to be particularly obvious in species of the family Cyatholaimidae (Chromadoroidea), where they are arranged in longitudinal rows running down the lateral, dorsal and ventral surfaces of the body. I have examined these pores in species of the genera *Cyatholaimus*, *Longicyatholaimus*, *Choniolaimus* and a new genus closely related to the two latter genera (all belonging to the Cyatholaimidae) and the structures are not simple pores but complex organs very similar to the campaniform organs of insects. I have found two types of organ of which the most common and widespread is that shown in Fig. 1, 3–5. It takes the form of an elliptical cup into which projects a thin sheet of cuticle which is attached to the bottom and two sides of the cup. This Type-1 organ occurs in longitudinal rows running the full length of the body in all the genera studied and in all, except the *Longicyatholaimus* species, the sheet of cuticle runs transversely—in *Longicyatholaimus* species it runs anteroposteriorly (this may be an artefact or possibly a functional distortion). The second type of organ (Fig. 1, 1–2) is found only on the *Choniolaimus* species, where it is restricted to a short row of seven organs running posteriorly from the amphids on each lateral side of the body and two similar rows, one on each side of the body, running posteriorly from about the level of the cloacal or anal opening. This Type-2 organ is circular in plan (Fig. 1, 1) with a central dot, which is the raised central part of a domed cap which lies within the sense pore and below the surface of the cuticle (Fig. 1, 2). Each organ is supplied with a nerve and apparently with a gland cell; but the condition of my material does not allow of a more detailed study. The association of a gland cell and a sense organ is not unexpected since this condition has been found to hold in many other nematode sense organs.

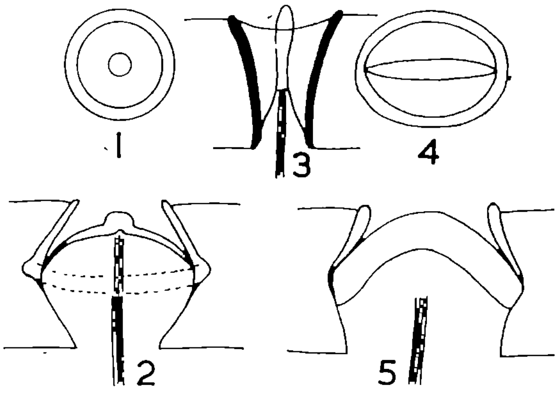

Fig. 1

It is reasonable to suppose that these organs function as mechanoreceptors in view of their morphological similarity to the campaniform receptors of insects, a suggestion supported by their distribution over the body. The distribution of Type-2 is particularly suggestive since it occurs on exactly the parts of the body which are subject to the most movement during the search for food and during copulation. The occurrence of such morphologically similar structures in both nematodes and insects does not necessarily lend weight to any idea of nematode/insect evolutionary relationships since they almost certainly represent the independent development of similar organs from similar materials to meet similar requirements and should be treated as an example of convergent evolution.

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Fine Structure of *Pythium de baryanum* Hesse and its Probable Significance

ELECTRON micrographs of young hyphæ of *Pythium de baryanum* reveal some striking differences from young hyphæ of other fungi, for example, *Rhizopus* spp.[1], *Neurospora crassa*[2], and vegetative cells of yeasts[3].

The most striking difference is in the structure of the cytoplasm. Young hyphæ of *Pythium* show more numerous and more regularly distributed cisternæ and vesicles than are seen in hyphæ of *Rhizopus* and other fungi examined, suggesting a more regular endoplasmic reticulum. In older vacuolate hyphæ the endoplasm is less complex. In the young hyphæ the cisternæ may occur in layers (Fig. 1) resembling the Golgi bodies (dictyosomes) of some algae, for example *Paraphysomonas vestita*[4] and green plants, for example, *Anthoceros*[5] and maize root meristem[6]. No such distinct Golgi bodies of plant type have been demonstrated in other fungi although irregular aggregations of cisternæ were shown by Blondel and Turian[7] in *Allomyces* and by Hawker and Abbott[1] in *Rhizopus*.

The mitochondria of *Pythium* also differ from those of *Rhizopus* and certain yeasts in having shallower, undulating cristæ of irregular thickness and depth of penetration into the mitochondrial cavity. Tubules are also sometimes present in the centre of the cavity. They are closely similar

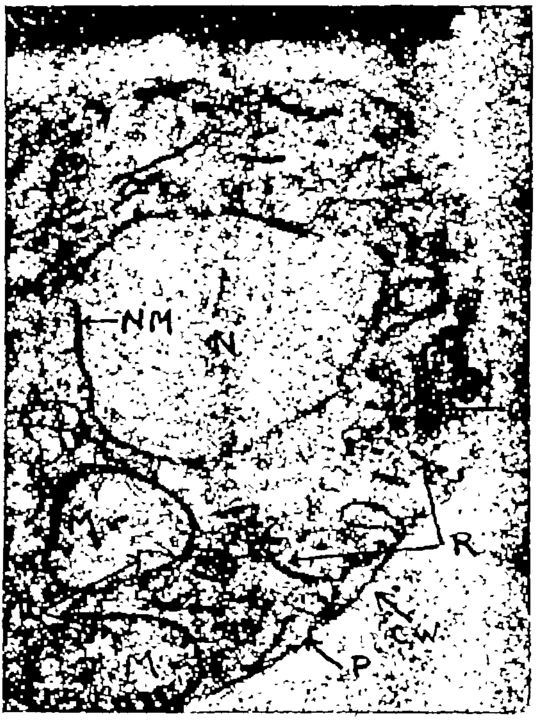

Fig. 1 Transverse section of part of a young hypha of *Pythium* (× 25,000). *CW*, Cell wall; *P*, plasmalemma; *R*, endoplasmic reticulum; *G*, Golgi body; *M*, mitochondrion; *MC*, irregular cristæ. *N*, nucleus; *NM*, nuclear membrane (note pores)

in appearance to those seen by Manton[5] in the liverwort *Anthoceros*.

The nuclear membranes are less regular in combined thickness and show more numerous pores and larger discontinuities than do those of other fungi studied.

The cell wall of *Pythium*, usually considered to consist of cellulose, is not electron dense and shows no structure in section, thus resembling the wall of *Allomyces*[7] but contrasting with that of *Rhizopus* which is electron dense and finely striate in section.

Nutritional investigations[8], the structure of the flagellæ of the zoospores[9] and the well-known morphology of the sexual reproductive organs already show that the Oomycetes differ widely from other Phycomycetes and from the higher fungi. The present study of the fine structure of *Pythium* confirms this difference and, in addition, indicates a closer affinity with certain algae and green plants than is shown by fungi in general.

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Grass Tetany and Histamine

THE occurrence, distribution and ætiology of grass tetany in cattle and sheep have already been adequately described[1,2]. The most consistent symptom in all cases is a low serum magnesium-level, values below 1 mg per 100 ml. being common. The condition is sometimes accompanied by hypocalcæmia[2]. Nevertheless, severe hypomagnesæmia can be present in some cases without the appearance of clinical symptoms of tetany, which raises the question of the possible occurrence of other causative factors. The high incidence of grass tetany, for example, on young, rapidly growing pastures has given rise to views that some factor may be introduced by the herbage, and Seekles[4] has even suggested the term 'nutrition-tetany' for the condition. This factor may be independent of those which act by reducing availability of magnesium to the animal.

Death in grass tetany is usually attributed to shock. A paper by Schayer[3] would ascribe all shock phenomena to rapid histamine release into the blood. The histamine-levels in blood serum in cases of grass tetany have been shown by Seekles[4] either to be abnormally high or abnormally low. Abnormally low values were attributed to the dilating action of histamine on blood capillaries causing increased permeability of the capillary walls. Among compounds known to cause histamine release on injection both aliphatic and substituted aromatic amines and the ammonium ion have been recorded[5]. Whether dietary factors could produce similar effects is not yet known but Parrot[6] has been able to cause the death of guinea pigs by parenteral administration of histamine and has also shown that prior administration of putrescine reduces the minimum lethal dose to one-tenth of its formerly required level. The guinea pig is, however, known to be abnormally sensitive to histamine. The presence of putrescine and cadaverine in spring grass has been reported by van der Horst[7] and histamine also is present[8].

The influence of low- and high-calcium diets in guinea pigs on death from histamine has been investigated[9]; low calcium diets reduce the minimum lethal dose administered into the stomach. Similarly, diets high in potassium lead to greater susceptibility to histamine when administered intravenously to mice[10] and the influence of heavy potash fertilization in conditioning the production of tetany-prone swards has been frequently reported[1].

The treatment of grass tetany by the use of antihistaminics in conjunction with magnesium–calcium therapy has been reported by Hendriks[11] and some preliminary observations using 'Antiallergicum Vetag' (Veterinaria S.A. Zurich), both with and without magnesium–calcium injections have given results which justify further investigation.

It seems desirable, therefore, to widen the scope of investigations in grass tetany from the field of magnesium availability alone to the manner in which magnesium deficiency, either alone or with other factors, causes tetany. In this wider investigation the relationship of magnesium deficiency to histamine metabolism may play an important part and deserves closer examination.

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Possible Presence of a Phytotoxin in Red-ring Infected Trees

RED-RING disease of coconuts is caused by the nematode *Rhadinaphelenchus cocophilus* (Cobb 1919) Goodey 1960, which colonizes the stem in a ring 3–5 cm inside the periphery where the fibro-vascular strands are densest, and causes the formation of a distinctive red-orange band in this zone. Characteristic symptoms are yellowing and wilting of leaves.

Although the causal agent and its method of spread have been examined[1], little or no work has been reported on the damage mechanism. The symptoms of red-ring disease are almost identical with those of bronze leaf wilt, which has been clearly shown to be due to vascular failure[2]; it is therefore possible that the damage mechanism of red-ring disease is similar in nature. This might result from blockage of vessels in the infected zone, but injection experiments using dyes into healthy and into diseased trees do not support this hypothesis, since the dye is detectable in the stem right up into the bud and out into the leaves[3]. Moreover, growing, healthy coconut trees can suffer very extensive damage to the stem and therefore to the vascular strands without obvious deleterious effects.

It is also possible, however, that wilt symptoms can arise from other causes such as the production of toxins. This may result in decreased efficiency of leaf cells with a consequent reduction in maintenance of the full transpiration stream[4–6]. Gross observations indicate that this may be the case in coconut trees infected with red-ring.

Preliminary experiments on seedlings and cuttings of tomato confirm this latter hypothesis. Hot- and cold-water extracts of red-ring infected tissue induce rapid and severe wilting of these plants, the effect being more rapid in the case of cuttings. The effect appears to be independent of the age of the extract and of the presence or absence of tissue fragments or *Rh. cocophilus* in the extract. The behaviour of tomatoes in extracts of healthy tissue is different, in that freshly prepared cell-free extracts do not produce wilting unless the extracts are allowed to stand for several hours before removal of tissue fragments. If tissue fragments be removed, it is necessary to stand the cell-free extracts overnight before they can induce wilting. There is, therefore, reason for believing that the toxin is not produced directly by the nematodes but is the product of tissue disintegration as a result of nematode invasion.

Exposure of extracts to temperatures of 100° C for 30 min does not affect their ability to induce wilting, and it is justifiable to suppose that the toxin, if present, is relatively thermostable.

Other tests have been carried out using three palms, *Ptychorapis augusta*, *Licula spinosa* and *Thrinax morissii*, as indicator plants to show the presence of a possible vivo-toxin. The results of tests with these plants are similar to those using tomato, although wilting is less pronounced. Wilting in diseased tissue extract does not occur in less than 72 h after immersion, while wilting in cell-free, healthy tissue extracts, takes far longer. These palms are far too small to observe either the external symptoms, such as yellowing, or internal symptoms, such as discoloration. In tomato plants, however, a distinct pattern of yellowing is observed. Yellowing precedes wilting in these plants. The yellowing begins along the midrib, extends along the lateral veins, then the interveinal spaces yellow and finally necrosis of the leaf occurs.

It is suggested that these observations are consistent with the production of a toxin or toxins in coconut tissues, and that the toxin or toxins are produced indirectly as a result of tissue breakdown following nematode invasion.

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Effect of Treatment of Seeds with 2-Chloroethanol on the Resistance to Boron Toxicity in Wheat Seedlings

It has been shown that treatment of seeds with 2-chloroethanol increased the resistances to high salt concentration[1] and herbicide[2]. The present study is concerned with the influence of seed treatment on the boron toxicity.

Table 1. Effect of Seed Treatment with 2-Chloroethanol on the Resistance to Boron Toxicity in Wheat Seedlings

Seed treatment	Boron application (mg boron/200 g soil)	Wilted plants (per cent) h after applic. of boron 48	72	96
0·2 per cent 2-Chloroethanol (14 h)	12·5	1·9	37·5	90·3
,, ,, ,,	9	0	22·8	78·9
,, ,, ,,	5	0	4·7	23·5
Control	12·5	18·4	69·2	100·0
,,	9	4·5	43·6	92·7
,,	5	0	23·1	66·3

Wheat seeds (variety, Carstens VIII) were soaked in 0·2 per cent 2-chloroethanol for 14 h at room temperature. After that, the seeds were spread on a filter paper and dried for 4 h. The control seeds were treated in the same way using water instead of 2-chloroethanol solution. 200 g sandy soil (*p*H 5·85) was placed in a Neubauer container and moistened by 25 ml. of water. Forty seeds were placed in the soil. At 6 days after the seeding, varying amounts of boron in the form of 5,722 p.p.m. H_3BO_3 solution (1 mg boron/1 ml. solution) were applied on the surface of soil. The application of solution increased the water content of soil temporarily, and further irrigation of water was made after the moisture content of soil reduced to the original level. The first visible symptom of the toxicity was the wilting of the tips of leaf blades. The numbers of wilted seedlings were recorded. Results are shown in Table 1. Tests were replicated three times and the averages were calculated. From the results indicated in the table, I have arrived at the conclusion that the seed treatment increased the resistance.

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Growth-Inhibiting Substances in Xylem Sap

A wide range of substances is already known to occur normally in xylem sap[1]. Experiments in this laboratory have now shown that growth-inhibiting substances are also present in xylem sap extracted from a range of woody plant species.

Xylem sap was collected from woody shoots using a method previously described[2], which allows quantities of up to 100 ml. to be extracted readily. To examine extracted sap for growth-inhibiting substances a volume of this material was first acidified to approximately *p*H 2·0 with dilute sulphuric acid and shaken three times with equal volumes of ether. The combined ether washings were then reduced in volume, samples placed on Whatman No. 1

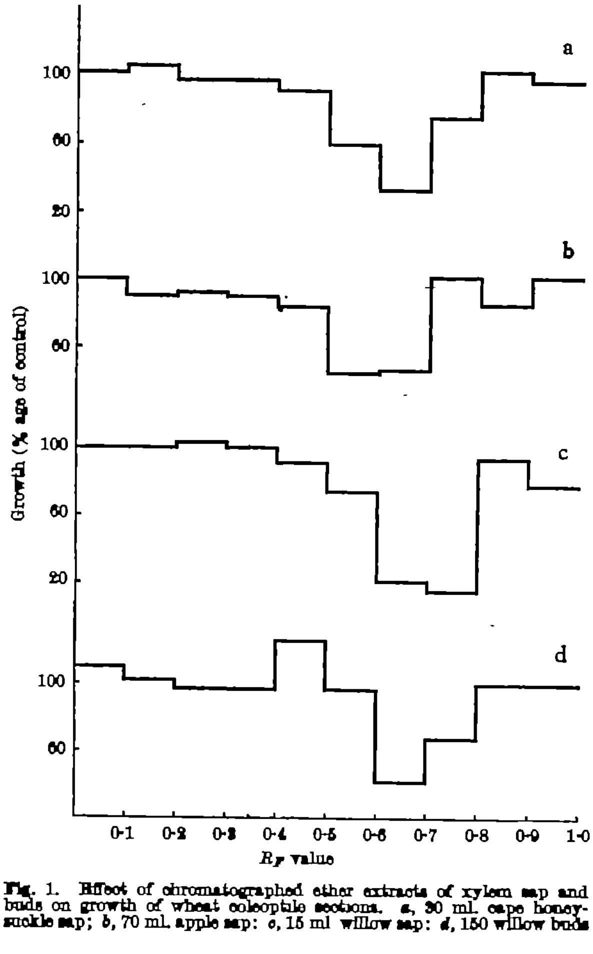

Fig. 1. Effect of chromatographed ether extracts of xylem sap and buds on growth of wheat coleoptile sections. *a*, 30 ml. cape honeysuckle sap; *b*, 70 ml. apple sap: *c*, 15 ml willow sap: *d*, 150 willow buds

chromatographic paper and chromatographed in a solvent of isopropanol/ammonia/water (10 : 1 : 1). The presence of growth-inhibiting substances on the resulting chromatogram was detected by the wheat coleoptile test using small rotating assay tubes containing 0·4 ml. of 2 per cent sucrose plus the paper section[3]. Elongation of coleoptiles was measured after 18–20 h. Fig. 1*a*–*c* show the results of tests with xylem sap of three species—Cape honeysuckle (*Tecomaria capensis* Spach), apple (*Malus sylvestris* (L) Mill. var. Sturmer pippin) and willow (*Salix fragilis* L.).

A region of marked inhibition is present on each chromatogram at an R_F value of about 0·5–0·7, suggesting the presence of similar substances in each sap.

Comparable assays of the acidic fraction of an ether extract of dormant willow buds (Fig. 1*d*) also show an inhibitory zone at approximately the same R_F values. This would suggest that the sap and leaf extracts contain similar inhibitory substances. The position of the zone of inhibition seems generally similar to that of the widely distributed 'inhibitor β'[4].

The present demonstration of such inhibitory substances within a transport stream of the plant raises the question of their possible regulatory significance in various growth phases of the plant. Such a means of free movement of inhibitors could be, for example, of importance in correlative phenomena such as control of dormancy and bud break[5–7]. Some relationship is certainly suggested because of the apparent similarity of the inhibitors present in xylem sap and in buds. Clearly, further investigations of the origin, nature and role of the inhibitors present in xylem sap are required. It is notable that no substances with growth-promoting activity could be detected in the sap extracts along with the inhibitors but this is not unexpected in view of the known strictly polar movements of naturally occurring auxins.

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Embryology of the New Zealand Onchidiidae and its Bearing on the Classification of the Group

THE Onchidiidae are a group of the Gastropoda, possessing both Pulmonate and Opisthobranch characters. This fact has led them to be classified sometimes as Pulmonates and at others as Opisthobranchs. It is rather surprising to find, in a group that could throw some light on the origin of Opisthobranchs and Pulmonates, and the classification of which is debatable, that the embryology of only one species, *Onchidella celtica* Forbes and Hanley, has been examined. The two papers published on the embryonic development of this species have both resulted in important conclusions being drawn as regards the classification of the Onchidiidae.

Joyeux-Laffuie published, in 1882[1], a long paper on the organization and development of *O. celtica*. His embryonic section was based on the examination of whole mounts, and this, combined with his taxonomic conclusions some of which were erroneous, would cause the systematist to remove the family from the Pulmonata to the Nudibranchia. The second paper was that of Fretter[2] on the functional morphology and post-veliger embryology of *Onchidella celtica* and their bearing on its relationships. Fretter discusses the Opisthobranch and Pulmonate affinities of the Onchidiidae and concludes that the Opisthobranch tie is the strongest.

During 1961 I investigated the embryology of the three species of Onchidiidae found in the New Zealand region. (Six species are described in the literature, but in a paper now in preparation this number has been reduced to three.) The investigation was concentrated on *Onchidella nigricans* Quoy and Gaimard, but embryonic material pertaining to the other two species was also examined.

Onchidella nigricans breeds at Lyttelton (43° 40′ S.) during the months of December, January and February. As are all Opisthobranchs and Pulmonates, it is an hermaphrodite. Copulation takes place and this may be unidirectional, that is, one individual acts as a male and the other as a female, reciprocal or in the form of a ring. So far as is known, the last method has not been observed in any other gastropod, but a slight modification of chain copulation would produce ring copulation. About 500–11,000 eggs are deposited in each egg mass, these being of the same general type as found throughout the Opisthobranchs. The early embryonic development is very similar to that of other gastropods and results, after approximately 25 days, in the formation of a veliger. Unlike *O. celtica*, *O. nigricans* has a pelagic veliger. This has the appearance of a typical Opisthobranch veliger. The duration of its pelagic life is unknown but it is probably short, lasting about seven days. This is based on a number of facts: (1) in the laboratory the pelagic period lasted about 7 days; (2) the shell of the veliger consists of about three-quarters of a whorl, the simple outer lip of the shell is scarcely incurved and the velum is not large; (3) there was little change in size and organization of the veligers during their planktonic life. The fact that *O. nigricans* possesses an egg mass containing a large number of eggs which hatch into free-swimming veligers increases the number of points in which these molluscs agree with Opisthobranchs.

The Onchidiidae are intertidal in habitat, and so have been regarded as having re-invaded the marine zone from a terrestrial habitat. If this is the case, then *O. nigricans* has developed a free-swimming veliger which is almost identical to that of many Opisthobranchs. As Needham[3] states, evolution is reversible in the sense that structures or functions once gained can be lost, but irreversible in the sense that once lost they can never be regained. Thus, it is unlikely that a free-swimming veliger, showing the characteristic structures of an Opisthobranch larva, would reappear in evolution.

Fretter[2] has listed the Opisthobranch and Pulmonate characters possessed by the Onchidiidae. To her list of Pulmonate characters may be added another one, the spiral form of the spermatozoa. So far as is known this character is only found in Pulmonates. In view of the mixture of Pulmonate and Opisthobranch characters found in the Onchidiidae, the group must always occupy a debatable position in any clear-cut classification. It is generally considered that Pulmonates and Opisthobranchs arose either from closely related Prosobranch groups, or that the Pulmonates may have originated from an early group of the Opisthobranchs themselves. Whatever is the case, the Onchidiidae lie very close to the stem of these two groups. In agreement with Labbe and Fretter[4], it appears that the most suitable place to assign the group is with the Opisthobranchs.

The other two species, *Onchidella flavescens* Wissel and *Onchidella campbelli* Filhol, both possess direct development. Their egg masses are basically the same as those of *O. nigricans* but they are larger and contain fewer eggs. Little embryological material was available for *O. campbelli* as this species occurs only on the Sub-Antarctic islands of New Zealand. The development of *O. flavescens* and *O. celtica* is very similar.

Throughout the animal kingdom invasion of the land is usually considered to have taken place via estuaries. Various species of the Onchidiidae found in the Australasian region constitute an excellent series exemplifying this. *O. nigricans* appears to possess the strongest marine affinities, as it has a free-swimming veliger, while species such as *O. flavescens* and *O. campbelli* have taken one step towards the invasion of a terrestrial habitat, in that they possess direct development. *Onchidium damelli* forms the next link in the invasion of land, this species being restricted to estuarine waters and mangrove swamps. Finally, *Onchidina australis* can be found up to 30 ft. above high-tide mark.

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MICROBIOLOGY

Sensitization of Bacterial Spores to Lysozyme and Hydrogen Peroxide with Reagents which Rupture Disuphide Bonds

VINTER[1–4] showed that the coat fractions of bacterial spores contain a high concentration of cystine disulphide bonds. Spore coats also contain a protein component[5,6] with which cystine could be associated. We have therefore subjected spores to reagents known to rupture protein disulphide bonds, and have investigated the results of these treatments.

Spores of *Bacillus cereus* NCTC 945 were incubated at 37° C with performic acid (2·5–25 per cent v/v); thioglycollic acid (1–25 per cent v/v) in the presence or absence of 8 M urea; 2-mercapto-ethanol (10 per cent v/v) at *p*H 3 in 8 M urea; sodium sulphite (12·5 per cent w/v) in the presence of 0·05 M cuprammonium ions and 8 M urea. Formic acid (2·5–25 per cent v/v) and hydrochloric acid (*p*H 3) were used as controls. None of these treatments altered the brightness of spores viewed by phase-contrast microscopy. Treated spores were washed with water and incubated with lysozyme at 37° C. Lysozyme caused phase darkening of the chemically treated spores resembling that which occurs during normal spore germination. The rate of phase darkening in lysozyme was quicker when urea was included with the disulphide bond-breaking reagents. Lysozyme did not alter the phase-contrast appearance of control or untreated spores.

Sensitization of spores to lysozyme with thioglycollic acid could be reversed by slow oxidation of the spores in aerated water. When attempts were made to reverse the effect of the thioglycollic acid treatment with hydrogen peroxide (0·04–4·0 M) it was found that the peroxide also caused phase darkening of the treated spores. Hydrogen peroxide had the same effect on spores treated with performic acid and 2-mercaptoethanol whereas up to 6 M hydrogen peroxide did not affect control spores. Phase darkening was followed by almost complete solubilization of the spores leaving only cell ghosts, presumably spore coat material, and therefore differed from the phase darkening which occurs during normal germination.

The *p*H activity curves of the lysozyme and hydrogen peroxide reactions showed important differences. The lysozyme reaction only occurred between *p*H 6·0 and 10·2 whereas the hydrogen peroxide reaction was most rapid above *p*H 10 and in the presence of metal ions and was inhibited by sequestrants. This suggested that the lysozyme reaction was physiological but the hydrogen peroxide reaction was non-physiological and depended on catalytic decomposition of the peroxide.

The reactions appeared to be general in their effects on bacterial spores. Spores of *Bacillus cereus* (three strains), *B. megaterium*, *B. polymyxa*, *B. brevis*, *B. fusiformis*, *B. mesentericus*, *B. licheniformis*, *B. subtilis*, *B. subtilis* v. *aterrimus* and *B. globigii* which were treated with performic or thioglycollic acids all became susceptible to lysozyme and to hydrogen peroxide, as also did spores of *Clostridium butyricum* (two strains), *C. saccharobutyricum*, *C. tertium*, *C. septicum* and *C. bifermentans*.

These results are compatible with: (1) Vinter's observation[1] of the high content of disulphide bonds in the coat fraction of spores; (2) chemical rupture of these bonds; (3) consequent disclosure of new substrates aided by the hydrogen bond breaking activity of urea; (4) action of lysozyme on previously protected lysozyme substrate which is present in spore mucopeptide[7]; (5) action of hydrogen peroxide on previously protected but unknown substrates. These results are also compatible with a spore structure in which the cystine disulphide-rich[1] and proteinaceous[5,6] coat surrounds the lysozyme-sensitive mucopeptide[8] and the calcium dipicolinate[8,9] of the cortex.

Normal germination of bacterial spores may involve similar reactions to those described here. Vinter[3] has suggested that spore disulphide bonds may not be solely concerned with resistance to irradiation but may also be involved in the sudden activity during germination. He demonstrated a qualitative reduction of spore disulphide bonds during germination[2]. Enzymatic rupture of spore disulphide bonds, followed by activation of a lytic system, could invoke changes in permeability[10] and swelling[11] of the spore typical of germination. For example, enzymatic rupture of disulphide bonds in yeast pseudokeratin has been described[2,12], and a spore enzyme capable of attacking spore mucopeptide was discovered by Strange and Dark[13]. Changes induced by such enzymes could parallel changes induced by disulphide bond-breakers and lysozyme. It is clearly possible that during normal spore germination endogenous enzyme systems could bring about changes similar to those induced by the exogenous systems we have described. The relevance of these reactions to normal germination is therefore now being examined.

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CYTOLOGY

Chromosomal Dynamics of *Gastrimargus*

CHROMOSOME number and morphology have been regarded as most constant properties of a species both within and between different individuals. This is also[1–4] true of short-horned grasshoppers The concept of absolute karyotypic stability entails an evolutionary cul-de-sac, and therefore a relative stability alone can afford scope for further genotypic change. Though it is known that spontaneous chromosomal mutations in stable genetic systems is low[5–10], yet chromosomal variations within an organism is as common as between organisms within a population[11]. However, a re-organization of a karyotype to attain a better homeostatic stability depends not only on the incidence of structural and numerical anomalies but also on their subsequent survival[12]. Such selective incorporation of anomalies are claimed in orthopterans[13]. The incorporation of anomalies into a genetic system is as much a measure of karyotypic plasticity as it affords the starting point of further evolutionary change. Cytological examination of the two species of *Gastrimargus*, *G. transversus* and *G. africanus orientalis* (Family, Acrididae; Order, Orthoptera) collected locally (Mysore, India) has clearly shown the karyotypic dynamism.

In both species of *Gastrimargus*, the diploid chromosome number in the male is 23 ($2n = 22A + X$) as judged from chromosome counts of the gonial metaphases. Further, while a majority of spermatocytes in metaphase I show eleven bivalents and one X, a few cells distinctly show nine bivalents and an X chromosome. The latter indicates a diploid number of 19. Thus far, no gonial metaphase plates with 19-chromosomes have been found. However, the presence of cells in anaphase I with 9 and 10 chromosomes at the two poles strongly suggests the existence of cells with 19-chromosomes in the germ-line. This variation in the diploid number has to be explained in terms of karyotypic instability which might involve the presence of supernumerary chromosomes in the population. In the cells so far examined, the occurrence of sister-chromatid bridges in anaphase I is found more frequently in *G. transversus* than in *G. africanus*. Although many cells contain a single bridge, some cells with 2 or 3 bridges are also found. The frequencies are being worked out. The presence of non-staining gap in the long bivalent in the two species of *Gastrimargus* is of special interest. Most of the cells in metaphase I show 'heteromorphic' non-staining gap in *G. transversus* and 'homomorphic' non-staining gaps in *G. africanus* in the centric ends of the chromosomes. Similar non-staining gaps have been described in rye[13], *Pyrgomorpha kraussii*[14], *Mecostethus grossus*[15] and *Tetrix ceperoi*[16]. Whether these non-staining gaps are due to structural heterozygosity[15] or symptomatic of genotypic unbalance[14] or mere phenotypic expressions comparable with the starved regions of chromosomes[13] is not clear at the present moment. Specific association between autosomal bivalents and between X and autosomal bivalents in pachytene-diakinesis-metaphase I suggests the existence of translocations in *G. transversus*.

As a result of failure of cytokinesis in the last gonial division, tetraploids result which manifest as binucleate spermatocytes. There is normal consummation of meiosis in these tetraploid spermatocytes with 22 autosomal bivalents and $2X$ univalents. The resulting diploid spermatids are distinguishable from the haploid spermatids, particularly among the X-bearing ones. The size differences between $2X$- and X-bearing spermatids is significant. An occasional occurrence of two X's with eleven autosomal bivalents in metaphase I suggests the premeiotic non-disjunction of X in the last gonial division. Two of the autosomal bivalents exhibit positive heteropycnosis in zygotene-pachytene-diplotene, both in *transversus* and *africanus*. One of them always shows precocious segregation in metaphase I, though occasionally both show. The behaviour of these bivalents recalls the behaviour of supernumerary chromosomes. The formation of ring bivalents in grasshoppers has been shown to be due to the presence of two chiasmata, one in the short and other in the long arm of the chromosome. The number of ring-bivalents varies from 0 to 5 per cell between cells of the same individual and between different individuals. In *G. transversus*, though most of the cells show 2 ring-bivalents, cells with 1 or 3 are also present. Some of the cells show 4 but none showed 5 ring-bivalents. On the other hand, in *G. africanus*, cells with 1, 2, 3, 4, and 5 ring-bivalents occur but those with 3 and 4 occur more frequently than others. Details of the cytology and chromosomal dynamics of *Gastrimargus* will be published elsewhere.

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VIROLOGY

Isolation of Individual Strains of Beet Yellows Virus

IN the programme of breeding for tolerance to beet yellows virus (SBYV) in sugar beet, it became desirable to obtain individual SBYV strains so that breeding material could be tested against them separately or in known combinations.

It has now proved possible to isolate single SBYV strains from plants infected with a complex of strains by a local-lesion technique. Leaves of the infected plants were ground in a mortar with 'Celite', and the sap was immediately inoculated with a very soft brush on to the upper surface of leaves of *Claytonia perfoliata* Donn. The test plants required no special treatment before or after inoculation; but best results were obtained if the temperature did not exceed 70° F between grinding and inoculation; washing the treated leaves immediately after inoculation tended to reduce mechanical damage.

Most of the inoculated leaves showed well-defined red or brown necrotic lesions about 10–14 days after treatment; uninoculated leaves and leaves inoculated with healthy sap showed no lesions. The lesions were very similar to those reported by Björling on leaves of *C. perfoliata* systemically infected with SBYV following aphid inoculation[1], but with mechanical inoculation the virus was restricted to the lesions and no systemic spread occurred. *Myzus persicae* (Sulz) did not acquire the virus from the lesions or from other parts of the plants during short or long acquisition feed times.

Single local lesions were excised with the minimum of surrounding leaf tissue, ground with 'Celite' in a drop of water and inoculated to *C. perfoliata* leaves; single lesions from these leaves were used for further inoculations. This process was repeated at least three times so that each resulting virus isolate had passed through several single-lesion transfers. Repeated passage of tobacco mosaic virus through single local lesions has usually given genetically pure strains of virus[2,3] and it seems reasonable to suppose that this technique has also given a similar separation of SBYV strains.

The final stage of obtaining sugar beet plants systemically infected with the single-lesion SBYV isolates and from which aphids could acquire the virus, was carried out using a technique based on that of Kassanis[4]. Sap from single *C. perfoliata* lesions or, preferably, from a leaf bearing many lesions derived from one original lesion, was brush-inoculated with 'Celite' on to both upper and lower surfaces of the older leaves of young sugar beet plants (4–6 leaf stage) which had been kept in the dark for

2 days previous to inoculation. The inoculated leaves of many of the sugar beet plants showed large numbers of very small brown or purple lesions about three weeks after inoculation, and some of these plants later became systemically infected. Some sugar beet breeding lines were more susceptible to systemic infection than others.

The virus strains isolated in this way from field plants infected with strain complexes were tested for virulence on *Chenopodium capitatum* Aschers, and this showed that many strains of differing virulence had been obtained. Some of these have since passed through several aphid transfers from sugar beet to sugar beet without apparently changing in virulence and they therefore appear reasonably stable *in vivo*.

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Spread of Yellows Virus by *Myzus persicae* in Sugar Beet Crops

Wingless *Myzus persicae* were reared on sugar beet plants (provided by Dr. G. E. Russell, Plant Breeding Institute, Cambridge) infected with sugar beet yellows virus (SBYV), sugar beet mild yellows virus (SBMYV)[1], or both viruses. Eight widely separated plants in a 7½-acre field of sugar beet each received three adult and six young aphids from these cultures on May 31, 1962, and nearby plants were examined for aphids and virus symptoms at weekly intervals thereafter.

In this exceptional season study of the spread of the wingless aphids was not impaired by the arrival of winged aphids from elsewhere. The apterae moved readily from plant to plant, even across rows 21 in. apart when the plants were less than 9 in. diameter. During June they spread over patches of the crop more than 10 ft. in diameter, and infected them with virus. Although heavily attacked by adult ladybirds which sometimes outnumbered them, dispersal enabled the aphids to maintain their populations. These populations (less than one aphid per plant) produced eight- to sixteen-fold increases per month in the incidence of yellows virus.

In late June 1961 populations of *M. persicae* in sugar beet seed crops averaged 600,000 per acre (15–50 aphids per plant[2]). With such populations the rate of spread of yellows virus should be much greater than the increases now found in the root crop. With such rapid spread, as yellows symptoms are not visible until three weeks after infection[3], nearly all the seed crop could be infected and infective although only 2·9–10 per cent of the sugar beet seed crop and from 7–31 per cent of the mangold seed crop shows symptoms of yellows virus in June[4]. Thus, although the measures taken in 1950[5] reduced heavy visible infection in the seed crop to these much lower levels, they may not have reduced their efficiency as sources of the disease for the root crop. This would explain why the incidence of the disease in the root crop has not decreased (mean August incidence, 1946–50 = 18·9 per cent; 1957–61 = 21·8 per cent), although there is much evidence that the seed crop is the principal source of the disease[6]. Effective control would be obtained if the seed crops were only grown in alternate years[6].

Experimentally infected plants received SBYV only, SBMYV only, or both viruses. SBMYV spread to more than twice as many plants as SBYV. The least rapid spread was from plants which received both viruses; analysis shows that either virus spread to twice as many plants when it was introduced alone as when the two viruses were introduced together. Also, there is some evidence that when both viruses were present the two infections were usually introduced into a plant at different times.

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ANTHROPOLOGY

Pigmy Tools from the Andaman Islands

Relatively little is known about the archæological work in Andaman Islands except for the work of Stoliczka[1], Holland[2], Cipriani[3], and Dutta[4], and practically no information is available about any stone industry. Some small stone artefacts were recovered by excavation from a kitchen-midden site situated at the Beehive Island (between lat. 12° 24′–26′ N. and long. 92° 55′–56′ E.) in Middle Andaman. The kitchen-middens are numerous in these islands and can be found whenever the elevated ridges are near to the coast or the creek.

The aborigines of the land are considered to be the survivors of the primitive negrito race, subsisting chiefly on hunting and collecting.

The collection comprises a large number of small stone artefacts fabricated out of the siliceous rocky material. They are well preserved. The collection represents a flake and blade industry belonging to the mesolithic stage of culture (the term 'mesolithic' is used here only to indicate the cultural mode of life, and does not imply dating). The industry is primarily based on the utilization of small flakes and blades and the preponderance of primary flakes is worthy of notice. When classifying the material, it emerges that the major types that contributed towards the formation of this industry are blades, points, scrapers, flakes and numerous waste products. The blades, however, are in the majority. Besides these there are some true microliths showing steep retouching. The artefacts are rather primitive, exhibiting a crude technology. The industry is of less antiquity and of recent origin. The artefacts indicate a society based on hunting and collecting economy. From a preliminary scrutiny of the artefacts, it seems that this industry had broad similarities in character with the Toalean and allied flake and blade industries developed in the Far East Islands and South-East Asia.

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MISCELLANEOUS

Possible Role of Meteorites in the Origin of Life

It has been postulated by Oparin[1] that life originated in the seas of the primitive Earth, at a time when the atmosphere was reducing. The dominant sources of free energy for biopoesis are presumed to have been lightning discharges in the atmosphere and ultra-violet radiation from the Sun[2]. However, both agents tend to deposit organic compounds relatively high in the atmosphere, rather than in the seas. Advection, convection, and diffusion must be invoked as the mechanisms depositing the reaction products in the hydrosphere. The purpose of this communication is to suggest a physical process capable of yielding organic compounds of high complexity in the primitive atmosphere and providing a relatively high efficiency of transfer to the hydrosphere.

When a large meteoroid enters the Earth's atmosphere, its minimum speed (11·3 km/sec)[3] corresponds to hypersonic conditions, and a detached shock-wave forms ahead which accompanies it in flight. The temperatures behind the shock are greatest in the stagnation region, and fall off gradually with distances behind the meteoroid and away from the roughly hyperboloidal envelope of the shock. Computations have been carried out by one of us[4] for the concentrations of atomic, molecular, and free radical species at various points behind such a shock front in a standard atmosphere. The calculated concentrations presume local thermodynamic and chemical equilibrium. As such, they cannot yield final concentrations of neutral species after passage of the shock, since these depend on the various rates of reaction involved (which are poorly known, in general).

Direct computations for an ambient reducing atmosphere have not been made. However, the results for normal air are very suggestive. So long as the velocity of a body impinging on the top of the atmosphere exceeds about 7 km/sec, the corresponding stagnation temperature at equilibrium behind the shock varies from 5,000° to 10,000° K for altitudes ranging from 100 km to sea-level, respectively. These temperatures are sufficient to yield dissociation, essentially complete for oxygen and partial for nitrogen, with some ionization of the products to O^+ and N^+. For higher velocities, double ionization and other processes occur. The equilibrium concentration of a particular species (not present in the ambient atmosphere) rises with the distance behind the meteoroid and then falls off.

Qualitative extrapolation of these results to a reducing atmosphere containing hydrogen, methane, ammonia and water is easily made. Passage of a hypersonic meteoroid should yield a wake containing the monatomic species H, C, N, and O, the diatomic species CH, CN, CO, NH, OH, and NO (charged and uncharged in these cases), triatomic species, electrons, etc. As the temperature downstream of the meteoroid drops, the original ambient composition will be approached asymptotically, with a possibly small amount of complex compounds residual in the wake, as permitted by the kinetics of the situation.

However, the process of reversion to the original composition can be arrested, at least partially, if the meteoroid (and its initial velocity) are sufficiently large for it to survive passage through the atmosphere and to impinge explosively in the sea. In this event, the phenomena occurring should be similar to those in the near-surface explosion of a nuclear bomb in water[5], as in the Baker series of tests by the Atomic Energy Commission at the atoll of Bikini[5]. A hollow column of water and spray should shoot up into the atmosphere. Afterward, a dome-shaped cloud of droplets of water (the condensation cloud) should form, and finally the characteristic mushroom-shaped cloud above the water should appear. Thus, hot water mixed with steam will engulf the hot descending remnants of the wake, preventing reversion to the original composition of the ambient atmosphere, and providing the possibility of further reactions in a gaseous and aqueous medium to form complex compounds. The fall-out of the water from these clouds should provide an efficient mechanism for direct transfer of complex organic compounds to solution in the hydrosphere. For infall on the primitive atmosphere of meteorites able to reach the terrestrial surface, the process should be operative in at least two-thirds of the cases, since this fraction represents the present ratio of sea to total surface of the Earth.

It might be argued that the complexity of the compounds involved in the origin of life would imply a concentration, after passage of the shock, so low that it would be unimportant in biopoesis. However, consider a meteorite with diameter 1 km impinging on the Earth. Viewing the wake in the atmosphere as cylindrical with a diameter at least equal to that of the meteoroid, one computes that at least 10^{10} kg of air would have passed through the wake of the shock. From results[4] for normal air, the yields of NO, O, CO_2, CO, NO_2, and N_2O in the wake where the temperature is 2,000° K correspond to approximate molar fractions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}, respectively; hence passage of the meteoroid creates at least 10^8 kg of NO, 10^7 kg of O, 10^6 kg of CO_2, 10^5 kg of CO, 10^4 kg of NO_2 and 10^3 kg of N_2O in the atmosphere at this temperature. These numbers hold only for air in equilibrium, but suggest strongly that formation of complex compounds by reactions following passage of the shock may be quantitatively significant. Note that the meteoroid diameter selected corresponds closely to that of the minor planet (1566) Icarus, which would have an atmospheric velocity of 32 km/sec if it encountered the Earth[3].

Two further points should be kept in mind. It is generally believed that an intermediate phase occurred in the formation of the planets from gas and dust, consisting in the condensation of solid bodies or planetesimals at low ambient temperature. The planets owe their origin to accretion of these planetesimals. It follows that the rain of meteorites must have been of much greater intensity on the primitive Earth than is observed at the present time. Further, one of us[6] has proposed that the ocean basins are merely large meteoritic craters, produced by explosive impact of large planetesimals in the presence of a hydrosphere, far back in Precambrian times. If this hypothesis emerges as true, it obviously will imply a profound importance of the effect of meteorite falls in biopoesis. Furthermore, it would make highly reasonable the presence of organic compounds on the Moon, as suggested by one of us[7], in view of the hypothesized pristine presence of an atmosphere and hydrosphere.

A direct experimental check on the production of organic compounds in this manner has been undertaken by Dr. W. J. Hooker of this laboratory, by firing a hypersonic projectile through an atmosphere into a tank of water. One might also quench in water the plasma issuing from a plasma jet. An experimental check is difficult, since it involves microanalytical chemical techniques.

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Sensitive Ionization System for the Detection of Permanent Gases and Organic Vapours by Gas Chromatography

THE two most commonly used high-sensitivity detection devices for gas chromatography, the 'flame ionization'[1] and the 'argon ionization' detectors[2], are insensitive to permanent gases. During an investigation into the various mechanisms responsible for ionization phenomena within the latter class of devices it was noted that under certain conditions of field and radioactive source strength, detector geometry and operating temperature, a simple ionization system using argon as carrier gas could be utilized to detect extremely low concentrations of permanent gases. The limit of sensitivity of this new mode of detector operation is up to 1,000 times greater than that reported with an earlier device[3]. In addition to this extraordinary response to fixed gases this same detector when operated at a different potential was found to retain certain characteristics, including the high sensitivity toward organic molecules as that noted by Lovelock[2] for the conventional small 'argon detector'.

The particular ionization chamber which provided optimal results is of an annular design as shown in Fig. 1. The outer cathode is a brass cylinder the internal dimensions of which are 10 mm wide and 10 mm high. The central anode is a solid brass rod 8 mm in diameter and 10 mm high, insulated from the cathode by polytetrafluoroethylene and containing two 1/16-in. dia. orifices which serve as gas inlets. The source of radiation is provided by a 200 mc. tritiated titanium foil (28 mm long × 10 mm wide) which snugly fits against the wall of the cathode. The total volume of this particular version of detector is 280 µl.

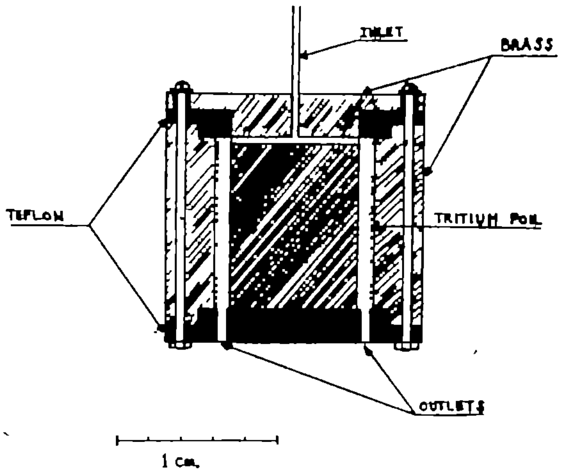

Fig. 1. A cross-section of the coaxial micro-ionization detector

While operating the device between 150° and 225° C, an investigation of the effect of variation in the applied field on the response to known volumes of permanent gases revealed an area of high sensitivity with a maximum occurring between 1–2 V (field strength = 10–20 V/cm.). At these low voltages (conveniently available from either a battery or a closely regulated power supply) all gases examined (He, Ne, CO, CO_2, H_2, O_2, N_2, CH_4, C_2H_6, C_2H_4) when passed through the detector provided an increase in current. At 165° C with an applied voltage of 1·34 V and pure argon flowing through the chamber, the standing current was $3 \cdot 7 \times 10^{-9}$ amp—about 1/2 of the saturation current obtained at 100 V. The noise-level was low at $1 \cdot 2 \times 10^{-13}$ amp. The minimum limit of detectability of these gases under the stated conditions is given in Table 1. No appreciable change in sensitivities were observed as the temperature was varied from 150° to 225° C.

Table 1. DETECTION OF PERMANENT GASES USING A CO-AXIAL MICRO-IONIZATION DETECTOR AND ARGON AS THE CARRIER GAS

Gas	µl./sec	g/sec	mole/sec
He	$7 \cdot 3 \times 10^{-3}$	$1 \cdot 3 \times 10^{-9}$	$3 \cdot 2 \times 10^{-10}$
Ne	$2 \cdot 6 \times 10^{-1}$	$2 \cdot 2 \times 10^{-7}$	$1 \cdot 1 \times 10^{-8}$
H_2	$5 \cdot 5 \times 10^{-4}$	$4 \cdot 8 \times 10^{-11}$	$2 \cdot 4 \times 10^{-11}$
CO	$4 \cdot 0 \times 10^{-4}$	$5 \cdot 0 \times 10^{-10}$	$1 \cdot 8 \times 10^{-11}$
CO_2	$1 \cdot 7 \times 10^{-4}$	$3 \cdot 3 \times 10^{-10}$	$7 \cdot 6 \times 10^{-12}$
O_2	$2 \cdot 8 \times 10^{-4}$	$3 \cdot 8 \times 10^{-11}$	$1 \cdot 2 \times 10^{-11}$
N_2	$1 \cdot 0 \times 10^{-3}$	$1 \cdot 5 \times 10^{-9}$	$5 \cdot 5 \times 10^{-11}$
CH_4	$1 \cdot 0 \times 10^{-4}$	$8 \cdot 8 \times 10^{-11}$	$5 \cdot 5 \times 10^{-12}$
C_2H_6	$3 \cdot 4 \times 10^{-4}$	$4 \cdot 8 \times 10^{-10}$	$1 \cdot 6 \times 10^{-11}$
C_2H_4	$2 \cdot 8 \times 10^{-4}$	$3 \cdot 4 \times 10^{-10}$	$1 \cdot 2 \times 10^{-11}$

The range of linearity did not substantially vary from one gas to another. For example, with methane it extended up to approximately 2 µl./sec above which the slope, although slightly decreased, continued in a linear fashion to at least 12 µl./sec. Ethane and ethylene, though included in Table 1, could have provided an additional 10-fold increase in response when analysed at normal argon detector voltages, which in the case of this detector are between 150–250 V.

The phenomenon of a maximum in sensitivity at such low applied voltages was observed to be a characteristic of small electrode separations (coaxial or parallel plate geometry) and the specific use of argon as a carrier gas.

The mechanism of ionization of permanent gases the ionization potentials of which lie above the energy of the argon metastable atoms (11·6 eV) appears to involve, in a unique manner, these neutral highly excited metastable atoms since an argon–ethylene (0·3 per cent) mixture used as a carrier gas completely quelled the excellent response.

It is believed that increased ionization in the chamber is due to the interaction of the argon excited atom (A^*) with the permanent gas molecule (M) resulting in a complex ion formation with a concomitant production of an electron, for example:

$$A^* + M \rightarrow AM^+ + e^- \qquad (1)$$

The stability of the complex ion AM^+ promotes the foregoing reaction and more than compensates for the lack of sufficient energy required for the direct ionization of the permanent gas molecules ($I.P. > 11 \cdot 6$ eV) via argon metastable atoms.

The use of the mass spectrometer and other appropriate devices has in the past established analogous reactions involving metastable atoms of the various inert gases[4–6]. In these experiments molecule ions such as A_2^+, He_2^+, $XeCH_4^+$, XeO_2^+, AN_2^+ and others have been reported. The lowering of the sensitivity observed when the applied voltage is increased is apparently due to a competition between reaction (1) and other processes which may include some form of the reverse reaction and also electron attachment for certain permanent gases. Formation of negative ions with oxygen and dissociative attachment for hydrogen are well known. Nitrogen, however, appears as an exception and will be discussed in a full report elsewhere.

Several other interesting and useful observations associated with this system were the following:

(1) This system can provide sensitivities similar to those noted in Table 1 without any potential applied to the chamber provided the cathode is suitably grounded and the device maintained at 160°–200° C. However, in these circumstances, where very small voltages generated by a thermoelectric effect are responsible for the flow of current within the chamber, the device becomes more susceptible to overload phenomena.

(2) Under comparative experimental conditions when ordinary commercial grade helium is used as a carrier gas the sensitivity of this detection system to permanent gases is reduced by at least a factor of 5.

(3) By further decreasing the internal volume of the detector, capillary columns can be readily utilized without the necessity of scavenger flow[7].

(4) When this device is operated at higher potentials (150–250 V) for the analysis of organic substances, the phenomena of electron attachment ordinarily exhibited by certain classes of compounds is minimal.

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Note added in proof. Preliminary analysis of Dr. Hooker's samples by Dr. C. A. Ponnamperuma (of the Exobiology Division, N.A.S.A., Ames Research Center, Mountain View, California) has indicated the presence of materials absorbing ultra-violet light of wave-length 260 mµ and appearing to have chromatographic characteristics similar to some of the known biological purines.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, February 11

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. G. Lipsey: "Problems of Scientific Discovery in Economics".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. D. R. Shearman: "Ionospheric Sounding by Incoherent Scatter at Very-High Frequencies".

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Dr. M. F. Perutz, F.R.S.: "Structure of Proteins"* (John Coffin Memorial Lecture).

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The High-Speed Diesel Engine—Higher Performance or Lower Cost ?"

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"North America in the Making" (programme of award-winning films).

Tuesday, February 12

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Carbon^{14} in Agricultural Research".

BRITISH SCHOOL OF ARCHAEOLOGY AT ATHENS (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. John Evans: "Neolithic Knossos—New Facts and Problems".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3), at 5 p.m.—Mr. R. Cook and Mr. N. Fleming: "Aspects of Noise Reduction in Merchant Ships".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the National College of Rubber Technology, Northern Polytechnic, Holloway Road, London, N.7), at 5.30 p.m.—Dr. W. McG. Morgan: "Chemical Promotion and Heat Treatment". 7 p.m.—Papers by members of the National College of Rubber Technology.

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Lecture on "High Speed Resonance Searching with Automatic Recording".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the SIR JOHN CASS COLLEGE CHEMICAL SOCIETY, at the Sir John Cass College, Jewry Street, London, E.C.3), at 7 p.m.—Prof. H. C. Longuet-Higgins, F.R.S.: "Some Applications of Electron Spin Resonance in Chemistry".

ROYAL SOCIETY OF MEDICINE, PSYCHIATRY SECTION (at 1 Wimpole Street, London, W.1), at 8 p.m.—Dr. G. A. Foulds: "Basic Personality and the Symptom Complex in Psychiatry".

Wednesday, February 13

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Prof. J. L. Gowans: "Some Problems in Cellular Immunology".*

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. D. J. Shearman: "Recent Anhydrite, Gypsum, and Dolomite from the Trucial Coast, Persian Gulf"; Dr. D. C. Turner "Ring-Structures in the Sara-Fier Younger Granite Complex, Northern Nigeria" (to be read by Prof. W. S. Pitcher).

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. S. L. Bragg: "Combustion Noise"; Dr. E. G. Masdin and Prof. M. W. Thring: "Combustion of Single Droplets of Liquid Fuel".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION P.G.P8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. P. Brock and Dr. J. McKeown: "Some Improved Lead Alloys for Cable Sheathing".

UNIVERSITY OF LONDON (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. G. R. Wadsworth: "Dental Conditions and Nutritional Status".* (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation).

ROYAL SOCIETY OF ARTS (at John Adam Street, London, W.C.2), at 6 p.m.—Dr. R. G. Allen: "Water Supplies and Conservation".

UNIVERSITY OF LONDON (in the Physics Lecture Theatre, Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. D. J. Bohm: "On Problems in the Basic Concepts of Physics"* (Inaugural Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Miss A. M. Copping: "The Nutritional Education of the Food Technologist".

INSTITUTE OF METAL FINISHING, ORGANIC FINISHING GROUP (joint meeting with the CORROSION GROUP of the SOCIETY OF CHEMICAL INDUSTRY, at the Borough Polytechnic, Borough Road, London, S.E.1), at 7 p.m.—Mr. U. R. Evans and Mr. C. A. J. Taylor: "Metal-Pigmented Paints for the Protection of Steel".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. K. F. H. Murrell: "The Quality and Quantity of Information"; Mr. J. Spencer: "The Design of an Instrument Display"; Mr. A. Cowan. "Process Plant Control—Information Centre Concept".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m.—Mr. M. G. Wilde: "Supersonic Airliners".

Thursday, February 14

PLASTICS INSTITUTE (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 9.30 a.m.—Symposium on "Design for Profit".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. Honor B. Fell, F.R.S.: "The Role of Membrane Systems in the Response of Cells to some Chemical Agents" (Special Review Lecture).

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. F. Bergel, F.R.S.: "The Therapeutic Future of Larger and Larger Molecules".*

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. H. Koch (University of Louvain): "Osmotic Regulation and Salinity Preference in the Stickleback (*Gasterosteus aculeatus* (Cuv. & Val.))".* (Further lectures on February 15 and 18.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Hannah Steinberg: "Measuring Effects of Drugs Upon Behaviour".* (Eleventh of twelve lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

CHEMICAL SOCIETY (at the Royal Institution, Albemarle Street, London, W.1), at 6 p.m.—Prof. R. Kuhn: "Problems of Cumulenes and Acid Hydrocarbons" (Centenary Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at the Central Hall, Westminster, London, S.W.1), at 6 p.m.—Dr. E. Eastwood. "Electronics—The Key to Air Safety" (Faraday Lecture).

INSTITUT FRANÇAIS DU ROYAUME-UNI (at Queensberry Place, London, S.W.7), at 6.15 p.m.—M. Etienne Wolff (Professeur au Collège de France): "Les Agents Provocateurs de Malformations" (La Production Expérimentale de Monstruosites).

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts. John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Sir Bernard Lovell, O.B.E., F.R.S.: "Investigation of Space by Probes and Radio".*

Friday, February 15

PHOTOELECTRIC SPECTROMETRY GROUP (in the Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 2 p.m.—Meeting on "The Collaborative Test of Recording Ultraviolet Spectrophotometers".

BRITISH COMPUTER SOCIETY (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Mr. R. A. Scriven and Mr. D. Benson: "Analogue Computer Study of a Long Catenary in a High Wind".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Doad, London, W.12), at 4 p.m.—Dr. D. R. Laurence: "The Problem of the First Clinical Trial of a Drug".*

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Discussion on "Why Ships Collide" lead by Captain F. J. Wylie, R.N. (Retd.) and Captain J. P. Stewart.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION P.G.S8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Teaching Materials Engineering". opened by Dr. T. J. Lewis and Dr. D. W. F. James.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (joint meeting with the HEAVY ORGANIC CHEMICALS GROUP, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. B. Sully: "Plasticisers".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. Marshall Nixon: "Conservation of Water Resources".

Friday, February 15—Saturday, February 16

BIOCHEMICAL SOCIETY (at the National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7), at 10 a.m. on Friday—Colloquium on "The Biochemistry of Animal Viruses"; at 2 p.m. Friday and Saturday—Scientific Papers.

Saturday, February 16

SOUTH-WEST ESSEX TECHNICAL COLLEGE, with the support of the CHEMICAL SOCIETY and the BRITISH BIOPHYSICAL SOCIETY (at the South-West Essex Technical College, Walthamstow, London, E.17), at 9.45 a.m.—Symposium on "The Evaluation of the Degree of Hydrogen Bonding in Biological Molecules".

Monday, February 18

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDE GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. K. S. Hocking: "The Work of the Tropical Pesticides Research Institute, Arusha".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should an Undergraduate Mechanical Engineering Course Include Production Engineering ?"

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. R. W. Beattie and Mr. N. Fairweather: "Electroplating and Inspection of Electrical Contacts".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Sheffield (February 16).

MAP CURATOR (preferably geography graduate with an interest in maps and bibliographic work) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds 2 (February 18).

LECTURER or ASSISTANT LECTURER (preferably with research interests in solid state physics, particularly the Mössbauer effect, low temperatures and N.M.R.) **in Physics**—The Registrar, The University, Manchester 13 (February 20).

Lecturer (with a degree in civil engineering and at least five years recent approved industrial experience) **in Civil Engineering** at Bendigo Technical College, Bendigo, Victoria, Australia—The Council for Technical Education and Training for Overseas Countries, 13 Lincoln's Inn Fields, London, W.C.2 (February 20).

Chair of Civil Engineering—The Secretary, The Queen's University, Belfast, Northern Ireland (February 28).

Demonstrator in Geology—The Registrar, University of New England, Armidale, New South Wales, Australia; and The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 28).

Associate Professor in Soil Mechanics in the Department of Civil Engineering, University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 1).

Browne Research Fellow in Botany or Zoology—The Provost, The Queen's College, Oxford (March 1).

Leverhulme Postgraduate Fellows (3) two in the Faculty of Science and one in the Faculty of Engineering—The Registrar, The University, Liverpool, quoting Ref. No. RV/240/N (March 1).

Research Fellow (with a good honours degree in physics or engineering) **in Textile Engineering**, to investigate problems concerned with the division of heat between two rubbing surfaces—The Registrar, The University, Leeds 2 (March 1).

Research Chemist (well-qualified organic chemist, preferably with an interest in microbiology and enzyme chemistry), for research relating to the removal of wool from sheepskins—The Director, Wool Research Organization of N.Z. (Inc.), Private Bag, Christchurch, New Zealand (March 1).

Chair of Physics in the University of Ghana—The Registrar, University of Ghana, P.O. Box 25, Legon, Accra; or The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (March 4).

Lecturer in Inorganic Chemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 4).

Senior Lecturer (preferably with training and research experience in biochemistry and enzymology, and some teaching experience) **in Agricultural Chemistry** at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 6).

Assistant Lecturer in Biochemistry—The Registrar, University College of Wales, Aberystwyth (March 9).

Lecturer or Senior Lecturer in the Department of Physics, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 16).

Reader in the Department of Physics, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 18).

Lecturer in Meteorology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

Associate Professor (with strong research interests in the application of advanced analytical and synthetical techniques to machine design and the kinematics of machines) **in Theory of Machines** at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 15).

Agricultural Chemist (aged 25–45, an honours degree in chemistry with, if possible, two years postgraduate training in agricultural chemistry and soil physics) in the Ministry of Natural Resources and Surveys, Nyasaland, for duties which include analyses of soils and crops in relation to fertilizer needs, and assessing water requirements of irrigated crops—The Director of Recruitment Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting T/RC 213/110/02.

Assistant Lecturer, Grade B (with a good honours degree and an interest in developing some branch of applied physics in H.N.C. and H.N.D. courses) **in Physics**—The Principal, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex.

Biology Teacher, to deputise for one year for the Senior Biology Master—The Headmaster, Bishop's Stortford College, Bishop's Stortford, Herts.

Junior Scientific Officer (female, with an honours degree in biology, physiology or chemistry, and preferably experience of blood grouping and a knowledge of genetics), for work on blood group serology—The Director, Blood Group Reference Bureau, c/o Lister Institute, Chelsea Bridge Road, London, S.W.1.

Laboratory Technician (competent in section-cutting and staining, and in photography) **at the Marine Biological Station**, Port Erin, Isle of Man—The Registrar, The University, Liverpool 3, quoting Ref. 252.

Male Laboratory Technician (experienced), for teaching unit—The Administrator, Department of Biochemistry, South Parks Road, Oxford.

Master to teach Chemistry and some Biology—The Master, Wellington College, Crowthorne, Berkshire.

Master to teach some Sixth Form Biology and Science in the Lower and Middle School—The Headmaster, Bootham School, York.

Postdoctoral Assistant, for work on a problem in inorganic chemistry involving the use of proton magnetic resonance spectrometry—Dr. J. A. Elvidge, Organic Chemistry Research Laboratories, Imperial College of Science and Technology, London, S.W.7.

Research Assistant (preferably with experience in cytology) **in the Department of Genetics**, to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

Senior Technician II (male, F.I.M.L.T., or equivalent qualification in hæmatology and blood transfusion), for duties which will include the care and maintenance of scientific equipment—The Director, Blood Group Reference Laboratory, c/o Lister Institute, Chelsea Bridge Road, London, S.W.1.

Technical Officer (with either a degree in physics or engineering, Grad.I.E.E., Grad. Inst.P., or similar qualification) **in the Medical Engineering Department**, to develop electronic and other equipment for use in medical research—The Secretary, Postgraduate Medical School of London, Ducane Road, London, W.12.

Theoretical Physicist (preferably with interests in solid state or nuclear physics); and an **Experimental Physicist** (preferably with interests in radio frequency spectroscopy)—Head, Department of Physics, Assumption University of Windsor, Windsor, Ontario, Canada.

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REPLANNING GOVERNMENT ORGANIZATION FOR SCIENCE

THE study of the influence of Government policy on *Science in Industry*[*], issued by the Conservative Political Centre, is the outcome of the work of a committee, with Mr. R. Carr as chairman. The committee was appointed in June 1960 to examine, in the light of the rapid scientific and technological development of other advanced countries, the role of the Government in stimulating and assisting research and development and the application of scientific methods and manpower to production in industry. Particular reference has been made in the report to scientific research and development and the application and dissemination of the results of research. It thus takes its place with such contributions as J. S. Dupré and S. A. Lakoff's recent book, *Science and the Nation: Policy and Politics*, surveying conditions in the United States, and with the report on *Industrial Research in Manufacturing Industry 1959–60* issued in 1962 by the Federation of British Industries. In fact, it was at a conference at Eastbourne in April that the last-mentioned was discussed, and at which a member of the Carr Committee, Mr. Aubrey Jones, made an important contribution. The report is the outcome of initiative by a political party and is noteworthy in the extent to which it reveals that the recommendations of the Plowden Report on the control of public expenditure and of the Gibb–Zuckerman Report on the management and control of research and development have penetrated the thinking of a major political party.

Primarily the report is concerned with the problems of Britain's industrial *future*. Indeed it is only concerned with the problems of science and Government in so far as they relate to that *future* and determine the extent to which Britain's scientific and technological effort is translated into effective action. It starts with the assumption that the solution to the problems lies in not only turning out new products, with a high technical content, beyond the competence of all except a few countries, but also in producing traditional goods at a very high level of efficiency. Above all, it is essential that these are of first-class quality and design and supported by vigorous salesmanship. No one could wish for clearer recognition of the imperative need of making the fullest possible use of modern science and technology than is to be found in this report. No less explicit is the recognition that an adequate educational programme is the vital factor.

The scientist will welcome other features of the report. For example, there is no illusion that the problems will be solved simply by spending vast sums of money on science, and it is recognized that the quality of management, old-fashioned trade union attitudes, and unwillingness to fit the products to the real requirements of the purchaser have all contributed to the difficulties. Further, it is recognized that more flexible instruments of policy can also have a specific influence on the course of research and development: it is a real advance to find in a report of this type the admission that it would be possible to modify the tax structure in such a way as to encourage inventiveness and the efficient use of labour.

True, Britain has yet to see all-party support for any concrete proposals to that end. However, the four main lines of action for Government proposed in this report and on which its attention is mainly focused are such as should command that support, whatever the differences in view as to the means by which such action should be effected. To create background conditions which can be expected both to stimulate industrial science and to make it as efficient as possible would be a reasonable remit for the Council for Scientific and Industrial Research. Add to that the responsibility for: (1) equipping the machinery of Government to fit its actions in detail to the need for proper scientific development in industry; (2) ensuring that the administration of the Government's own expenditure on science is organized to secure full value for money in the light of Britain's needs and her resources; and (3) seeing that the 'ground-level' contacts between this administration and particular parts of industry are used to the best effect, we have a reasonably comprehensive description of the functions of the Minister for Science and his Advisory Council up to the level at which they merge with the responsibilities of the Cabinet itself.

There is, in fact, a refreshing realism about this study and a freedom from preconceived ideas that may well have been fostered by some of the recent discussions in this field at the Eastbourne Conference already mentioned and at meetings of the British Association and other bodies. Questions of competition, of large- and small-scale enterprise, and of co-operative undertakings are considered with more detachment and a fuller sense of the public rather than individual interest. It is frankly admitted that a scientific policy, in the sense of a conscious effort to obtain the maximum economic advantage from scientific resources must be part of the core of the economic policy. The view of Prof. A. K. Cairncross that economic growth in a highly developed society depends more on the pace of technological development than on the actual amount of capital investment is accepted. Accordingly, the National Economic Development Council must be concerned with relating both the targets and the flow of information to scientific and technical possibilities and needs. This involves including on the Council, and in its secretariat, men who understand science and scientists who have a real knowledge of the factors which are important to research, development and innovation in industry and who can apply that knowledge continuously in the work of the Council.

One could scarcely expect to find in a survey of this type any searching review of the organization of Government and the reactions between science and public policy, but the Committee was led to conclude that a much more detailed appraisal of the industrial repercussions of policy is required than the present structure of Government can provide. Some new way must be found for bringing industrial, scientific and technical considerations into the determination of policy over a wide range of departmental activity. It recommends that every Government Department dealing with industry should have a new kind of 'scientific–economic staff' to act as a forward policy planning unit, advise the Minister on the technical and industrial implications of the Department's future policy plans, to gather economic and technical information from the relevant industries, and generally provide a technical intelligence service to the Department.

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[*] *Science in Industry: The Influence of Government Policy.* Pp. 46. London: Conservative Political Centre, 1962.) 3s.

This is manifestly in line with the whole trend of the Plowden Report and even when a Department, like the Treasury, has no call to make specific technical judgments, the Committee believes that it would be useful for Ministers to have such a staff with a technical bias. In particular, it recommends strengthening scientific representation on both the National Economic Development Council and the secretariat and close liaison between the secretariat and the proposed staffs of the Departments. While it strongly supports the recommendations of both the Plowden Report and the Gibbs–Zuckerman Report for greater interchange of staff both in the Civil Service itself and between the Service, industry and the universities, it recommends that part of the proposed staffs should comprise scientists and economists recruited from industry and the universities on temporary contracts or secondment. It is essential that the staff should be of high quality, whether recruited from the Civil Service itself or from those with industrial experience or specialized training in science and economics, and the two-way flow of such men elsewhere in the Civil Service and in industry and in the universities is a valuable subsidiary effect of the proposal.

For the rest, Mr. Robert Carr and his Committee take a long and somewhat critical look at scientific administration generally, and while it would be unreasonable to expect the Committee's recommendations to secure extensive discussion or executive action pending the report of the Trend Committee, some of their criticism can scarcely be ignored by the Minister for Science. At the outset, it recommends the establishment at Cabinet level of a Technical Policy Committee to formulate the main lines of a scientific policy related to military and economic strategy. The membership of this Committee would include the Minister for Science, the Minister of Defence and a senior economic Minister, but no convincing evidence is advanced as to what advantage this proposal would have over making the Minister for Science a member of the Economic Policy and Defence Committees of the Cabinet, or indeed why it should prove effective in the absence of insufficient appreciation of scientific and technical factors, among others, on the part of the Cabinet generally and ultimately of the electorate.

Where the Committee appears to be most critical is in relation to defence, and here it would seem that it would prefer to strengthen the responsibilities and influence of the Minister for Science. Like the Gibb–Zuckerman Report, the Committee is critical of the functioning of the Defence Research Policy Committee, and it suggests that that Report should be followed by an investigation of the relation between the Service Departments, the Ministries of Aviation and Defence and the Treasury. It formed the impression that the Ministry of Defence required stronger powers in relation to research and development and that the Minister's Chief Scientific Adviser required a stronger staff. Although it suggests that as much defence research and development work as possible should be contracted to industry, and that improved liaison is required between the defence and civil sides, it makes no comment on the relations between the Defence Research Policy Committee and the Advisory Council on Scientific Policy either through the device of a common chairman or through the Minister for Science. Further, if the separation between defence and civil research and development is maintained, the Committee points out, care must be taken to ensure that by-products of the defence programme, once identified, are developed and exploited, and this implies that additional resources must be available for civil and industrial research generally.

The Committee's analysis in this context of the use of contracts, and especially of development contracts, which it develops later in the section of the report "Services for Industry" is sound. However, it might have gained in depth by consideration of American experience as unfolded, for example, by Prof. J. S. Dupré and S. A. Lakoff in their recent book, *Science and the Nation: Policy and Politics*†. Some of the dangers and implications of the American system indicated in the book are highly relevant even in the different conditions prevailing in Britain and underlie some of the *malaise* which, for example, has led to the appointment of the Nye Committee. The ease with which the military side can dominate an advisory scientific body, as for example in the experience of the Advanced Research Projects Agency, may afford valuable guidance when Britain's scientific organization is under reconsideration. Above all there are the apt remarks of Dr. J. R. Killian, jun., in a foreword that a technological society requires an adequate complement of scientists and engineers in the public arena if it is to deal wisely with great policy matters arising out of science and technology, and that the interface between science and political science is an area worthy of careful attention by both scientists and political scientists.

It is here, Dr. Killian considers, that the most effective bridges between science and the social sciences can be built, and while the Carr Committee does not enter into any profundities of political science its report contains enough practical suggestions to warrant serious and impartial appraisal. On balance it supports the strengthening rather than any decrease in the authority and responsibilities of the Minister for Science, and it is especially critical of the present staffing of his Office. The Committee would transfer to the Vote for that Office those for the four Research Councils—the Overseas Research Council is again not mentioned. This omission is rather curious in view of a subsequent observation on the importance of stimulating more contacts and exchanges with scientists overseas and introducing a scheme of research fellowships for young scientists from abroad. Such a project would provide his Office with a body of highly qualified scientific advisers as part of its full-time staff, including scientists temporarily seconded to Government service from industry and the universities. The Committee recognizes that finance for university research cannot be brought under the Minister for Science in the same way, but regards the absence of any formal consultation with the Minister in the process of allocating funds for scientific research at the universities as a defect in the present system. Accordingly, it emphasizes the need for effective cross-representation and consultation between the University Grants Committee and the Office of the Ministry for Science.

To a certain extent, developments in that direction have already been forced on the Advisory Council for Scientific Policy, but the Carr Committee does not appear to discount the value of that Council. It considers that full-time advisers are also required, though a group of eminent and representative scientists such as is constituted by the Council could continue to provide a stimulus and an independent judgment of great value. The Committee does not, however, appear to appreciate the difficulty of the manpower issue at that level or the co-ordinating influence which such a Council can contribute in reviewing the work of the several Research Councils, etc.

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† *Science and the Nation: Policy and Politics*. By J. Stefan Dupré and Stanford A. Lakoff. (Spectrum Book.) Pp. x+181. (Prentice-Hall, Inc., Englewood Cliffs, N.J., and London, 1962.) 16*s*.
[See also p. 635 of this issue of *Nature*.]

Once again, strong support is forthcoming for the National Research Development Corporation, although it is admitted that its role is slighter than was at first expected. It is suggested that the basis of its finance should be reconsidered to see if better allowance could be made for the exceptionally long time likely to elapse between the provision of money for a project and the receipt of any substantial returns. The Committee also favours the establishment of an Industrial Guarantee Corporation and suggests that the Department of Scientific and Industrial Research should be provided with a specified sum for expenditure on civil development contracts over a five-year period. Both civil and defence contracts should provide the contractors with rights in exploiting the end product with the emphasis on rapid development rather than wide distribution of rights.

As might be expected, the Department of Scientific and Industrial Research receives more detailed examination, and it is recommended that the Department's research stations should, wherever possible, be progressively more closely integrated with the industries to which they are related. Welcome stress is laid on the value of greater mobility and interchange of staff, both by way of visits, exchanges and contracts between their staff and scientists in Britain and in other countries, while it is suggested that the research stations should regard the training of industrial scientists as one of their functions. This educational function has already been urged as one of the most important functions of the research associations, but its advocacy in respect of the research stations appears to be novel. Such a function, however, should become important particularly if the extent to which fundamental research is pursued in the research stations is severely limited. The Carr Committee makes no particular criticism of the research associations themselves, and although it recognizes the limitations and inherent dangers of the system, it considers that their existence could prove a valuable asset if Britain joins the European Economic Community. The educational and other advantages, particularly in the dissemination of information, outweigh the drawbacks, but the Committee would like to see the Department's grant policy towards established associations directed towards underwriting unusually interesting and adventurous projects rather than towards subsidizing routine work.

This seems rather a naïve view which ignores the inherent drawback in the finance of research from public funds, and Sir Henry Tizard's caution against the dependence of venture and enterprise on public finance may appropriately be recalled. The propping of a conservative or decadent industry, as distinct from the encouragement of a developing and enterprising industry, is as likely to be to the public disadvantage as to its advantage. Criticism of the information services of the Department of Scientific and Industrial Research is equally somewhat hesitant and overlooks the extent to which the Department fails to describe and report on such activities. On the other hand, there is a welcome emphasis on the importance of personal contact. Particularly, with the smaller firms and in encouraging firms to make full use of available facilities, such contacts are the vital factor and are the first step towards securing the benefits which mechanical developments in the handling of information have now made possible.

It is interesting to put the report alongside an address by the chairman of the Council for Scientific and Industrial Research, Sir Harold Roxbee Cox, to the Motor Industry Research Association on November 28 on taking the lead in technology. Sir Harold desiderated four great changes needed if Britain were to strengthen her dispositions for the development of invention, where the present weakness lay. First, Britain needs many more scientifically and technologically trained people. Secondly, from these Britain must encourage an increasing proportion of the best talent to adopt design and development as their professional ambition. Thirdly, more of the scientists and technologists in industry, more of the research workers, developers and designers, must become interested in administration and policy and reach positions of command. What is needed here is a different distribution of disciplines at the top, in which the applied scientist trained in management could pull his weight. Fourthly, a clearer definition of objectives is required. Too much research is carried out with no clear idea of where it may lead, and while fundamental research should always be encouraged, those objectives should be more precisely defined which are likeliest to offer technological advantage: thus it would be possible to recognize the most promising lines of applied research and development.

The resemblance between the lines of Sir Harold's address and the whole trend of the Carr Report on science in industry is remarkable. How far the analysis will commend itself to the Minister for Science is another matter. In a recent address to the National Association of British Manufacturers, Lord Hailsham claimed the achievement of two major political objectives as Minister: one was that policy had been determined by scientists on scientific grounds, and the other that he had been able to avoid building up an expensive and useless Ministry of Science. Neither objective, however, seems incompatible with the recommendations of this report, which is equally in harmony with Lord Hailsham's version of a society in which those trained minds, in science, in arts and social sciences, who put public service in front of enjoyment, profit or leisure, have a dominant part in the administration.

Apart, however, from raising, at least by implication, the question of how much should be spent on scientific research, whether pure or applied, the report is usefully considered in the context of the book by Dupré and Lakoff, which also makes incidental reference to that question. Important as is that question, however, a rather more pragmatic approach is needed, for example, than those of G. Holton in his "Notes Toward the Design of Proper Scales for Scientific Research and Scholarship" (spring 1962 issue of *Daedalus*) or of S. Dedijer in his paper on "Measuring the Growth of Science" (*Science*, 138, 781; 1962). Dupré and Lakoff, in fact, seem to give a sounder lead in their comment that a research partnership between public and private institutions does not necessarily work easily and automatically. Such a partnership, for example, is involved in the distinguishing characteristic of organized research in the United States since the Second World War—extramural Government research.

To perform its tasks effectively, the research partnership must be the object of thoughtfully constructed policies and of mutual accommodation between the partners. Dupré and Lakoff display very fairly the conflicts that arise between Government and industry, between Government and universities, the clashes of loyalty involved which are frequently independent enough of the specific characteristics of national institutions but derive from the nature of the university, or of industry, or of Government itself. Whether they are concerned with the formulation of policy or with the stuff of politics, the major challenge thrown down to organization if the relations between science and Government are to function smoothly is as manifest in this book by Dupré and Lakoff as in the Carr Report: it can neither be mistaken nor ignored by the scientist.

THE NERVOUS SYSTEM

Experimental Neurology

By Prof. Paul Glees. Pp. xii + 532. (Oxford: Clarendon Press; London: Oxford University Press, 1961.) 75s. net.

THIS book is a translation of a book published in German by the author in 1957. The lapse of time has made some amplification possible. Dr. Glees is well known for his contributions to neurohistology. The book begins with a description of the methods used to investigate the finer anatomy of the nervous system with particular reference to the tracing of the degeneration resulting from experimental lesions, and goes on to review the methods used for the study of nervous activity, including the investigation of evoked potentials by electroencephalography and the study of behaviour. Nowadays, the old distinction between anatomy and physiology has disappeared in the sense that in a large field of experimental neurology the study of the one without the other would be meaningless. Hence Dr. Glees takes the reader through the anatomo-physiology of the nervous system from the peripheral receptors by way of nervous conduction and the synapses to the spinal cord and thence to the various anatomical structures at higher levels until he reaches the cerebral cortex.

Clearly a book, even of this size, cannot hope to cover all aspects of both neuroanatomy and neurophysiology. There are therefore gaps. For example, an account of the differing rates of conduction in fibres of different sizes in the peripheral nerves does not mention the attempt to correlate these with particular forms of sensibility. It is a little difficult to know how speech comes into experimental neurology, but if it is to be included it should not be limited to a description of 'centres' for motor speech and writing even with the admission that "the word 'centre' for these areas tends to give [a] false impression".

Nevertheless this book, which is well produced, well illustrated and very well documented with references, fills a real need and will be of value to a wide range of readers who want to know what is happening in the important, but nowadays highly technical, field of experimental neurology.

LORD BRAIN

FRÉDÉRIC JOLIOT-CURIE

Frédéric Joliot-Curie et l'Energie Atomique

Par Pierre Biquard. (Savants du Monde Entier, Nr. 3.) Pp. 255. (Paris: Éditions Seghers, 1961.) n.p.

THIS is a paper-back volume, in French, in the series *Savants du Monde Entier*. The standard of the biographies which have appeared already is high and the promised volumes, which range from Aristotle to Leriche, Fermi, Cuenot and Branly, will be awaited with interest.

The author of the book is pleasantly objective and, so far as possible, allows the story to tell itself in terms of original documents. Joliot was born on March 19, 1900, entered the laboratory of Marie Curie in October 1925, married her daughter Irène a year later, was awarded the Nobel Prize in Chemistry in 1935, claimed the fission of uranium in 1938, and started up the small atomic pile *Zoé* in 1948. He died in 1958, two years after his wife.

Joliot, the son-in-law of Marie Curie, owed much to mother and daughter; but he had an original mind, great technical skill and an inventive genius. He was a man of wide attainments: he played the piano and painted effectively. He read widely and, surprisingly, was devoted to the works of Kipling, some of which had been translated by his wife. The rational, anti-clerical outlook of the Curies suited his socialism. He was 'a citizen of the world' and was active in the movements for the use of science in the cause of peace and the improvement of the lot of man. There is a danger that his activities as a communist may obscure, in the Western mind, his real achievements as a physicist. There can be no doubt of his absolute sincerity in his political ideas in relation to the catastrophic possibilities for mankind in the applications of nuclear physics.

The stories of the beryllium rays, which were an important step in Chadwick's discovery of the neutron; the production of artificial radioactivity; the sorting out of the real nature of the so-called trans-uranic elements are well told. Joliot's favourite instruments were C. T. R. Wilson's cloud-chamber, which he improved, and the Geiger counter. Unlike so many atomic scientists in other parts of the world, he always remembered with gratitude the sources of these simple and beautiful fundamental instruments of atomic research.

The documentation is excellent, and the appendixes contain lists of all Joliot-Curie's publications, jointly with his wife and separately, a list of his lecture courses, a bibliography of works devoted to him and a useful glossary, in French, of terms used in nuclear physics. The illustrations are varied and informative. Among others, these include reproductions of pages of the laboratory note-books of Joliot-Curie, a picture of him with his favourite cloud-chamber apparatus, an example of his landscape painting and a photograph of the crowd at his funeral.

W. L. SUMNER

ASPECTS OF PHYSICAL CHEMISTRY

Annual Review of Physical Chemistry

Vol. 13. Edited by H. Eyring, Associated Editors: C. J. Christensen and H. S. Johnston. Pp. vii + 528. (Palo Alto, California: Annual Reviews, Inc., 1962.) 7.00 dollars (United States); 7.50 dollars (elsewhere).

THE present volume of this highly valued review of physical chemistry contains 21 articles covering the usual proportion of old and new titles. This means that individual chapters are fairly short and when it is found that there are some 3,285 references in the bibliographies it will be appreciated that the treatment, apart from being selective, is concise. All the same, most of the authors have contrived to make their reviews interesting. A new feature, which is to be repeated, consists of a chapter of reminiscences by a veteran physical chemist. In view of the great compression of the factual articles it is questionable whether this will be welcomed by all readers.

It is obvious that not even a list of titles can be provided and only a few aspects of the volume can be mentioned. There are many useful tables of results. The discrepancy in the values of the bond length C—H found by two different groups of methods is not resolved. The chapter on cryogenics includes an interesting discussion of the Mössbauer effect, which is having an influence in various fields. In the high-temperature field, which this year is confined to moderate temperatures, it is said that the melting-point of gold is still uncertain by one or more degrees, and the accepted heat of formation of silica is apparently too low. The intervention of the solvated electron in radiolytic oxidations seems to be more probable than that of the H_2^+ ion. In water the OH' ion is probably more highly solvated than the H^+ ion, and in acid solutions the H^+ ion is more solvated than H_3O^+. The production of isotopes in meteorites by spallation reactions induced by cosmic rays is likely to throw light on the question of the constancy of cosmic rays in time and space.

The chapter on solutions of non-electrolytes seems to suggest that in this field some stagnation has set in and that what is called for is a new approach. That on electrolyte solutions repeats once more the familiar criticism of measurements with cells involving liquid contacts, without throwing much light on the validity of the thousands of measurements made daily with such cells in laboratories of all kinds; a more constructive approach would have been of more interest. In studies of gas kinetics, it is said, gas chromatography (on which some 1,500 publications appear annually) is replacing mass spectrometry. The mechanism of acid-catalysed reactions is much less simple than has been supposed.

J. R. PARTINGTON

SPECTROSCOPY IN SCIENCE AND TECHNOLOGY

Fundamentals of Infrared Technology

By Marvin R. Holter, Sol Nudelman, Gwynn H. Suits, William L. Wolfe and George J. Zissis. (Macmillan Monographs in Applied Optics.) Pp. xv + 442. (New York and London: The Macmillan Company, New York, 1962.) 79s.

Theory and Applications of Ultraviolet Spectroscopy

By Prof. H. H. Jaffé and Prof. Milton Orchin. Pp. xv + 624. (New York and London: John Wiley and Sons, Inc., 1962.) 113s.

Standardization of Spectrophotometric Data in the Near Ultraviolet, Visual and Near Infrared

By Dr. Jørgen Fog. (Institute for Medical Biochemistry and Physiology, University of Oslo.) Pp. 64. (Oslo: Johan Grundt Tanum Forlag, 1962.) n.p.

THE interaction of matter with electromagnetic radiation of all wave-lengths is assuming an ever-increasing importance in both pure and applied science. The nature of the absorption of wave-lengths 5–50 times as long as those of visible radiation has become of great industrial as well as academic interest as a result of the successful application of infra-red spectroscopy to the analysis of organic materials. The military applications of infra-red radiation, particularly its use for the guidance of missiles, are so important that the fate of a nation might well depend on the state of its infra-red technology.

The first of these books deals mainly with the latter aspect of the technology of infra-red radiation. It contains the substance of a course of lectures given at the University of Michigan by its five authors. Its first section covers basic radiation concepts, radiation measurements and transmission through various media with particular emphasis on the Earth's atmosphere. The second part is devoted to optical materials and instruments. The next section deals with the theory of detectors, noise in semiconductor detectors and detector measurements. The final chapters are concerned with typical applications, with atmospheric spectra and the major sources of infra-red information. While the growth of this subject has undoubtedly received its greatest stimulation from its use in warfare, its non-military applications such as the investigation of the radiation balance in the atmosphere are of great importance. This book will be very valuable to those who wish to profit from developments that have sprung from military requirements as well as those whose present responsibility it is to satisfy them.

The second of these books deals with the ultra-violet absorption spectra and electronic structures of molecules. It marks the enormous progress which has been made in the past thirty years in explaining chemical properties in terms of electronic structures. This achievement has resulted largely from extensive studies of the nature of the electronic spectra of a vast number of molecules. The early chapters of the book contain an excellent account of basic molecular orbital theory and the subsequent discussion of molecular spectra is developed logically within this theoretical background. Starting with the interpretation of the spectra of the simple chromophores the subject is expanded to include the whole range of organic molecules. In later chapters ligand field theory and the part played by '*d*' orbitals are discussed and applied to the spectra of inorganic complexes. Other topics treated less thoroughly in this book include fluorescence, phosphorescence and equilibrium constant determination. It is clear that the modern chemist and spectroscopist has to be steeped in quantum theory if he is to have any real understanding of his subject. At times the going is hard, but the reward is well worth the effort required for its comprehension.

The third book deals with some of the practical details of ultra-violet spectrophotometry. That its author is a professor of medical biochemistry is indicative of the way in which spectroscopic techniques have proved of great analytical value in diverse fields. The content of the book is, however, very limited, and most of what it contains can be found in the manuals issued by the instrument manufacturers.

W. C. PRICE

CHEMICAL PHYSICS

Determination of Organic Structures by Physical Methods

Vol. 2. Edited by F. C. Nachod and W. D. Phillips. Pp. xiii + 771. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 114s. 6d.

VOL. 1 of this work (1955) dealt with recent developments of established methods of elucidating molecular structure and the present volume supplements this by a series of 50–90 page review essays dealing with rapidly developing physical techniques of recent origin. Each writer assumes that interested readers are well acquainted with the most modern, expensive, type of American equipment that is available and that they are primarily concerned in understanding further the significance of recent precise data.

Chapter 1, on optical rotatory dispersion, will appeal most to the conventional organic chemist, for it surveys the very considerable achievement of C. Djerassi and his colleagues and can be used as a précis of his book. For the organic chemist who is interested in reaction mechanisms, Chapter 2, on mass spectrometry, collects much significant information, for mechanisms of formation of mass spectra are essentially similar to routes of unimolecular decomposition of organic molecules and give knowledge about bond strengths and of the tendencies of activated molecules to undergo molecular rearrangement. Very soon organic chemists will be demanding mass spectrometers as essential tools in studies of molecular stability and not merely as equipment for finding molecular weights.

The remaining chapters, three on optical spectroscopy and six on various aspects of magnetic resonance spectroscopy, will interest those scientists who have made 'chemical physics' their domain of interest. The individual subjects are introduced mathematically and deal mainly with refinements of knowledge of the structures of fairly simple organic or organometallic compounds. All the chapters have been well planned with data selected so as to show the importance of developments of theoretical significance, but organic chemists who wish to make but empirical use of spectroscopic or magnetic resonance data may find it difficult to glean helpful information from these pages until they have mastered older and simpler texts.

Anyone who picks up this volume can see that 'chemical physics' has now become a major science in its own right: physical chemists have had the vigour to conquer the old realm of 'properties of matter' from conventional physicists as a younger generation of the latter has emigrated to the new world of sub-atomic studies. This progressive shift of interest from pictorial organic chemistry to quantum physics should inevitably lead to major developments in physical biology as more and more natural products become amenable to exact measurement and books such as this are needed to publicize technical progress. Unfortunately, perusal of the listed references throughout the whole volume shows only too clearly that Britain is lagging in scientific repute by providing too little and too late the equipment and technical services that chemists should now be using in their laboratories.

W. A. Waters

SELF-REGULATION OF ANIMAL POPULATIONS

Animal Dispersion in Relation to Social Behaviour

By Prof. V. C. Wynne-Edwards. Pp. xi + 653 + 11 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 55s.

The author of this book has been impressed with the large number of behaviour patterns among animals, especially in the higher organized groups, that do not seem to be satisfactorily explained although their widespread occurrence indicates that they must have importance for the species. He also wished to find a satisfying theory about the natural regulation of numbers. It is certainly true that there is a very large amount of animal behaviour the ecological meaning of which has not been explored, partly because the attention of ethologists has so far been chiefly concentrated on sexual and breeding relationships, rather than on the relation of animals to the whole of their habitat and its resources. It is also the case that ecology is just now in a considerable state of confusion about the relative parts played by different processes concerned in the natural regulation of average population-levels and of population fluctuations.

The theme of this book is that over-exploitation of food resources by an animal population will lead to dissipation of the resources and deterioration of the population; that there exists an optimum level of any food resource for allowing sustained productivity; that this needs to be matched by the maintenance of an optimum level of the animal population exploiting it; and that this optimum is attained by means of the many behaviour patterns and devices (especially social ones) found among animals. The author further postulates that this end-result of evolution has been brought about in the past not by individual natural selection, but by group-selection of populations—that is, by some local populations of a species making, as it were, serious short-term mistakes in population control that were punished by starvation or other consequences of food shortage, while other populations with better fortune (and appropriate genes or traditional changes) evolved suitable behaviour 'signals' for limiting increase beyond the optimum population-level. An analogy is drawn between the position in our sea fisheries when there is overfishing, and that facing any species in Nature.

The theory is set forth with enthusiasm, often pontifically (if a bishop can wear blinkers), sometimes in a sort of Messianic exaltation which admits of no other important processes affecting population-levels. The language is usually lucid and the great array of facts that has been unearthed very interesting in itself; but the reasoning behind the language is extremely involved, and (in spite of the elaborate apparatus of decimal-numbered paragraphs) rather woolly, and the new dogma incorporated in it safeguarded by many careful side-steps that make one rather uneasy, and certainly unconvinced at present.

The incontrovertible virtue of this book is that it brings together in one place a concentration of information about the nature of social behaviour and its co-ordination by means of behaviour signals, territorial patterns and the methods by which animals determine and keep them, sexual displays, communal roosting, movements and so on. But Prof. Wynne-Edwards has not, so far as I know, ever carried out any field research on natural population control, and he therefore perhaps tends to over-simplify the way in which animals live. Thus, most species fluctuate greatly in numbers, some of them violently, a few of them with remarkable regularity, and it is difficult to imagine how there can be a set of behaviour signals elaborate enough to take care of all these different levels of numbers, or similarly of their fluctuating resources, so as to gear at all closely the populations to their supplies of food.

Moreover, what of the numerous predators that depend on scores or hundreds of different other animals for food? Or herbivores that depend on irregular and unpredictable crops of tree fruits and seeds? All we can really say is that most species do not usually starve even locally, and therefore their numbers do usually remain below danger limits. Phrases like "feed-back for the homeostatic machine" do not cover up such difficulties, which are not to be solved by any sweeping arm-chair notion. Furthermore, the author states openly that the method of group-selection in populations is an essential assumption for his theory. On p. 21 it is stated that "Group-selection is not by any means a new concept, though it has never been accorded the general recognition that its importance deserves. Particular attention has been paid to it in the present work, because it is fundamental to the dispersion hypothesis."

The enormous weakness of this enormous book, so full of fascinating information, so impregnated with one particular teleological bias, is that it gives no single case-history of group selection. I have no doubt that the process does occur and may be quite important, but this kind of selection does not only have to be related to population density and resources: it could also relate to predator pressure, disease and many other things. It is stated early in the book that predation, parasites and pathogens "will be discussed sufficiently fully". The author's views can be inferred from the fact that he devotes less than 3 out of 601 pages to these topics. In fact, this new theory, though more pleasantly clothed for display than some of the other exclusive dogmas about the natural control of populations with which the subject has been cursed during the past decade or so, cannot be a universal and exclusive explanation of such a tremendously complex phenomenon in all the animal species on Earth. Whether it occurs widely at all the courageous reader had better decide for himself. Once acclimatized to the persistent bias of the text, he will learn a great many unexpected things about animal dispersion and behaviour that certainly do require explaining as adaptations.

C. S. Elton

Science and the Nation

Policy and Politics. By J. Stefan Dupré and Sanford A. Lakoff. (Spectrum Book.) Pp. x+181. (Prentice-Hall, Inc., Englewood Cliffs, N.J., and London, 1962.) 16s.

PROF. J. S. DUPRÉ and Prof. S. A. Lakoff's readable study of the relations between science and the Government in the United States is a natural supplement or continuum of A. H. Dupré's *Science in the Federal Government*, which outlines the development of those relations up to 1940 where the present volume takes up the story. It is equally a counterpart to D. K. Price's *Government and Science*, though essentially it indicates and illustrates the problems rather than analyses them in detail or in depth. In the first part, the authors outline briefly the changes in the relations between Government and science, both in industry and in the universities, which have taken place in the United States during the past twenty years and some of the implications of those changes, notably of the development of extramural Government research. Further, the contribution which scientists have made to the formulation of policy is described in a way which assists the British reader to appreciate the significance of characteristic features of the American system, such as the position of the President's Special Assistant for Science and Technology, the President's Science Advisory Committee, or the Advanced Research Projects Agency, in a British context.

In the second part of the book the authors outline lucidly the part which scientists have played in the political scene from the general issue of defence, the development of the atomic bomb and nuclear warfare, and the issues of security, responsibility and decision which arise. The nature of politics and the process of decision could have been a little more clearly displayed. However, the book makes abundantly clear two points to which Dr. J. R. Killian, jun., refers in a foreword: the extent to which the interface between science and political science should repay study from both sides; and the imperative need for public understanding of what is to-day involved in the relations between science and Government. If such study yields constructive results in the way of new methods or institutions, if the difficult and changing problems of organization are solved, those methods and institutions will not be used, nor those solutions applied if political prejudice or dogma are allowed, in default of public understanding, to bar the way. [See also p. 629 of this issue of *Nature*.]

Progress in Inorganic Chemistry

Vol. 3. Edited by F. Albert Cotton. Pp. 551. (London and New York: Interscience Publishers, a division of John Wiley and Sons, 1962.) 113s.

THIS volume maintains the high standards of its predecessors. Hydrothermal synthesis of crystals is discussed in some detail by Laudise in an interesting article in which I was surprised to learn that sapphires may be grown hydrothermally. The degree of 'Americanization' in this particular article is unfortunate: "criteria . . . must be compromised"; "validation of . . . data"; "methods . . . categorized"; "unsystematized field", and the very prevalent "iron-containing compounds" when "compounds containing iron" would be so much clearer to anyone not familiar with the language.

There are two very long articles—one on vinyl compounds of metals by Seyferth running to nearly 500 references, and one on primary solid hydrides by Gibb. In the latter case not many conclusions were evident. Liehr has a fairly brief chapter on vibronically coupled inorganic systems, where it is clear that the author is under the impression that it is written down to the level of the average inorganic chemist. Unfortunately, this is not true. Those who struggle through may find the statement about the "rigorous crystalline field solution . . . here pictorially discussed" a little depressing. The difficulty of theoreticians to appreciate how far behind many inorganic chemists still remain does not help the keen laggards to catch up. Perhaps *Progress in Inorganic Chemistry* will be able to produce chapters on group theory and crystal field theory, for example, to lay some foundation for these chemists. The last review is on high-temperature inorganic chemistry by Searcy, who defines high temperature as "any temperature at which entropy plays a significant role in determining the reaction equilibria of interest". This is a fascinating article, particularly where dissociation energies of dimers of the elements are discussed in terms of multiple bonding and the anomalously low single bond energies for N–N and O–O. So much present-day inorganic chemistry is concerned with co-ordination compounds that this review is a refreshing change.

The general standard of presentation in this series is high, and there are few errors. J. R. BEATTIE

The Chemistry and Technology of Leather

Edited by Fred O'Flaherty, William T. Roddy and Robert M. Lollar. Vol. 3: 'Process Control of Leather Quality. (American Chemical Society Monograph Series, No. 134.) Pp. ix + 518. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1962.) 120s. net.

THIS third volume in the authoritative series on leather technology maintains the high standard of its predecessors. Full descriptions of tanner dye application, dyeing mechanism and chemistry of fat-liquoring emulsions are given as well as a discussion of the application of organic solvents for tannery practice. For the specialist there are separate sections on modern procedures for treatment of calfskin and other leathers such as industrial, glove and novelty leathers.

The text is well illustrated by photographs and the bibliography is exhaustive. It is unfortunate that in an otherwise excellent production the indexing is inadequate, but the work (which will be complete with the issue of Volume 4) is an essential part of the library of the leather technologist. A. I. SCOTT

Missile Guidance (Temple Press Monographs on Rockets and Missiles)

By J. Clemow. Pp. vii+87. (London: Temple Press Books, 1962.) 15s.

THIS book is one of a series, covering all aspects of guided weapons, intended to be a concise and authoritative introduction to the subject. The present volume discusses fundamental principles and the effects of mechanical imperfections in beam-riding, homing and command control systems; inertia navigation and other forms of long-range weapon guidance are to be treated in a later monograph. The author has been engaged in research and development of guided weapons systems for many years and has been responsible for Services text-books on this topic. It is a great pity, therefore, that the present work contains a large number of typographical errors, apparently due to too much haste in writing and in correcting proofs. These are not at all serious in themselves, but they interrupt the flow of the arguments presented.

Apart from this, the material is well arranged and a nice balance is maintained between the exposition of theory and descriptions of equipment, especially in the sections on beam-riding and homing. The particular choice of subject-matter is ideally suited to the needs of the young engineer or scientist just entered on work involving the study and analysis of guidance systems, and thus requiring a relatively brief but balanced explanation of the technology as a whole. For this reason it would probably be helpful if the bibliography were extended to include some of the text-books covering the same field. G. J. HERRING

THE IONOSPHERIC TOP-SIDE SOUNDER

THE *Alouette* satellite, containing an ionosonde capable of sounding the ionosphere from above, was launched on September 29, 1962. It was designed and built by the Canadian Defence Research Telecommunications Establishment and the satellite was put into orbit by the U.S. National Aeronautics and Space Administration. Other co-operating institutions include the U.K. Radio Research Station and the U.S. Central Radio Propagation Laboratory. The following three articles present some early results from the three co-operating institutions.

SOME PRELIMINARY RESULTS OF SOUNDING OF THE TOP SIDE OF THE IONOSPHERE BY RADIO PULSES FROM A SATELLITE

By E. S. WARREN

Defence Research Telecommunications Establishment, Defence Research Board, Ottawa, Canada

MOST of the present knowledge of the ionized upper atmosphere, the ionosphere, has been obtained by examination of radio waves reflected from the bottom of the ionization layer. Except by the incoherent scatter technique the top side of this layer is inaccessible for examination by radio waves from the ground, since the waves which penetrate to these heights are not reflected but continue outward into space. The concept of sounding the top side of the ionosphere using a sounder in a satellite was an obvious consequence of the ability to launch artificial earth satellites. As long as four years ago the idea was current among ionospheric research groups in the United States, United Kingdom, Germany and Canada. Because radio sounders, using vacuum tubes, are heavy and consume kilowatts of power, it was necessary to use solid-state devices in order to produce a sounder suitable for use in a satellite. The first top-side sounder to operate in orbit was launched at 0605 G.M.T. on September 29, 1962, as part of the *Alouette* satellite, 1962 $\beta\alpha 1$.

The *Alouette* satellite contains, in addition to the sounder, apparatus for counting cosmic-ray particles (National Research Council, Ottawa), for observing the very-low-frequency radio spectrum, and for monitoring the engineering performance. A measure of the cosmic noise-level as a function of frequency is provided by the automatic gain control voltage of the sounder receiver. The sounder data only will be discussed here.

The top-side sounder consists of a transmitter, receiver, timing circuits, antennæ, and antenna matching networks[1]. The power radiated during a pulse is of the order of 10 W. Two orthogonal antennæ are used, a 150-ft. dipole, operating at frequencies below 4·5 Mc/s, and a 75-ft. dipole for the range 4·5–11·5 Mc/s. The sounder is operated on command. Once started, it functions for 10 min, generating thirty transmissions in which the radio frequency changes linearly with time over the frequency-band 0·4–11·5 Mc/s. It then shuts off automatically until commanded on again. Nickel–cadmium batteries, recharged by solar cells, permit about 5 h of operation per day.

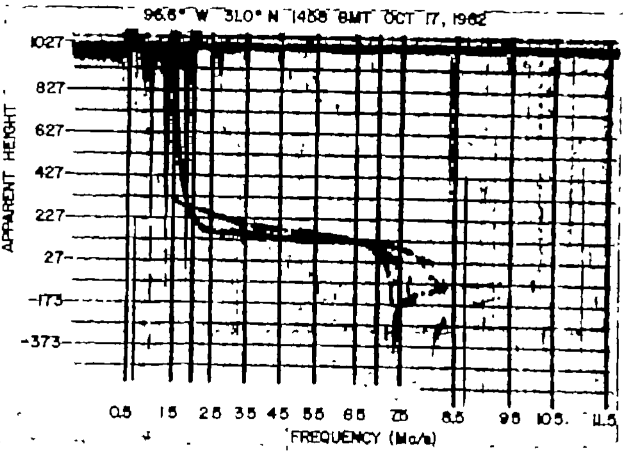

Fig. 1. A typical top-side ionogram

The *Alouette* orbital parameters as of October 17, 1962, provided by the National Aeronautics and Space Administration Computation Centre, are:

Anomalistic period		105·4137 min
Inclination		80·463 deg.
Argument of perigee		2·5649 deg.
motion	minus	49·531 deg./day
Right ascension of ascending node		158·890 deg.
motion	minus	0·984 deg./day
Semi-major axis		1·15892 Earth radii
Eccentricity		0·00225
Perigee		996·38 km
Apogee		1031·05 km
Velocity at perigee		26,498 km/h
Velocity at apogee		26,374 km/h

The height of the orbit was chosen by compromise. The lower the height, the smaller is the region explored. The greater the height, the smaller is the signal-to-noise ratio of the signals reflected near the height of the maximum ionization density. The inclination of the orbital

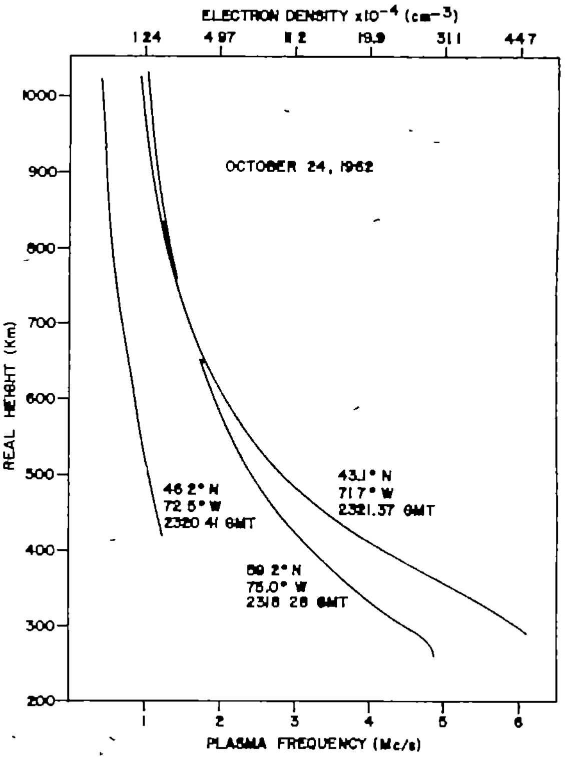

Fig. 2a. Distributions in height of free electrons showing depression of density at Ottawa latitudes

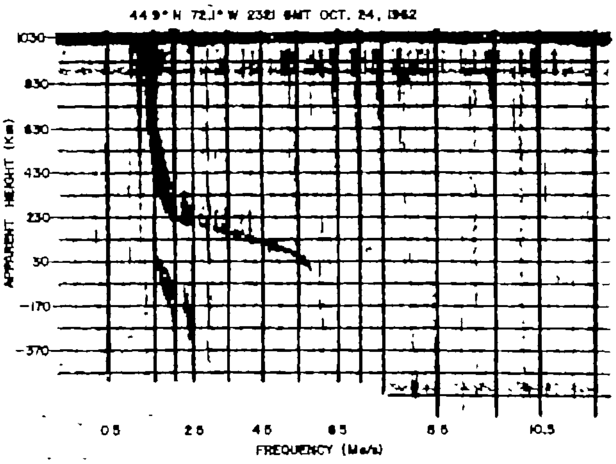

Fig. 2b. Ionogram showing abruptness of transition at the boundary of the low density region

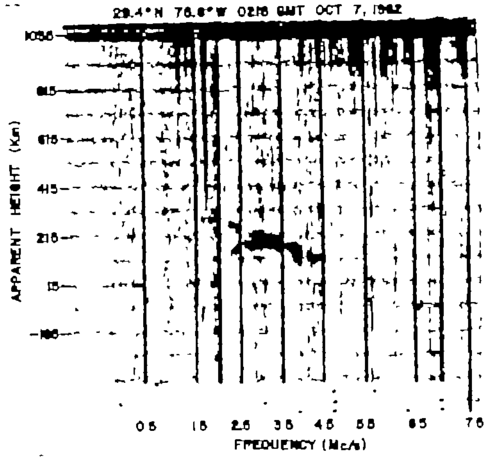

Fig. 4a. Ionogram showing cyclotron spike at 1·7 Mc/s.

plane to the equatorial plane was chosen to provide rotation of the orbital plane relative to the galaxy sufficient to distinguish diurnal variations of the ionosphere from seasonal variations, while retaining coverage of as great a range of geographic latitudes as possible.

The satellite sounder emits pulses of radio waves which travel to a reflecting surface in the ionosphere and back to the receiver in the satellite. The depth of penetration of the waves into the ionosphere and the travel time required for the return trip of the pulse is a function of the radio frequency of the wave. It is customary to express the travel time in terms of an apparent height of the reflecting surface computed using the velocity of the pulse in free space. Recordings of the apparent height as a function of frequency (ionograms) are generated from information telemetered to the ground. This information consists of the frequency calibration, of the modulation of the transmitter, and of the amplitude and time delay of the received pulses.

A top-side ionogram is shown in Fig. 1. The satellite is at a height of 1,027 km above ground. At the lowest frequencies the plasma is too dense for propagation except in the whistler mode, which is not observed on the ionograms. As the frequencies are reached for which propagation in the other modes becomes possible, vertical marks appear on the ionograms. These plasma spikes result from the generation of pulses for which the group velocity is zero. The extraordinary wave spike occurs at 1·35 Mc/s. At higher frequencies pulses reflected from below the satellite are observed. The apparent height of reflexion of the extraordinary wave falls rapidly from the satellite height at 2·0 Mc/s and penetrates the ionosphere at 8·1 Mc/s. The pulses at higher frequencies are returned from the Earth. The ordinary wave reflexion trace is similar, displaced to lower frequencies by about 0·7 Mc/s.

The apparent reflexion depths measured from the satellite sounder provide adequate data to determine the dependence of the free electron density of the ionosphere on the three spatial dimensions and time. From the distribution in height of the electrons, an effective temperature and a mean ionic mass may be deduced. A preliminary investigation of this kind has been carried out for mid-latitudes by Nelms[2]. On the assumption of an isothermal atmosphere in diffusive equilibrium, with one predominant ionic constituent, the ion mass of the constituent at 400 km is twice that of the constituent at 800 km.

A minor ionospheric storm was in progress during the first few days after launch, causing a significant change in the latitude dependence of the ionospheric electron density, as described by Hagg[3]. During this storm the electron densities were reduced at all ionospheric heights over a 25° range of latitudes centred on Ottawa. Muldrew[4] has found that the ionospheric electron density in this range of latitudes was strongly correlated with the particle counting rate in the outer Van Allen belt at latitudes between about 55° and 62° N. At 2320 G.M.T. on October 24 the ionization density of the ionosphere was again observed to have decreased dramatically over a limited range of latitudes. Fig. 2a shows the distribution in real height of the free electrons in the depressed region and at its boundaries. On the ionogram of Fig. 2b there are two reflexion traces, one each from the regions of high and low density. Their occurrence on the same record shows that

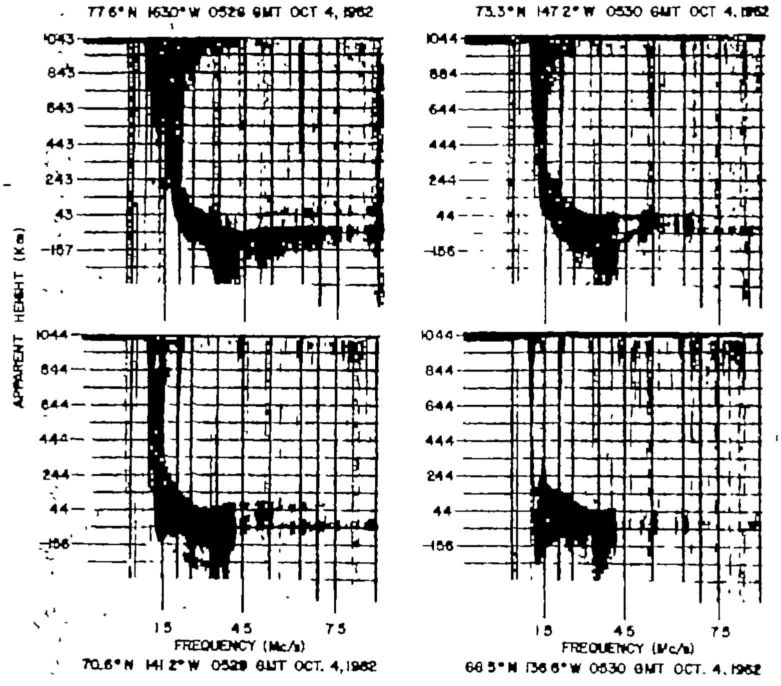

Fig. 3. Ionogram series showing spread-F and sporadic E

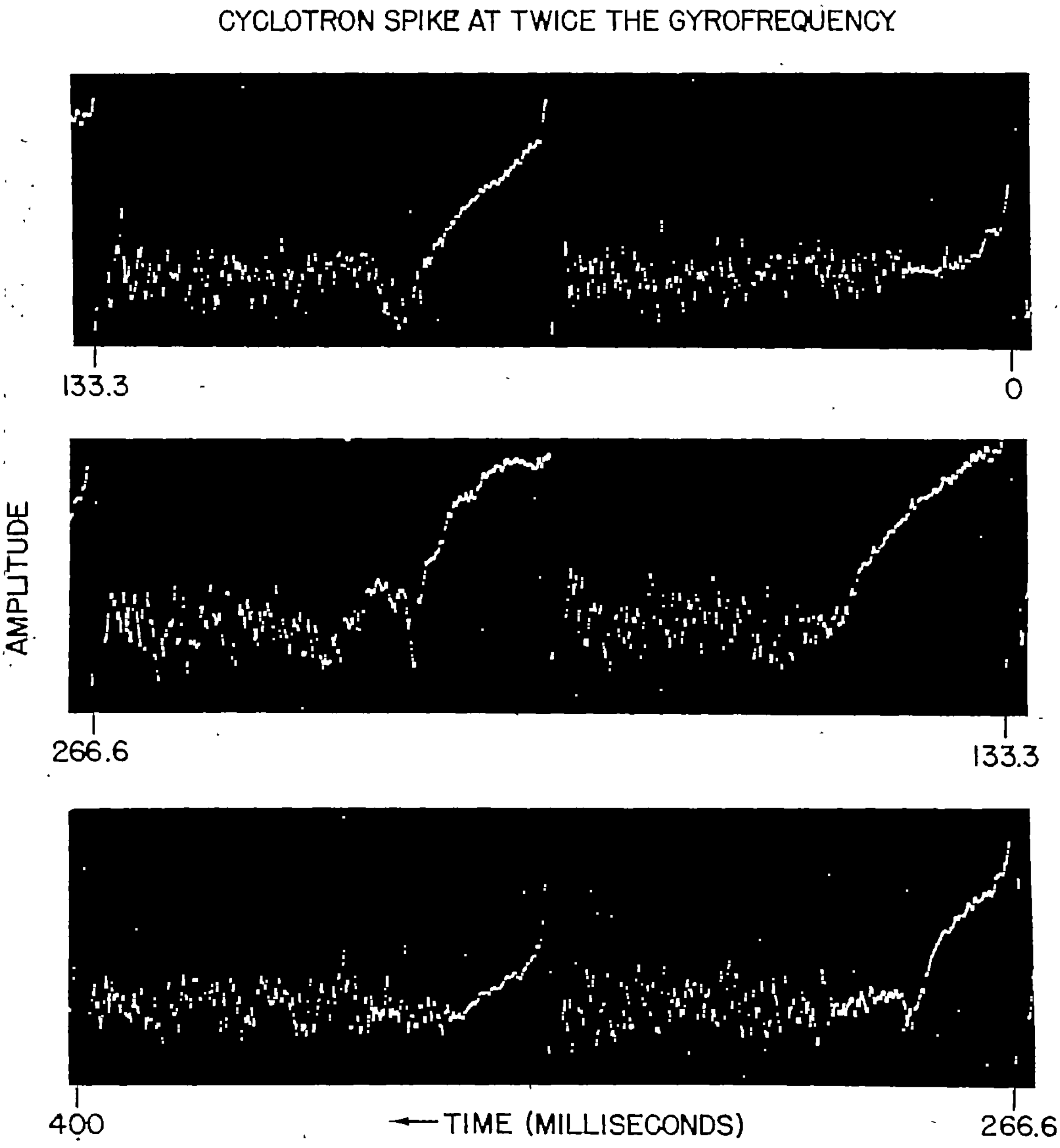

Fig. 4b. The time dependence of signal amplitudes forming a cyclotron spike

the transition from one region to the other as the latitude changes is very abrupt. This rather extreme change of the electron density was probably produced by particles ejected from the Sun with the solar flare of October 23.

At high latitudes the ionospheric reflecting surfaces are rough, producing a diffuse echo on the ionograms. The diffuseness of the echo is called spread-*F* after the same phenomenon observed on bottom-side ionograms. Fig. 3 consists of a set of night-time ionograms obtained within the auroral zone, showing, as Petrie[5] has reported, spread-*F* decreasing at lower latitudes. These ionograms show another feature that at first sight seems like a double echo from the Earth. The additional echo is reflected from a thin sporadic *E* layer about 100 km above ground. This particular patch of sporadic *E* ionization had a horizontal extent of 1,700 km. Petrie has also reported the existence in the auroral zone of diffuse clouds of ionization several hundred kilometres thick, through which the satellite passes.

The electron distributions, temperatures, and effective ion mass are parameters of great value in investigations of the ionosphere, particularly during storm times. However, some of the most interesting phenomena were unexpected. Among these are the existence of cyclotron spikes and the displacement of the plasma spikes from the associated reflexion trace.

When the transmitter is operating at multiples of the gyrofrequency of electrons about the Earth's magnetic field, cyclotron resonance occurs which causes a receiver response similar to the plasma spikes. The cyclotron spike at 1·7 Mc/s in the ionogram of Fig. 4a is that for twice the gyrofrequency. Fig. 4b shows amplitude-time curves for the signals that form this spike. These succes-

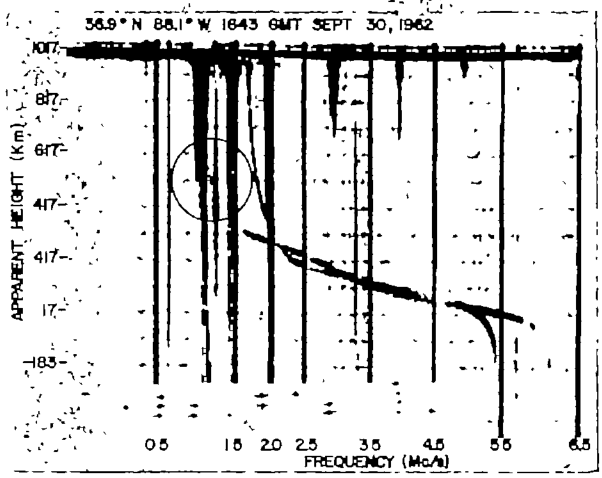

Fig. 5. Ionogram showing absence of ordinary wave reflexions between 1·25 and 1·6 Mc/s

sive pulses of the sounder transmitter are separated in frequency by 15 kc/s. Lockwood[6] has described the phenomenon and suggested that bunching of the electrons occurs when they are driven at multiples of the gyro-frequency, and that these electrons re-radiate energy to the receiver when the transmitter is shut off.

The separation of the plasma spikes from the associated reflexion traces are explicable in terms of a perturbation[7] of the ionosphere by the satellite in which the electron density behind the satellite is lowered and that ahead increased. In Fig. 5 the extraordinary wave plasma spike appears immediately to the left of the 1·5-Mc/s frequency marker. This spike forms at the lowest frequency for which pulse propagation of the extraordinary wave from the satellite can occur. This frequency is determined by the lowest density in the vicinity of the satellite. The reflexion trace commences at the lowest frequency for which the pulse can travel vertically downward from the satellite. For the extraordinary wave this is about 1·7 Mc/s. The difference in frequency between the spike and the reflexion trace is therefore a measure of the amplitude of the perturbation. The circled reflexion trace between 1·1 and 1·25 Mc/s results from coupling between the Z-wave and the ordinary wave. This identification is based on the fact that it is bounded in frequency in all the observed examples by the lowest frequency for ordinary wave reflexions and by the highest frequency for which Z-waves can propagate at the satellite. This upper limit for Z-wave propagation is indicated by the Z-plasma spike[6]. The gap in ordinary wave reflexion trace between 1·25 and 1·6 Mc/s is providing an additional means of studying the perturbation of the plasma.

In conclusion, the top-side sounding technique has been shown to be a valuable new addition to the methods available for investigation of the upper atmosphere.

The contribution of many people to the success of this experiment, particularly our colleagues at this Establishment who designed and built the spacecraft and those members of the staff of the U.S. National Aeronautics and Space Administration Goddard Space Flight Center and Marshall Space Flight Center who were responsible for placing the spacecraft in orbit, tracking the satellite, and obtaining telemetered data, is gratefully acknowledged.

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INVESTIGATIONS OF THE UPPER IONOSPHERE DEDUCED FROM TOP-SIDE SOUNDER DATA

By J. W. KING
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THE Defence Research Board of Canada, the ionosphere top-side sounder of which has been described by Warren (preceding communication) has generously agreed that the Radio Research Station at Slough should record telemetry from the satellite as it passes over the Department of Scientific and Industrial Research stations at Winkfield near Slough (51° N., 1° W.), Singapore (1° N., 104° E.) and in the Falkland Islands (52° S., 58° W.), and make deductions from the recorded top-side ionograms. This article gives an account of some of the preliminary results obtained in this way. The Budden matrix method applied to a *Pegasus* computer as described by Thomas and Vickers[1] has been used to reduce the ionograms to $N(d)$ curves which show the electron concentration, N, as a function of the depth, d, below the satellite. An example is shown in Fig. 1 in which the scale of N is logarithmic. As this example shows, it is often possible to separate the log $N(d)$ curve into two parts, both of which are sometimes closely rectilinear, and to ascribe a 'scale height', H, to each part so that:

$$N \propto \exp(-h/H)$$

Up to the present, five passes over Slough and eleven passes over Singapore, each producing about 30 $N(d)$ curves, have been analysed. These data have been used to investigate the way in which the scale height varies with height above the ground and with latitude, and also the way in which the electron concentration at fixed heights varies across the equator.

Fig. 2 shows the scale heights deduced by drawing the tangents to the curve in Fig. 1 at a series of different heights. It is at once obvious that the scale height is roughly constant in each of two regions, the first extending roughly from 300 to 550 km above the ground and the second from 750 to 1,000 km, and that the scale height in the upper region is greater than that in the lower. All the log $N(d)$ curves show similar results in that the scale height is always observed to increase with height, but not all show two regions in which the scale height is constant. In order to investigate the phenomenon further it is useful to average the scale heights for all the electron density distributions occurring in a single pass and to examine the results. Some typical averaged values are

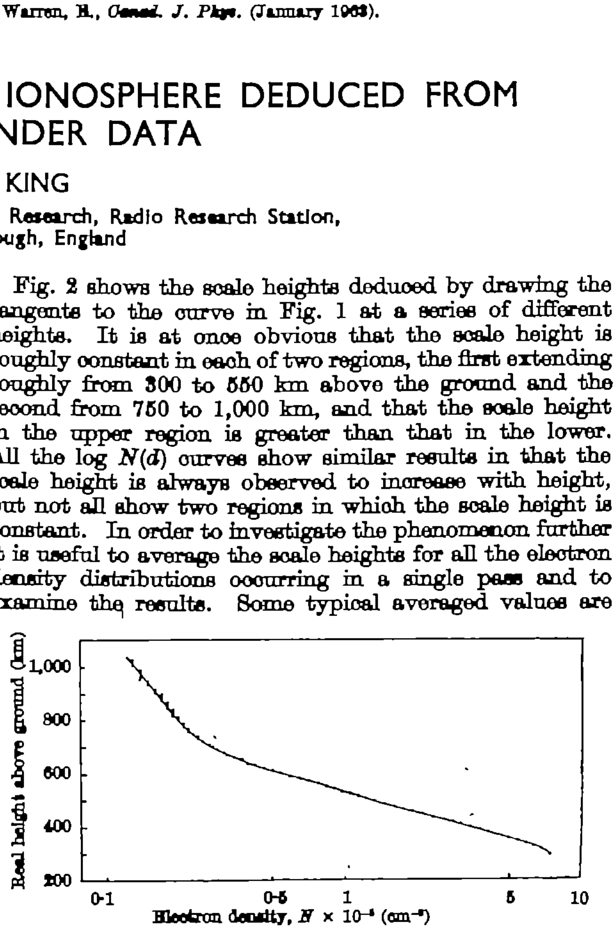

Fig. 1. Electron density distribution for a point 9° N., 88° E. recorded at Singapore at 1614 U.T. on October 1, 1962

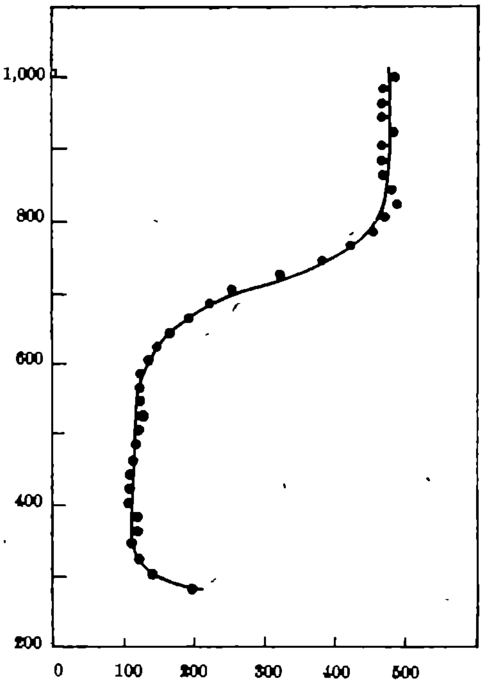

Fig. 2. Plot of scale height against real height for the distribution at a point 9° N., 88° E. recorded at Singapore at 1614 U.T. on October 1, 1962. The curved portion of the plot between 290 and 330 km represents the portion of the electron density distribution immediately above the layer maximum

listed in Table 1. It is noticeable that in the daytime the scale height in the lower region is greater at Slough than at Singapore, while that in the upper region is approximately the same. The ratio of the scale heights in the two regions is therefore smaller at Slough than at Singapore. In the light of these results it is interesting to examine the latitude variation of scale heights measured during a single pass covering a range of latitudes. Fig. 3 shows the results for such a pass extending from about 33° N. to 60° N., recorded at Slough. From Fig. 3 it is apparent that the scale height in the lower region increases as the satellite moves away from the equator, while that in the upper region remains roughly constant. These results, combined with those in Table 1, seem to indicate that the scale height near 500 km increases markedly as the distance from the equator increases, but that near 900 km does not.

The fact that the $N(d)$ curves usually indicate the presence of either one region (400–600 km above ground) or sometimes two regions in which the scale height is constant would suggest at first sight that in these regions the composition is uniform and the atmospheric gases are isothermal and in diffusive equilibrium. If these assumptions are made and if it is supposed that the electrons and ions are in thermal equilibrium and the predominant ion in the region 400–600 km is O^+, then it follows that the daytime temperature in this region over Slough is 1,530° K, a value consistent with that (1,600° K) suggested by the 1961 COSPAR atmosphere. If it is further supposed that the atmosphere at 900 km above Slough is at the same temperature, then it follows that the ions there must have a mean mass of 8 atomic units, as would be expected if they were a mixture of O^+ and He^+.

If corresponding calculations based on the same assumptions are made for the results from Singapore, they lead to a temperature of 800° K by day in the lower region, and to a mean ionic mass of 4·6 atomic units at 900 km by day. It is obvious that the higher night-time scale heights at Singapore would have to be explained by a decrease in the height of the O^+ to He^+ transition region, with consequent reductions of the mean ionic masses at 500 and 900 km, resulting from a lower night-time temperature.

Table 1. SCALE HEIGHTS OF THE ELECTRON DENSITY DISTRIBUTION

	Scale height at about 500 km above ground (km)		Scale height at about 900 km above ground (km)		Ratio	
Slough Rev 17 (day)	225	mean 190	390	mean 370	1·7	mean 2·0
Slough Rev. 30 (day)	145		370		2·5	
Slough Rev. 58 (day)	200		370		1·9	
Slough Rev. 248 (day)	190		360		1·9	
Singapore Rev. 26 (day)	100	mean 100	320	mean 350	3·2	mean 3·5
Singapore Rev. 40 (day)	100		350		3·5	
Singapore Rev. 53 (day)	100		380		3·8	
Singapore Rev. 34 (night)	120	mean 110	540	mean 600	4·5	mean 5·5
Singapore Rev. 128 (night)	100		650		6·5	

The magnitude of the daytime temperature deduced from the Singapore results scarcely seems feasible, and we must conclude that in these equatorial regions the simple assumptions made here are probably not valid. The observations outlined in the next paragraph also suggest that the simple assumption of diffusive equilibrium cannot be expected to be valid near the equator; it may

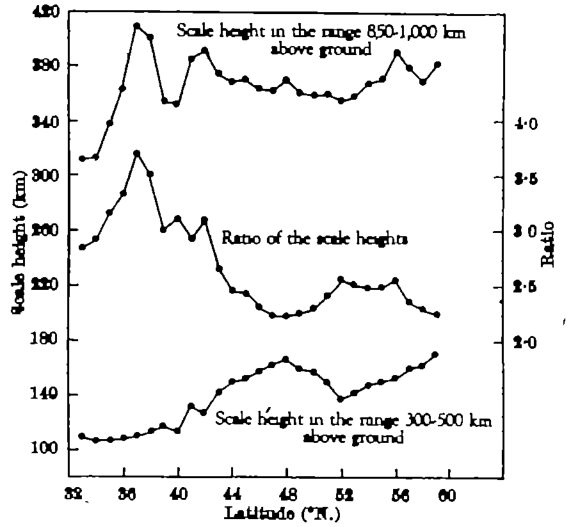

Fig. 3. Plots showing the variation of scale height with latitude as observed during revolution 30 recorded at Winkfield between 1017 and 1027 U.T. on October 1, 1962

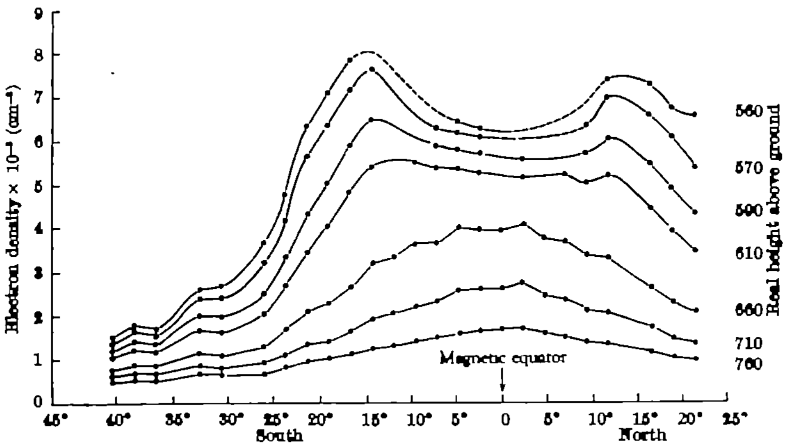

Fig. 4. Contours of electron density at a series of fixed heights above the ground. These results were obtained from revolution 25 recorded at Singapore between 0301 and 0312 U.T. on October 1, 1962

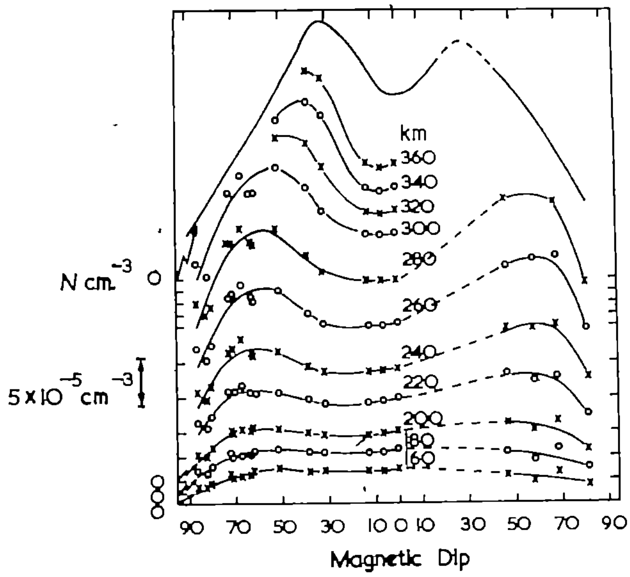

Fig. 5. Variation with magnetic dip of the mean quiet electron density *N* at a series of fixed heights at local noon in September 1957. The zero of the scale for the electron density at each height is marked. The average peak electron density *N*m *F*2 is shown as the top curve in the diagram. (After Croom, Robbins and Thomas (ref. 2))

be that the gradual decrease of scale height as the latitude decreases is a consequence of this.

The curves in Fig. 4 show how the electron density at a series of fixed heights above the ground changes with latitude during a typical pass over Singapore. These should be compared with the corresponding curves from bottom-side sounders as given, for example, by Croom *et al.*[2], whose results are reproduced in Fig. 5. The structure of the equatorial geomagnetic anomaly is clearly seen and it appears that the separation between the two peaks becomes less as the height increases until at about 700 km they merge to form a single maximum on the magnetic equator. It thus appears that the structure is such that the equatorial anomaly takes the form of an arch with its top about 700 km above the ground. Further plots such as that shown in Fig. 4 show that the top of the arch formed by the sub-equatorial peaks appears to be farthest from the ground, approximately 900 km, at about 20 h L.M.T. At this time the contours also show that the anomaly is most pronounced. During the subsequent hours the arch gradually falls until near midnight the top is only 400 km above ground and the anomaly has virtually disappeared. It should be realized that this latter representation of the diurnal variation of the anomaly is based on only the eleven Singapore passes at selected times which have so far been analysed, and extended observations might produce somewhat different results.

Another phenomenon, which is frequently observed on passes recorded at Slough, is related to sudden apparent variations in the scale height of the observed top-side electron density distributions. These usually occur, for the cases so far investigated, over a very narrow range of latitudes between 67° N. and 70° N. In this region the scale height appears to increase drastically, by as much as 50 per cent, and it is seen from Fig. 6 that the increase occurs mostly at heights greater than 700 km above the ground. It seems possible that this effect may be the result of some kind of interaction between the outer Van Allen belt and the atmosphere, but the fact that the latitude at which the phenomenon is observed is rather high may mean that the anomalous ionograms result from indirect or oblique observations of the affected region of the ionosphere.

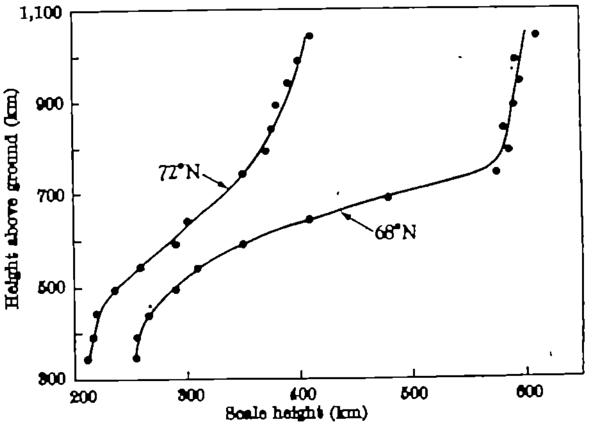

Fig. 6. Plots showing the variation of scale height with height for two electron density distributions obtained 4° apart. The first one was obtained at 72° N. and is normal and the second one was obtained in the middle of the abnormal region at 68° N. The results were recorded at Winkfield at 2227 U.T. on October 3, 1962

The satellite *Alouette* is part of the top-side sounder programme the overall management of which is the responsibility of the United States National Aeronautics and Space Administration. *Alouette* was designed and built by the Canadian Defence Research Telecommunications Establishment and was launched into orbit by the United States.

The present article represents co-ordinated effort on the part of data acquisition, processing and reduction groups at the Radio Research Station, and is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research, England.

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SOME EARLY RESULTS FROM THE IONOSPHERIC TOP-SIDE SOUNDER SATELLITE

By R. W. KNECHT and T. E. VAN ZANDT
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This article presents some early results, using *Alouette* data, on the latitudinal variation of ionospheric structure and of the geographical extent of high latitude spread *F*.

Ionospheric structure. In Fig. 1 is presented a latitudinal cross-section of the top side of the ionosphere constructed from an evening pass of *Alouette* on October 1 (U.T.), 1962. This pass is mapped in Fig. 2. A moderate magnetic and ionospheric storm was in progress during this period. The 35 ionograms taken at the positions marked by open circles in Fig. 2 (and by ticks along the line showing the height of the satellite in Fig. 1) were reduced to electron density profiles using a computer programme developed by G. L. Nelms of the Canadian Defence Research Telecommunications Establishment. The accuracy of the electron density profiles is thought to to be better than ± 20 km. The contours of constant plasma frequency (isopycnic contours) were drawn through the data points with little smoothing. The contours are incomplete because of occasionally poor

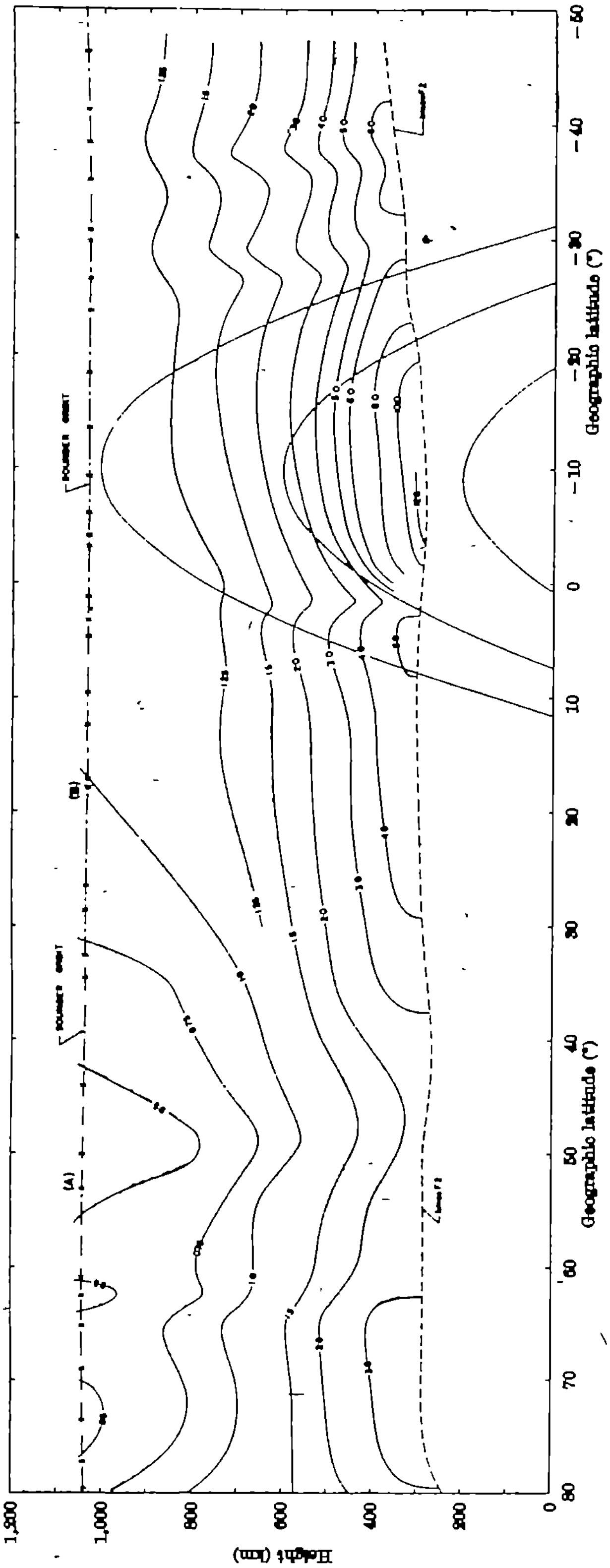


Fig. 1. A latitudinal cross-section through the ionosphere constructed from the pass mapped in Fig. 2. The contours are of constant plasma frequency in Mc/s.

ionograms, the 11·8-Mc/s upper frequency limit of the sounder, and inadequate bottom-side data.

Among the several noteworthy features along this 15,000-km cross-section are the three narrow 'troughs' in the isopycnic contours. The troughs are not magnetically conjugate, as can be seen by comparison with the lines of force also plotted in Fig. 1. (The computed lines of force all pass over 90.00° W. and 8.75° S.) Nevertheless, the two troughs at about 2° N. (+23° dip) and 27° S. (−27° dip) appear to bound different regions of the ionosphere. Poleward of these two troughs, f_oF2 (the largest plasma frequency in the $F2$ layer) is 5 or 6 Mc/s and decreases slowly with increasing latitude. Equatorward of the troughs, f_oF2 increases rapidly to at least 10 Mc/s and $h\max F2$ (the height of f_of2) decreases. The slight maxima of the isopycnic contours on the poleward sides of these two troughs extend up to at least 1,000 km, as can be seen in Fig. 3, which shows the plasma frequency at the satellite.

Although these data are equinoctial, the electron densities south of the dip equator were conspicuously larger and the structure of the ionosphere was much more complex than north of the dip equator.

The electron densities at all heights reached a minimum between 47° N. and 53° N. The electron density profiles

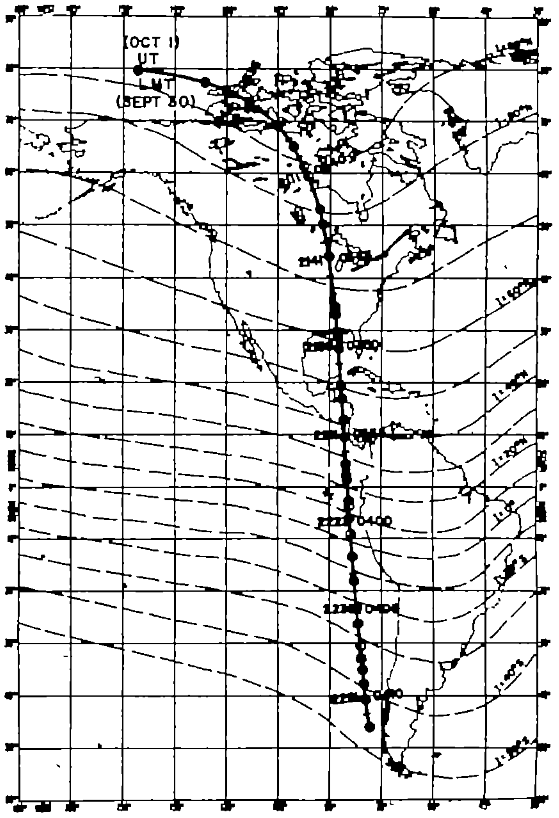


Fig. 2. Map showing the location and time of top-side soundings used to construct the ionospheric cross-section (Fig. 1). Isoclines are also shown

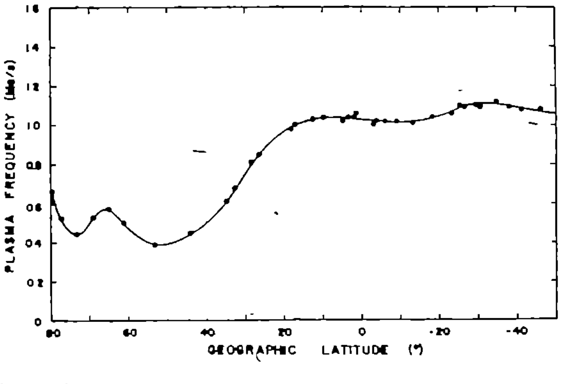


Fig. 3. Plasma frequency at the height of the satellite (1,033–1,043 km) as a function of geographical latitude

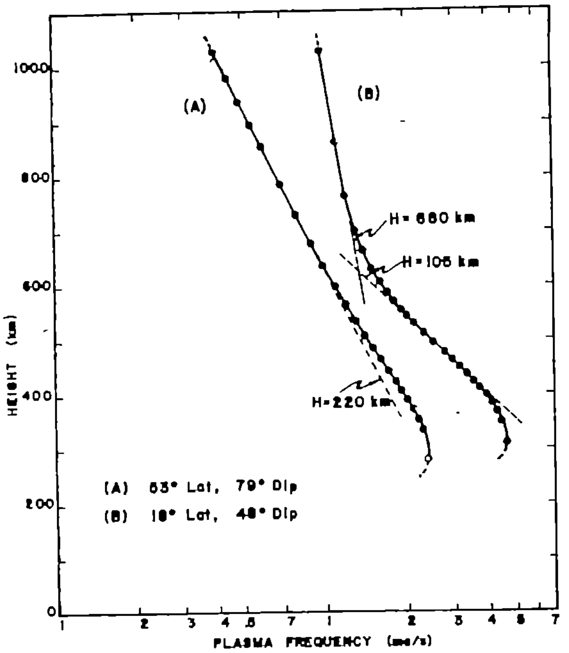


Fig. 4. Electron density profiles at points (*A*) and (*B*) on the latitudinal cross-section shown in Fig. 1

in the minimum and to the north had a markedly different shape from those to the south (Fig. 4). The profiles at latitudes higher than 45° N. (of which profile *A* is typical) consist of a long exponential region extending downward from 1,000 km, with a slight bulge just above *h*max*F*2. The scale height of the plasma, which is defined by $H = -(1/2)/(d/dh)(\ln f_N)$, where f_N is the plasma frequency, is 220 km in the exponential region. The long exponential region suggests that the ionosphere in this region is composed mostly of a single ionic species in diffusive equilibrium. Then, according to whether the species were H^+, He^+ or O^+, the temperature would have to be about 110, 440 or 1,750° K, respectively. Earlier observations show that the temperature could scarcely be as small as 440°, hence the ionosphere in this region must be composed mostly of O^+ at a temperature of about 1,750° K.

Below 45° N., the shape of the top side changes rapidly to that of profile *B*, which is typical of all latitudes below 20° N., including the region of the magnetic equator. Profile *B* contains two exponential parts, as indicated, with scale height of 105 km in the lower part and 680 km in the upper part. If these exponential parts corresponded to regions of single ionic species in diffusive equilibrium and if the ions were H^+, He^+ or O^+, the temperature would have to be 52·5, 210 or 840° K respectively, in the lower part, and 340, 1,300 or 5,440° K, respectively, in the upper part. At night at these heights the ionosphere is expected to be nearly isothermal at a temperature which could not be much smaller than 840°. Therefore, the lower part of profile *B*, which constitutes the usual *F*2 layer, must consist almost entirely of O^+ at 840°.

But the upper part must also be at a temperature of 840°, which leads to a mean ionic mass of 2·5. Therefore this region must be part of the transition toward a preponderance of H^+. Its apparent exponential form (based on only three points) is probably spurious. Comparison of these results shows two striking differences on this occasion between the ionosphere at latitudes higher and lower than 45°. First, at the higher latitudes the temperature is about twice as large as at the lower latitudes. Secondly, at the higher latitudes the ionosphere consists mostly of O^+ up to at least 1,000 km, whereas at the lower latitudes the transition to lighter ions begins at about 600 km. Since a moderate magnetic and ionospheric

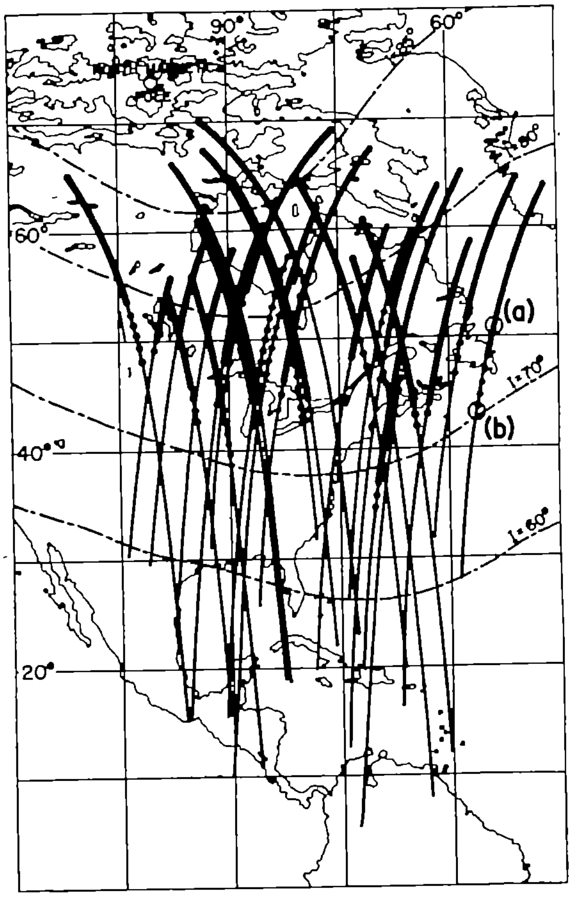
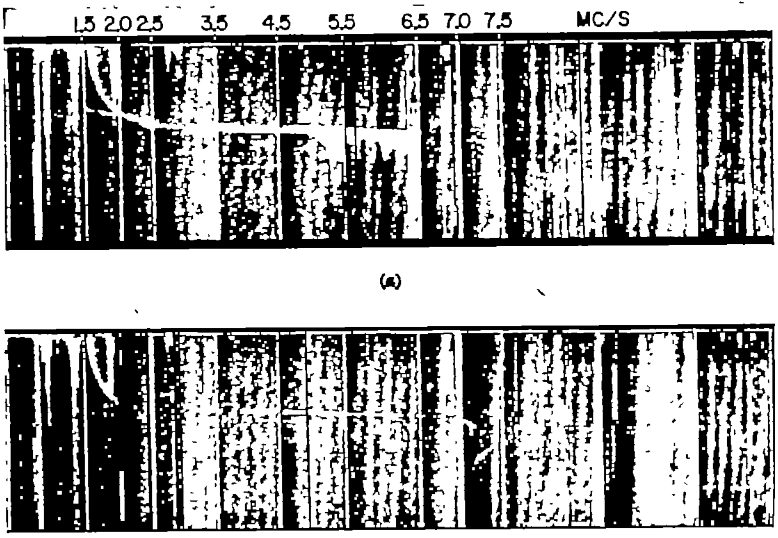


Fig. 5. Occurrence of spread *F* on 27 passes of *Alouette* recorded at Blossom Point, Maryland (see text)



(*a*)

(*b*)

Fig. 6. Topside ionograms taken at points (*a*) and (*b*) on Fig. 5. Ionogram (*a*) taken at 1336 U.T., October 9, 1962, shows considerably spread traces. Ionogram (*b*), taken at at 1334 U.T. on October 9, 1962, shows sharp traces

storm was in progress during this period it cannot yet be said whether the structure observed on this occasion was a normal condition or a storm effect. However, the previous results are not inconsistent with previous observations.

The ion composition as a function of height and a more accurate temperature might be determined by a detailed comparison between the observed profiles and calculated profiles based on diffusive equilibrium[2]. Ion compositions determined without such detailed comparisons may be seriously in error, except where the electron density is exponential for more than a scale height.

Geographical extent of spread F. Several studies of the temporal and geographical variations of spread *F* have been made using bottom-side ionospheric sounding data[3]. Spread *F*, which is believed to be due to the presence of field-aligned irregularities in the ionosphere, manifests itself on ionograms as a spreading of the ionospheric echo over a range of delay times, in contrast with the distinct echoes in the absence of spread *F*. The studies referred to here have shown that spread *F* is most common in polar and equatorial regions. However, the boundaries of these regions of maximum occurrence have been difficult to describe owing to the sparsity of the sounding network. The *Alouette* top-side sounder, on the other hand, takes soundings approximately every 120 km along trajectories that regularly traverse the equatorial and polar zones.

The observations reported here have been taken from 27 passes of *Alouette* recorded by the U.S. National Aeronautics and Space Administration telemetry station at Blossom Point, Maryland, during the period September 30–October 12, 1962. During this period the southward passes occurred between about 1930 and 2330 h 75° W. time and the northward passes between 0830 and 1255 h 75° W. time.

Each ionogram taken during these 27 passes was put into one of three groups, according to the degree of spread *F*. The results are shown in Fig. 5. The trajectory is represented by a thin line where sharp traces were observed; filled circles represent ionograms on which a moderate degree of spread *F* was observed or which were otherwise uncertain; and trajectories represented by a thick line indicate that considerable spread *F* was present. Two top-side ionograms, taken at points (*a*) and (*b*) on the easternmost pass, shown on Fig. 5, are reproduced in Fig. 6*a* and 6*b* to illustrate considerable spread and sharp traces, respectively.

Several tentative conclusions can be drawn from this study of two weeks' data. First, in the area of observation (see Fig. 5), spread *F* was almost always present at magnetic dips greater than about 75°, and it was rarely present below about 70°. Secondly, spread *F* was equally prevalent during the mid-morning and the evening periods.

The refined description of the temporal and geographical extent of spread *F* which *Alouette* makes possible should be of considerable help both in propagation studies and in understanding the mechanism of spread *F*.

This work was supported by the National Aeronautics and Space Administration. We thank G. L. Nelms for providing us with his true height programme. The assistance of Wynne Calvert, E. E. Ferguson, G. B. Goe, D. E. McKinnis and other members of the U.S. Central Radio Propagation Laboratory staff was invaluable.

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THE NATIONAL BOTANIC GARDENS OF SOUTH AFRICA, KIRSTENBOSCH

By PHILLIDA BROOKE

DURING 1963 the National Botanic Gardens of South Africa at Kirstenbosch, near Cape Town, celebrate their Golden Jubilee with a series of events which will interest and attract scientists and laymen from many parts of the world. It has long been accepted that the 30-mile long Cape Peninsula, which boasts more species of flora than the entire British Isles, is one of the richest and most interesting botanical regions in the world. Yet had it not been for the establishment of Kirstenbosch Gardens in 1913 it is likely that many rare and beautiful species of native South African plants would now be extinct.

When William J. Burchell, botanist and explorer, arrived in Table Bay in 1810 he had heard much of the botanical wonders of the Cape. "All that I had pictured to myself," he wrote soon afterwards, "was far surpassed . . . At every step a different plant appeared and it is not an exaggerated description if it be compared to a botanical garden, neglected and left to grow to a state of Nature; so great was the variety everywhere." Burchell found 105 distinct species of plants in a single mile not a stone's throw from white-washed, compact little Cape Town, though the season was late summer when, for three months, the Earth had been scorched by the Sun and seared by the south-east wind. In the wooded ravines and gorges of Table Mountain scarlet and gold disas crowded the mossy edges of late-running streams; in autumn the march lily (*Amaryllis belladonna*) appeared, deep pink and heavily scented. In winter the mountain slopes were purple with heath and carpeted with fragile oxalis which gave place, in the glory of the Cape spring, to Compositae, Iridaceae, Proteaceae and *Mesembryanthemum*, breath-taking in their vivid colours and abundance. Aware of a ready market for their wares in the streets of Cape Town and uninhibited by any legislation, flower sellers helped themselves generously to indigenous flora, tearing bulbs and roots from the ground with careless abandon. Not far away, farmers ploughed up the earth or, worse still, burnt it, so destroying the natural vegetation in favour of transient spring grazing: and all the while the city of Cape Town grew, laying down streets, building houses where once there were fields of wild flowers that could be cut down with a scythe. Well-known for their complacency, residents in Cape Town seemed little concerned when botanists deplored the situation and begged them to do something active to protect their magnificent floral heritage. True, certain necessary—if tardy—laws were passed which forbade the gathering of rarer species, but it soon became clear that this protection of what already existed was not enough; what was required was a means of bringing to cultivation and preserving for all time those plants which were facing the threat of annihilation.

No one was more keenly aware of this urgent necessity than Prof. H. H. W. Pearson, of the South African College in Cape Town. He had arrived from England in 1903 with a distinguished academic record at Cambridge and considerable practical experience both in Ceylon and at Kew, where he had been assistant to the Director. His appointment to the Harry Bolus chair of botany in the South African College brought him to the Cape for the first time at the age of thirty-three and gave him the opportunity to

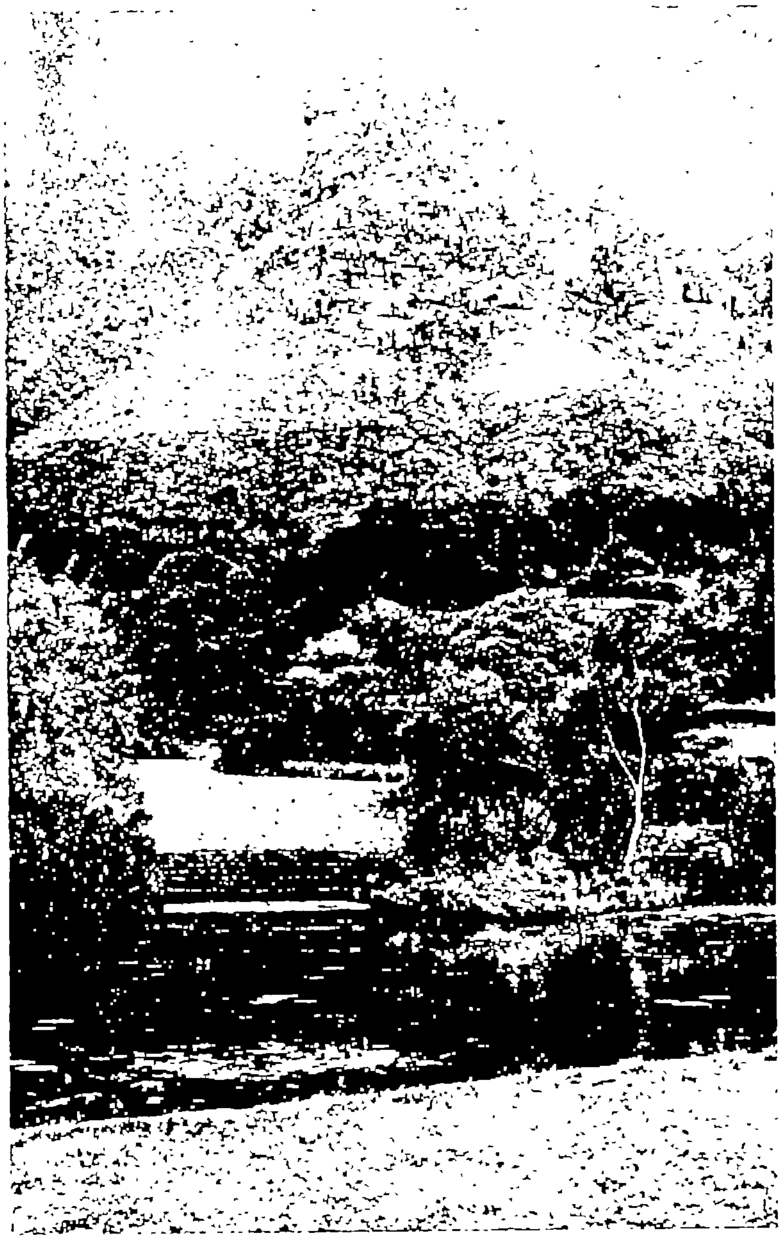

Fig 1. Castle Rock dominating the scene at Kirstenbosch (*Photo Richard Simons*)

study in the field the tremendous wealth of native flora of which he, like Burchell, had heard so much. However, disappointment awaited him. The Cape, he found, was a botanically impoverished land with a crying need for a fully equipped institution devoted to the scientific cultivation and study of indigenous plants. Ever since the Cape's earliest Commander, Jan van Riebeeck, had planted vegetables for passing seamen in the mid-seventeenth century, this had been a country renowned for its splendid gardens where *Araucaria excelsa* (Norfolk Island pine) and roses, *Magnolia*, oaks and sweet peas grew in abundance. But no one had ever troubled to create a garden solely devoted to the fascinating flora of South Africa, the ecology of which botanists had scarcely begun to explore. As he grew familiar with the country, Prof. Pearson began to foresee an establishment on the lines of the Royal Botanic Gardens, Kew, a natural garden, supported by the Government, where South African gardeners could be trained to cultivate and preserve South African plants under South African conditions. In addition, it should be an expression of the intellectual and artistic aspirations of a nation whose duty it was to appreciate the rare and the beautiful with which Nature had so lavishly endowed it.

It was not until November, 1910, that Pearson had the opportunity to offer his suggestion to the public in a concrete form. The South African Association for the Advancement of Science was to meet in Cape Town; six months earlier the four provinces of South Africa had been united under the British flag, and Pearson realized that the time was ripe to present a proposal that would appeal to the strong spirit of patriotism then prevailing and, at the same time, be of the utmost scientific interest and importance. It was in his presidential address, therefore, that he invited his associates to consider with him the establishment of a national botanic garden.

Response was immediate. A meeting was held under the presidency of Lord de Villiers, Chief Justice of South Africa, and a deputation was sympathetically received by the Prime Minister, General Louis Botha. Eventually, on May 16, 1913, a happily unanimous Parliament resolved that a national botanic garden, subsidized by the Government, should be established without delay.

Prof. Pearson had already chosen the site of his garden. Government-owned Kirstenbosch Estate lay on the eastern slopes of Table Mountain approximately seven miles from Cape Town and covered an area of some four hundred acres. Its soil was rich and varied; it was well watered by mountain streams, and indigenous plants—including seven acres of rare and beautiful silver trees (*Leucodendron argenteum*)—grew there in abundance. South and east of the estate there spread a wealth of woodland stretching towards the Cape Flats, the blue waters of the Indian Ocean and the shimmering peaks of the Hottentots' Holland mountains some forty miles away. Behind, cleft by deeply wooded ravines, towered the ramparts of Castle Rock, 2,000 ft. high, with the limitless blue of the African sky beyond. It was of this corner of the Cape Peninsula that Burchell wrote: "The view from this spot, and indeed all the scenery around, is the most picturesque of any I have seen in the vicinity of Cape Town". Without doubt Kirstenbosch, supported by the Government and developed under the expert scientific guidance of Prof. Harold Pearson, could become one of the great botanic gardens of the world.

But, according to a newspaper of the day, it had been allowed to degenerate into a "melancholy monument to neglect". Originally an outpost of van Riebeeck's seventeenth century Dutch colony at Cape Town, Kirstenbosch had been occupied by a garrison of soldiers who protected the town against the depredations of neighbouring Hottentots. A hedge of wild almond (*Brabeium stellatifolium*) was planted crescent-wise across the lower eastern slopes of Table Mountain and remnants of this one-time southernmost boundary of civilization may still be seen at Kirstenbosch, a national monument, 15 ft. high and in parts impenetrable. Later, a Government official named Kirsten owned land in the vicinity and it is thought that it was from him that the estate acquired its name. When the British occupied the Cape early in the nineteenth century Kirstenbosch was divided into two portions and allocated to Henry Alexander and Colonel Christopher Bird, both senior officials in the Colonial Service. Bird built himself a homestead high against the mountain on

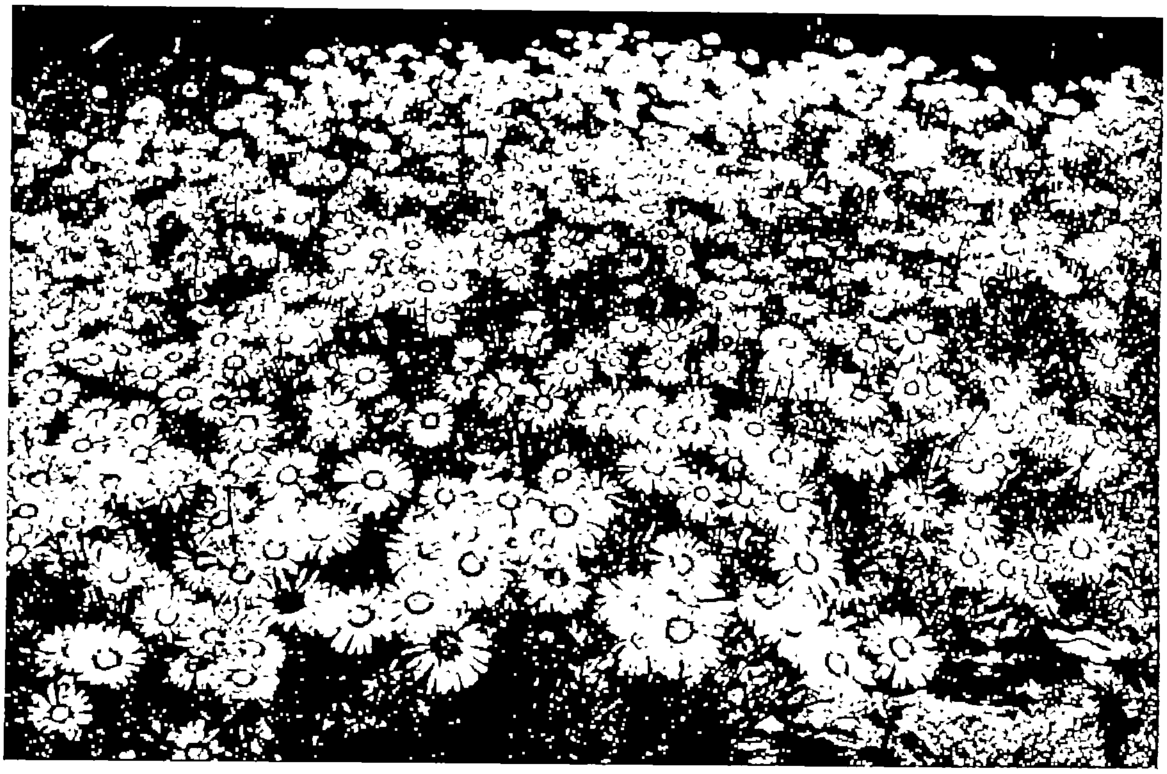

Fig. 2. A display of *Felicia tricolor*. In September the terraces at Kirstenbosch are vivid with brightly coloured spring annuals. (*Photo: Richard Simons*)

the site of the original barracks; he planted Spanish chestnuts and, it is thought, paved the beautiful little bath which now forms the heart of the Gardens and from which rises a perpetual, crystal-clear spring. Alexander built a house lower down the hillside and, according to legend, refused to put windows into the bedrooms, maintaining that where one slept there was no need to look at the view.

In time Bird returned to England, Alexander died and in 1823 his unhygienic abode was transformed by Dirk Gysbert Eksteen, member of an old Cape family, into a gracious Cape-Dutch homestead with lofty windows and wide-welcoming doors. Eksteen planted the oaks which remain to this day; he laid out vineyards and orchards and lived according to the genial, generous pattern which characterized nineteenth century Cape Town. But Eksteen died; his descendants moved away and the house became a ruin. It was at this time that Cecil John Rhodes bought Kirstenbosch Estate and, on his death in 1902, bequeathed it to the people of South Africa. At first little was done to develop it; pigs rooted among the fallen acorns, weeds grew rampant in vineyard and orchard and undergrowth obscured the dimpled beauty of the little paved bath.

When Prof. Pearson assumed honorary directorship of Kirstenbosch in August, 1913, he faced a formidable task. Despite the financial aid of the Government and the support of the newly formed Botanical Society of South Africa, funds were dangerously short. Moreover, the Professor was obliged to fulfil his duties at the College in addition to restoring an ordered garden out of a chaotic wilderness. "It will be a burden," he wrote to his friend and colleague, Prof. A. C. (later Sir Albert) Seward, "but it is worth carrying even if it never falls to me to exploit its contents."

Those were prophetic words. Scarcely three years later, after an almost incredible amount of work had been accomplished against all odds—most of it in war-time—Prof. Pearson died at the age of only forty-six. His grave lies on the hillside overlooking the Gardens and his headstone bears an inscription taken from the epitaph of Sir Christopher Wren: "If ye seek his monument, look around".

It was not until the First World War was over that Pearson's successor, Prof. R. H. Compton, was able to assume duties and in the lean years between—during which the already inadequate Government grant was reduced—Kirstenbosch Gardens were maintained by the combined efforts of the Botanical Society and of the curator, Kew-trained J. W. Mathews. Under Compton's directorship they expanded to the very summit of Table Mountain by an additional grant of 600 acres in 1922. Paths were laid, swamps were drained, hillsides were terraced and planted and the work begun by Prof. Pearson developed to maturity. In 1923 natural history classes for local school children were inaugurated, first entirely in the field then, with the completion of the thatched lecture hall, partially indoors. The Gardens' academic work extended further with the establishment, in 1938, of the Compton Herbarium, where 200,000 specimens of South African flora are preserved and where plants are brought for identification by both amateur and professional botanists. More than 30,000 packets of seed, gathered at Kirstenbosch, are despatched, free of charge, each year to the 4,000 members of the Botanical Society, while many others are sold to nurserymen all over the world. Seed collection and preparation is therefore an important part of the work carried out at Kirstenbosch, and it is largely due to the Gardens that South African flowers are now cultivated as far afield as the U.S.S.R. and New Zealand, Japan and California.

After thirty-five years at Kirstenbosch, Prof. Compton was succeeded by Prof. H. B. Rycroft under whose energetic and enthusiastic guidance the National Botanic Gardens—one of the glories of South Africa and famous throughout the world—are now entering on their Golden Jubilee Year.

A most ambitious programme of festivities, extending over ten months, has been planned. In February a garden party will be held on the lawns at Kirstenbosch and at this the State President, Mr. C. R. Swart, as patron-in-chief of the celebrations, will be guest of honour. Exhibits from America, Europe, Australia and the East will be on view at the International Flower Show planned for March, while the national spring and wild flower show in September promises to be of spectacular beauty and of great botanical and horticultural interest. Museums, libraries and art galleries throughout the country are collaborating with the Jubilee Council to organize displays of books, paintings and other exhibits, botanical in character and interest, and an international exhibition of floral stamps—"Flora 63"—has been sponsored by the Lions Club of South Africa and the Thematic Stamp Club. In the spring a week has been set aside for the young people of South Africa, who will hold their own flower show, a procession of (artificial) flower-decked floats through the streets of Cape Town and orchestral and choral concerts with "Flowers" as the theme.

Most important and serious of all the festivities will be the assembly at Kirstenbosch of fifty or more of the world's most distinguished botanists and horticulturists, including Sir George Taylor, director of the Royal Botanic Gardens at Kew; Dr. W. Domke, of the Botanical Gardens in Berlin; Prof. H. Merxmüller, director of the State Museum, Munich; Prof. H. Weimarck and Mr. Rolf Dahlgren of the Botanic Institute and Museum, Lund; Prof. W. Robyns, director of the Botanical Gardens, Brussels; Prof. Reed C. Collins of the Gray Herbarium, Harvard University; Prof. H. P. Riley of the University of Kentucky and many others.

These specially invited guests of the Jubilee Council will be entertained on day excursions to places of particular botanical interest near Cape Town and will attend lectures on South African flora arranged for their benefit. Finally, they will be taken on a tour of South Africa which will last for more than three weeks and during which they will have an opportunity to study in the field a wide variety of vegetational types, and, at the same time, to see some of the country's most splendid scenery. Headquarters of all these events will be Kirstenbosch Gardens, superbly beautiful throughout the year but at their most colourful in the southern spring months of September and October.

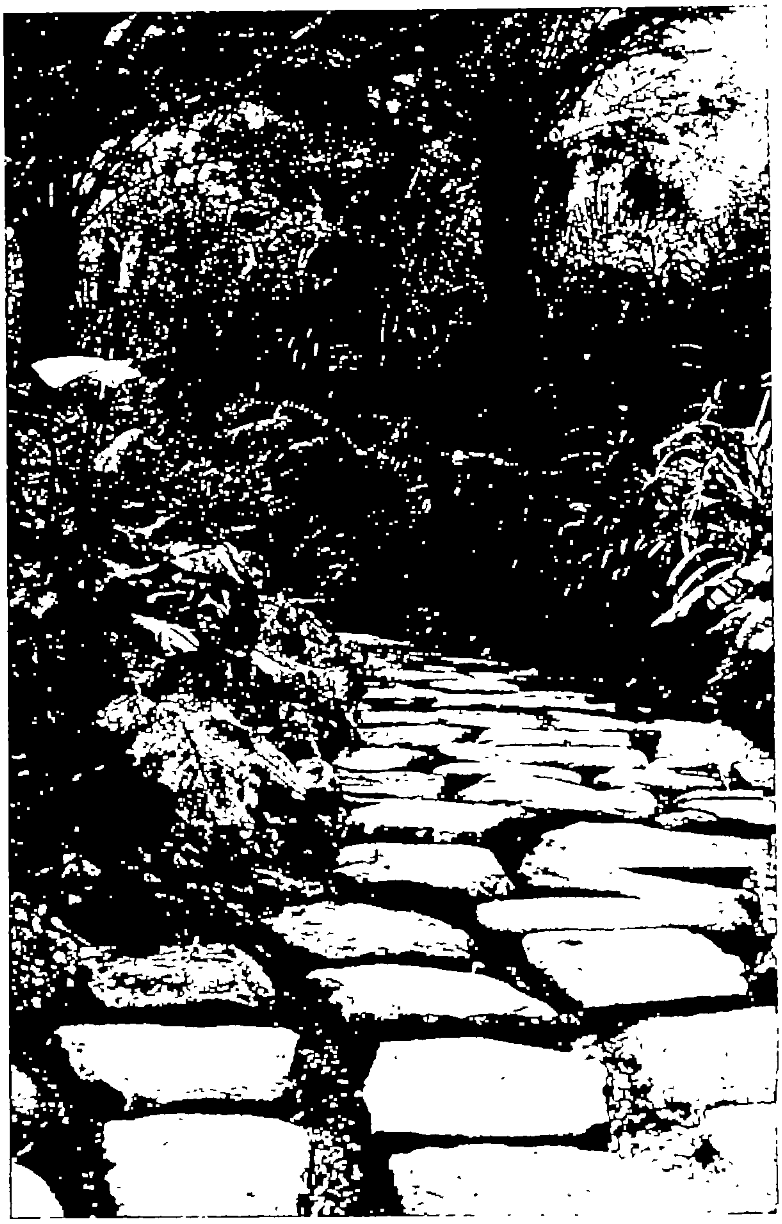

Fig. 3. A leafy dell at Kirstenbosch. Clear spring water filters through paths of stepping stones. Arum lilies grow in profusion here. (*Photo: Richard Simons*)

Primarily the purpose of these celebrations will be to make known to the world in general and to botanists and horticulturists in particular the work and functions of Kirstenbosch. At the same time it is earnestly hoped to raise funds which are urgently needed for immediate and necessary development. Since its inception fifty years ago, Kirstenbosch has struggled to establish and maintain a scientific foundation without which it could not rightly claim the title 'Botanic'. Financially, the Gardens are dependent on grants from various local authorities and funds accumulated by the Botanical Society of South Africa. But Kirstenbosch, situated as it is in the heart of a floral region second to none in scientific interest, has much to offer the world and it is for the world to make that contribution to botany a reality. When Prof. Pearson conceived the simple but magnificent idea of the National Botanic Gardens he foresaw, as its heart, a botanical research institute staffed by scientists who could set about the thorough exploration—ecological, morphological and systematic—of South African flora. In his presidential address to the South African Association for Advancement of Science on November 2, 1910, he said: "Science has not finished with a plant when it has been labelled and placed in a herbarium. . . . Research should be undertaken with the single object of discovering the truth regardless of the consequences. These may at any time assume a practical and economic importance which no one has been less inclined to expect than the investigator himself." This holds true in 1963 as it did fifty years ago and thus far, at least, Pearson's dream has not been realized for there is, as yet, no research institute at Kirstenbosch. It is estimated that the establishment and maintenance of such an institute would cost a minimum £43,000 while a further sum of £22,000 is urgently needed to enlarge the totally inadequate lecture hall where 12,000 children attend biology lessons each year. Though the extent of Kirstenbosch is more than 1,000 acres only a relatively small area is under cultivation. To develop the Gardens further would cost an estimated £9,000. It is to the public—national and international, scientific and lay—that the National Botanic Gardens of South Africa appeal for these funds.

EVIDENCE THAT THE Xg BLOOD GROUP GENES ARE ON THE SHORT ARM OF THE X CHROMOSOME

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FROM an examination of two women with ovarian dysgenesis, and of their families, evidence has emerged which suggests that the locus for Xg, the X-linked blood group system[1], is on the short arm of the X chromosome.

The two patients were consistently sex chromatin positive and each displayed, in the tissues sampled for cytological study, chromosome mosaicism of two cell lines: one, the minority, had 45 chromosomes including a single X and the other, the majority, had 46 chromosomes. In the cells of the latter line there was one normal X chromosome and a metacentric chromosome resembling No. 3 of the set; this abnormal chromosome has been interpreted[2], in these and in other patients with similar karyotype, as an isochromosome for the long arm of the X chromosome. While other interpretations are possible the suggestion that these metacentric chromosomes are isochromosomes for the long arm of the X seems likely from the combined cytological and clinical evidence. A recent observation[3] supports this interpretation: the sex chromatin masses of five such patients have a greater amount of DNA than do those of normal females. Furthermore, the presumptive isochromosomes have been shown[4,5] to be symmetrically 'hot' along their whole length when labelled with ^{3}H-thymidine late in the period of DNA synthesis.

The argument to follow concerning the site of the Xg locus hinges on this interpretation—that the structurally altered chromosome consists of two long arms of the X chromosome without a short arm.

Family J. The pedigree of this family is shown in Fig. 1. The proposita, III-3, briefly reported by Lindsten[6], is aged eighteen; she has primary amenorrhœa and the output of pituitary gonadotrophin in her urine is increased while that of œstrogens is decreased. She is of short stature (4 ft. 6 in. or 138 cm). She has neither an unusual number of pigmented nævi nor webbing of the neck. Her father was thirty-three and her mother twenty-five at the time of her birth.

Cytological investigations showed that the proposita was sex chromatin positive in cells from buccal mucosa smears, skin sections and in cells from two skin biopsies grown *in vitro*. Cells were cultured from one blood sample and from two different skin biopsies and chromosome mosaicism was observed: the majority of cells had 46 chromosomes, including an apparently normal X and a presumptive isochromosome for the long arm of the X; the minority of cells had 45 chromosomes with an XO karyotype. The parents and sibs of the proposita have sex chromatin corresponding to their phenotypic sex.

From the family history it is clear that the maternal grandfather, I-2, was colour-blind. By Ishihara and anomaloscope tests III-2 and III-5 are deuteranopic: the proposita, III-3, is deuteranopic according to Ishihara but has a normal Raleigh equation with an increased contrast. By both tests II-3 and II-4 and III-1-4-6 were normal, and by Ishihara II-1 and II-2 were normal.

The Xg groups of the family are shown in the pedigree. The proposita lacks her father's Xg^{a} antigen and this indicates that her normal X is from her mother, as does the fact that she has her mother's colour-blindness gene but not her father's gene for normal colour vision: the isochromosome would therefore have to be of paternal origin. (The family illustrates crossing-over between the loci for Xg and for deutan colour vision; this is to be expected for the cross-over rate between the two genes is known to be high[7].)

Family R. The pedigree of this family is shown in Fig. 1. The proposita, II-2, is aged twenty-two years, has primary amenorrhœa with markedly increased output of pituitary gonadotrophin, is of short stature (4 ft. 9½ in. or 146 cm) and has numerous pigmented nævi but no webbing of the neck. At the time of her birth her father was thirty-one and her mother twenty-six. She has a normal elder brother.

The patient is sex chromatin positive in cells from the oral mucosa, from skin biopsy grown *in vitro* and in the leucocytes of the blood smear; some of the sex chromatin masses in the oral mucosa cells appeared to be unusually large. The patient's chromosomes were examined in cells from two blood cultures and one skin culture and showed her to be a chromosome mosaic: the majority of her cells had 46 chromosomes, including an apparently normal X and a presumptive isochromosome for the long arm of the X, and the minority of cells had 45 chromosomes with an XO karyotype. The presumptive isochromosome in the cells with 46 chromosomes was found[5] to be symmetrically 'hot' and to label with ^{3}H-thymidine later than the other chromosomes in the course of DNA synthesis. The father and mother had a normal chromosome complement in cells from blood cultures.

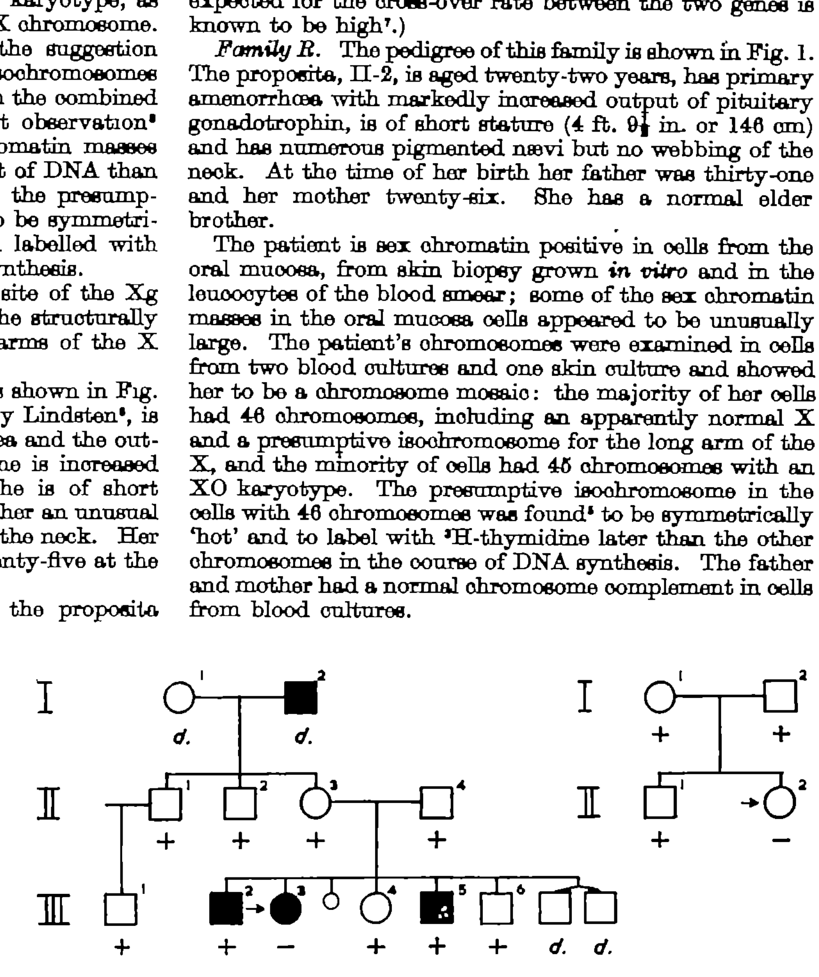

Fig. 1. The family J. (left) and the family R. (right). Arrow = proposita. Xg groups: + = Xg(a+), − = Xg(a −). Colour vision: solid black = colour-blind (deuteranopic), hollow = normal (for details see text). d. = dead and not tested for Xg or for colour vision

The proposita, her mother and father have normal colour vision; the first two were tested on the anomaloscope and the Ishihara, the father only on the latter.

The Xg groups of the family are shown in the pedigree: because the proposita is Xg(a −) while her father is Xg(a +) it must be considered that the morphologically normal X which she has in both her cell lines is from her mother (who must be heterozygous Xg^aXg). It follows that the presumptive isochromosome present in the cells with 46 chromosomes is of paternal origin—yet the patient is Xg(a −).

Other cases. The families of 12 other patients who have presumptive isochromosomes for the long arm of the X have been tested, but the Xg groups did not segregate in the informative way they did in the J. and R. families. The groups of these other families are recorded in Table 1: in four of the 12 patients the presumptive isochromosome appears to be of paternal origin (families Fra., All., And., Eri.; see discussion).

Table 1. TWELVE FAMILIES IN WHICH THE PROPOSITA HAS A PRESUMPTIVE ISOCHROMOSOME FOR THE LONG ARM OF THE X BUT IN WHICH THE Xg GROUPS ARE LESS INFORMATIVE THAN THOSE OF THE FAMILIES J. AND R.

Investigator	Identification	Xg groups of				
		Father	Mother	Proposita	Brothers	Sisters
Court Brown and Whyte	Hig.*	+	+	+	−	
	Tho.*	+ c.b.	+	+ c.b.		+
Motulsky	Sea.	+	+	+		
Polani and Hamerton	Fra.	−	+	+		
	Gib.*	+	+	+	+	+
	Rog.	+	+	+	−	
Lindsten	Las.*	−	+ ‡	−	+ + +	
	All.*	−	+	+		+
	And.	−	+	+	+ +	
	Eri.	−	+	+		+
	Sjo.	−	+	−		+
	Sve.	+	+	+		

+, Xg(a +). −, Xg(a −). c.b., colour blind.
* not found to be a mosaic (XO/X iso-X).
‡ Her mother, sister and 2 brothers are all +.

Discussion. Analysis of the two families, J. and R., indicates that the presumptive isochromosome of both patients is of paternal origin. If this abnormal chromosome found in the Xg(a −) daughters consists of two long arms of the X chromosome from the Xg(a +) father then these arms do not contain the Xg locus, which should therefore be located on the short arm. (The evidence that family J. gives for the deutan locus being on the short arm has already been discussed[2,3].)

Two possibilities could upset this argument. The hypothesis of Lyon[10], which postulates that in the mouse and possibly in all mammals only one X chromosome is 'active' in any one cell, makes it theoretically possible that the isochromosomes of our two patients are 'inactive' in all cells. If this were so the Xg(a −) reactions of the two patients could merely reflect lack of gene action and would not afford evidence of the arm on which Xg is located. The fact that the isochromosome is 'hot' when labelled with ^{3}H-thymidine late in the period of DNA synthesis[4,5] indicates that it synthesizes later than the other chromosomes: whether this means that it is also 'inactive' remains to be demonstrated.

The second possibility is that the two different cell lines found in both patients might be so distributed that those producing the Xg groups have 45 chromosomes including the single, maternal, X carrying the silent *Xg* gene. In this event again the Xg locus could be on either arm of the X chromosome. However, an identical, non-random distribution of two cell lines in two unrelated individuals seems most unlikely.

Thus, though the evidence is suggestive, definite proof that the Xg locus is on the short arm of the X chromosome must await clarification of these problems.

In some of the patients, the Xg findings can help in tracing the origin of the presumptive isochromosomes, but there are certain limitations and two sets of factors have to be taken into consideration: the 'activity' or otherwise of the presumptive isochromosomes and the situation of the Xg locus on the short or long arm of X chromosomes generally. If the presumptive isochromosomes of the patients were 'inactive' in all cells, a paternal origin could be assumed in six of the 14 patients (R., J., Fra., All., And., Eri.) irrespective of the situation of the Xg locus: on the other hand, if they were 'active', one could conclude a paternal origin for the six only on the assumption that the Xg locus is on the short arm. In the two patients J. and R., 'activity' of the isochromosomes would imply that the Xg locus is on the short arm (if we disregard mosaicism), and indeed this is the object of the present article.

We thank Dr. P. M. F. Bishop, London, who referred the proposita of family R. for investigation, Dr. M. Svenmar, Råvemåla, for help in obtaining blood samples from the family J., and Prof. I. Kugelberg, Stockholm, for the colour vision investigation of that family. We also thank Dr. W. M. Court Brown, Edinburgh, Dr. E. G. Oastler and Dr. W. G. Whyte, Glasgow, and Dr. A. Motulsky, Seattle, for samples from three of the families listed in Table 1. We thank Dr. J. D. Mann, Grand Rapids, and Dr. A. Cahan, New York, for generous supplies of the anti-Xg^a serum.

One of us (J. L.) gratefully acknowledges a grant from Nordisk Insulinfond.

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[5] Giannelli, F. (in preparation).

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NEWS and VIEWS

The Academy of Sciences of the U.S.S.R.: Academician V. A. Kirillin

ACADEMICIAN VLADIMIR A. KIRILLIN, director of the High Temperature Laboratory of the Department of Technical Sciences, has been elected a vice-president of the Academy of Sciences of the U.S.S.R. in succession to Academician A. V. Topchiev, who died in Moscow on December 27. Academician Kirillin is a graduate of the Moscow Power Institute, where he has taught for the past twenty years. Ten years ago he was elected corresponding member of the Academy, and in 1962 he became a full-member. He has been awarded a State Prize and a Lenin Prize for his participation in theoretical and experimental investigations of the thermal properties of water and steam. He is also chairman of the U.S.S.R. Co-ordinated Committee on the Properties of Steam.

Academician B. E. Paton

ACADEMICIAN BORIS PATON, director of the Electrical Welding Institute, has been elected a new member of the Presidium of the Academy. He became a full-member of the Academy in 1962. Academician Paton has been working for some twenty years in the Institute, which is named after his father, Yevgeny Paton. Last year he was elected president of the Academy of Sciences of the Ukrainian S.S.R.

Engine Research and Development at the Ministry of Aviation: Dr. John Remfry

Dr. John Remfry has been appointed director of engine research and development at the Ministry of Aviation, in succession to Dr. J. W. Drinkwater, who is now at the National Gas Turbine Establishment, Farnborough. Born in 1904, he held a Foundation Scholarship at Sir Joseph Williamson's Mathematical School, Rochester, before entering H.M. Dockyard, Devonport, as an indentured apprentice to Marine Engineering and Shipbuilding in 1919, where he gained the Admiralty Prize for first place in school subjects in all dockyards in 1923 and a Whitworth scholarship in 1924. He graduated with the degree of B.Sc.(Eng.) with honours in mechanical engineering and motive power at the City and Guilds Engineering College in the University of London in 1926 and was awarded the Diploma of the Imperial College in aeronautics in 1927 after post-graduate study at the Royal College of Science. After a year at the National Physical Laboratory, Dr. Remfry was until 1936 a research engineer with Messrs. Rowntree and Co., York. During the next year in the Chief Engineer's Department of the London County Council he was concerned with the efficient generation and utilization of steam and electrical power. In 1937 he joined the Engine Department of the Royal Aircraft Establishment, Farnborough, and worked on problems of heat interchange for aircraft engine radiators, oil coolers and charge coolers. In 1944 he was transferred to the Directorate of Engine Research and Development, which was successively under the Ministries of Aircraft Production, Supply and Aviation, where he was engaged on the performance estimation and approval of reciprocating and gas turbine engines and the supervision of research at universities and contractors' works on aerodynamics, thermodynamics, heat transfer and engine materials. He has been an assistant director for aero engine projects and performance since 1950.

International Academy of Astronautics: Honorary Members

Prince Louis de Broglie, Miss Jacqueline Cochran and Mr. Harry F. Guggenheim have been elected honorary members of the International Academy of Astronautics. The first honorary member of the Academy was the late Prof. Niels Bohr.

Prince Louis de Broglie, the well-known French physicist, has made major contributions to the development of modern physics and to the fostering of international scientific co-operation. He is a member of the French Academy of Sciences and has been its secretary since 1942. Prince Louis de Broglie has received many honours and awards, including the Nobel Prize for Physics in 1929. For his efforts to explain aspects of modern physics to the layman, he was the first recipient of the Unesco Kalinga Prize in 1953. He has been professor at the Institut Henri Poincaré in Paris since 1928, and in 1943 he founded a Centre for Studies in Applied Mathematics. In 1945 he was counsellor to the French High Commission on Atomic Energy.

Miss Jacqueline Cochran (Mrs. Floyd B. Odlum), the leading woman in American aviation, has done much to prove that women are as capable as men to achieve success in the field of practical flying. She was the first woman to fly in the Bendix transcontinental race in 1934, and has since won numerous Bendix trophies, as well as the Clifford Burke Harmon Trophy of the International League of Aviators. Miss Cochran was the first woman pilot to pierce the sonic barrier in 1953, and she holds several national and international flight records. Since 1959 she has been president of the International Aeronautical Federation, an organization the functions of which now include the verification and maintenance of manned space flight records.

Mr. Harry F. Guggenheim, American philanthropist and publisher, has played an important part in the promotion of aeronautics and astronautics both on a national scale and on an international level. His interest in aviation goes back to the 1920's. He was U.S. delegate to the International Conferences on Civil Aeronautics in Washington, D.C., in 1927 and 1928. As president of the Daniel and Florence Guggenheim Foundation, he founded the Daniel and Florence Guggenheim Jet Propulsion Centres at Princeton University and at the California Institute of Technology, where his father had previously founded the Guggenheim Aeronautical Laboratory. Mr. Guggenheim was U.S. Ambassador to Cuba during 1929–33, and he has been honoured with numerous awards and decorations.

Anglo-Belgian Company for Marine Reactor Development

The United Kingdom Atomic Energy Authority and a syndicate of Belgian industrial companies headed by Belgonucléaire have jointly formed a company to own all the results of their development programme for the *Vulcain* reactor. The formation of the Company follows an announcement in May 1962 that the Authority had signed an agreement with Belgonucléaire to undertake a joint research and development programme on a design of nuclear reactor suitable for marine propulsion—given the name of *Vulcain*—on which Belgonucléaire had already done much preliminary work. This reactor is an advanced version of the pressurized water reactor embodying the principle of variable moderation. The Authority's participation in this joint effort is part of the programme of research announced by the Minister of Transport in November 1961 to develop a reactor system economically attractive to a wide range of shipping. This research is being undertaken with the approval and support of the Working Group on Marine Reactor Research, appointed jointly by the Minister for Science and the Minister of Transport. After assessing the relative merits of a number of reactor types most suitable for further development for marine propulsion, the Working Group's recommendation was accepted in November 1962, that the programme of research be concentrated in future on two types. One of these is the *Vulcain* reactor, and the other is the Integral Boiling Reactor, which is an advanced reactor concept originating within the Authority.

Fuel Elements for *Scorpio* Reactor

The Metallurgy Division at Harwell has recently completed the production of 400 plutonium-bearing fuel elements for *Scorpio* (Sub-critical carbon-moderated reactor assembly for plutonium investigations) experiments at the Atomic Energy Establishment, Winfrith. They are being used to simulate the partially used uranium fuel elements of a power reactor, in which plutonium has been formed by neutron bombardment of uranium-238; this plutonium build-up profoundly affects the behaviour of a reactor, especially its response to temperature variations. The fuel elements take the form of cylindrical bars, 28 in. long by 1·2 in. diameter, of depleted uranium containing 0·43 per cent uranium-235 with 0·25 per cent plutonium added; they are sheathed in stainless steel 0·015 in. thick. It is hoped that the *Scorpio* experiments will not only improve the basic understanding of plutonium reactor physics but will also help to check the accuracy of theoretical calculations of the effects of plutonium build-up in power reactors. The *Scorpio*'s are sub-critical graphite-moderated reactor assemblies of widely variable lattice geometries and capable of being heated to temperatures of up to 400° C. Precise measurements of neutron flux distribution throughout such an assembly (in *Scorpio* 1), or within individual lattice cells (in *Scorpio* 2), enable the reactivity of a system to be studied at different temperatures.

Astronomy in the United States

A PANEL to estimate the probable need for major new facilities in astronomy in the United States during the next 5–10 years has been set up by the National Academy of Sciences. Dr. Albert E. Whitford, director of Lick Observatory, Mount Hamilton, California, is chairman of the panel. Chairman of the Committee on Government Relations is Dr. George B. Kistiakowsky, professor of chemistry, Harvard University, who was special assistant on science and technology to President Eisenhower.

The panel will concern itself mainly with solar and galactic astronomy, optical and radio astronomy, ground-based and space astronomy, independent research observatories and those connected with universities. The astronomer members of the panel will also discuss the needs of astronomy with respect to manpower and the use of new devices such as high-speed computers for the automation of telescopes and the processing of data, electronic-image tubes as substitutes for photographic plates, and radio-astronomy receivers. Other members of the panel are: Prof. R. N. Bracewell, Stanford University; Prof. Frederick T. Haddock, jun., University of Michigan; Dr. Frank D. Drake, National Radio Astronomy Observatory, Green Bank, West Virginia; Dr. William Liller, Harvard College Observatory; Dr. W. W. Morgan, Yerkes Observatory, University of Chicago; Mr. Bruce H. Rule, California Institute of Technology; and Dr. Allan R. Sandage, Mount Wilson and Palomar Observatories.

The Institute for Medical Research, Federation of Malaya

THE annual report of the Institute for Medical Research, Federation of Malaya, for 1960, which includes a report from the United States Army Medical Research Unit (Malaya) and statistical appendixes, as well as a list of publications and reports, includes also a report on the eighth and final year of continuous filariasis investigations (Pp. vi + 184. Kuala Lumpur: Institute for Medical Research, 1962. 2.50 Malayan dollars). This report envisages a co-ordinated campaign of control by mass treatment with diethylcarbamazine, starting in 1961, of all persons living in endemic areas, estimated at 5 per cent of the population. Research on *Salmonella typhi* phage types in Malaya continued, but routine biochemical estimations occupied most of the time of the staff of the Division of Biochemistry. The Division of Entomology finished its intensive collection of culicine mosquitoes, and in wall tests with *Aedes aegypti* a new formulation of benzene hexachloride containing a resin gave similar results to the normal wettable powder formulation. An investigation into monkey malaria in the country was commenced, and a notable advance was made in co-operation by Government agencies to improve nutrition in rural areas. A valuable development was the opening of a plant for the production of tuna fish meal by a commercial firm in Penang. Further evidence was obtained that leptospirosis is a common infection in humans in Malaya.

Salary Re-scaling for Colleges of Advanced Technology

IN replying for the Government in the House of Commons on January 23, the Parliamentary Secretary to the Ministry of Education, Mr. C. Chataway, said it was intended, as one consequence of the transfer of the colleges of advanced technology to direct grant, that salaries of the staff in the Colleges should be related to those of university staffs. By the end of summer 1962, broad agreement had been reached between the Ministry and the national teachers' bodies concerned on the main outlines of the negotiating procedure to be followed for this transition from the Burnham system to one more closely related to the university system. The Government, however, was at this time also considering the position with regard to university salary negotiations, and eventually concluded the review of university salaries was most appropriately referred to the National Incomes Commission and that college of advanced technology salaries should be simultaneously referred to the Commission. The Commission had not yet settled the procedure for the submission of evidence, but when it had done so the various bodies concerned would be fully informed. When it was decided to authorize an immediate overall increase of 10 per cent in university salaries it was also decided, in view of the very substantial increase which college of advanced technology staffs had received as a result of the last Burnham settlement in January 1962, that any adjustments in their salaries should be limited to the more senior grades. The precise adjustments will be a matter for discussion with the teachers' associations and the representatives of the chairmen and principals of the colleges as soon as it is known what increases are to be approved for the various grades of university staff. Mr. Chataway added that he fully shared the view that the colleges must be recognized as equivalent in status to universities but that decision on that must await the Robbins report.

Films on the Peaceful Uses of Atomic Energy

As part of its continuing programme to make information available in its Member States, the International Atomic Energy Agency has established a film library and has recently published a catalogue of the 167 films that are now available for loan (Film Catalogue No. 1: *Films on the Peaceful Uses of Atomic Energy available through the International Atomic Energy Agency*. Pp. v + 51. Vienna: International Atomic Energy Agency, 1962). In the catalogue, films are listed under subject headings such as geology, safety, instrumentation, space, physics, radioisotopes, reactors, waste disposal. Each film is briefly described in a few sentences of text. Indicated in tabular form are country of origin, date of production, length, languages available and whether the film is in colour or black-and-white. Although most of the films at present available are in English, about fifteen are available in French, seven in Russian, four in German and one each in Spanish, Italian and Japanese. Several films are available in more than one language. Films are loaned to organizations in the Agency's 78 Member Nations for only the cost of return shipment. Copies of the catalogue are available from the Film Library, the International Atomic Energy Agency, Kärntnerring 11, Vienna I.

Bibliography on Direction Finding and Related Ionospheric Propagation

FOLLOWING a conference held at the University of California at Los Angeles in June 1960, at which the aspects of long-range high-frequency radio propagation that affect radio-location and direction-finding, and the related problems of measurement and analysis, were discussed, it was decided to launch a *Bibliography on Direction Finding and Related Ionospheric Propagation Topics* (Edited by Olaf D. Remmler. National Bureau of Standards Technical Note 127. Pp. 115. U.S. Government Printing Office, Washington 25, D.C. 1962. 60 cents). A group of the papers presented at the conference was published in *Radio Propagation*, Section D of the *Journal of Research of the National Bureau of Standards*, May–June issue, 1961. This bibliography, though not exhaustive, includes more than 850 titles on direction finding and related topics including instrumental details, observations and data analysis, and theories of propagation. In connexion with the conference, the Numerical Analysis Research Staff of the University of California at Los Angeles prepared a bibliography of works on the conference subject published during 1955–59. This has since been edited and extended to include references to individual papers resulting in the present publication published during 1955–mid-1961. In addition, references to bibliographies, proceedings of conferences, reviews, surveys and texts as far back as 1940 have been incorporated to provide access to the earlier literature of the subject and to related fields of research.

University News :

Keele

Since its foundation in 1949 as the University College of North Staffordshire, one of the special features of the University of Keele has been the maintenance of close links between different disciplines and the avoidance of departmental specialization. Although there has been no Department of Sociology, considerable emphasis has been placed on the development of sociological studies in a number of Departments, particularly those of Economics, Geography and Political Institutions. Moreover, for some years past the University has been responsible for the running of the *Sociological Review*. The further development of sociological studies within the University will be greatly helped by a gift of £50,000 from the Nuffield Foundation to provide a permanent endowment for a Statistical Research Unit in Sociology. It is hoped that the director of the Unit will not only develop new sociological studies based on accurately recorded information, but will also forge fresh links between sociological studies already in progress in several Departments of the University.

Sussex

The third annual report of the University of Sussex, covering the year ended July 31, 1962, which includes the formal birth of the University when its Royal Charter took effect on August 16, reviews briefly the progress in buildings and administration (Pp. 22+xii. Stanmer, Brighton: The University of Sussex, 1962). Capital expenditure amounted to more than £645,000, met mostly from £629,000 in non-recurrent grants from the University Grants Committee, which also made a recurrent grant of £40,500. The recurrent grants for the quinquennium 1962–67 were announced in April 1962, but additional funds from Government sources will be required if the University's target of 1,700 students by 1966–67 is to be achieved. Further substantial donations were received during the year and amounts received and promised against the original target figure of £500,000 exceeded £650,000. During the year, addition of 23,000 volumes and pamphlets, 16,000 by purchase, brought the total in the library to 33,000. In October 1961 fifty-two undergraduates were admitted to the B.A. degree course and three part-time research students to courses leading to a higher degree. For 1962–63, 2,453 applications were received for the B.A. and 627 for the B.Sc. course, and 202 and 159 students, respectively, were accepted.

The Clough Memorial Research Fund

Part of the Clough Fund has, in former years, been used in the form of research grants for the furtherance of geological research in Scotland and Northern England. The Edinburgh Geological Society has now decided to use this money to institute the Clough award. This is to be presented, biennially, to the person or persons normally in their early thirties and of British nationality whose geological work in Scotland or Northern England (as defined in the regulations for the administration of the Clough Fund) is of outstanding merit. The first Clough Award will be made during 1963. Further information can be obtained from the Honorary Secretary, the Edinburgh Geological Society, c/o the Grant Institute of Geology, King's Buildings, West Mains Road, Edinburgh 9.

The National Institute of Sciences of India

At the twenty-eighth anniversary general meeting of the National Institute of Sciences of India, held on December 30, the following were elected officers and members of Council of the Institute for 1963: *President*, Prof. H. J. Bhabha; *Vice-Presidents*, Dr. A. C. Joshi and Prof. T. R. Seshadri; *Treasurer*, Sri S. Basu; *Foreign Secretary*, Prof. P. S. Gill; *Secretaries*, Prof. R. C. Majumdar and Prof. B. R. Seshachar; *Editor of Publications*, Prof. P. Maheshwari; *Members of Council*, Dr. Atma Ram, Prof F. C. Auluck, Prof. Ram Behari, Prof. K. A. Chowdhury, Dr. S. N. Dasgupta, Prof. A. K. Dutta, Prof. S. Gupta, Dr. B. S. Kadam, Prof. S. Kilpady, Prof. D. S. Kothari, Dr. L. S. S. Kumar, Dr. B. Mukerji, Dr. C. Radhakrishna Rao, Dr. M. L. Roonwal, Prof. M. R. Sahni, Prof. T. S. Sadasivan, Prof. N. R. Tawde and Prof. W. D. West.

Sir Lawrence Bragg, director of the Davy Faraday Laboratory, the Royal Institute, London, was elected an Honorary Fellow.

The Night Sky in March

Full moon occurs on Mar. 10d. 07h. 49m. U.T. and new moon on Mar. 25d. 12h. 10m. The following conjunctions with the Moon take place: Mar. 6d. 22h., Mars 3° N.; Mar. 22d. 10h., Saturn 1° N.; Mar. 22d. 13h., Venus 2° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Saturn on Mar. 20d. 23h., Venus being 0·9° N. Mercury is too close to the Sun for observation. Venus is a morning star, rising about an hour before the Sun. Mars is above the horizon most of the night, setting shortly before sunrise. It is at a distance of 77 million miles on March 15, when its stellar magnitude is $-0{\cdot}2$ and it is in Cancer. Jupiter and Saturn are too close to the Sun for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Mar. 3d. 23h. 21·6m., χ^1 Ori. (*D*); Mar. 6d. 03h. 09·0m., 85 Gem. (*D*); Mar. 7d. 00h. 46·8m., δ Cnc. (*D*); Mar. 16d. 04h. 18·5m., θ Lib. (*D*). *D* refers to disappearance. The vernal equinox occurs on Mar. 21d. 08h.

Announcements

The British Small Animals Veterinary Association and the Universities Federation for Animal Welfare are jointly sponsoring a symposium on "Small Animal Anaesthesia" at the Middlesex Hospital Medical School during July 23–24. Further information can be obtained from Mrs. J. G. Wright, 114 Maida Vale, London W.9.

A symposium on "The Natural Radiation Environment" will be held at William Marsh Rice University, Houston, during April 11–13. Subjects under discussion will include: instrumentation and techniques; cosmic radiation; terrestrial gamma radiation; natural environmental radiation studies. Further information can be obtained from Prof. J. A. S. Adams, Department of Geology, Rice University, Houston 1, Texas.

"Bird Protection in Scotland" is the title of the Acorn Lecture to be given by Mr. G. Waterston, the Scottish Centre for Ornithology and Bird Protection, at the Arthur D. Little Research Institute on February 25. Further information can be obtained from the Secretary, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian.

A symposium on "Cell Electrophoresis", sponsored by the British Biophysical Society, will be held at the Chester Beatty Research Institute during May 7–8. The types of cells under discussion will include those from normal tissue, cultures, blood and bone marrow, bacteria and plants. Further information can be obtained from Dr. E. J. Ambrose, Chester Beatty Research Institute, Fulham Road, London, S.W.3.

Erratum. In the article entitled "Adaptive Teaching Machines", which appeared on p. 1276 of the December 29, 1962, issue of *Nature*, the word 'minimize' in the first sentence of the penultimate paragraph should read 'maximize'.

THE ASSOCIATION OF UNIVERSITY TEACHERS

THE Association of University Teachers were the guests of the Southampton Association of University Teachers and the University of Southampton during December 17–20, and their meetings were presided over by Prof. W. W. Chambers of the University of Glasgow.

The deliberations were dominated by the effects of the Government's financial policy towards universities. The effects which were reported to the Council included losses of staff, failure to provide places for some 2,500 well-qualified candidates and postponement or changes in plans for expansion affecting every institution in the United Kingdom and Northern Ireland. Although most attention was given to problems of expansion, the long-delayed issue of salaries gave rise to heated debate when representatives learned that the claim for increases of about 30 per cent, substantially agreed with the vice-chancellors, had not been discussed with the University Grants Committee, as provided for in the procedure laid down by the Treasury. The University Grants Committee had not arranged the necessary meeting. This news, together with Press reports and rumours that the matter was to be referred to the not yet constituted National Incomes Commission, led to demands that the subject be taken up with the Chancellor of the Exchequer through Parliament. The Council in a resolution objected to the discrimination inherent in the National Incomes Commission but left the Association free to present data to the Commission if university salaries were referred to them by the Government.

The discussion of university expansion was centred on what was described as the "18+ Crisis", and the Council passed a policy resolution submitted by Liverpool:

"That the policy of the A.U.T. on expansion should be based on the following principles:

"(1) The A.U.T. recognizes and welcomes the necessity for a substantial expansion of the universities for reasons both of national need and social justice. It insists, however, that neither purpose will be served if expansion leads to deterioration in the quality of the education offered.

"(2) The special quality of university education derives from the active participation of university teachers in fundamental research and scholarship. Therefore, the A.U.T. regards the provision of adequate time and facilities for these activities to be essential to the successful fulfilment of the universities' role.

"(3) The A.U.T. considers that the maintenance of the quality of university education must have precedence over expansion if lack of money or other resources forces a choice. No lowering of essential standards can be contemplated.

"The first duty of the A.U.T. in the present situation is to intensify the campaign for an immediate and substantial increase in the resources available to the universities.

"Further, it is the duty of the A.U.T. to warn the public that grave injury to the national economy will result if university research is sacrificed in an attempt to expand undergraduate teaching without the necessary resources."

The Council then went on to discuss proposals for making the problems of university expansion widely known to the general public.

The growth of the Association to a membership of some 10,000, almost all non-medical university teachers, in universities other than Oxford and Cambridge had created problems of organization which were the subject of further discussion, and proposals were made for changing the form of one of the half-yearly meetings.

Other matters which were discussed included proposals to improve the status of university technicians, the problems of student selection and the improvement of general degrees and possible ways of raising the popular esteem of the general degree. D. CHAPMAN

ZOOLOGICAL EXPEDITIONS

THE twenty-eighth annual meeting of the Association of British Zoologists was held at the Offices of the Zoological Society of London on January 5. The president for 1963, Dr. F. Fraser Darling, was unable to attend, and the chair was taken by Prof. C. F. A. Pantin. After the business meeting several papers were read on "The Scientific Aims, Planning and Results of Zoological Expeditions".

Mr. N. A. Holme (Plymouth) outlined his present work investigating the bottom of the English Channel. Since the pioneer dredging activities of the *Porcupine* and *Challenger* expeditions the faunistic exploration of the benthos is now fairly complete, except for the deep sea. In the English Channel the present surveys are concerned with assessing biomass and productivity, dispersal and aggregation, and the factors which limit the distribution of bottom-living animals. There is no ideal sampling apparatus, either qualitative or quantitative: trawl, dredge, Petersen bottom-sampler, anchor-dredge and automatic camera all have their disadvantages. On a rapid survey only one type of gear can be used at each station, and Mr. Holme stressed the importance of careful planning to make the most of the ship's time. After dealing with the practical aspects of sorting, preserving and working-up samples of benthos, Mr. Holme made a plea for the early publication of results.

Dr. Anna Bidder (Cambridge) gave a lively account of her trip to the South Seas to study living specimens of the pearly nautilus in 1960–61. Local people in New Caledonia, the Loyalty Islands and New Britain brought her *Nautilus* caught in traps baited with putrescent fish and meat. In this way she was able to study, in the public aquarium at Nouméa or, elsewhere, in aquaria improvised from slop-basins on the foreshore, specimens of three species of this nocturnal, bottom-living mollusc. The chambered shell of the pearly nautilus has been sung by poets and worked over by every zoology student, and yet we have known scarcely more than Aristotle could tell about the habits of the animal within. Now Dr. Bidder has not only been able to confirm the Aristotelian account, but answer much which was speculative concerning one of the most bizarre forms of cephalopod radiation.

Prof. C. F. A. Pantin (Cambridge) described how, during a war-time holiday, his chance find in an English oakwood of the terrestrial nemertine worm *Geonemertes* led him in subsequent years to pursue studies on the taxonomy and world distribution of this genus, and on its anatomy, physiology and ecology, whenever opportunity could afford during visits to Europe, Africa, New Zealand and various oceanic islands. Prof. Pantin made the point, which was further emphasized in discussion by Prof. G. C. Varley (Oxford), that whereas the objectives of an expedition should be carefully defined, the members of the expedition should have a sufficiently wide background to take advantage of the unexpected opportunity or the sideline which sometimes may lead to the most rewarding results. Prof. Pantin also suggested that in all universities the young graduate proposing to undertake research should first be given the opportunity of an 'expedition year', a year in the field which would yield experience of real value irrespective of the kind of research eventually pursued.

The University of Cambridge Expedition to British Honduras in 1959–60 was a considerable enterprise where ten undergraduates worked in the field for eleven months on a comprehensive programme including archaeology, botany, zoology and physiography. Mr. P. K. Bregazzi (Cheltenham College) described the zoological work which was mostly concerned with the faunal ecology of the corals and the land ecology of the cays which lie scattered within a broad channel between the shore and a long fringing reef. Using mostly skin-diving methods the party lived much of the time in a small sailing-boat with which they managed to explore 150 miles of the channel. Interesting results were obtained on the diurnal activity of coral polyps and on the origin of 'beach-rock' formations on the islands, but Mr. Bregazzi concluded that the work was chiefly significant as the first step in a long-term programme and he noted that the area had since been visited by at least five expeditions from Britain and the United States.

An expedition with a careful programme in applied science was described by Mr. P. D. Fry (Royal Veterinary College). In 1962 eight undergraduates visited East Africa to study so far as they could the diseases which affect wild mammals and birds and domestic cattle, and to collect material which might show how these diseases affect man. Special attention was directed to game animals likely to be included in cropping programmes. Travelling in 'Land-Rovers' the party in ten weeks got through a great deal of work, mostly in Uganda, where they were able to examine more than 200 animals ranging from the spiny mouse to the elephant. Systematic autopsies yielded pathological material and parasites, and there were collections of blood-smears and blood sera for studies on protozoal disease and on immunology. Some of the collections were from game anaesthetized with the 'capchur' gun. A notable feature of this expedition, to which Prof. Varley directed attention, was its active co-operation in the field with medical and veterinary research organizations of Uganda and Kenya, and the approval and patronage it won from the World Health Organization.

Dr. Robert Clarke (National Institute of Oceanography) summarized the discussion and concluded with some general observations. He recalled that the exploratory expedition in zoology had, in the past thirty years, mostly given way to specialized objectives in evolution, physiology and especially ecology. The emphasis was now on productivity, both by land and sea, and he welcomed the kind of expedition described by Mr. Fry which had avowed economic ends in a world hungry for proteins. The planning of expeditions should take realistic account of possible hitches in the field, so that, as mentioned by Mr. Holme, any lengthy or costly expedition should have alternative programmes which (combined, as it may be, with the readiness to improvise) can in the event turn some impending failure into success. Only the enthusiast like Dr. Bidder, going out alone, could afford a single objective and be almost sure to achieve it. Working up results at home, an expedition always brings back more material than it can deal with. Great collections build up over the years, and there was an urgency in Prof. Pantin's plea for more taxonomists in professional zoology. Even an expedition with the most radical aims in applied science must know what the animals are—be they a food resource or a disease vector.

Reviewing the general trend of expedition activity, Dr. Clarke noted that the major expeditions were national undertakings until about ten years ago. Their era was one of great achievement, and by example he mentioned the work of the vessels of the *Discovery* Expedition and the National Institute of Oceanography which between 1926 and 1951 had made the remote Southern Ocean the best known scientifically of the world oceans; but he welcomed the present trend towards international co-operation in the expeditionary field. Such activity has mainly been at sea, and in its modest way a beginning was made by the *Enern*'s voyage to the Antarctic in 1953 when Norwegian, Dutch and British scientists collaborated in a whale-marking expedition. Development of the large-scale venture has been assisted by the growth of international organizations like the International Council of Scientific Unions and the specialized agencies of the United Nations which have provided the administrative machinery. The International Indian Ocean Expedition, in which ships of thirteen nations are engaged, will be especially active in 1963, and in this year also five nations will co-operate in surveying the equatorial region of the Atlantic. International relations could not but benefit from such work, and it was heartening to mention in conclusion another aspect of the co-operative expedition which he illustrated from a recent mission with the Food and Agriculture Organization to the west coast of South America. No traveller could have failed to notice that past expeditionary work in the countries of Asia, Africa and Latin America has mostly been the enterprise of Europeans and North Americans. But it was desirable that the nationals of these countries should explore their own deserts and rain forests and seas. Enthusiasm was not lacking, and now, with the development of technical assistance programmes of the United Nations and of bilateral organizations, the necessary aid and training had become available and was already in the field.

ROBERT CLARKE

THE GALE OF FEBRUARY 16, 1962, IN THE WEST RIDING OF YORKSHIRE

By C. J. M. AANENSEN and J. S. SAWYER

Meteorological Office, Bracknell, Berks

VERY many people living in and around Sheffield will remember for a long time the disastrous westerly gale of February 16, 1962. The gale commenced at about 0430 G.M.T. in the West Riding and continued for the next 8 h. In Sheffield alone 101,500 dwellings out of a total of 161,000 dwellings were damaged in one way or another, and 98 dwellings (including 69 prefabricated houses) were damaged beyond repair. Elsewhere throughout a broad belt running northwards parallel to the Pennines from North Derbyshire to Northumberland very considerable damage occurred to both buildings and trees.

As soon as the scale of damage became known, Meteorological Office staff in the area made a survey of the type and distribution of damage and later some theoretical and climatological aspects of the storm were considered

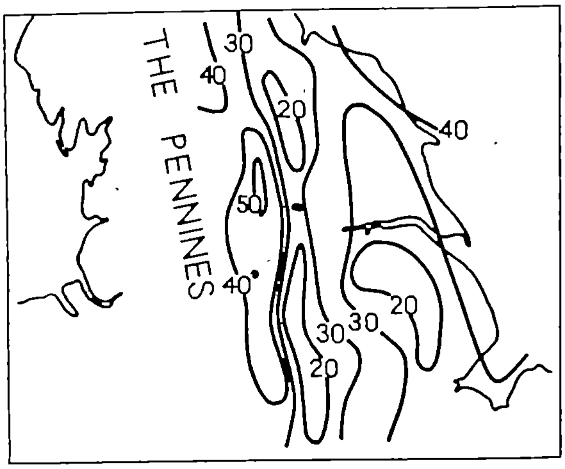

Fig. 1. Mean surface wind speed in knots at 0900 G.M.T. February 16, 1962, in the area east of the Pennines. ● Indicates Sheffield

at the Meteorological Office headquarters. It is proposed to publish a report on this work in detail in the future, but the following summary may be of interest now.

Without doubt the worst hit area was in and around Sheffield, and it was fortunate that a record of the wind strength and direction was obtained from the pressure tube anemometer mounted above the Weston Park Museum in Sheffield itself. The anemometer head is some 35 ft. above the flat roof of the Museum and only slightly sheltered by rising ground to the west. The record shows a maximum gust of eighty-four knots with at least five other gusts of eighty knots and more and thirty-six gusts of more than seventy knots. The recorded direction of the wind was 280°–300°.

An analysis of the various wind speeds recorded throughout Great Britain showed that on the whole this gale was not particularly noteworthy, especially west of the Pennines. In fact the most outstanding feature was that major wind damage to structures and trees occurred in a relatively narrow belt in the lee of the Pennines running approximately from Chesterfield through Leeds to Tyneside. This feature of the wind speed is brought out in Fig. 1, which shows the distribution of surface wind speed east of the Pennines at 0900 G.M.T. It will be seen that as well as the belt of maximum winds close to the Pennines there is a further belt of maximum wind some 40 km to the east with a marked line of lighter winds between and another area of minimum winds in Lincolnshire and the East Riding.

The gale was associated with a strong pressure gradient between a depression moving from Iceland to the Baltic and an almost stationary anticyclone over the eastern North Atlantic. The gale was at its height while the depression was moving across Scandinavia. Inspection of normal synoptic charts showed evidence of a very distorted pressure field over Northern England and this was analysed in detail by Mr. D. C. E. Jones. Of the various charts available, that for 0900 G.M.T. is based on most observations and shows the distortions most clearly (Fig. 2). The interesting features are: (*a*) a trough of low pressure extending north to south on the east side of the Pennines with separate low-pressure centres at Sheffield, Dewsbury and Ilkley; (*b*) a small centre of high pressure to the west of the Pennines between Bolton and Blackburn; (*c*) the intense pressure gradient across the Pennines. The wind was generally from west-north-west over the whole area and was far from a geostrophic balance with the pressure field. The air was accelerated as it passed from regions of high to low pressure and reached its maximum near the centre of low pressure as expected from Bernoulli's equation.

It is well known that the deformation of airflow in the neighbourhood of hills and mountains may give rise to lee wave phenomena and that large variations of surface wind and disturbance of the pressure field occur in association with wave effects aloft[1]. It was natural, therefore, to inquire whether this gale was in some way associated with the topography of the region, particularly in view of the fact that Sheffield lies to the east of the highest part of the southern Pennines. The present state of the theory of lee waves is not sufficient to allow a three-dimensional calculation to be made; but a calculation was carried through for a steady-state, two-dimensional gravity controlled flow over a ridge similar to the cross-section of the Pennines upwind of Sheffield, using the procedure developed by Sawyer[2]. This calculation revealed the existence of a powerful lee wave downwind of the Pennines with the unusually long wave-length of 22 km. In Fig. 3 is shown the calculated streamlines across the Pennines but with the vertical scale exaggerated sixteen times compared with the horizontal scale. It is noteworthy that a large vertical amplitude of perturbation is attained very rapidly as one proceeds from the surface, thus producing a marked packing together of the streamlines below the troughs of the waves. In particular it appears that Sheffield was beneath the second wave trough downwind of the Peak and that theoretically surface winds of thirty-five knots in the undisturbed stream would be increased in the vicinity of Sheffield to seventy-three knots. Thus, in spite of the restriction of the calculation to two-dimensional airflow and the neglect of condensation effects, it is clear from the calculations that intensifications of surface wind in association with lee waves aloft can provide a perfectly feasible mechanism for the exceptional winds at Sheffield.

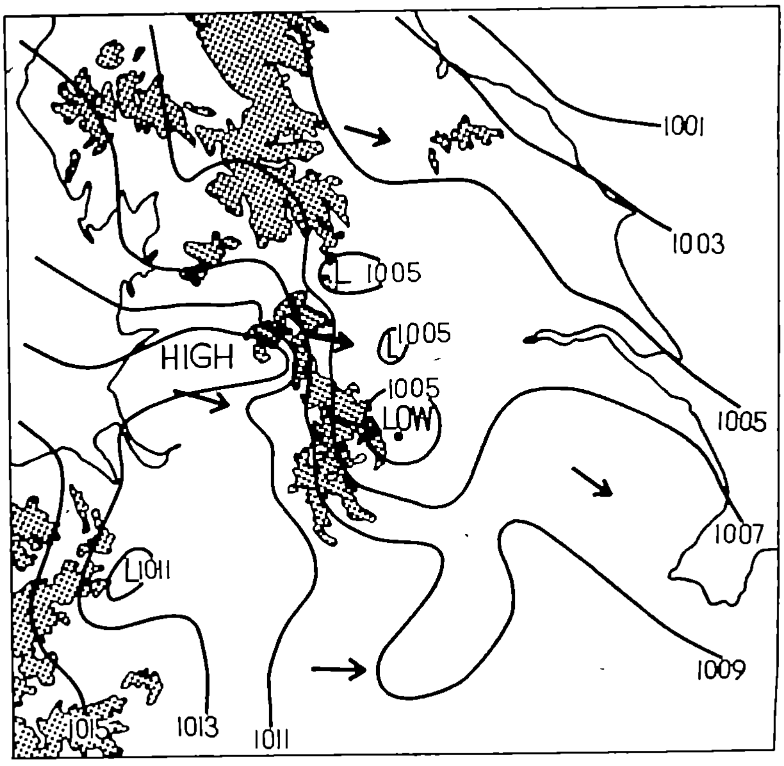

Fig. 2. Mean sea-level pressure (millibars) for 0900 G.M.T. February 16, 1962. Land above 1,000 ft. shaded. ● Indicates Sheffield. Arrows indicate surface wind direction

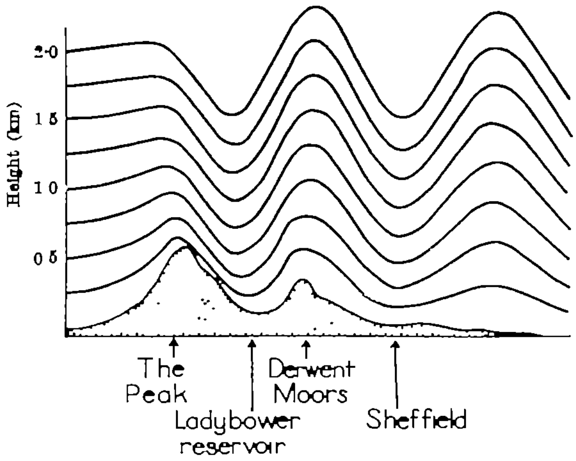

Fig 3 Calculated streamlines for the flow across the Pennines in the vicinity of Sheffield

Damage by westerly gales has been experienced in the West Riding on other occasions, notably only four days earlier on February 12, 1962. Damage on this occasion was by no means so severe in Sheffield but was widespread along the east of the Pennines and was of severity equal to that of the later gale in places from Leeds northwards. The large-scale weather situations on these two occasions were remarkably similar, but on February 12 the air, though stable, did not have such large inversions. Lee waves aloft were again possible and the theoretical calculations again indicated a powerful lee wave system with the wave-length having the exceptionally large value of thirty-eight kilometres. Because of the longer wave-length Sheffield did not lie beneath the trough of the second lee wave downward of the Peak, as on February 16, but somewhat downwind of the first trough. However, there was still a sufficient concentration of low-level streamlines at Sheffield to cause a substantial increase in surface wind speed, as actually happened.

Engineers, builders and meteorologists alike need some idea of the probable frequency of occurrence in Sheffield of severe gales such as that of February 16, 1962. Considerable damage in Sheffield by earlier gales occurred on March 1, 1956, and on December 22, 1894. The Sheffield anemograph records date from March 1958 only, and there are no other anemographs in the area. It is perhaps uncertain whether the usual climatological methods of estimating the frequency of near extreme values are applicable, but comparison by Mr. H. C. Shellard with the long period records for Manchester Airport and Cranwell gives a tentative estimate that a storm of the severity of that of February 16, 1962, would probably come in the 'once in 150 years' category.

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[1] Queney, P. *et al.*, *World Meteorol. Org., Geneva Tech. Note* No. 34, *WMO*-No. 98 *TP*43.

[2] Sawyer, J. S., *Quart J Roy Met. Soc.*, **86**, 326 (1960).

SEMICONDUCTIVITY AND CEMENTING ACTION IN HYDRAULIC-BOND TYPE CEMENTS

By A. K. CHATTERJI and T. C. PHATAK

Central Building Research Institute, Roorkee, India

THE variation of electrical conductivity with temperature has been measured of some hydraulic-bond type cements, such as ill-crystallized calcium silicate hydrate, calcium sulphoaluminate hydrate, calcium sulphate dihydrate. Results indicate that these materials may be impurity semiconductors. A probable mechanism of cementing action as a semiconductor surface reaction is suggested.

Electrical conductivity of calcium silicate hydrate, calcium sulphoaluminate hydrate and calcium sulphate dihydrate has been measured at different temperatures. Calcium silicate hydrate was prepared by the interaction in solution of analytical reagent grade calcium nitrate and sodium silicate. Calcium sulphoaluminate hydrate was prepared by the second method mentioned by Jones[1] (precipitation from aluminium salt solution). Calcium sulphate dihydrate was prepared as a precipitate from the interaction of analytical reagent quality calcium carbonate and sulphuric acid. The materials were washed with absolute alcohol to remove any free calcium and excess moisture and were identified by chemical, X-ray diffraction and differential thermal analysis methods. Dehydration of calcium silicate hydrate on heating and the dehydration and phase changes occurring in calcium sulphoaluminate hydrate when heated at different temperatures were also examined. These results are not given here.

For measurements of electrical conductivity the compounds were made into tablets using a 20-mm cylindrical mould and plunger and were compressed in a hand-operated hydraulic press at a 140-kg pressure. The surface of the tablet was cleaned with a fine emery paper, and its dimensions were noted. It was then placed between two spring-loaded platinum electrodes mounted at the end of a tube of fused quartz. The complete assembly was inserted in a tubular furnace with natural atmosphere. The temperature of the furnace was raised at a rate of about 20° C/min through manual control of the auto-transformer. The lead to the electrodes, which consists of two wires of platinum and platinum–rhodium, served the dual purpose of measuring temperature and resistance. These quantities were simultaneously measured with a pyrometer and a megohm bridge.

Curves (*abcd*), (*pqr*) and (*klmno*) (Fig. 1) are respectively for calcium silicate hydrate, calcium sulphoaluminate hydrate and calcium sulphate hydrate and show points of sharp inflexions. The figures at the inflexion points indicate the temperatures in degrees centigrade.

For calcium silicate hydrate the conductivity increases with temperature in the range 50°–150° C (*ab*), decreases in the range 150°–325° C (*bc*) and again increases in the range 325°–750° C (*cd*) and above. For calcium sulphoaluminate hydrate, the portion (*pq*) of the curve corresponds to the portion (*bc*) of calcium silicate hydrate (*qr*) to (*cd*) and the portion corresponding to (*ab*) is absent. It would perhaps have been obtained if the measurements were extended to lower temperatures. In the curve for calcium sulphate hydrate the portions (*lm*), (*mn*) and (*no*) correspond to the (*ab*), (*bc*) and (*cd*) respectively of the calcium silicate hydrate curve. Portion (*kl*) represents rise in resistance owing to the removal of surface moisture.

Change in conductivity with temperature of these materials may be attributed to the escape of water molecules during heating and to the phase changes occurring at the inflexion temperatures. These considerations, however, leave the question regarding the nature of current carriers open. Further, there is always a tempera-

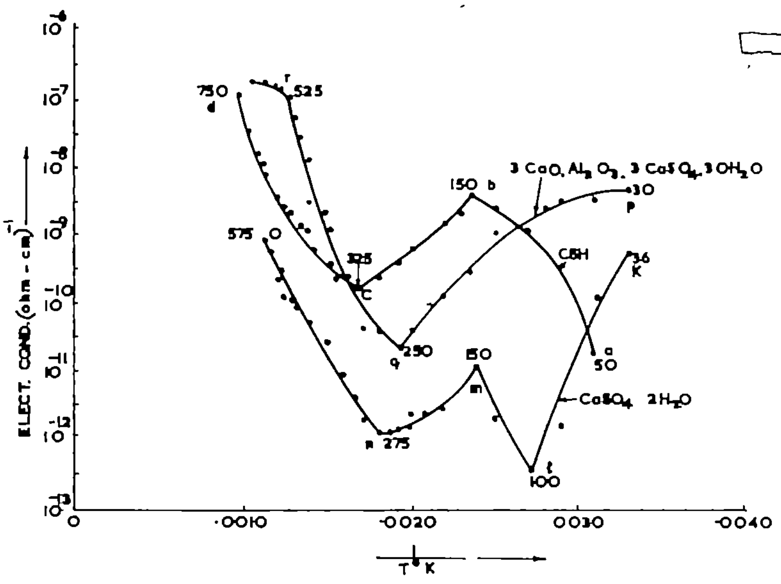

Fig. 1. Variation of electrical conductivity with temperature of the three hydrates

ture-lag in the phase-changes occurring by the two modes of heating—static and dynamic. The following approach provides an answer to the question of carriers and avoids the other issue.

An explanation of foregoing observations may also be given in terms of the possible change in concentration and mobility of the carriers with temperature considering these materials as impurity semiconductors. There is a marked similarity of these curves with those showing temperature-dependence of conductivity for arsenic-doped silicon samples[2]. The explanation for the latter phenomenon is that at low temperatures the conductivity is low as the carriers are frozen and with the rise of temperature ionization of carriers occurs which results in an increase in conductivity. This goes on for calcium silicate hydrate up to 150° C and for calcium sulphoaluminate hydrate possibly close to 30° C (as the curve becomes practically flat about this temperature). For calcium sulphate dihydrate this temperature is 150° C. Drop in conductivity with further rise in temperature—portions (*bo*), (*pq*) and (*mn*) of the curves—occurs owing to the decrease in mobility of the carriers with the rise in temperature[2]. Conductivity again rises with temperature—portions (*cd*), (*qr*) or (*no*) of the curves—owing to intrinsic semiconductivity[2] (or ionic conductivity or superimposition of both depending on the material).

As regards the nature of carriers involved in these materials, it is recalled that hydrogen atoms in solid interstitial solution in a crystal may act as 'donors'. In a similar manner it is assumed that in these hydrates hydrogen ions act as 'holes' and are mobile. It need not be assumed that the hydrogen ions from all the water of hydration act as 'holes'. The hydrogen ions which act as 'holes' are considered to be derived from water molecules retained at higher temperatures at least up to the temperature at which intrinsic semiconductivity begins. Some of the molecules of water may provide for 'holes' and these can act as 'impurities'. On this basis, these hydrates should be considered as impurity semiconductors with 'hole' conduction. This is the basic assumption of the present approach and needs direct verification. (It may be of interest to note that, under certain conditions, water may produce surface states of acceptor type on surface of germanium[3].) From dehydration experiments one can find out the number of water molecules retained at high temperature. This is found to be two for calcium sulphoaluminate hydrate and less than one for calcium silicate hydrate and calcium sulphate dihydrate per molecule of the hydrate. This gives an idea of the number of hydrogen ions, acting as holes, involved in cementing action. That the hydrogen ions from some of the water molecules in the hydrate may behave differently may be the reason why all the inorganic hydrates are not cementitious.

Assuming that the hydraulic bond type cements are '*p*' type semiconductors, cementing action of such cements (that is, the adhesion between such cement and aggregate) is considered to be a semiconductor surface reaction with a 'donor' type bond formed through the capture of 'holes' by the aggregate. Inside the cement crystal, the 'holes' constitute negative valencies which are free and are able to move about throughout the crystal and may appear at any point on the surface of the crystal. Thus the questions of choice or of relative orientation of crystallographic planes involved in adhesion do not arise. As such a reaction will depend on the position of the Fermi-level in the aggregate also, the aggregates with the Fermi-level relatively lying deep will have poor bond. In the present view, adhesion is a semiconductor surface reaction involving capture of 'holes' by the aggregate. This view of adhesion, due to free valencies within the crystal, is opposed to the prevalent view of adhesion due to localized valencies on the cement surface.

It is useful to have the structure of the cementing phase as a gel structure (or glass-like random structure) in order to maintain three-dimensional continuity; but in contrast with the concept held at present that the gel structure is essential for cements, emphasis in the present view is on their semiconductivity. A cementing phase will be preferred as a cement if it has a gel structure, but to behave as a cement at all the essential requirement is its semiconductivity. As in the present case, semiconductivity is considered to be due to 'impurities' and since adhesion, though weak, is developed even at the initial stages of setting of cement, the present view seems to indicate that the cementing crystals are formed from the very beginning of the setting process. This supports Le Chatelier's views.

Hydraulic action may now be considered. Cement clinker minerals react with water—whether in paste or in suspension—at different rates. Tricalcium aluminate reacts very quickly, tricalcium silicate less quickly, β-dicalcium silicate very slowly and the ferrite phase hydrates at a rate slightly lower than tricalcium aluminate. The difference in the rates of reaction with water is attributed to the difference in the basicity of the cationic reactant. The higher the basicity the higher the rate of reaction. For example, tricalcium silicate may be considered to be a basic salt $Ca_2(CaO)SiO_4$ and the high rate of reactivity of tricalcium silicate with water may probably be attributed to the facility for reaction with water possessed by the loosely held calcium oxide part of the structure of tricalcium silicate[4].

There are two views regarding the mechanism of hydraulic action of cement clinkers. According to one, the hydraulic action is considered to be a solution precipitation process. The clinkers go into solution (whatever slight solubility these may have) and the reaction occurs in the water medium with the formation of corresponding hydrates which, because of their still lower solubility, precipitate out[5]. There is another view which considers that the growth of hydrates occurs by solid-state reaction though the solution precipitation process can occur in favourable conditions. From the study of the gel formed during hydration it is held that the hydration process is a solid-state reaction which occurs by diffusion[6].

If it is taken that the hydraulic action is a solid-state reaction, then one would like to identify it with the known types of solid-state reactions. For example, it is known that the chemical reaction on the surface of a semi-conducting crystal is controlled by the position of the bulk Fermi-level in the crystal. Position of the Fermi-level can be raised or lowered by introducing suitable impurities in the crystal. Reactions which occur through transfer of electrons from the crystal are favoured by raising the Fermi-level and its lowering favours the reactions which occur through transfer of holes.

If hydraulic action were a semiconductor surface reaction, then, by introducing suitable impurity in the crystal, say, a 'donor' type impurity, the hydraulic action could be altered. To check this point Portland cement clinker (of composition tricalcium silicate, 37 per cent; dicalcium silicate, 35 per cent; tricalcium aluminate, 11 per cent; the ferrite phase, 8 per cent; free lime 0·65 per cent) was used for the following experiment as individual clinker minerals were not available in sufficient quantities. The clinker was powdered to a size finer than 53μ. A portion of the powder was heated to 800° C for 4 h. Another portion was mixed with 1 per cent of zinc oxide (an '*n*' type semiconducting oxide) and heated similarly. The rates of reaction with water of the clinker powder, clinker powder heated and of the clinker powder heated with the admixture of zinc oxide were obtained by estimating the lime liberated in a given time. It was found that the amount of lime liberated and hence the rate of reactivity with water was highest in clinker heated with zinc oxide and lowest in unheated sample.

When clinker was heated alone, it was in reality heated with calcium oxide—an '*n*' type semiconducting oxide. If some of the lime enters in solid solution into any or all of the clinker minerals, the effect of calcium oxide in solid solution would be to raise the Fermi-level of the crystal. Addition of zinc oxide would raise it further. With rise in Fermi-level more free electrons would be available for reaction on the surface provided such reaction is an 'acceptor' type reaction. The observed enhancement of rate of hydraulic action on heating the clinker may mean that: (*a*) the clinker minerals are semiconductors; (*b*) hydraulic action is an 'acceptor' type semiconductor surface reaction.

There are no published data regarding electrical properties of the clinker minerals. It is only known that $CaO.SiO_2$ is probably an ionic conductor[7], that is the electronic part in any electrical conductivity shown by this material is negligible. Water is practically without any action on this mineral[8]. This may indicate that electronic conductivity of the clinker is a necessary condition for hydraulic action. Though it is not known if clinker minerals are semiconductors there is no bar for these minerals to be impurity semiconductors through either non-stoichiometry or through trace impurities, and clinker minerals are known to have both. Glassy phase clinkers may be glassy phase semiconductors.

In a water molecule the hydrogen and the hydroxyl group are connected by a slight polarized bond with the positive pole on hydrogen atom and the negative pole on the hydroxyl group. The molecule of water on approaching the crystal with appropriate crystallographic structure may get attached to it by 'acceptor' bond through a capture of electron from the conduction-level of the crystal.

Increasing the calcium oxide content in clinker crystals plays a part in influencing their hydraulic action by making more electrons available, for the presence of calcium oxide raises the Fermi-level of the crystals. Rate of hydraulic action thus becomes dependent on the position of Fermi-level in the clinkers and the higher it is the greater is the rate. The difference in the rates of hydraulic action in different clinker crystals may be due to the difference in the position of the bulk Fermi-level of these crystals. Thus tricalcium aluminate should have a Fermi-level higher than tricalcium silicate and the lowest should be in dicalcium silicate. The position of the Fermi-level in the ferrite phase should be controlled by two opposite factors, calcium oxide raising it and ferric oxide ('*p*' type semi-conductor) lowering it. The resultant position should be close to that of tricalcium aluminate and its rate of hydraulic action should also be close to tricalcium aluminate as is actually observed, while from the point of view of basicity the ferrite phase should have a higher rate of hydraulic action than tricalcium aluminate.

When gypsum is 'overburnt' it either does not hydrate or hydrates after a long time. When it is heated more than 1,000° C a part of it breaks up into calcium oxide and sulphur trioxide and the resulting calcium oxide goes in solid solution in the residual calcium sulphate. The product thus formed readily hydrates and this fact is utilized in producing a type of gypsum cement. The explanation of this may also be along the lines given here and parallelism between hydration of cement clinkers and calcium sulphate is obvious. This should be true for hydration of lime, and high reactivity with water for lime may be due to being an '*n*' type semiconductor with relatively high Fermi-level.

Puzzolans are materials which react with lime in presence of water and harden. These fall on the borderline between hydraulic- and reaction-bond-type cements. The reaction between a puzzolan and lime in the presence of water is known to be a chemisorption process. Since a chemisorption process depends on the electrical characteristics of the reactants, it had been suggested that absorption of lime by puzzolan in the presence of water may be a semiconductor surface reaction and for a material to behave as a puzzolan it has to be a semiconductor[9]. These suggestions are made on the basis that the variation of electrical conductivity with temperature of puzzolans like kaolin and kaolinitic clay on firing with and without admixture of zinc oxide was found to be characteristic of semiconductors[9] and an increase in the electrical conductivity of these materials resulted in the decrease of lime absorption[9,10]. Work on lime reactivity of ferric oxide (a '*p*' type semiconductor) has shown that lime absorption increases with the electrical conductivity of ferric oxide and thus the reaction is a '*p*' type reaction on a '*p*' type semiconductor[11]. Applying these results, it has been possible to improve the puzzolanic activity of kaolin and kaolinitic clay[12] and of fired laterite[13].

It appears that the cementing behaviour of a large section of cements—hydraulic-bond type and puzzolans—may find explanation from the point of view of semiconductor chemistry and the cementing action may be governed by a few general principles. It may be that organic cements would also fall in line but nothing can be said yet.

The present view regarding cementing action of hydraulic-bond-type cements may be summarized as follows: cement clinkers and puzzolans are probably semiconductors. The hydraulic action of cement clinkers and the reactivity of puzzolans with lime in the presence of water are semiconductor surface reactions and the rates of such reactions are governed by the position of bulk Fermi-level in these crystals—the higher the Fermi-level the greater the rate. The reaction rate can be altered by changing the position of Fermi-level. Cementing hydrates owe their cementing property to their semiconductivity and these may be '*p*' type semiconductors with some of the hydrogen ions from the water molecules in these hydrates behaving as 'holes' and this distinguishes the cementing hydrates from other inorganic hydrates. Adhesive property of cements is due to these 'holes' acting as 'free' negative valencies which can move throughout the crystal and can appear at any point on the cement surface so that the bonds are not localized on the surface. Adhesion between cement and aggregate is a semiconductor surface reaction and the bond involved is a 'donor'-type bond.

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EQUALITY OF VELOCITY OF GAMMA-RAYS OF OPPOSITE CIRCULAR POLARIZATION IN A TRANSVERSE MAGNETIC FIELD

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THE influence of the magnetic field on the propagation of light has been a subject of a number of investigations. Among more recent ones, those of Farr and Barnwell[1] may be mentioned. Using an interferometer they observed an apparent increase of the velocity of light in passing through a transverse magnetic field of 20,000 *G*. However, the increase observed, about 34 cm/sec, was comparable with the estimated error of the experiment, 19 cm/sec.

A much more accurate experiment was performed recently by Prof. R. V. Jones[2]. He passed a beam of light through a transverse magnetic field of prismatic shape, so that a change in the velocity of light would result in a deflexion of the light beam, just as in an ordinary optical prism. A very sensitive optical lever arrangement was then used to detect any deflexion that may occur. No change in the velocity of light was observed. By pushing the sensitivity of the optical lever to extreme value and by taking very considerable precautions to eliminate or reduce various disturbing effects, such as pressure and temperature variations, the experimental error was minimized so that an upper limit on the change in velocity as low as 10^{-2} cm/sec could be established. This is an improvement of several orders of magnitude as compared with the experiment of Farr and Barnwell. Jones's apparatus, as he pointed out, does not test the possibility of the magnetic field 'splitting' the velocity of light, that is, affecting different circular polarizations in opposite ways. Such an effect is theoretically possible owing to the polarization of the vacuum predicted by quantum electrodynamics[3,4].

Different velocities for two circularly polarized components would, of course, mean the rotation of the polarization plane and could be detected by the usual optical methods—with an arrangement consisting of a source of light, polarizer, analyser and an intensity-sensitive detector of the light passing through the analyser.

What the analyser, polarizer and detector should be depends on the choice of radiation. In choosing the wave-length of the radiation, one has to consider the relative merits of various experimental techniques applicable for respective wave-length regions and the accuracies attainable. If the difference in velocities of two opposite circular polarizations is Δc, and if it is maintained through the time t, the polarization plane will be rotated by an angle $2\pi ct/\lambda$, where λ is the wave-length of the radiation in question.

It would thus at first sight appear clearly advantageous to use short wave-length, that is, γ-radiation. This is, of course, not so simple, the difficulties in polarizing and analysing γ-radiation being comparatively great. For visible light, much less rotation of the polarization plane is compensated by much higher accuracy in measuring this rotation.

The Mössbauer effect, however, offers a new possibility of increasing the accuracy in the determination of the polarization plane and in polarizing γ-radiation.

To obtain polarized γ-rays, one can utilize nuclear reactions, where the outcoming γ-rays can show various degrees of polarization; radioactive decay (selecting the angle of emission of the preceding radiation); or Compton scattering. These are, obviously, not very convenient ways; either one has to face the intensity problem if scattering is used, or an accelerator is needed when producing polarized γ-rays through nuclear reactions.

Now, if one looks at the radiation pattern of a dipole placed in a magnetic field, one can see that the radiation emitted in a direction perpendicular to the magnetic field is, in fact, linearly polarized. Why not then use the magnetic field to polarize γ-rays, dipole transitions being readily available in a radioactive decay? A process of resonant absorption of this radiation by nuclei placed also in an external magnetic field could serve to analyse the polarization. The answer is, of course, simple. Dipole radiation consists of the $m = 0$ and the $m = \pm 1$ components, both linearly polarized in the direction perpendicular to the applied field, and also to each other.

If we attempted to analyse the polarization of dipole radiation in a way suggested here, no change in the intensity of resonant absorption would be seen though the relative orientation of the external polarizing and analysing field is changing. While this statement can be verified for any orientation, it becomes very obvious if one considers the polarizing and the analysing fields to be perpendicular to each other. The $m = \pm 1$ components cannot then excite identical components in absorbing nuclei, as their polarization is wrong; however, their polarization is correct to excite the $m = 0$ component. Similarly, the $m = 0$ can excite the $m = \pm 1$ components. However, although the tacit assumption of this reasoning, that the difference in energy between the $m = 0$ and the $m = \pm 1$ components is small compared with a width of the resonance, is certainly more than fulfilled for the Doppler broadened γ-rays, it can be questioned under the conditions of recoilless emission and absorption when the resonance width is of the order of natural width.

To take the best-known example of a dipole Mössbauer transition, the energy spectrum of 14·3-keV γ-rays emitted without recoil by cobalt-57 nuclei in an iron lattice shows a clear separation in energy of the $m = 0$ and the $m = \pm 1$ components; the strong internal field at the nuclei of such a lattice (of the order of 330 k*G*) affects the energies of the magnetic substrates sufficiently to produce energy differences of several natural line-widths between these components, and external polarizing and analysing fields can serve to orient the magnetic domains and therefore internal magnetic fields. The $m = 0$ and $m = \pm 1$ source components can excite only those of identical energy in the absorber, so we have in effect a source of linearly polarized γ-rays and an analyser with a strong dependence of resonant absorption on the relative orientation of external fields, maximum absorption occurring for parallel external fields and minimum for perpendicular ones.

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A general plan of arrangement would then be as follows. The source and the polarizer are replaced by cobalt-57 nuclei in the iron lattice, the domains of which are oriented by the external field. The analyser is an iron lattice to which the external orienting field is applied. A proportional counter detects changes in transmission of radiation. This can be done with a good accuracy, up to 0·1 per cent, and consequently small changes in the orientation of the polarization plane can be detected.

Another argument for choosing γ-rays for the detection of the effect of the magnetic field comes from quantum electrodynamics; the effect of the magnetic field on light can occur through the virtual pair production and this is, of course, more probable at higher energies, that is, more probable for γ-rays than for visible light.

The effects of vacuum polarization on the velocity of light are proportional to the square of the field intensity and to the path of the photon through the field[3]. Thus, in order to bring the upper limit as low as possible, one should aim at using the largest and strongest magnetic fields available. At the time the new radial-ridge cyclotron was nearing completion, which offered the opportunity to use a large magnet with little outlay. The maximum field in the magnet was over 18,000 oersteds, falling to 15,500 oersteds at 20 in. diameter and rather more rapidly at larger diameters. The field in the ridge was about 15,000 oersteds. The use of this large magnet (pole diameter 55½ in.), the space available and the source strength determined the experimental arrangement to a considerable degree.

The arrangement with the best sensitivity to the rotation of the polarization plane would be a polarizer with its field parallel (or perpendicular) to that of the main magnet and an analyser with its field at 45°. The experimental procedure with such an arrangement would consist in observing the counting rates in the proportional counter with the field of the main magnet switched on and off.

However, the strong field from the main cyclotron magnet, extending to a considerable distance from the machine, creates serious difficulties for such an arrangement. Unless the analysing magnet was removed several metres from the machine a rotation of the analysing magnetic field would occur when the cyclotron magnet is switched on and off—because of the 45° between the analysing and strong magnetic field. Unless this rotation could be reduced to well below 1° the advantage of this arrangement would be lost. However, removal of the analysing magnet to sufficient distance was inconvenient and would have meant a reduction of the counting rate by a large factor. Thus, it was judged better to avoid the difficulty at the expense of sensitivity to the rotation of polarization plane, by aligning both the polarizing and analysing fields with the main field. Once the higher sensitivity of the first arrangement is sacrificed, a further possibility of improving the geometry appears, that is, the possibility of using the main magnet also to serve as polarizing and analysing magnet. Such a set up, finally adopted for the experiment, gives the minimum distance between the source and the absorber, and the best alignment between the analysing, polarizing and the main field, as one field performs all three functions.

In this arrangement one, obviously, cannot compare the counting rates with the field switched on and off—the main field switched off will not perform its polarizing and analysing function. Instead, one can compare the counting rates at half and at full field. At half field the fringing field is still sufficiently strong to perform its polarizing and analysing function, while any effect depending on the square of the field intensity is reduced to one-quarter of the full field value. This was the procedure used in the experiment.

The source, about 80 mc. of cobalt-57 diffused into the iron foil and giving the Mössbauer radiation of 14·3 keV, and the pure natural iron absorber were placed in front of the two opposite melanex windows on the cyclotron vacuum chamber in which a pressure of 10^{-5} mm mercury was maintained through the experiment.

The absorber was followed by a *PX* 130 xenon-filled proportional counter, the output of which was fed into a single-channel pulse height analyser selecting the 14·3-keV γ-ray peak. The scaler input was gated with a crystal controlled timing circuit, accurate to better than one part in 10,000.

In the course of the experiment, the half-field absorber transmission was compared with the full-field transmission in frequent alternation and the corresponding counting rates—hereafter called *HF* and *FF*—recorded.

To detect any possible effect of the change of the magnetic field on the source and the counter, control readings with the absorber removed were also taken. The effect of the magnetic field on the proportional counter was further investigated in separate tests.

In the arrangement adopted, the polarizing and analysing field changes and one must know whether this could affect the counting rates, possibly due to differences in the degree of polarization of the source and the absorber material. This effect was indeed observed and therefore the experimental procedure had to be such as to enable one to separate such spurious effects from any real ones. This was done by test runs in which the absorber was placed immediately after the source, before the main field, everything else being unchanged. Such test runs were performed in alternation with the normal sequence: source—main field—absorber—counter.

Denoting the test and the normal source—field—absorber sequence with *SAF*, respectively *SFA*, the experiment essentially consisted in repeating the following set of measurements:

Arrangement:	*SFA*	*SFA*	*SAF*	*SAF*
Field:	Half	Full	Full	Half
Counting rate:	*HF*	*FF*	*FF*	*HF*

Results are here expressed as the ratios of counting rates at half and full fields. A total of about 2×10^7 counts were recorded and the following values obtained:

$$SFA \text{ arrangement: } HF/FF = 1{\cdot}0071 \pm 0{\cdot}0015$$
$$SAF \text{ arrangement: } HF/FF = 1{\cdot}0094 \pm 0{\cdot}0015$$

Removing the absorber the following ratio was obtained:

$$\text{No absorber: } HF/FF = 1{\cdot}00017 \pm 0{\cdot}0016$$

The errors quoted are standard deviations, obtained from the spread of the actual readings—the standard deviations calculated from the number of counts being smaller by a factor of two.

One should first note that both *HF*/*FF* ratios deviate from unity; there is an increase of absorption at full field. As can be seen from the *HF*/*FF* ratio when the absorber is removed, the increase is not due to the effect of the magnetic field on the counter. This effect is due to the fact that, although at half field the absorber material reaches a high degree of magnetization, the complete saturation is not yet attained. Thus, there is an increase in absorption when the polarizing and analysing field changes from its 'half field' value of 1,250 oersteds to the 'full field' value of 2,500 oersteds, when the material has reached the magnetic saturation. The existence of the change of the *HF*/*FF* ratio due to the incomplete saturation must also be considered as a possible source of error; should there be an axial asymmetry in the fringing field in two absorber positions, then a different ratio *HF*/*FF* could be obtained for two source—field—absorber arrangements. The field in these positions was carefully measured and it was found that the polarizing fringing field when the field is at its half value is 1 per cent smaller on the source side. The difference in the degree of magnetization in the two positions due to this field difference should be of the order of 0·01 per cent, and the corresponding change in absorption (and in the ratio HF/FF) would be completely negligible in comparison with the standard deviations given.

From the ratios one obtains the increase in HF/FF due to the effect of the magnetic field on the radiation:

$$\Delta(HF/FF) = 1{\cdot}0071 - 1{\cdot}0094$$
$$= -0{\cdot}0023 \pm 0{\cdot}0020$$

that is, $\Delta(HF/FF)$ is in the limits of the values $-0{\cdot}0043$ to $-0{\cdot}0003$. Since the polarizing and analysing fields are parallel, any real effect must give a positive value of Δ, so we shall take the upper limit of the increase in ratio to be $-0{\cdot}0023 + 2 \times 0{\cdot}0020$ or 0·0017.

Assuming, in analogy to optics, that the absorption of linearly polarized radiation is dependent on the square of the cosine of the angle θ between the polarization planes of radiation and of the analyser, the intensity of transmitted radiation can be written in the form:

$$I = A + B \sin^2 \theta$$

For small angles θ, the fractional change of the intensity $\Delta = \Delta I/I = 2B/A\,\Delta\theta^2$ corresponds to the change $\Delta\theta$ in the angle between the polarization planes.

The ratio B/A was determined in a separate calibration experiment, by observing the transmission for parallel and crossed polarization planes. A value $B/A = 1{\cdot}05$ was obtained. With this value, and with the upper limit for the intensity change $\Delta = \Delta I/I = 0{\cdot}0017$, one obtains the upper limit for the rotation of the polarization plane $\theta = 1{\cdot}6°$. The corresponding upper limit on the velocity splitting is obtained from:

$$\Delta c = c \cdot \frac{\lambda}{S} \cdot \frac{\Delta\theta}{360}$$

where S is the path of the photon through the magnetic field and λ its wave-length. Inserting the values ($S = 50$ in., $\lambda = 8{\cdot}6 \times 10^{-9}$ cm) one obtains for the upper limit to the velocity-splitting:

$$\Delta c = 9 \times 10^{-3} \text{ cm/sec}$$

or:

$$\Delta c < 10^{-2} \text{ cm/sec}$$

which happens to be the same as Jones's limit to the changes of mean velocity:

$$\tfrac{1}{2}[(c + \Delta c) + (c - \Delta c)]$$

One might add that this is by no means the limit of accuracy at present available using the Mossbauer radiation. With a stronger source, so as to be able to use the most sensitive arrangement (45° between the polarizer and the analyser), an improvement by a factor of at least 10 will be possible.

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POSSIBLE USE OF ARGON-39 IN THE POTASSIUM-ARGON METHOD OF AGE DETERMINATION

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In the determination of ages by the potassium–argon method the generally accepted technique in present practice is to measure the amount of radiogenic argon present in the sample by the method of isotopic dilution, with argon-38 as the diluting isotope. In the present research a new technique has been investigated in which argon-39 is used as the diluting isotope, and is added dissolved in an artificial silicate which approximates the pertinent properties of the rock being analysed. The ease of handling and measurement involved in such a procedure is apparent.

Fechtig *et al.*[1] have reported the use of neutron generated argon-39 in natural silicates and in potassium chloride to measure the diffusion characteristics of argon in these substances. Reynolds[2] has also applied this technique to similar measurements on potash–lime–silica glasses.

For the exploratory experiments a sample of potassium silicate glass was prepared as a melt from high-purity silica glass powder and analysed grade potassium carbonate, and was homogenized by heating at 1,450° C for 3 h. The potassium content of the product was 10·8 per cent. The resultant clear glass was crushed to 40–60 mesh size in a diamond mortar, and was activated in the Brookhaven graphite reactor in the fast neutron section. Argon-39 was produced by the reaction:

$$^{39}\text{K}(n,p)^{39}\text{Ar}$$

After removal from the reactor, and 'cooling' of short-lived activities, the samples were coloured purple from neutron structural interactions. Annealing at 200° C for 2 h removed this colour.

The annealed samples were carefully analysed for argon-39 content by melting in an ultra-high vacuum system. The evolved gases were purified by condensation at liquid nitrogen temperatures and by gettering with titanium sponge. Isotopic dilution with an argon-38 enriched spike was used, and final determination of argon was made with an inert gas mass spectrometer[3]. The argon-39 content was $8{\cdot}54 \pm 0{\cdot}17 \times 10^{-7}$ standard c.c./g.

In use the argon-39 spike was added, dissolved in a weighed amount of ground and sized artificial silicate, to a known weight of similarly sized rock or mineral being analysed for radiogenic argon-40. The contamination of the sample with air argon-40 is the factor which limits the accuracy and usefulness of the potassium–argon method, especially for young minerals. This may amount to as much as 10^{-6} standard c.c. and can completely swamp the radiogenic argon-40 for rocks of ages less than ten million years. Therefore, as a first step the combined samples should be subjected to a treatment to preferentially remove or reduce this contamination. One such treatment is discussed here. For analysis, samples were placed in an apparatus to remove the required representative portion of the argon by melting or crushing, and to purify this gas by suitable freeze-out and gettering[4]. Extraction by melting was carried out in a molybdenum crucible at temperatures of 1,600–1,800° C. It should be emphasized that since only a representative sample of gas from the mineral–artificial glass mixture is needed for analysis by this method, the degree of completeness of extraction of the gas from the sample is not important.

For vacuum crushing the apparatus used was a narrow mortar of stainless steel attached to the ultra-high vacuum system through a glass-to-metal seal. The pestle was a stainless steel piston loosely fitted into the mortar, and attached by a rod to a soft iron armature within the glass portion of the system. The pestle was lifted once a second by this iron core actuated by a solenoid situated outside the apparatus. It was allowed to fall under its own weight to crush the sample. The temperature of the crusher could be controlled by external baths or furnaces for degassing or for temperature-controlled runs. After a typical run of 17 h, a sample was found to have been reduced from 40–60 mesh to 60 per cent through a 200-mesh sieve. Gas was copiously evolved during this crushing process, although the crusher was not as efficient as would be desirable.

Measurement of the argon peaks of the purified gas was made with the inert gas bakeable mass spectrometer. By determination of the 36, 39 and 40 peak ratios, a correction for air argon-40 was applied, and the volume of radiogenic argon-40 in the mineral sample calculated according to the method of isotopic dilution.

The radioactivity of the argon-39 suggests an alternative method of measurement which is at present under investigation. If the air argon contamination can be made negligible by a suitable pretreatment (see later), the total volume of the purified argon in a representative gas sample can be measured, and radioactive counting used to determine the argon-39 content. These two measurements would give the argon-40 to argon-39 ratio necessary for the calculation of the radiogenic argon-40 content of the original mineral sample by the dilution method.

The validity of the application of the isotopic dilution procedure in these instances depends on the identity of behaviour of the argon-39 in the spike, and the radiogenic argon-40 in the mineral. After intermingling by melting there would be little question of this; but during pretreatment for removal of air contamination, or during heat-up to the melting point, there may be some uncertainty that the behaviour of the isotopes of argon will be the same in the different silicates. The property of importance would be the rate at which argon-39 diffuses from the artificial samples relative to the diffusion rate of radiogenic argon-40 in natural silicates at similar temperatures. Therefore, the diffusion constant D was used to measure the efficiency of the annealing of the structural damage produced by neutron bombardment, and for comparison of the diffusion of the various argon isotopes. The particles of the powdered and sized samples were assumed to be spheres of effective radius a, and diffusion was evaluated as D/a^2, thus including the particle size factor and eliminating the uncertainties in its determination. This function could be calculated from the fraction of argon removed during a heating treatment according to well-established models for the solution of the Fick diffusion equation[4].

The heating for diffusion measurements was carried out in the extraction system at temperatures between 500° and 1,000° C for known periods of time. The gas removed was spiked, purified and measured with the inert gas mass spectrometer as was done for total argon determinations. The diffusion values calculated from these measurements are shown in Fig. 1, Section *a*. It will be noted that the diffusion of argon-39 in the artificial samples was the same, within experimental error, as that of radiogenic argon-40 from the natural glasses and microcrystalline rock (dolerite) measured during this work. It can also be seen that there is little significant difference for the diffusion of atmospheric argon-40, despite the intuitive assumption that this argon would be held on surfaces or in microcracks, and would be released at an entirely different rate.

The release of gas on crushing was also investigated in a similar manner. If we assume that the rate-limiting step for the release is the desorption of gas from the surface newly exposed by crushing[5], we may again use the D/a^2 function as a convenience in plotting. D is now a desorption coefficient for the gas leaving the newly exposed surface and a is the radius of the particles resulting from the crushing. We can also plot this as a function of the temperature at which the crushing was conducted. This has been done as shown in Fig. 1, Section *b*. We note that there is little dependence on temperature for gas removal by this process, particularly for the air argon-40. Evidently the rate of removal depends mainly on the rate at which new surface is exposed; in other words, on the efficiency of the crushing process. Most significant is the similar desorption coefficients of radiogenic argon-40 and argon-39, and the dissimilarity in the coefficient for air argon-40 release from these same substances. Again using the analogy with diffusion release, the 'half-life' for removal of a gas is equal to 0·03 a^2/D for particles assumed to be spherical[4]. For air argon-40 removal in this particular crushing machine this is about 80 h, as opposed to 8,000 h for the other argon isotopes. One can be optimistic, then, that these can be decreased to such a value as would make it possible preferentially to remove the air argon-40 contamination within a reasonable time by improvement in the design and efficiency of the vacuum crushing device.

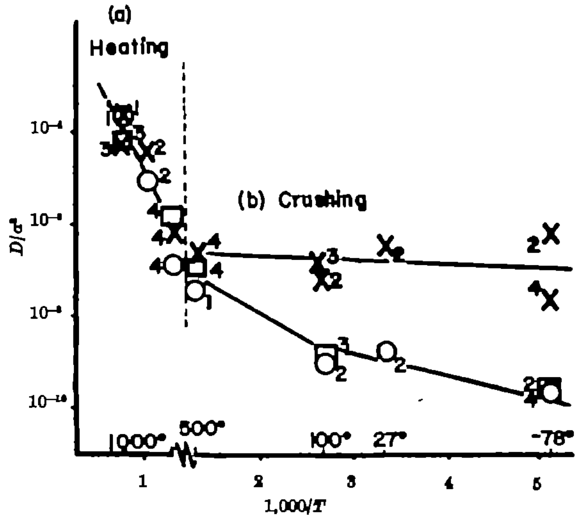

Fig. 1. Temperature dependence of the release of argon isotopes by (*a*) heating and (*b*) crushing. (1) Volcanic glass; (2) dolerite; (3) artificial glass; (4) artificial glass–volcanic glass mixture
○, ^{40}Ar$_{\text{radiogenic}}$; ×, ^{40}Ar$_{\text{air}}$; □, ^{39}Ar

Using the method of isotopic dilution with argon-39, measurements of the radiogenic argon-40 content of two test materials were made (Table 1). These samples were a young volcanic glass (*KA*-40) and a very old pegmatite muscovite mica which represented extremes in mineral types and argon contents. Both samples were kindly supplied by W. A. Bassett, as were the results for the conventional analyses of the mica. For the argon-39 spiked samples each group represents fractions from a single sample while heating at the temperatures indicated, with or without crushing. The results show order-of-magnitude agreement. The poor agreement, both in precision and accuracy, seems to be a result of the very small gas samples being handled and measured.

For these measurements rock and silicate samples were taken of such a size as to be handled conveniently in

Table 1. Volume of Radiogenic Argon-40, Standard c.c./g of Sample

	Argon-39 spike heating only	Argon-39 spike crushing	Argon-38 spike conventional
Volcanic glass *KA*-40	1·67, 1·79 × 10^{-7} (500°) 6·38 × 10^{-7} (melt)	1·87 × 10^{-7} (250°) 2·53, 1·36, 5·74, 4·90 × 10^{-7} (500°) 1·43, 1·47, 4·11 × 10^{-7} (500°)	2·12, 2·23 × 10^{-7} (melt)
Muscovite mica	1·45 × 10^{-5} (melt) 0·71 × 10^{-5} (melt)	1·09, 3·68 × 10^{-5} (500°)	1·76, 1·85 × 10^{-5} (melt)

existing equipment, with little regard for optimum size of the radiogenic argon samples. Natural glasses are probably most like the spike-containing artificial glass in pertinent properties, such as argon diffusion, but unaltered samples suitable for age determinations are of young ages, hence low in radiogenic argon. For radiogenic argon gas samples in this low range of about 10^{-7} standard c.c., the standard deviation for measurement in the instrument used, without electron multiplier, was about 20 per cent. By the nature of the technique described here, fractional gas samples were taken which varied from 1 to 60 per cent of the total gas, so that the results obtained with these, in turn, exhibited corresponding lower precision. Also, for a material structurally as different from the spike-containing artificial glass as is the mica, the discrepancy observed here was not surprising.

It is believed that the measurements with these two samples were encouraging, but were an insufficient test of the method. As yet, unfortunately, no opportunity has been available to repeat the measurements with equipment designed to give radiogenic argon samples of a size more suitable to this technique. At present, the technique of measurement, using the radioactivity of the samples as described here, is under investigation.

I thank Oliver A. Schaeffer for assistance and other members of the geochemistry group, Chemistry Department, Brookhaven National Laboratory, where this work was done, for discussions.

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INTRINSIC ELECTRIC STRENGTH OF POLYTHENE

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RECENT experiments[1] with sodium chloride and potassium bromide single crystals have shown that their intrinsic electric strengths decrease slowly with increase in specimen thickness, and also that there are statistical time-lags. The average time-lag decreases rapidly with over-voltage, and it depends on the cathode material—but the electric strengths are independent of this. The foregoing are characteristics of the single electron avalanche breakdown mechanism[2]. The collective electron theories[3], which predict electric strength independent of specimen thickness, cannot apply to these materials. The intrinsic electric strengths of solid dielectrics have previously been considered independent of thickness of the specimen[4] provided this exceeds a few electron mean free paths; but in fact the possibility of there being a small effect is not completely excluded by the available evidence. Measurements made with comparable specimens usually yield appreciable variance of electric strength. A small rate of decrease of electric strength with increase in specimen thickness cannot be established for statistical reasons unless great numbers of specimens are used, or the range of thickness is great. Examination of the literature shows that these conditions seldom have been satisfied, and the question of whether or not the intrinsic electric strength of solid dielectrics depends on specimen thickness needs to be re-opened.

Statistical time-lags have previously been observed[5] in the breakdown of polythene; but, according to Oakes[6], the electric strength is independent of specimen thickness. This conclusion is based on the comparison of a group of specimens with thicknesses between 0·0005 in. and 0·002 in., and a group having thicknesses between 0·002 in. and 0·008 in. However, close inspection of Fig. 7 of ref. 6 suggests that this conclusion is false. In this diagram, breakdown voltage is plotted against thickness for specimens of grade 20 polythene at room temperature. There is a difference in the slopes of the lines which best fit the points at the two extremities of the range of thickness. A detailed analysis of Oakes's measurements yields the following results. There were 41 specimens in the thickness range 0·002 in.–0·008 in. and of these 22 exceeded 0·005 in. The mean electric strength of these 22 specimens was 16·2 MV in.^{-1} and the variance was 1·51 $(\text{MV in.}^{-1})^2$. Values of electric strength were also obtained for 40 specimens of the same grade of polythene in the thickness-range 0·0005–0·002 in. The mean electric strength was 17·8 MV in.^{-1} and the variance was 1·53 $(\text{MV in.}^{-1})^2$. The probability of the difference between the means of the two groups being due to chance variations in the samples is shown, by application of Student's t test, to be less than 0·1 per cent.

We have sought confirmation of the thickness effect by measuring the electric strength of 'Alkathene 7' in the thickness-range 0·001–0·018 in. The specimens were of the plane-recess type, prepared by moulding in a manner similar to that described by Oakes[6], and as in his experiments the electrodes were of colloidal graphite. The diameter of the recess was 3/8 in. for specimens less than 0·006 in. thick, but it was increased to 1 in. for the thicker specimens in order to ensure adequately uniform field at the base of the recess. In Oakes's experiment, the voltage was increased at the rate of about 300 V sec^{-1} until the specimen broke down; in the present investigation a series of 1 : 8,000 μsec voltage impulses was applied to each specimen of thickness less than 0·006 in. and a series of 1 : 120 μsec impulses to the thicker specimens. (The wave-front of 1 : 8,000 μsec impulse increases from 10 to 90 per cent of peak value in 1μsec and the tail decays to half-peak value in 8,000 μsec.) In both cases the amplitude of each impulse exceeded that of its predecessor by 3 per cent. An independent experiment was performed using specimens of thicknesses between 0·002 in. and 0·004 in. which established that the average electric strength was the same for both voltage wave-forms. The results of the experiment is illustrated by Fig. 1 in which electric strength at 20° C is plotted against specimen thickness on a logarithmic scale. The best straight line drawn through the 128 points obeys the law $E = 12{\cdot}8 - 3\ (\log_{10} t)$ where E is in MV in.^{-1} and t is in units of 10^{-3} in. The product moment correlation coefficient is $-0{\cdot}79$, and the probability of this occurring by chance is less than 10^{-3}. The thickness effect present in Oakes's observations is therefore confirmed. The mean electric strength obtained with impulse voltages is, however, only about 80 per cent of the corresponding value obtained by Oakes using slowly increasing voltages. This effect does not appear to be due to the use of different grades of polythene, or to faults in the specimens used for the impulse tests, or to systematic errors in the measurement of voltage and thickness.

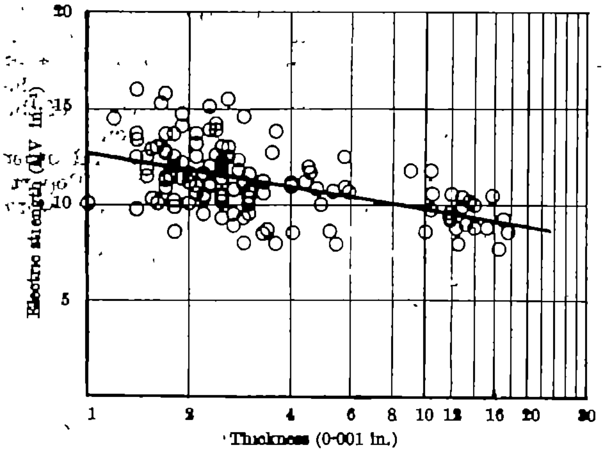

Fig. 1. Electric strength of polythene at 20° C

Measurements using our equipment yielded maximum, average and minimum values of respectively 15·2 MV in.$^{-1}$, 12·7 MV in.$^{-1}$ and 10·6 MV in.$^{-1}$ for the impulse electric strength of 8 specimens of 'Alkathene 7' of thicknesses between 3·5 10^{-3} in. and 4·5 10^{-3} in. The variance was 3·0 $(\text{MV in.}^{-1})^2$. Corresponding values obtained from a group of seven similar specimens tested with slowly rising voltages were 16·8 MV in.$^{-1}$, 15·3 MV in.$^{-1}$ and 12·8 MV in.$^{-1}$. The variance was 1·6 $(\text{MV in.}^{-1})^2$. The difference between the means is significant at the 1 per cent level. Oakes used seven specimens of grade 20 polythene in the above range of thickness. The maximum, average and minimum values of electric strength were respectively 18·3 MV in.$^{-1}$, 16·1 MV in.$^{-1}$ and 13·1 MV in.$^{-1}$, and the variance was 3·0 $(\text{MV in.}^{-1})^2$. The difference between the mean values of 15·3 MV in.$^{-1}$ and 16·1 MV in.$^{-1}$ obtained with slowly increasing voltages is not significant. The cause of the impulse effect is not known but the mean impulse electric strength was also found to be about 80 per cent of the mean value measured with slowly increasing voltage at − 74° C and − 195° C. This fact seems to eliminate an explanation involving the mechanical properties of the material since these change appreciably when polythene is cooled from about 20° C to − 195° C.

An investigation of the influence of cathode material on the mean statistical time-lag at 20° C was made using groups of not less than 50 specimens of 'Alkathene 7'. The specimen thicknesses were within the range 0·002–0·004 in. The voltage-wave rose to its peak value in 0·3 μsec and remained within 1 per cent of this for the subsequent period of 5 μsec. Mean statistical time-lags of 0·9 μsec and 0·5 μsec were obtained using 1 per cent voltage increments and cathodes respectively of colloidal graphite and vacuum-evaporated aluminium. When the voltage increment was 3 per cent the mean time-lag was 0·5 μsec for specimens with graphite cathodes. The difference between the comparable mean statistical time lags of 0·9 μsec and 0·5 μsec is appreciable, but because of the great variance it is barely significant. The experiment was repeated using similar groups of specimens prepared from a fresh supply of 'Alkathene 7', but the mean time-lag for graphite electrodes was about 20 times the value already quoted and the mean electric strength was about 5 per cent less, so that combination of the two sets of results cannot be justified.

The mean electric strength of alkali halide crystals does not depend on the cathode material but Oakes[6] observed a small effect with polythene. In the present experiments mean electric strengths were obtained of 12·4 MV in.$^{-1}$ when the cathodes were of colloidal graphite and 11·2 MV in.$^{-1}$ when they were of evaporated aluminium. The difference between these mean values is significant at the 0·1 per cent level. The specimens were those used for time-lag studies with 1 per cent voltage increments at 20° C. A cathode effect may be accounted for plausibly by assuming the formation of electronic space charge. The possibility of this depends on the magnitude of pre-breakdown current, electron mobility and density of traps, and little is known about these quantities in polythene. If space charges form, the basic assumption of uniform electric field is of course invalid.

We thank Mr. W. G. Oakes, of Imperial Chemical Industries, Plastics Division, for his co-operation in providing records of his experiments and Mr. W. Reddish of the same organization for assistance with the supply of 'Alkathene 7'.

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INVESTIGATION OF SMALL CYCLIC STRAINS BY ETCH PITS AT DISLOCATION SITES

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Of the several methods at present available for the more or less direct observation of dislocations, the chemical etching technique is the simplest and most versatile for those materials for which it is appropriate. The development by Livingston[1] and Young[2], independently, of etchants capable of revealing dislocation sites on copper single crystals has extended the scope of this technique greatly, and should lead to an increased understanding of the important dislocation mechanisms involved in the deformation of face-centred cubic materials.

This article describes some of the preliminary results of an investigation in which chemical etching is being used to study dislocation densities and arrangements in face-centred cubic metals in the early stages of cyclic deformation at very low stress amplitudes. The materials used are copper and α-solid solution copper–aluminium alloys, chosen because available evidence[3,4] indicates a definite relationship between free-electron concentration and stacking fault energy within the α-range of this type of alloy system. Since much of the recent work on fatigue in crystalline materials stresses the importance of cross-slip in the development of a fatigue failure[5-7], the stacking fault energy should be an important parameter in determining the fatigue behaviour of face-centred cubic metals. We find, in fact, distinct differences in dislocation arrangement which can be related to stacking fault energy effects, even during the first few cycles of a fatigue test at a stress amplitude considerably less than the macroscopic yield as determined by single crystal tensile tests.

Annealed single crystals of pure copper and copper–aluminium alloys were cycled in a four-point reversed bending apparatus attached to a standard Instron tensile machine. The alloy compositions and maximum shear stress amplitudes on the primary slip system are given in Table 1. Livingston's (111) etchant was used in this investigation and, following the chemical polishing procedure described by Erdmann[2], was effective in producing dislocation etch pits in alloy crystals containing up to 14·8 atomic per cent aluminium. The axial orientation of the crystals was controlled during growth from the melt to ensure a predominance of slip on the primary system during cycling.

Table 1. STRESS AMPLITUDES IN CYCLIC BENDING

Composition	Critical shear stress (kg/mm^2)	Max. shear stress (cyclic) (kg/mm^2)	Stacking fault (ref. 3) energy (ergs/cm^2)
Cu	0·10	0·07	160
Cu + 2·7 Al	0·81	0·51	65
Cu + 5·3 Al	0·95	0 57	20
Cu + 14·8 Al	1·20	0·80	1–3

Some of the observations of interest, representative of the differences between pure copper and the alloys, are illustrated in Figs. 1–7. Differences in etch-pit arrangement among the alloys were very slight during the initial stages of cyclic strain at comparable stresses.

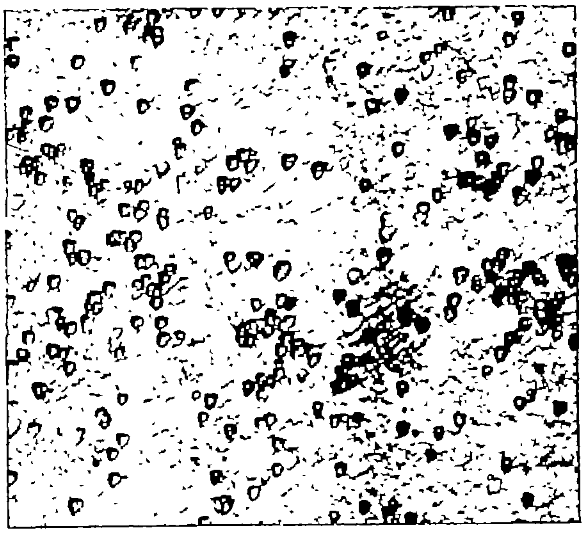
Fig. 1. 'As grown' dislocation arrangement in 99·999 per cent copper (× 135)

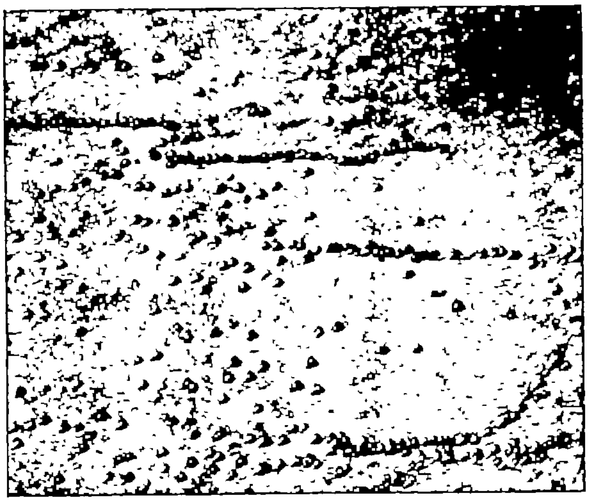
Fig. 2. 'As grown' dislocation arrangement in copper + 2·7 atomic per cent aluminium (× 135)

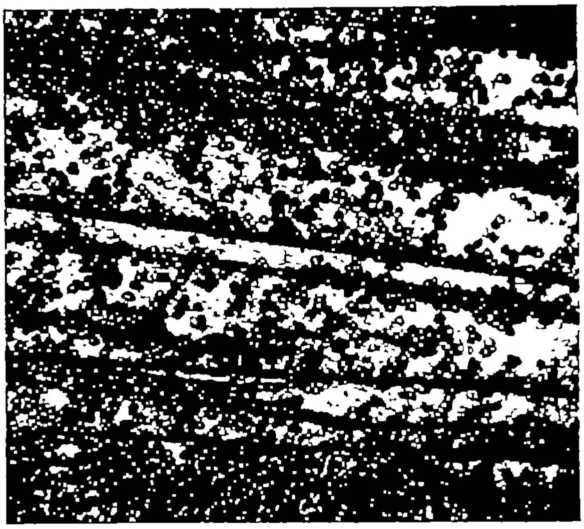
Fig. 3. Dislocations assembly at sub-boundaries after six alternate bending cycles in copper (× 67·5)

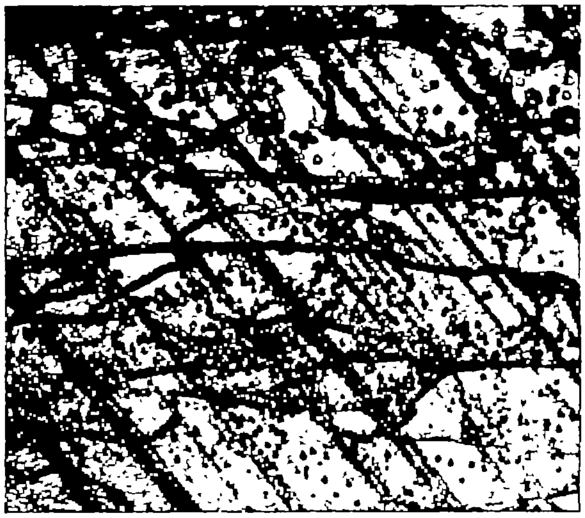
Fig. 4. Slip-band structure in copper–aluminium after six cycles of reversed bending (× 67·5)

Figs. 1 and 2 show the (111) faces of a copper crystal and a copper + 2·7 atomic per cent aluminium crystal, respectively, in the as-grown (annealed) conditions. No great difference in dislocation density is evident, both compositions showing between 10^5 and 10^6 etch pits/cm^2. The arrangement, however, is somewhat less uniform in the alloy, where the pits show a greater tendency to line up in the form of striations and subgrain boundaries. (This tendency becomes even more pronounced in the alloys of higher solute content.)

After 6 cycles of reversed bending at a maximum shear stress of 70 g/mm^2 in the copper specimen, a general increase in dislocation density has occurred, and rather diffuse and regularly spaced slip lines have formed. The etch-pit picture in Fig. 3 illustrates this arrangement quite clearly; a comparison with the more pronounced slip-band structure of Fig. 4 suggests a much greater restriction of the newly generated dislocations to their own slip planes in the alloy, and more freedom of movement of both the grown-in and the new dislocations in the copper. There is an apparent 'assembling' of dislocations along sub-boundaries in the copper, whereas the pit density between slip bands in the alloy remains virtually un-

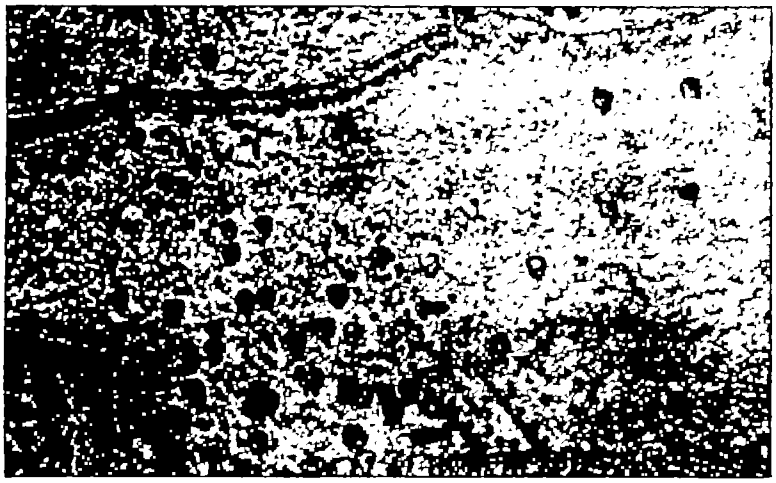

Fig. 5. Dislocation pile-ups in copper + 2·7 per cent aluminium crystal during reversed bending test at maximum shear stress of 0·51 kg/mm², after 3 cycles (× c. 225)

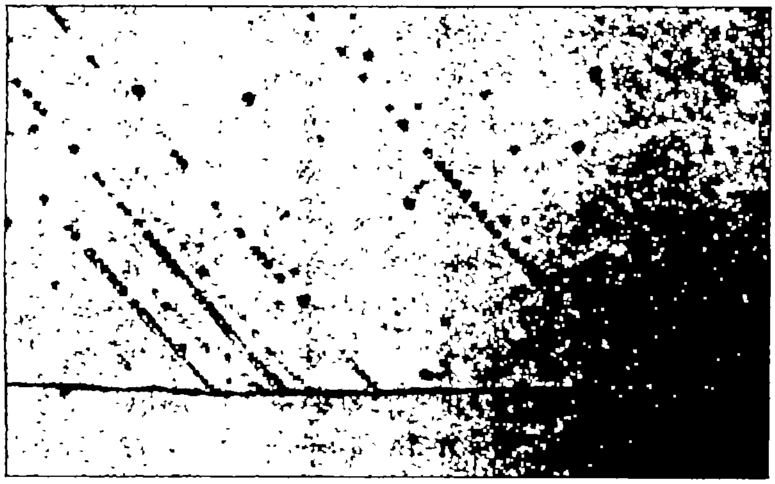

Fig. 6. Same as Fig. 5, after 15 cycles (× c. 290)

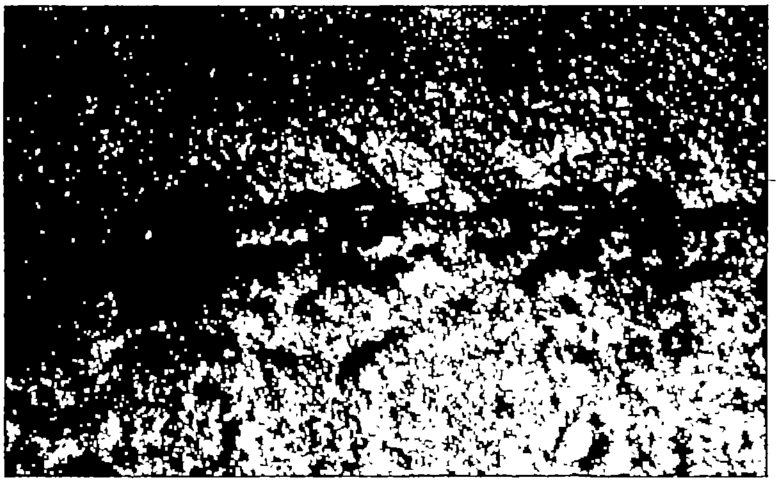

Fig. 7. Same as Fig. 5, after 40 cycles (× c. 200)

changed with increased cyclic strain. There is evidence of a limited amount of slip on secondary planes (the conjugate slip system) after six bending cycles in both the pure copper and the alloys.

Because of the restriction of the dislocations to their primary slip planes in the alloys, pile-ups at barriers such as striations and sub-boundaries have become quite numerous after as few as three cycles at a maximum shear stress of about two-thirds of the macroscopic critical shear stress (Figs. 5, 6 and 7). A study of a large number of these pile-ups indicates that they are in a relaxed condition and consist of small groups of equally spaced dislocations at intervals along the slip plane. It appears that, on release of the applied shear stress, the dislocations tend to run back toward the source, finally arriving at an equilibrium distribution of small groups bounded by minor obstacles on secondary slip planes. The average spacing of these obstacles (about 12 microns) indicates a density of about 7 × 10^5/cm², in reasonable agreement with the original dislocation density in the unstrained specimen. No significant decrease in this spacing is evident within the first 40 cycles of reversed bending.

Etch-pit distributions and densities have been observed at intervals up to a total of 100 cycles of bending. There is little change in the patterns illustrated here between the 6th and 100th cycles. In the alloy crystals the slip bands become more densely populated and somewhat more closely spaced with increased cycling. Some evidence of glide polygonization is apparent at about 100 cycles in the copper specimens, corresponding to a total cumulative strain of about 0·003.

These experimental results reveal significant differences in dislocation behaviour between a pure metal and an alloy of face-centred-cubic structure even in the very early stages of cyclic straining. The observed differences, including the increased pinning effect in the alloys, are in general agreement with a previous suggestion based on tensile test data, namely, that initial hardening of alloys of low-stacking fault energy is largely attributable to a Suzuki-type mechanism wherein solute atoms are concentrated at stacking faults because of their higher solubility in the hexagonal phase[8].

The apparent restriction of the dislocations in the alloy crystals to their own slip planes is thought to be a direct result of the decrease in stacking fault energy with alloying; the more homogeneous increase in dislocation density in pure copper should result in a more rapid initial work-hardening rate (as observed in tension tests) and conceivably a more rapid approach to saturation hardness, with crack initiation at smaller total strain. The limited data available in the literature are in apparent agreement[9,10]. In both tensile and fatigue tests less source activation on secondary slip systems is apparent in the alloys, which is probably due to a latent hardening of secondary slip planes by the intersecting dislocations of primary slip.

The frequent occurrence of pile-ups observed in the alloy crystals at these small strains was somewhat unexpected and lends support to present-day theories that stress the importance of pile-ups in the later stages of hardening.

A more quantitative analysis of these results and the extension of this investigation to include fatigue tests to failure at various stress-levels and distributions is in progress and will be the subject of a later report.

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CÆSIUM-137 FROM FALL-OUT IN HUMAN MILK

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IT is well known that in human milk the ratio between strontium-90 and calcium is lower than in the diet of the mother[1]. Only sparse information exists as regards a comparison of the contents of cæsium-137 (the other long-lived biologically significant fission product) in a mother's milk and in her diet. Several animal experiments have been performed by Wasserman, Comar and Twardock[2] and have shown that the ^{137}Cs/K ratio in goats' milk is 1·4 based on a dietary ^{137}Cs/K ratio of unity.

The following measurements were performed with milk from a primipara 24-year-old Danish woman, who bore a child October 15, 1961. The milk was collected daily during the period November 1, 1961–September 10, 1962. Until the last half of August 1962 the daily mean production of milk was about 1¼ l.; of this, about ¾ l. per day was consumed by her baby; the rest was obtained for analyses. The milk used for analyses was identical to the milk used for the feeding of the baby, as the mother drew out all milk before each feeding and fed the baby from a feeding-bottle. Most of the daily samples were dried and ashed (at 400°–450° C), and the ash was bulked into monthly samples before measurement. These ashed samples have been measured in a 3 in. × 4 in. well-type sodium iodide (Tl) crystal (Harshaw) with a 50-ml. well. Several samples were, however, measured as fresh milk in a 1·6 l. can, which fits around a 3 in. × 3 in. sodium iodide (Tl) crystal (Harshaw). The pulses were analysed in a *TMC*256 channel analyser.

Table 1 shows the results of the monthly measurements and gives furthermore, for comparison, the levels of monthly collected dried milk samples from dairy cows living in the same part of the country as the woman.

Table 1 shows that the human milk (as well as the cows' milk) displays a variation in content of cæsium-137 with time. In the winter and in early spring, before the dairy cows started grazing, the human milk-levels were rather constant: 8·4 ± 0·5 pc. ^{137}Cs/g K. In May the level rose by a factor of nearly four, and in the following three months, June–August, the level was nearly constant: 34 pc. ^{137}Cs/g K. Already, in September, Danish dairy cows start to have an increasing amount of food other than grass, and the effect of this decreasing contribution from grazing was observed both in the human milk and in the cows' milk collected in September.

Table 1. CÆSIUM-137 AND POTASSIUM IN MILK

Sampling period	Human milk: pc. ^{137}Cs/g K	Human milk: g K/g ash	Cows' milk: pc. ^{137}Cs/g K
November 1–December 31, 1961	8·1	0·23	4·5
January 1–31, 1962	9·2	0·21	1·5
February 1–28, 1962	7·1	0·26	3·0
March 1–31, 1962	7·4	0·25	3·5
April 1–26, 1962	10·5	0·26	4·0
May 23–31, 1962	30·6	0·28	22·0
June 1–30, 1962	34·0	0·27	33·0
July 1–31, 1962	34·0	0·28	38·0
August 1–31, 1962	33·7	0·29	37·0
September 1–10, 1962	28·1	0·31	29·0

The relative *S.D.* due to the measurements of the human milk is less than 10 per cent.

As the content of cæsium-137 in the human milk appeared to be nearly constant in the period November–March (Table 1), and as the mother was on a regular diet in this period (1 kg dairy products, 0·15 kg grain products, 0·3 kg fruit and vegetables, 0·2 kg meat, fish and eggs, and 0·6 kg water as tea or coffee), it was anticipated that a steady state between the mother, her diet and her milk had been reached. The mother's diet and her milk were gathered for one week in March. The diet-sampling was performed by having the mother prepare double portions of everything she consumed in the week the experiment ran. The duplicates were put into a pail, which was sent to the laboratory. Six months later, in the beginning of September, the experiment was repeated, but unfortunately the milk-production was decreasing rapidly in this period, and after September 10 only very little milk was secreted. Table 2 shows the results from the two periods.

Table 2 shows that the ^{137}Cs/K ratio is higher in the donor's milk than in her diet. The ratios between cæsium-137 secreted by the milk and ingested with the diet are related to the amount of milk secreted. Thus the percentages of ingested cæsium-137 secreted per litre milk only differ slightly for the two experimental periods, March and September. As probable explanations for the relatively large differences between the observed ratios between milk and diet from the two months, beside the differences due to variation in milk production, one could mention that the daily average intake of potassium with diet in March was 2·8 g against 2·2 g in September, and that the concentration of potassium in the human milk from September was about 20 per cent higher than in the milk from March; but most important, that the September period was just before the end of the lactation period and thus could not be assumed as representing a steady state.

To get an estimate of how much and how long a time it would take for a single oral cæsium-137 dose given by the food to be excreted in the milk, 260 g of reindeer meat from Greenland was given to the milk donor on April 26. Lidén[3] has shown that reindeer meat has a relatively high cæsium-137 content, and thus is suited as a tracer in

Table 2. A COMPARISON BETWEEN THE LEVELS OF CÆSIUM-137 IN HUMAN MILK AND IN THE DIET OF THE DONOR

Sampling period	Sample	pc. ^{137}Cs/g K	pc. ^{137}Cs secreted daily in the milk	pc. ^{137}Cs ingested daily with diet	$\frac{\text{(pc. }^{137}\text{Cs/g K) milk}}{\text{(pc. }^{137}\text{Cs/g K) diet}}$	$\frac{\text{pc. }^{137}\text{Cs secreted}}{\text{pc. }^{137}\text{Cs ingested}}$	Ingested ^{137}Cs secreted per l. milk (per cent)
March 10–16, 1962	Milk	9·2 ± 0·2	6 ± 1	—	1·9 ± 0·1	0·43 ± 0·08	32 ± 6
March 9–15, 1962	Diet	4·9 ± 0·2	—	14 ± 1			
September 4–10, 1962	Milk	27·4 ± 1·5	9 ± 2	—	1·1 ± 0·1	0·17 ± 0·04	28 ± 7
September 3–11, 1962	Diet	24·2 ± 0·5	—	53 ± 5			

The error terms in the table indicate one *S.D.* due to the measurement.

Table 3. Caesium-137 Levels in Human Milk after a Single Oral Intake of 8,300 pc. Caesium-137

Sampling period	pc. ^{137}Cs/g K	pc. ^{137}Cs/l.	Estimated ^{137}Cs content in the milk from the fall-out background: pc. ^{137}Cs/l.	Accumulated excretion in the milk corrected for fall-out background: pc. ^{137}Cs	Accumulated excretion percentage
April 27–29	220	97	4	372	4·5
April 30–May 2	93	42		524	6·3
May 3–5	60	33		640	7·7
May 6–8	65	28	5	732	8·9
May 9–11	55	25		812	9·8
May 12–14	44	20		872	10·5
May 15–17	48	23	14	922	11·1
May 18–22	30	14		922	11·1
May 23–31	31	15	15	—	—
June	34	16	16	—	—

The relative *S.D.* of the results due to measurements is less than 10 per cent.

human biological experiments. The total content of caesium-137 in the 260 g of meat was measured at 8,300 ± 55 pc. The milk was collected and measured (mostly as fresh milk) during the month following intake. Table 3 shows the results.

This experiment was complicated by the fact that the dairy cows in the part of the country where the milk donor is living began grazing about the middle of May this year and consequently the 'normal' caesium-137 content in the mother's milk increased by a factor of nearly 4 (Table 1) during the experiment. However, it is evident from the figures in Table 3 that about 7 per cent of the ingested dose of caesium-137 was excreted in the milk within the first week after the intake of the reindeer meat, and within a fortnight about 10 per cent was excreted by the milk. For dairy cows, it has been shown[4] that about 10 per cent of a single oral dose of radiocaesium will, in a 7 days collection period, be recovered in the milk.

The present investigation indicates that babies fed on human milk could be exposed to a greater caesium-137 body burden than if they were fed on milk from dairy cows, provided that the diet of the mother shows a greater (about 2–3 times) ^{137}Cs/K ratio than the cows' milk. It should be mentioned that the specific caesium-137 activity per litre cows' milk normally will be greater than in human milk, even if the ^{137}Cs/K ratio is greater in the human milk. This is due to the fact that the concentration of potassium in human milk is only about one-third the concentration of potassium in cows' milk.

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NITROGEN METABOLISM OF AFRICAN CATTLE FED DIETS WITH AN ADEQUATE ENERGY, LOW-PROTEIN CONTENT

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In recent years increasing interest has been directed at the protein requirements and nitrogen metabolism of ruminants kept under adverse nutritional conditions[1-3]. Much of this interest has been centred on the urea excretion of these animals[1-3], since ruminants can utilize urea for protein synthesis, and so any mechanism which they may possess to conserve urea is of prime importance. The work of Schmidt-Nielsen *et al.*[1] showed that the camel (*Camelus dromedarius*) excretes very small amounts of urea when deprived of water and fed diets low in protein. Schmidt-Nielsen and Osaki[2] have shown that the excretion of urea nitrogen by sheep kept on a very low crude protein diet is also low. More recently Livingston *et al.*[3] reported a reduction in the amount of urea nitrogen excreted by *Bos taurus* and *Bos indicus* cattle fed rations progressively decreased in crude protein-level and which were also deprived of water.

Studies in this laboratory on the basal nitrogen catabolism and protein requirements of African cattle have shown that low urea nitrogen excretion may be related to the energy as well as the protein content of the ration. Concomitant work has shown also that steers fed rations with an adequate energy content appear to utilize dietary nitrogen much more efficiently than previously recorded. These experiments and the results are briefly described in this article. The nitrogen metabolism of the animals concerned was examined by means of balance trials and the determination of the percentage distribution of nitrogen in the urine.

Twelve Afrikander steers and twelve Mashona steers (a local indigenous breed) were used in the trials. Each breed comprised three groups of four animals aged approximately one, two and three years respectively. Four different rations (crude protein contents 5, 7, 9 and 11 per cent) were fed to each group with the treatments arranged factorially. The rations were formulated to be approximately iso-caloric and consisted of 60 per cent concentrates (a mixture of molasses, cassava, maize and cotton-seed cake) and 40 per cent roughages (lucerne and Rhodes grass hay). The variation of crude protein content was obtained by adjusting the relative amounts of cassava and cotton-seed cake in each feed. The feeds were milled, mixed and cubed so it was impossible for any of the animals to select the more palatable components of the diet. The amount of feed offered to each animal was related to its body-weight and based on the finding of Blaxter *et al.*[4] that the voluntary feed intake of ruminants is directly related to $W^{0.73}$. The actual weight of feed offered (in lb.) was equal to $0 \cdot 152\ W^{0.73}$, where W was the live-weight of the animal in pounds.

In general, the feeds were readily eaten, and only very small mounts of waste were recorded except with the 5 per cent crude protein ration, where feed refusals were appreciable. Access to water was provided at all times. Each nitrogen balance trial consisted of a ten-day preliminary period followed by a seven-day collection period, when total fæcal and urinary collections were made. The total nitrogen intake, fæcal excretion and urinary excretion of each animal was determined for the seven-day collection periods. In addition, the urine samples were analysed for urea, ammonia, creatinine, uric acid, allantoin and hippuric acid content. In every nitrogen balance trial all the 24 animals retained nitrogen, that is, were in positive nitrogen balance. This indicates that the amount of nitrogen supplied was always in excess of the animals' maintenance requirements. The apparent nitrogen digestibility of the feeds did not vary to any significant extent with the age or breed of the animal. The detailed results of these two aspects of the work, together with the feed consumption of the cattle as a function of body-weight, will be presented in another communication.

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* Seconded from the Federal Ministry of Agriculture.

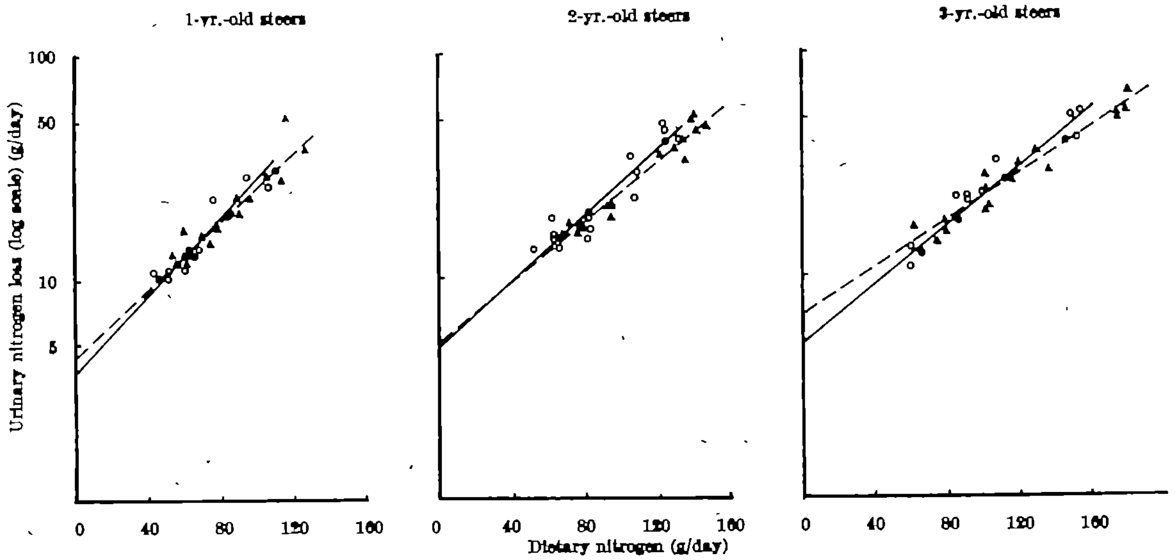

Fig. 1. Urinary nitrogen loss as a function of intake of nitrogen of six groups of African cattle. ○, Mashona; △, Afrikander

In Fig. 1 the logarithm to the base ten of daily urinary nitrogen excretion is plotted against the total dietary nitrogen for each animal in the six breed/age groups. For all groups there is a very high correlation between the two sets of values and the relationship in each case is apparently linear. Extrapolation to zero nitrogen intake gives an estimate of endogenous nitrogen catabolism of the cattle of varying body-weight under trial. These estimates are given in Table 1 together with the mean body-weights of the four animals in each group and the endogenous nitrogen calculated from the following well-used relationship of Brody[7]. Endogenous nitrogen equals 146 $W^{0 \cdot 72}$, where it is expressed in milligrams per day, and W is the body-weight of the animal in kilograms. Examination of the values in Table 1 shows that the endogenous nitrogen obtained is approximately 50 per cent of that given by the Brody relationship.

Table 1. MEAN BODY-WEIGHTS AND ENDOGENOUS NITROGEN (E.N.) CATABOLISM (DIRECTLY ESTIMATED AND CALCULATED USING THE BRODY FORMULA) FOR THE SIX GROUPS OF STEERS

Animal	Mean weight (kg)	E.N. estimated (g/day)	E.N. (Brody) (g/day)
1-yr.-old Mashona	242·9	3·91	8·06
1-yr.-old Afrikander	284·5	4·44	9·03
2-yr.-old Mashona	341·0	4·95	10·31
2-yr.-old Afrikander	401·9	5·06	11·62
3-yr.-old Mashona	394·3	4·96	11·46
3-yr.-old Afrikander	484·5	6·80	13·32

The results of the distribution of nitrogen in the urine of the 24 steers, which are detailed in Table 2, may be summarized as follows. A progressive reduction in the crude protein content of the ration produced a decrease in the amount of urea, ammonia, uric acid, hippuric acid and, in general, allantoin excreted. Creatinine excretion was fairly constant and not affected by a decrease of nitrogen intake. It was, however, closely related to the body-weight of the animal, which agrees with the conclusions of Brody[7]. The largest relative decrease was shown by urea. During the periods of feeding the 5 per cent crude protein ration the urea excretion of the steers was 3 per cent or less of their output on the 11 per cent crude protein feed. In fact, the decrease of urinary nitrogen with protein-level of the ration was mainly due to this declining output of urea.

Table 2. AVERAGE DISTRIBUTION OF NITROGEN (g/DAY) IN THE URINE OF FOUR STEERS OF SIX BREED/AGE GROUPS FED FOUR DIFFERENT DIETS

Animal	Per cent crude protein of feed	Total N	Ammonia N.	Urea N	Creatinine N	Hippuric acid·N	Uric acid N	Allantoin* N	Unidentified N
1-yr.-old Mashona	5	10·872	0·877	0·324	2 099	1·168	0·451	4 912	1·041
	7	12·246	0·736	1·310	2·385	1·713	0·931	4·051	1·120
	9	15·555	0·786	4·561	2 267	1·410	0·695	5·653	0·184
	11	26 665	1·742	11·341	2·434	1·661	0 826	8·100	0·561
1-yr.-old Afrikander	5	12·211	0·527	0·480	2·404	1 096	0·344	5·296	2·064
	7	16·136	0·785	3·023	2·898	1·352	0·767	5·077	2·234
	9	20 225	1·044	5·981	2·514	1·613	0·728	8·250	0·095
	11	36·618	1·856	18·359	3·013	1·371	1 033	10 246	0·740
2-yr.-old Mashona	5	14·117	0·492	0·387	2·654	1 486	0·472	7·261	1·366
	7	17·084	1·349	2·184	3·116	1 445	0·911	7·569	0·510
	9	26·184	1·375	9·967	2·967	1·985	0·749	8 520	0·621
	11	44·080	2 459	22·840	3·069	1·736	1·030	9·257	3·749
2-yr.-old Afrikander	5	16·446	0·496	0 611	3·583	1·749	0·565	9·781	−0·289
	7	20 466	1·367	3·092	3 720	1·265	0·964	9·423	0 635
	9	37·267	1·456	16·252	3·619	2·220	0·799	11·601	1 320
	11	49·329	2·650	26·025	3·769	2·942	0·985	10·573	2·386
3-yr.-old Mashona	5	16·287	0·717	0·787	2·969	1·274	0 693	7·185	2·664
	7	19·347	0·868	4·724	4·125	0·791	1·044	6 659	1 138
	9	26·181	—	—	3 167	—	—	—	—
	11	45 896	3·220	24·945	3·750	0 545	1·524	8·571	3 340
3-yr.-old Afrikander	5	18·703	0·775	0 515	4·059	1·347	0·686	8·460	2·862
	7	23·062	1·338	4·726	4·734	0 954	1·037	8 032	2 241
	9	30·802	—	—	3 900	—	—	—	—
	11	56·499	2 926	30·640	4 010	0 997	1·348	12·848	3 732

* Determined by the Larson method. Young and Conway (ref. 9) showed that this method gives values averaging 33 per cent higher than estimates by the Rimini-Schryver reaction.

Table 3. AVERAGE PERCENTAGE DISTRIBUTION OF NITROGEN IN THE URINE OF 24 ANIMALS FED FOUR DIFFERENT DIETS AND THE MEAN BIOLOGICAL VALUES OF THE PROTEIN IN THE DIETS

Per cent crude protein of feed	Biological value of dietary N	Ammonia N	Urea N	Creatinine N	Hippuric acid N	Uric acid N	Allantoin N	Unidentified N
5	82·5 ± 3·19	3·84	3·52	20·02	9·40	3·64	48·49	11·08
7	78·7 ± 3 78	5 96	17·04	19·29	7 54	5·28	37·35	7·45
9	73 2 ± 4·55	4·84*	35·14*	12·01*	7 64*	3·27*	35·20*	1·89*
11	62·9 ± 6·25	5 79	50·97	7·85	3·80	2·66	23 70	5 22

*Mean values for 16 animals.

From Table 3, it will be seen that urea is only a minor constituent while allantoin is the major nitrogenous constituent of the urine during the periods of lowest nitrogen intake. Table 3 also lists the mean biological values of the protein in the four diets as a measure of the efficiency of protein utilization by the 24 steers. The biological values were calculated by the Thomas–Mitchell method using the values obtained for metabolic faecal nitrogen (0·366 g nitrogen per 100 g of dry matter intake) and endogenous nitrogen (endogenous nitrogen in mgm = 95·6 $W_{\text{kgm}}^{0\cdot 675}$). A reduction in biological value as the protein-level was increased is apparent from the results. Further, it is interesting to note that the highest biological values are obtained when the urea excretion is low and the amount of allantoin excreted relatively high.

Two important conclusions may be drawn from the results of the trials. The two breeds of African cattle studied appear to have a low endogenous nitrogen catabolism. This suggests that their protein requirements for maintenance are also low, which supports the recent work of Elliott[5]. In addition, many people from field observations have suggested that indigenous cattle in Central Africa are better adapted to withstand a deficiency of protein than exotic or introduced cattle. It is possible that this adaptation may be manifested in a reduced protein requirement for maintenance. Secondly, cattle fed diets with an adequate energy but very low protein content excrete very small amounts of urea. Reasons for this low excretion are suggested by the work of Chalmers *et al.*[8] and that of Somers[4]. The former workers found that ruminal production of ammonia, the precursor of urea, is low when the diet fed contains adequate energy in the form of available or readily fermentable carbohydrate. Somers concluded that in sheep the return of nitrogen as urea to the rumen by the saliva is likely to be quantitatively important when the intake of nitrogen is low. If both these mechanisms are operating it is reasonable to deduce that the excretion of urea in the urine will be very small.

The results of the trials also showed that the most efficient protein utilization was associated with low urea excretion or alternatively a high percentage of the nitrogen in the urine present as allantoin. The ratio of urea nitrogen to allantoin nitrogen in the urine may be a useful index of protein utilization by the ruminant.

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REPRODUCTIVE PERFORMANCE OF FEMALE MINIATURE SWINE INGESTING STRONTIUM-90 DAILY

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A LONG-TERM investigation designed to determine the biological effects of daily strontium-90 ingestion in miniature swine has been in action here since 1958. The ultimate objective of the work is to provide data which will assist in evaluating the hazards of dietary strontium-90 for man or his progeny. The animals being used in these investigations are Pitman–Moore miniature swine which have a mature weight of 60–80 kg[1]. The dietary requirements, gastrointestinal tract, and bone mass of miniature swine are quite similar to man. These factors, along with a relatively long life-span, dictated their use in this work.

In considering the long-term effects of any toxic agent, one area of concern is the influence on the reproductive performance of the individual. As shown by the large amount of pertinent data presented at a recent conference on this subject[2], an extensive amount of work has been done on the effects of radiation on reproduction. For the most part, this work was performed on the smaller, commonly used laboratory species. Almost without exception, these experiments have utilized external whole-body γ-, neutron- or X-irradiation. This article summarizes some data on the reproductive performance of a large animal where the source of radiation is internal.

The basic experimental procedure as applied to each level of radionuclide feeding is shown in Table 1. A more detailed description of the experimental design and the procedures utilized has been presented elsewhere[3]. Feeding levels used are 0, 1, 5, 25, 125, 625 and 3,100 μc. of strontium-90 per day. At the two highest levels, the basic experimental procedure is not completed because of the early death of the original females and/or their offspring.

All animals are maintained in group housing, which is equipped with individual feeding facilities to control dietary intake. Strontium-90 is administered each day in a single large feed pellet with the morning aliquot of the ration.

Table 1. BASIC EXPERIMENTAL PROCEDURE

Original females	^{90}Sr feeding initiated at 9 months of age. Breeding to control male initiated at approximately 9·5 months of age.
F_1 generation	^{90}Sr available in utero via placental transfer; suckling period via the dam's milk; 6 weeks to 3 months of age, ^{90}Sr fed at one-quarter the dam's level; 3–6 months of age, ^{90}Sr fed at one-half dam's level; 6 months of age ^{90}Sr fed at dam's level. Breeding to control male begun at 9 months of age.
F_2 generation	Same procedure as F_1 generation, except animals are not bred.

All females scheduled for breeding are checked daily with a vasectomized male to determine if they are in oestrus. Bred females are removed from group housing to individual farrowing pens approximately one week before they are due to farrow. All offspring are weighed shortly after birth.

No apparent differences have been noted in the frequency and duration of the oestrus cycle or in the number of repeat breedings. Difficulties associated with parturition have been minimal in all groups.

Table 2 summarizes birth data on all litters born on the main strontium-90 experiment. No differences in litter size are apparent at levels up to 25 μc. ^{90}Sr/day. The differences which are seen at the 125 μc. and 625 μc.

Table 2. LITTER-SIZE AND BIRTH-WEIGHTS OF ANIMALS IN STRONTIUM-90 EXPERIMENT

	Adult level of ^{90}Sr feeding (μc./day)					
	0	1	5	25	125	625
No. of litters	10	45	19	22	6	2
Average litter size ± *S.D.*	5·6 ± 2·0	5·4 ± 1·5	5·2 ± 1·1	5·3 ± 1·7	4·0 ± 1·4	6·5
No. of offspring	56	242	98	117	24	13
Average birth weight in kg ± *S.D.*	0·73 ± 0·16	0·69 ± 0·15	0·75 ± 0·13	0·69 ± 0·13	0·71 ± 0·12	0·61 ± 0·14

^{90}Sr/day-levels may be real; however, insufficient numbers preclude definitive conclusions. No great differences in average birth weight are noted between levels. At the 625 µc. ^{90}Sr/day-level, there was a suggestion of change with one of the two litters born being of subnormal size, while the other was of normal size. Out of the litters (110) observed to date, only five offspring were stillborn, one control and two each at the 1 µc. and 5 µc. ^{90}Sr/day-levels; this is well within the normal range.

At the 3·1 mc. ^{90}Sr/day-level, the animals succumb at about 100 days after initiation of the feeding, which does not allow time for a term pregnancy. At the time of the death of the animals, developing fœtuses were observed. These fœtuses appeared to be of normal morphology and size. A blood sample obtained from one of these fœtuses was examined and exhibited normal values. Presumably, had the dam survived to term, viable offspring would have been born.

It can be generalized from the experimental data presented that for a large animal comparable in size to man, viable offspring of normal morphology will be born to females fed high levels of strontium-90 daily if the animals survive long enough to allow pregnancy to go to term. It should be recognized that the limited data presented here for higher levels might not permit detection of subtle changes in reproductive performance which would be readily apparent with a larger number of animals. In this regard, it is intended that a more comprehensive statistical analysis of the data will be undertaken when additional data are available for the higher feeding-levels.

These results are not surprising in view of the radionuclide under consideration and its metabolic and physical characteristics. Strontium, with a metabolic pattern analogous to calcium, concentrates almost exclusively in the osseous tissues of the animal. Further, strontium-90, with a half-life of approximately 28 years, decays with emission of a 0·54-MeV β-particle. Its daughter, yttrium, with a half-life of 64 h, decays with emission of a 2·27-MeV β-particle. The range of these β-particles in bone is not great, about 1 mm for the higher-energy β and about 0·2 mm for the lower energy; in soft tissue, the range is somewhat greater.

On the basis of these physical factors, it is readily apparent that in a large animal essentially all the radiation energy from absorbed strontium-90 would be released in bone or adjacent tissue. Furthermore, since the reproductive tissues are not contiguous to bone, the radiation dose from this source would be negligible. Perhaps the greatest source of radiation to the reproductive tissues of the female will be from unabsorbed strontium-90 in the gastrointestinal tract. Considering the thin wall of the gastrointestinal tract, the large percentage of ingested strontium-90 that is not absorbed (about 90 per cent in the adult), and the proximity of the ovaries to the gastrointestinal tract, this may be a significant source of radiation. Preliminary radiation dose measurements at the location of the ovary in an animal fed 3·1 mc. strontium-90 daily for about 100 days verified these assumptions.

Since the developing fœtus has the thickness of the uterine wall and the placental membranes and fluids between it and the gastrointestinal tract, it is doubtful if radiation originating from the gastrointestinal tract would be significant to the fœtuses. Undoubtedly, the greatest source of radiation to the fœtuses of a dam ingesting strontium-90 is the strontium-90 it incorporates into its own skeleton. The fœtuses have the advantage of the various discriminatory processes between the dam and her bone or plasma against strontium relative to calcium, and in addition have the further discriminatory process of the placenta. The overall effect is such that only about one-eighth as much strontium-90 relative to calcium is found in the fœtal bone as is found in the adult[4]. The radiation dose-rate to the fœtal bone reflects this discrimination, so that the average radiation dose-rate to the bone of a full-term fœtus at the 25 µc. per day-level is about 0·5 rad per day. Of significance, too, is that for earlier periods of fœtal life when ossification is less complete, the dose-rate would be much less. The same limitations of range of the β-particles that were pointed out for the adult animal apply to a considerable degree for the fœtuses, since at least in late stages in fœtal life, when ossification is progressing rapidly, the fœtuses have obtained considerable size.

It is well to point out, however, that the foregoing assumptions and the experiment so far only consider the effect of the radiation emitted by the strontium-90 on the reproductive tissues or fœtuses of the young adult swine. It is, of course, conceivable that if these females were bred later in life after a considerable radiation dose had been accumulated by the skeleton and associated tissues, the generalized debilitated condition of the animal might preclude normal reproduction or, possibly, if pregnancy were to occur, the associated stresses might jeopardize the life of the dam or offspring.

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DETERMINATION OF THE PARTICLE WEIGHT OF SPHERICAL VIRUSES

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RECENT advances in the sedimentation study of solutes resulted in the application of so-called short columns[1,2] which greatly reduces the time required to attain equilibrium conditions, especially for macromolecules. Further, the introduction of the Rayleigh interference optics permits the analysis of dilute solutions of the latter compounds[3]. This article describes the use of the Spinco analytical ultracentrifuge and the short-column method for the determination of the particle weight of plant viruses from sedimentation equilibrium conditions.

A Spinco model *E* ultracentrifuge equipped with Schlieren and Rayleigh interference optics and a rotor temperature control unit was used throughout these

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Table 1. PARTICLE WEIGHTS OF CERTAIN PLANT VIRUSES OBTAINED BY THE SHORT COLUMN SEDIMENTATION EQUILIBRIUM METHOD

	Conditions of centrifugation						Particle weight × 10⁻⁶	
Virus	Virus concentration (%)	Virus concentration (Fringes)	Observed column height (mm)	Rotor temp. (°C)	Speed of rotor (r.p.m.)	Concentration difference (fringes)†	Calculated (M_e)*	Literature (M)
TYMV	0·16	5·20	1·17	18·5	1,180	4·50	5·0	5·0 (ref. 4)
BMV	0·24	8·69	0·90	28·3	1,156	5·11	5·3	4·6 (ref. 5)
SBMV	0·18	6·36	1·27	16·5	1,495	9·80	6·0	6·6 (ref. 6)
BSV	0·15	5·69	0·98	20·0	1,240	7·50	9·7	8·9–10·6 (refs. 7–14)

*The partial specific volumes used for the calculation of the particle weights were 0·660 for TYMV (ref. 4), 0·710 for BMV (ref. 5), 0·696 for SBMV (ref. 6) and 0·712 for BSV (ref. 7).

† Concentration difference across the cell at equilibrium expressed in number of fringes.

M_e = particle weight obtained by sedimentation equilibrium.

studies. For the determination of the degree of homogeneity and the sedimentation coefficients of the viruses, rotor speeds between 12,590 and 35,600 r.p.m. were selected. A capillary type synthetic boundary cell and the interference optics were used for the measurements of the concentrations of the viruses. The rotor speed for these experiments was, initially, 7,000 r.p.m. and after 25 min was reduced to approximately 4,000 r.p.m. For the equilibrium runs carried out at very low revolutions the speed of the rotor could be satisfactorily controlled by using an *An-J* rotor, the weight of which is 22 lb. The average speeds were derived from the counter of the Spinco ultracentrifuge. The variation in speed amounted to ± 3 per cent. Again the Rayleigh interference optics was found advantageous as a means of recording the distribution of the virus in dilute solutions. The temperature of the rotor, although constant for each run, was in the range of 18°–28°. The calculation of the particle weights was carried out according to Richards and Schachman[8] using $c_b - c_m/c_0$ (Table 1) and also by the slope of the plot ln c versus x^2 (Fig. 1).

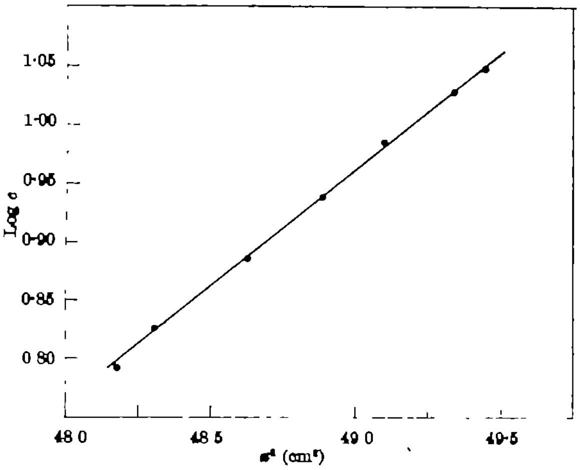

Fig. 1. Plot of log c versus x^2 from sedimentation equilibrium data for brome mosaic virus. The conditions of the experiment are given in Table 1

The following viruses were examined: turnip yellow mosaic virus (TYMV) and bushy stunt virus (BSV) (Dr. C. Knight, Virus Laboratory, University of California, Berkeley), brome mosaic virus (BMV) (Dr. M. Brakke, Department of Plant Pathology, University of Nebraska, Lincoln) and southern bean mosaic virus (SBMV) (Dr. W. C. Price, Department of Plant Pathology, University of Florida, Gainesville). On sedimentation velocity analysis these viruses, after dilution with 0·1 M sodium chloride to give a concentration between 0·2 and 2 per cent, appeared essentially homogeneous at the time of our experiments. The calculated sedimentation coefficients, corrected to standard conditions, were 98*S* (virus concentration 2·0 per cent) and 115*S* (0·3 per cent) for TYMV, 90*S* (1·0 per cent) and 91*S* (0·25 per cent) for BMV, 112*S* (0·7 per cent) for SBMV and 114*S* (2·0 per cent) and 131*S* (0·3 per cent) for BSV. These values are in good agreement with those reported in the literature[4-6,9]. TYMV, SBMV and BSV each contained a minor component which accounted for less than 1 per cent and the sedimentation coefficients of which were very similar to those of the viruses.

Table 2. ATTAINMENT OF SEDIMENTATION EQUILIBRIUM OF CERTAIN PLANT VIRUSES AS JUDGED BY THE CONSTANCY OF THE NUMBER OF FRINGES OF THE OPTICAL PATTERNS

Time of centrifugation (h)	No. of fringes SBMV	No. of fringes BSV
1	5·0	2·6
2	6·0	4·5
3	6·5	5·5
4	7·8	5·5
5	8·5	6·0
6	9·0	7·0
7	10·0	7·6
20	9·8	7·5

The conditions of centrifugation are given in Table 1.

The particle weights of the viruses investigated are given in Table 1 and are in close agreement with the known values. Further, the particle weight of BMV and BSV calculated from the slope of the plot ln c versus x^2 was found to be $5{\cdot}2 \times 10^6$ and $9{\cdot}4 \times 10^6$, respectively. For all determinations, rotor speeds between 1,000 and 1,300 r.p.m. and virus concentrations between 0·15 and 0·24 per cent proved to be optimal. A higher speed of 1,967 r.p.m. consistently resulted in accumulating virus material at the bottom, solvent-oil interface of the cell. A minute accumulation of virus at this interface could readily be detected by Schlieren and at low concentrations by the interference optics (Fig. 2). If solutions with concentrations above 0·35 per cent were analysed even at a speed of approximately 1,000 r.p.m., deposition of solute was again noted (Fig. 2). The observed height of the columns for the experiments reported in Table 1 was close to 1 mm. Under these conditions, equilibrium as judged by the constancy of the fringes of the optical patterns (Table 2) was obtained after 6–8 h of centrifuga-

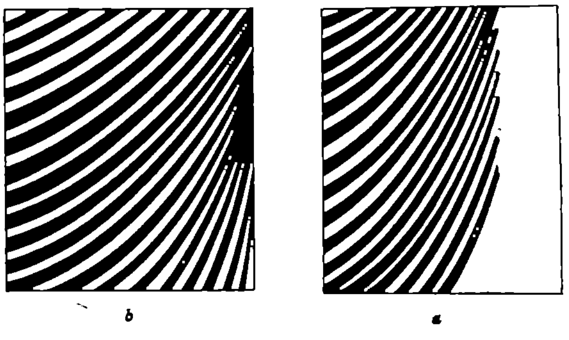
b *a*

Fig. 2. Interference patterns from ultracentrifugation analyses of SBMV. The pattern on the right (Fig. 2*a*) was obtained by centrifugation of a 0·35 per cent solution of SBMV at 1,495 r.p.m. Accumulation of virus particles at the bottom of the cell is indicated by the loss of fringes in this section. If the virus concentration was decreased to 0·18 per cent but the analysis performed at the same rotor speed (Table 1) no accumulation of virus occurred at the bottom of the cell (Fig. 2*b*)

tion. However, all runs were continued for a total of 20 h to ascertain that true sedimentation equilibrium had been attained and that no material was deposited at the bottom of the cell. For the viruses studied the time for attainment of equilibrium derived from the equation of Van Holde and Baldwin[1] for a column height of 1 mm and an assumed F^{α}-factor of less than 1·0 would be less than 24 h. Thus, for the short-column method, if applied to macromolecules of the size and shape of those of the viruses investigated, the rotor speed should probably not exceed 1,400 r.p.m. nor the virus concentration be in excess of 0·25 per cent.

The first report of the particle weight of a virus derived from sedimentation equilibrium is probably that of McFarlane and Kekwick[15], who investigated BSV using a column of approximately 2 mm and a speed of 1,500 r.p.m. Equilibrium was obtained between 48 and 96 h and a value of $7·6 \times 10^6$ was calculated. Recently, Beams *et al.*[16], using a magnetically supported ultracentrifuge, briefly reported the particle weight of BSV and SBMV to be $7·8 \times 10^6$ and $6·3 \times 10^6$, respectively. Although the value for BSV obtained by Beams *et al.* agrees with that of McFarlane and Kekwick, there is a discrepancy between these values and the value reported herein. Determination of the particle weight by other methods such as light scattering[10], direct particle counting[11], sedimentation and diffusion[7–9], sedimentation and intrinsic viscosity[12] and X-ray scattering[13,14] yielded values from 8·9 to $10·6 \times 10^6$ with an average of $9·7 \times 10^6$, which agree well with our data.

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DONOR AND HOST CONTRIBUTION TO SPLENOMEGALY IN HOMOLOGOUS MOUSE CHIMÆRAS

By IRMGARD M. ZEISS* and MILES FOX†

ANIMALS tolerating the presence of living homologous spleen cells in their organism may be described as 'homologous spleen cell chimæras'. Splenomegaly is a recognized phenomenon in the early stages of such chimærism. It is caused, or at least accompanied, by an immunological reaction of donor cells against their host, the so-called 'graft-versus-host' reaction. This may be concluded from observations in suitable donor–host combinations. Six relevant examples based on evidence published by Simonsen[1] and Michie, Woodruff and Zeiss[2] are summarized in Table 1. The apparent relation of the graft-versus-host reaction to splenomegaly, together with the fact that immunological reactions involve cell proliferation[3–5], suggests multiplication of donor cells as the direct cause of the spleen enlargement. Gorer and Boyse[6], in distinguishing between the host and donor component in chimæra mouse spleens by means of specific cytotoxic antisera, found that donor cells may constitute the vast majority of the total spleen cell population, which implies a considerable donor cell multiplication. However, the proportion of each component seemed to depend on the genetic relation of the host and donor strains used. Biggs and Payne[7] traced the donor cells in chimæra chick embryos by the sex chromosome difference, and found 44 per cent of the dividing cells to be of donor origin.

On the other hand, according to Davies and Doak[8], donor cells—if at all demonstrable—form a very small minority of the dividing cells in enlarged spleens of chimæric mice. Similarly, the analysis of mouse chimæra spleens by the discriminant spleen assay[9] nearly always revealed a donor cell minority[9]. Moreover, Howard, Michie and Simonsen[10] were able to show that in F_1 hybrid mice injected with spleen cells from a parent strain splenomegaly is mainly due to a host cell proliferation. The use of different test systems may to some extent account for the discrepancies in the various observations.

The evidence now seems to favour the view that at least in mouse chimæras splenomegaly is mainly due to host cell proliferation. But this does not rule out the possibility of a phase of intense mitotic activity of donor cells which must be expected to occur if the postulated immunological reaction does in fact take place. The present investigation was therefore designed to permit the detection of donor and host cell proliferation, even if one of these factors were relatively small. The study was based on the analysis of large numbers of dividing cells from chimæra spleens, utilizing the abnormally short chromosome pair of the *CBA*/*T*6 mouse as a marker for donor cells.

Induction of chimærism. Members of *A*-strain litters were injected intravenously within 24 h of birth with 15 million adult *CBA*/*T*6 spleen cells. Test animals and uninjected litter mate controls were killed 3, 5, 6, 7, 9 and 10 days after injection and their body and spleen weights recorded. These specimens formed the 'main experimental series', but in addition a one-day specimen and two specimens each for the second and sixtieth day after injection were available.

Chromosome preparation. The methods described by Ford and Hamerton[11] and Rothfels and Siminovitch[12] were combined and modified to allow analysis of a large number of dividing cells in each specimen. A detailed description of the procedure has been published elsewhere[13].

Evaluation of specimens. In all test and control preparations of the main series the number of metaphase nuclei

Table 1. PRESENCE OR ABSENCE OF SPLENOMEGALY IN DIFFERENT DONOR–HOST COMBINATIONS

	Recipients, strain and age	
Donor, strain and age	*A* new-born	(*B*/*C*) F_1 4–8 days old
B adult	splenomegaly	splenomegaly
A adult	no splenomegaly	no splenomegaly
B foetal	no splenomegaly	no splenomegaly

Splenomegaly only occurs if the donor is capable of reacting immunologically against the host, while the host is defenceless, or virtually defenceless.

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Table 2. SPLENOMEGALY IN THE HOMOLOGOUS CHIMÆRAS

Litter No.	Age		Relative spleen weights* Chimæras	Relative spleen weights* Controls	Ratios chimæras/control
G68	3 days	1	56	43	1·30
		2	67		1·56
G69	3 days		75	57	1·32
G68	5 days		103	56	1·84
G69	6 days		106	82	1·29
G67	7 days		143	78	1·83
G66	9 days		127	79	1·61
G64	10 days		161	79	2·04
G65	10 days		146	83	1·76

$$* \text{Relative spleen weight} = \frac{\text{spleen weight} \times 1{,}000}{\text{body-weight}}$$

in 10,000 nuclei was counted, and in each of the chimæra preparations the chromosomes of 363–790 metaphase nuclei, mostly divided into batches of 100, were screened for the presence or absence of the marker chromosomes. Only those nuclei were included in the analysis in which at least 39 of the 40 chromosomes could be clearly distinguished. The reliability in typing metaphase nuclei was checked by 151 blind tests in pure preparations of *A* and *CBA/T*6 spleen cells. In the first 52 tests 6 out of 17 *A* cells and 2 out of 35 *CBA/T*6 cells were incorrectly classified. In the following 99 tests only 1 out of 62 *A* cells was filed in the wrong category, while all 37 *CBA/T*6 cells were correctly identified. There was thus a possibility of overestimating the number of donor cells in the test spleens. To avoid this pitfall all cells in the chimæra specimens which could not be classified with certainty were excluded from the analysis. Of those cells thought to be of donor type the position on the slide was noted and the cells were rechecked after the discernment had been re-sharpened by studying pure preparations.

In four instances the numbers of dividing donor cells were observed in 50–70 batches of 10 cells, which were later combined to make units of 100. In these specimens the frequency distribution of dividing donor cells was examined for its agreement with that expected in a binomial distribution. The observed data gave a satisfactory fit with those predicted and it was thus assumed that the distribution was in fact binomial. With large samples the binomial distribution very closely approaches the normal distribution. In this study, therefore, the mean number of dividing donor cells in a specimen and the 95 per cent confidence interval were estimated from the results in batches of 100 cells according to the formulæ for normally distributed items.

Splenomegaly. The basic requirement of the study—that all chimæras should show splenomegaly—was actually accomplished, as may be seen from Table 2. It seems convenient and permissible for judging the significance of the spleen enlargement to adopt the standards elaborated by Michie, Woodruff and Zeiss[4], because the *A*-strain recipients of the present experiment were derived from one of the colonies on which the data of reference are based. Accepting that for a comparison of one test and one control animal a relative spleen weight ratio of 1.32 significantly exceeds the normal variability, only two of the chimæras—*G*68/1, 3 days; and *G*69/3, 6 days—fail by a little to comply with this standard. As both these animals were killed before splenomegaly reached its peak it may be assumed that by a later date they may well have had acquired a significant enlargement. It seems justifiable therefore to conclude that a graft-versus-host reaction was in process in every one of the chimæras studied.

Rate of spleen cell proliferation. The use of 'Colcemid'—though essential for suitable chromosome preparations—rendered the estimation of the true mitotic rate in chimæra and control spleens impossible. However, as both test and control animals had been exposed to the effect of similar doses of the drug, a comparison of the number of metaphase nuclei in 10,000 nuclei allows at least a relative evaluation of mitotic activity. From this it is apparent that during the 3–10-day period the mitotic activity in chimæra spleens always exceeds that of their litter mate controls (Table 3). The mean excess of metaphases in chimæras (77·22 per cent) was found to differ significantly from zero; $0·02 < P < 0·05$.

In the controls cell proliferation seemed to proceed at two markedly different levels. Statistical analysis of the data shows that the mean number of 99·33 metaphases/10,000 nuclei in the 3–6 day specimens differs significantly from the mean (231·20) of the 6–10 day specimens ($P < 0·01$). This division into two phases of mitotic activity is somewhat obscured in the chimæras (Table 3). The difference between the mean numbers of metaphases in the corresponding time intervals is suggestive but not statistically significant ($0·05 < P < 0·10$).

Donor and host contribution to cell proliferation. The fact that the chimæra spleen cells are a mixed population consisting of unknown quantities of host and donor cells makes the evaluation of the results in absolute terms impossible. In spite of this, two definite statements can be made; first, during the 3–10-day period, dividing donor cells, invariably demonstrable, constitute only a minority—though of varying magnitude—of all dividing cells in the chimæra spleens; and secondly, however high their absolute mitotic rate may be, depending on the unknown number of donor cells which settled in the chimæra spleen, donor cell multiplication alone cannot account for the increased overall mitotic activity of the chimæras. On the contrary, even after subtracting the donor contribution from the total number of metaphases there remains a considerable excess of host metaphases, which is probably significant (Table 3). The calculated average excess of 68 per cent of host metaphases underestimates the true rate of excess of host cell multiplication, as it is related to 10,000 chimæra (host plus donor) nuclei instead of 10,000 host nuclei. For this reason the difference between this average excess and zero (for which P was found to be $< 0·1$ but $> 0·05$) may be more significant than is apparent here.

Table 3. COMPARISON OF THE MULTIPLICATION RATE OF CELLS IN CHIMÆRA AND CONTROL SPLEENS AND CONTRIBUTION OF HOST MITOSES TO SPLENOMEGALY

Specimen	Age (days)	Metaphase nuclei/10,000 nuclei Chimæra	Metaphase nuclei/10,000 nuclei Control	Ratio chimæra/control*	Ratio host contribution† in chimæra/control
G68/1	3	143	101	1·42	1·34
G68/2	3	180		1·78	1·72
G69/3	3	141	123	1·15	1·13
G68/3	5	311	74	4·20	4·15
G69/4	6	271	229	1·18	1·18
G67/3	7	365	253	1·44	1·28
G66/2	9	471	216	2·18	2·02
G64/2	10	196	173	1·13	1·10
G65/3	10	369	285	1·29	1·24

* The average percentage excess (77·22 per cent) of chimæra metaphases over control metaphases differs significantly from zero; $0·02 < P < 0·05$.

† The calculation of the host contribution is based on the mean percentage of donor cells in all dividing cells of the specimen (Table 4). For the difference between the mean excess (68 per cent) and zero P was found to be $0·05 < P < 0·1$.

Table 4. DONOR CELL CONTRIBUTION TO SPLEEN CELL MULTIPLICATION IN CHIMÆRA SPLEENS

Specimen	Age (days)	Absolute spleen weight (mg)	Metaphase nuclei analysed Sample size	Metaphase nuclei analysed No. of samples	Mean percentage donor cells in analysed metaphases	95 per cent confidence interval
G68/1	3	12	100 108 113 469	4	5·51	+1·75 – +9·27
G68/2	3	15	50 164 63 102	4	3·93	+0·35 – +7·51
G69/3	3	19	100	7	1·43	−1·59 – +4·45
G68/3	5	26	100	5	1·40	−0·42 – +3·22
G69/4	6	47	100	6	0·50	−1·18 – +2·18
G67/3	7	47	100	6	11·33	+6·01 – +16·65
G66/2	9	42	100	6	7·33	+3·83 – +10·83
G64/2	10	66	100	5	2·60	−0·44 – +5·64
G65/3	10	50	100	6	4·67	−0·65 – +9·99

Table 5. EARLY AND LATE DATE CHIMÆRAS. COMPARISON OF SPLEEN WEIGHT AND MITOTIC ACTIVITY, AND ESTIMATION OF THE NUMBER OF DIVIDING DONOR CELLS

Age (days)	No. of mice chim.	No. of mice contr.	Relat. spleen weight ratio chim./contr.	*Mitotic rate per cent chim.	*Mitotic rate per cent contr.	No. of metaphases analysed	Percentage donor mitoses spleen	Percentage donor mitoses marrow
1	1	1	0·98	0·01	0·30	50	0	—
2	2	1	1·08	0·03	0·65	100	0	—
60	2	2	1·64	0·00	—	300	2	0·5

* These figures are based on the estimated total number of cells and total mitoses in the spleen.

Donor cell multiplication. A comparison of the absolute spleen weights and the percentages of donor cells in dividing cells of the 3–6-day specimens (Table 4) suggests a constant absolute division rate in the donor cell population in the face of increasing numbers of dividing host cells. A striking break in this process of 'dilution' of donor mitoses by increasing numbers of host mitoses is apparent at 7 days, when donor mitoses gain considerable ground in spite of intense host cell proliferation (Tables 3 and 4). After this sudden outburst of donor cell multiplication, 'dilution' of dividing donor cells by rapid host proliferation (9 days) and a decline in donor cell multiplication (10 days) jointly seem to reduce the proportion of donor mitoses. There is thus evidence, in spite of the difficulties of obtaining information about the true rate of division in donor cells, which suggests that at about 7 days an enormous multiplication of donor cells occurs.

Observation in 1-, 2- and 60-day specimens. As may be seen from Table 5, there was no indication of splenomegaly in the 1- and 2- day specimens, nor were donor mitoses observed in the spleen. However, it is interesting to note that in these chimæras the mitotic activity, compared with that in their controls, appears considerably depressed.

In the 60-day specimens a minority of donor mitoses persisted, and was found to occur even in the bone marrow. Both chimæras had survived runt disease, and although smaller than their uninjected litter mates, were apparently healthy. The high relative spleen weight ratio, which might suggest a persistence of a sub-lethal graft-versus-host reaction, is partly the result of the lower body weight of these animals.

Discussion. The results of this experiment once again demonstrate the predominant role of host cell proliferation in chimæra splenomegaly, but they also reveal a phase of intense donor cell multiplication which may well be a decisive factor in the timing of peak enlargement.

Recently, the immunological capacity of new-born mice[14] and the importance of host contribution to splenomegaly in hybrid/parent strain chimæras (that is, F_1 hybrids injected with spleen cells from an adult parent strain donor)[13] have been demonstrated. The latter observation implies that a factor other than a host-versus-graft reaction—possibly a mechanism of self-protection—is involved in the host cell proliferation. Therefore three factors must be considered when trying to interpret the increased mitotic activity, and the rise and fall of host and donor cell multiplication in the chimæras of the work reported here; first, a graft-versus-host reaction; secondly, a host-versus-graft reaction; and thirdly, a hypothetical protection mechanism, which is set into motion by the effects of the graft-versus-host reaction. As the observations in hybrid/parent strain chimæras quoted here suggest that this protection mechanism is in action as early as 6 days, graft-versus-host and host-versus-graft reaction, and cell proliferation of such ætiology must be expected to occur at an even earlier stage. The 'dilution' of donor mitoses by increasing numbers of host mitoses in the 3–6-day specimens of this experiment may thus possibly illustrate the waning of the donor and host cell multiplication resulting from the partners' reaction against one another, and the commencement of the host's protective activity. This interpretation is further supported by the finding of Fox[15] that in hybrid/parent strain chimæras—(*CBA* × *C57BL*)F_1/*CBAT6*—a majority of donor mitoses (60 per cent) at the second day after injection is reduced to a minority (8–10 per cent) by 4 days.

In comparing these high proportions of donor mitoses in hybrid/parent strain chimæras with the data presented here, it would seem that the generally low level of donor mitoses in this study, besides reflecting the degree of immunological antagonism in the strain combination, is characteristic of homologous chimærism induced in new-born mice which, although they will eventually become tolerant of the donor cells, are initially capable of at least weekly reacting against them. (Eventual tolerance in the present chimæras—had they been allowed to survive—may be assumed by analogy with the observation that the injection of 9–15 × 10^6 *CBA* spleen cells into new-born *A* mice induced tolerance in most cases[2].)

Depression of mitotic activity in the very early stages after spleen cell injection seems to be a common feature in both hybrid/parent strain chimæras[15] and the homologous chimæras of this experiment. However, as non-specific causes cannot be ruled out yet, it would be premature to describe the phenomenon as an essential part of the graft-versus-host reaction.

The sudden increase of donor mitoses at day 7 again raises the question as to the nature of the proliferation. Is it of graft-versus-host reaction type or do donor cells suddenly join in the host's protection efforts? As a little later (at 9 days) the proliferation of host cells also reaches a peak, the feature might be explained as an overlap of a second immunological assault of the partners on one another and the host's renewed protective response, but no definite interpretation can be offered at the present time. The distinction between the three phenomena which might possibly be involved may, of course, have an important bearing on the concept of the mechanism which leads to immunological tolerance. If this later stage of host and donor cell proliferation was in fact the expression of a secondary immunological response of host and donor to one another's antigens, it could be assumed that tolerance is not achieved before such a reaction has occurred.

Most of this experimental work was carried out in the Department of Surgical Science, Edinburgh, where both of us were guests; one of us (M. F.) while holding a clinical research fellowship from the Medical Research Council, and the other (I. M. Z.) while on study leave from the University of Otago, New Zealand. We thank Prof. M. F. A. Woodruff for his hospitality and guidance. We also thank Mr. R. S. Booth, Mathematics Department, University of Otago, for his advice on the statistical handling of the data, and Drs. B. F. Heslop and N. W. Nisbet (Department of Surgery, Otago University), and Dr. D. Michie (Department of Surgical Science, Edinburgh) for criticism of the draft manuscript.

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BREEDING OF THE CHILEAN OYSTER (*OSTREA CHILENSIS* PHILIPPI) IN THE LABORATORY

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THE 1948 Lund University Expedition to Chile[1] collected samples of *Ostrea chilensis* from the northern part of the Golfo de Ancud and in the neighbouring Bahia de Ancud, where they were found on stony beaches and on sand and mud down to depths of 11 m. In one of the samples collected in November 1948, several specimens brooding eggs and larvæ were found which were stated to measure 0·5–0·75 mm and 0·7–1·2 mm respectively. This is abnormally large for *Ostrea* eggs and larvæ and suggested that all the larval development would occur while being brooded in the mantle cavity and that the duration of the planktonic stage would be very short. A similarly reduced planktonic stage has been recently reported for the New Zealand oyster, *O. lutaria*[2].

A consignment of *O. chilensis* was received in good condition in this laboratory on September 17, 1962, and placed in glass tanks with running sea-water. Within a few hours a number of early embryos were aborted, indicating that some of the oysters were brooding when they arrived. Four liberations of larvæ were obtained in October during which period the temperature of the water gradually decreased from 15° to 11° C. This range is approximately that found in the late spring and early summer on their natural grounds[3]. Thirteen liberations occurred in November when the temperature was being maintained at 11°–14° C.

The larvæ at liberation had a well-developed foot and eyespot, and mostly metamorphosed within a few hours on the glass and slate sides of the tank and on mussel shells suspended for spat collection in the tank. The shell shape agreed with that figured by Ranson[4] although the size was less than that estimated by Soot-Ryen[1]. The mean and range of shell-length of six liberations are given in Table 1.

Table 1

	Mean	Range
1	435μ	390–510μ
2	477μ	430–500μ
3	475μ	450–490μ
4	490μ	460–510μ
5	492μ	450–520μ
6	491μ	460–510μ

The larvæ were sufficiently active to swim up to the surface film, although the velum is small compared with that of *O. edulis*. The gill rudiment, consisting of 6–8 projections on the left side, is more pronounced than in the mature larvæ of *O. edulis*. Some larvæ isolated in a glass beaker had not become attached 24 h later, suggesting that clean glass was not a favourable surface, although two specimens had metamorphosed without attaching—a phenomenon which has also been seen in *O. edulis*. Some of the larvæ which had not metamorphosed were placed on mussel shell and two became attached in the next 5 h, indicating that metamorphosis can be delayed for at least 24 h.

Two brooding adults have been opened and the number of eggs or embryos estimated (broods of *O. edulis* of this size would contain about 500,000 embryos) (Table 2) Early embryos are white in colour, but at the later stages the brood is light brown in colour instead of the grey or black of *O. edulis*.

Table 2

	Mean diam.	Internal vol.	Dry meat wt.	No. of embryos
1	60 mm	15·0 ml.	1·18 g	66,600
2	51 mm	11·5 ml.	0 94 g	62,800

One adult was observed to be spilling a few eggs in the exhalent current. When opened few were in the inhalent chamber but there were many in the exhalent chamber and filling the water tubes of each demibranch, and more were seen issuing from the genital pore. The eggs, which measured 323 × 264μ, were exceptionally large for a lamellibranch with pelagic larvæ. Thorson[5] noted that the largest lamellibranch egg to develop into a pelagic stage (*Yoldia limatula*) has a diameter of 150μ; those species with larger eggs have a direct non-pelagic development. In *O. edulis* the egg is 150μ in diameter and in *O. lurida* 100μ. A number of individuals of *O. chilensis* have been observed to spill either eggs or early embryos in the exhalent current, and it is suggested that this is caused by the difficulty of forcing these very large eggs through the gills into the inhalent chamber.

Some of the adults which had spilled eggs or early embryos over a period of 3 days were separated from non-breeding individuals. When one was opened 11–13 days later the larvæ had grown to a mean shell length of 331μ at a temperature of 13°–15° C. When another was opened after 27–29 days the larvæ had a mean shell length of 390μ; the temperature in the second part of the period had been 9°–14° C. A sample of this brood was cultured and at 13°–14° C the shell length of the larvæ grew at about 10μ per day. A rudimentary foot was seen on the third day and on the seventh day the foot was well developed, a row of rudimentary gill filaments was visible and the eye spot was clearly developing. On the eleventh day one spat was found attached to the wall of the vessel and many on the fourteenth day. It is probable then that the incubation period at temperatures of 13°–15° C is about 5–6 weeks.

The spat have grown well after settlement and some exceeded 1 mm in length within 12 days at 13°–15° C. The very short free-swimming larval life suggests that *O. chilensis* might be a valuable species to introduce into British waters, since the loss of larvæ by tidal currents and predators would be substantially reduced and most of the settlement would take place near to the parent stock. The temperature at which breeding occurs is lower than the 17°–20° C required by *O. edulis*, and this could result in an earlier production of spat and, since the larvæ at release are more mature than those of *O. edulis*, spatfall would presumably be more reliable. There is the possibility, however, that production of larvæ would continue for a large part of the year, resulting in thin oysters with a proportion 'white sick' during the normal marketing season. In this case *O. chilensis* might be suitable only for the cooler waters of the British Isles. In external appearance it closely resembles *O. edulis* and in the imported sample the oysters were in excellent condition.

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LETTERS TO THE EDITOR

PHYSICS

Perturbations in the S_2 Spectrum

The pressure effect on the predissociation of diatomic molecules is an experimentally well established phenomenon; but the detailed perturbation mechanism is not yet clear. In this communication, some new observations are reported on the well-known predissociation in the $B^3\Sigma_u^- \rightleftarrows X^3\Sigma_g^-$ system of S_2 (ref. 1). In absorption, the v'' progression shows, in pure sulphur vapour, a sudden intensity increase starting from $v' = 9$. In the emission spectrum the $v' > 9$ bands are missing. If a large amount of foreign gas is added to the absorbing vapour, new perturbations occur in the absorption spectrum, and in the emission[2] spectrum the missing ($v' > 9$) bands appear[3].

Lochte-Holtgreven[3] finds the $v' > 9$ levels to be pressure perturbed; but Kondratjew and Olsson observe modifications of the rotational structure in the bands $v' < 9$, which are usually not perturbed. The perturbations in emission are due to collisional removal of the predissociation according to Asundi[2], but to the population increase of the levels $v' > 9$ according to Christy and Naudé[4]. The experiment reported here may clarify the foregoing process.

We essentially repeated Kondratjew and Olsson's experiments increasing the collisional effect. Nitrogen gas at 70 cm mercury was replaced by xenon gas (atomic weight 9 times greater) at a pressure at 2 kg/cm². The absorption spectrum of sulphur obtained in the presence of 2 kg/cm² of xenon contained in a cylindrical silica vessel 15 cm long is illustrated by spectrum *B* of Fig. 1. It may be compared with spectrum *A* obtained in absence of foreign gas. The comparison between the two spectra shows that the vibrational perturbation is partially removed when the pressure is increased. In spectrum *B* all bands have the same apparent structure and are regularly distributed in the spectrum, as though there were no perturbation or predissociation. As a quantitative check, the relative intensities of the bands should be compared with the relative transition probabilities in the $v'' = 0$ progression. The Franck–Condon factors are estimated by direct calculation of the overlap integrals using the Morse wave function. The results obtained are given elsewhere. In spectrum *A*, an important discontinuity is seen at $v' = 9$.

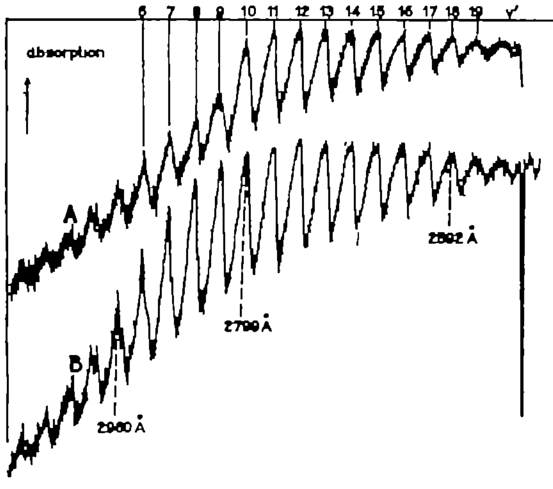

Fig. 1. Comparison of the microdensitometer tracing of spectrum *A* obtained in pure sulphur at low vapour pressure with that of spectrum *B* obtained in the presence of xenon at 2 kg/cm² pressure showing the removal of the perturbation by collision

The pressure-induced perturbation of the rotational lines was investigated with a 'JACO–EBERT' grating spectrograph and a 0·5 Å per mm reciprocal dispersion. The rotational absorption lines of the λ 2828·9 Å (0·9) band show a considerable broadening in the presence of xenon gas as compared with that in pure sulphur vapour. In the usual qualitative investigation of the effect of predissociation on the shape and the width of absorption lines, the inverse process of emission by atomic recombination is usually neglected. At low gas pressure the contribution of the emission is negligible; but, when the gas pressure is increased, the atomic recombination can no longer be neglected, for the intensity and shape of the emission lines depend not only on the relative values of radiationless and absorption probabilities but also on the atomic radiative recombination rate. It is indeed plain that if the atoms formed by photodissociation can partially recombine in a three-body collision process before they separate completely, the apparent radiationless transitional probability will be reduced.

In short, the foregoing experiments show that the pressure perturbations observed in the $B^3\Sigma_u^- \rightleftarrows X^3\Sigma_g^-$ band system of S_2 are of two kinds: (1) the vibrational perturbation closely connected with 'inverse predissociation' process; (2) the apparently independent rotational perturbation of the $v' < 9$ levels.

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Dependence of Angular Width of High-frequency Diffraction on Sound Wave-length

It has been well established[1,2] that, in the case of diffraction of light by very-high-frequency sound waves only the first order of diffraction appears in the field of view and it attains the maximum intensity when the sound wave-front is tilted to the corresponding Bragg reflexion angle. Any further tilt from this position results in gradual diminution of intensity and finally in the disappearance of the diffraction order. The angle over which the intensity of the diffraction order is distributed is called the 'angular width of high-frequency diffraction'.

David[3], following Brillouin's theory, gave for intensity I_{+1} of the first order the following expression, assuming the higher orders to be of negligible intensity:

$$I_{+1} = I_m \left[\frac{\sin \frac{\pi L}{\lambda^*} \varphi}{\frac{\pi L}{\lambda^*} \varphi}\right]^2 \qquad (1)$$

where I_m is the maximum intensity of the first order, L is the length of the sound path traversed by the light beam,

λ^* is the sound wave-length in the medium and φ is the angle of deviation of the sound wave-front from the Bragg position. It can be seen by differentiating the expression (1) that the angular width of high-frequency diffraction depends only on the value of the parameter $(\pi L/\lambda^*)$. That is to say, for very high frequencies and large lengths of the sound field, the phenomenon of characteristic reflexion of light gains importance. The angular width of diffraction does not depend on any other factor like the wave-length of the incident light or the intensity of the sound field.

It has been shown[4] that the intensity of the first order attains its maximum when $L = \mu_0\lambda^{*2}/\lambda$. This value of L makes the parameter $(\pi L/\lambda^*)$ equal to $(\pi\mu_0/\lambda).\lambda^*$—where μ_0 is the refractive index of the medium and λ is the wave-length of light used for diffraction—showing that the angular width of high-frequency diffraction is directly proportional to the sound wave-length in the medium.

Intense diffraction orders are produced at several ultrasonic frequencies and their intensity maxima at the corresponding Bragg angles are adjusted to be the same with the help of a photo-tube, by controlling the power input to the crystal. The crystal holder is tilted towards either side of the Bragg position and the exact position of disappearance of the diffraction order is judged by the photo-tube. The expression (1) clearly predicts the positions of disappearance, which occur whenever $\frac{\pi L}{\lambda^*}\varphi = n\pi$, where $n \neq 0$ is any integer. This can be simplified and written, in the case of the first order, as:

$$\varphi = \frac{\lambda^*}{L} \qquad (2)$$

The values of φ calculated from the relation (2) and those obtained experimentally by the photo-tube are tabulated in Table 1 for several sound frequencies utilized in this investigation using water as the medium for sound propagation.

Table 1. TEMPERATURE, 28° C; L, 1·5 CM

Frequencies of ultrasonics (Mc/s)	φ (in min of arc) Calculated from (2)	 Measured by photo-tube
23·06	15·0′	16·5′
49·97	7·0′	8·0′
73·04	5·0′	5·0′
91·20	4·0′	4·5′
144·0	2·4′	3·0′
164·0	2·1′	2·2′
184·0	1·9′	2·0′

Table 1 shows a remarkable agreement between theory and experiment. However, the measured values by the photo-tube are slightly higher than those calculated from expression (2) and this slight deviation is due to the fact that the sound field is not the ideal one with undamped amplitude as assumed in the theory.

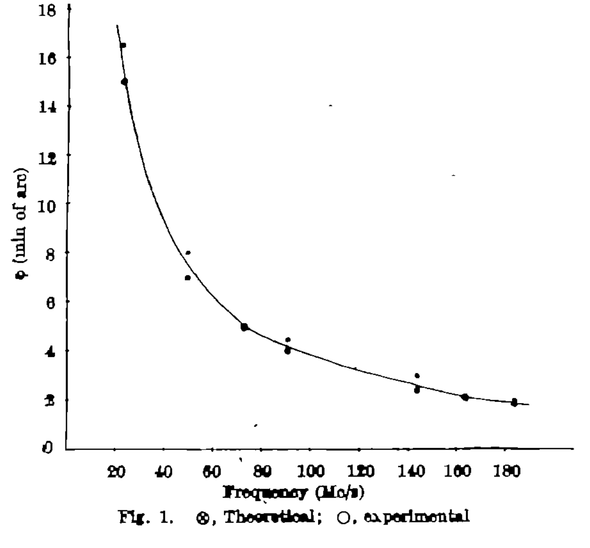

Fig. 1. ⊗, Theoretical; ○, experimental

A plot of the values of φ against sound frequency is shown in Fig. 1. The curve clearly indicates the dependence of the angular width of high-frequency diffraction on sound wave-length. The angular width is scarcely two min of arc and the sound wave-length is only about thirteen times the wave-length of light at the highest frequency used in this investigation.

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Pressure Calibration of a Tetrahedral Anvil Device

THE tetrahedral anvil high-pressure device was first described by Hall[1] in 1958. The pressure calibration of this device was based on the resistivity measurements of Bridgman[2]. Because the calibration points based on those measurements have been revised downward[3], the original linear plot of sample pressure versus applied force no longer has validity. In a preliminary determination of the shape of a calibration curve based on the newer values of the calibration points, curve A (Fig. 1) was obtained. These values were obtained with an apparatus containing 7-in. hydraulic rams and triangular tungsten-carbide anvils which were 0·781 in. in edge-length. The calibration wires were inserted in silver chloride cylinders 5/32 in. in diameter which in turn were inserted in the pyrophyllite tetrahedrons, which were about 25 per cent oversize (edge-length). A thick coating of iron oxide mixed with water was applied to the assembled tetrahedron before it was pressed. The use of tetrahedrons which were 35 per cent oversize caused the samples to be more distorted by the flattening of the ends of the silver chloride cylinders, as described elsewhere[4]. Tetrahedrons which were only 15 per cent oversize transmitted the applied force to the calibration wire less effectively. In this case about 10 per cent more ram force was necessary to attain 25 kbars on the calibration wire.

Curve B (Fig. 1) was obtained under the same experimental conditions as here, except that the pyrophyllite tetrahedrons, completely assembled, were dried at 90° C for 2 h after the iron oxide was applied[5]. In addition to this substantial increase in efficiency, the deformation of the sample was reduced. Results obtained following

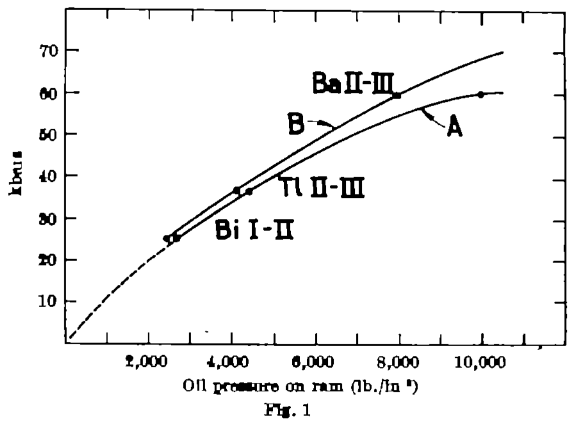

Fig. 1

this procedure were reproducible to about 1 or 2 per cent. It should be observed that the points on curve B can be connected by a nearly straight line which does not pass through the origin. Preliminary experiments indicate that sample porosity may reduce the efficiency of pressure transmission by 5–10 per cent.

The pressure-transmitting qualities of pyrophyllite can also be improved by heating the tetrahedrons to 650° C for several hours before the samples are assembled. This treatment causes the pyrophyllite to be about 10 per cent more efficient in pressure transmission. However, this procedure apparently reduces the elasticity of the pyrophyllite, because frequent blow-outs were observed on decreasing the applied pressure.

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GEOLOGY

Association of Dolomite and Anhydrite in the Recent Sediments of the Persian Gulf

A. WELLS[1] has recorded the presence of dolomite associated with aragonite and gypsum in the Recent sediments of the higher parts of the tidal flats around the Qatar peninsula in the Persian Gulf. A similar environment of coastal lagoons and tidal flats extends eastwards from Qatar along the Trucial Coast towards Oman. This communication refers to a short core collected from the higher part of the coastal flats (the sabkha) in the Sheikdom of Abu Dhabi, some 200 miles east of Qatar. The core, 20 cm in length, was taken from the surface of the sabkha on the mainland approach to the Abu Dhabi causeway.

Wells interpreted the dolomite and gypsum around the Qatar peninsula as having been "derived from sea-water left behind in the sediment on the upper part of the tidal flats and concentrated by evaporation during the time between spring or storm high tides, although it has not yet been established to what extent the dolomite is primary and to what extent it is a penecontemporaneous replacement of pre-existing carbonate". The distribution of dolomite in the core from Abu Dhabi, which is described in this communication, indicates that the dolomite is an early diagenetic replacement mineral.

In the Abu Dhabi core, aragonite, calcite, dolomite and halite have been identified. Gypsum, which occurs in the sample described by Wells[1] from Qatar, has not been found, but anhydrite occurs in considerable amounts. The vertical distribution of these components is shown in Fig. 1. The uppermost 8 cm consists essentially of an accumulation of sand- and silt-sized grains of quartz and carbonates, associated with evaporitic halite and anhydrite. This buff-coloured upper part rests on a cream-coloured, very fine-grained carbonate mud, which is well laminated at certain levels. Halite occurs throughout, but anhydrite, which characterizes the superficial parts of the core, has not been identified below 4 cm. At a depth of 9–14 cm from the surface the carbonate is almost exclusively aragonite, but below this level dolomite makes its appearance in detectable amounts. The ratio of dolomite to

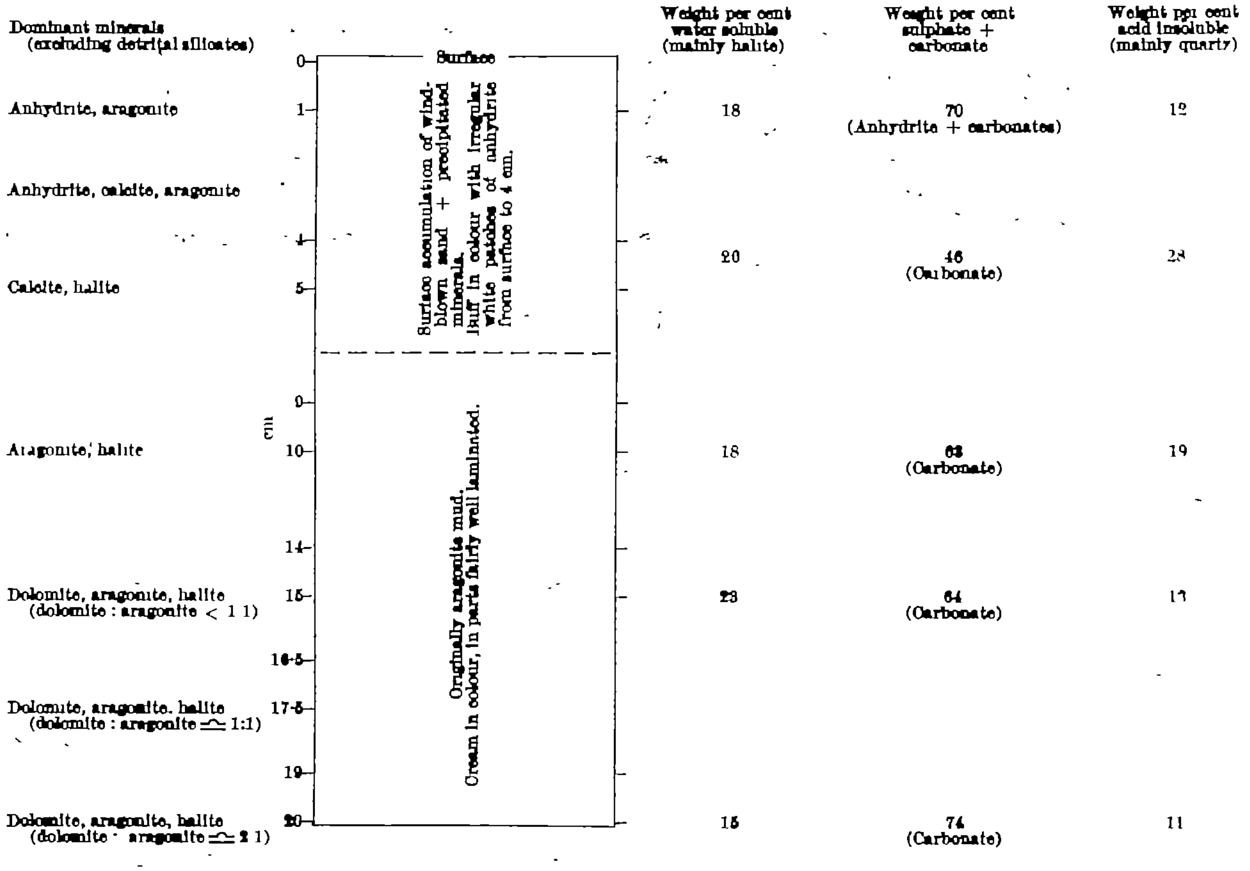
Dominant minerals (excluding detrital silicates)
Weight per cent water soluble (mainly halite)
Weight per cent sulphate + carbonate
Weight per cent acid insoluble (mainly quartz)
Surface
0
1
4
5
9
10
14
15
16·5
17·5
19
20
cm
Anhydrite, aragonite
Anhydrite, calcite, aragonite
Calcite, halite
Aragonite, halite
Dolomite, aragonite, halite (dolomite : aragonite < 1 1)
Dolomite, aragonite, halite (dolomite : aragonite ≃ 1:1)
Dolomite, aragonite, halite (dolomite : aragonite ≃ 2 1)
Surface accumulation of wind-blown sand + precipitated minerals. Buff in colour with irregular white patches of anhydrite from surface to 4 cm.
Originally aragonite mud. Cream in colour, in parts fairly well laminated.
18
70 (Anhydrite + carbonates)
12
20
46 (Carbonate)
28
18
63 (Carbonate)
19
23
64 (Carbonate)
13
15
74 (Carbonate)
11

Fig. 1

aragonite increases downwards from less than 1 : 1 at 15 cm to approximately 2 : 1 at 20 cm.

The anhydrite is clearly an evaporitic component, but the vertical distribution and mutual relationships of the dolomite and aragonite suggest that the dolomite is an early diagenetic replacement of aragonite. Although wind-blown grains are scattered throughout the length of the core, there is nothing to suggest that the dolomite is detrital. All the available evidence indicates that it was formed *in situ* and is chemical in origin.

Owing to the extremely low annual rainfall, the pore fluids in the sabkha are supplied by the high salinity sea water of the adjacent lagoons. The waters of the tidal lagoons are alkaline with pH values up to 8·0. In the sabkha, which extends inland for 7 miles near Abu Dhabi, the ground-water is acid with the pH varying between 6·0 and 6·2. Transitional pH values are found in a narrow zone along the seaward margin of the sabkha. During October 1962 the ground water-level in the sabkha stood at approximately 75 cm below the surface, although it varied slightly from place to place. The ground water temperature averaged 35° C. A lateral and vertical migration of these fluids within the sediment is caused by evaporation from the sabkha surface. It is thought that the pore fluids, probably rich in magnesium, bring about the transformation of aragonite to dolomite. However, until a more detailed field and laboratory study has been completed this conclusion can only be a tentative one.

The mineral identifications were made by the X-ray diffraction powder method. The dolomite consistently showed an absence of the superstructure reflexions (221) and $(11\bar{1})$. This feature is characteristic of protodolomites[2]. The dolomite identified by Wells is rich in calcium ($Ca_{54}Mg_{46}$) and has an expanded lattice which suggests that it may also be a protodolomite, although Wells did not record the absence of the superstructure reflexions.

It is of interest at this stage of the investigations, that the dolomite is associated in the Qatar region with gypsum and at Abu Dhabi with anhydrite, in what at first sight appear to be similar environments.

Recent work on a series of short cores taken across the seven mile width of the sabkha near Abu Dhabi has revealed the presence of abundant gypsum. A complex, and as yet not understood, series of relationships exists between the gypsum and the anhydrite. The results of these later investigations will be more fully described elsewhere.

This communication is based on work forming part of a programme of research on the Recent sediments of the Trucial Coast, Persian Gulf, financed by the Department of Scientific and Industrial Research.

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[2] Graf, D. L., and Goldsmith, J. R., *J. Geol.*, **64**, 173 (1956).

The Cabot and Great Glen Faults

IN his recent contribution to this journal, Prof. Tuzo Wilson[1] describes the Cabot fault in the eastern coastal region of North America and compares it with the Great Glen fault which bisects the Scottish Highlands from the Firth of Lorne to the Moray Firth. He finds that transcurrent movements on the line of the Cabot fault occurred during an interval extending from late Devonian to early Carboniferous times, and cites Kennedy's[2] similar findings for the age of movements on the Great Glen fault as additional evidence of the close similarity of the two faults.

I have been engaged in a detailed investigation of the history of movements on the Great Glen fault, and have reached conclusions which are relevant to the question of the equivalence and possible former continuity of the Cabot and Great Glen faults. I have found conclusive evidence that while a sinistral transcurrent movement of the type recognized by Kennedy[2] postdates the emplacement of the Morvern-Strontian and Foyers "Newer Granite" complex, this movement was completed prior to the deposition of the Middle Old Red Sandstone in the area about Loch Ness and the Moray Firth. The presence of sediments of undoubted Middle Old Red Sandstone lithology[3] among the sheared rocks of the fault-zone has resulted from further and distinctive transcurrent movements, of somewhat smaller magnitude than those of early Old Red age, which occurred in Tertiary times. These later movements, which were dextral in sense, are chiefly recognizable because they have caused the dislocation of the Skye Tertiary dyke swarm at the line of the Great Glen fault. Prior to these movements, the major outcrops of the Middle Old Red Sandstone astride of the fault showed a detailed correspondence, as regards both geometry and lithology, such as is not apparent at the present day.

The dating of the major sinistral movement on the Great Glen fault is thus more narrowly defined and is earlier than the age interval found by Kennedy[2] for the same dislocation. The comparability of this fault with the Cabot fault is therefore much less close than is claimed by Wilson[1].

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THE new information provided about the Great Glen fault by N. Holgate is naturally welcome. I am grateful to him for pointing out its possible bearing on my suggestion[1] that the Great Glen and Cabot faults might have been two ends of the same fault, if Europe and North America had been joined together at the time of the formation of these faults, and if the continents had afterwards moved apart. Granted that Holgate's views are correct, there are still at least three points to be settled before they can be held to have invalidated my suggestion, although they may have modified it.

First, if the supposed date of faulting can be changed in an area so well studied as is most of Scotland, so can the age of the Cabot fault. I hinted in my paper at the conflict in the views which have been published about the age of faulting in Massachusetts and Rhode Island. If there appears to be less conflict in northern Newfoundland, it is because the areas of fossiliferous rocks are small and they have been little studied. D. M. Baird and officers of the Geological Survey of Canada have been so kind as to confirm in conversation that the age of the Cabot fault is neither firmly nor precisely established.

Secondly, the argument has been made more complex by R. Dearnley's description[2] of the Minch fault between the Outer Hebrides and the mainland of Scotland. It is said to be similar in age and displacement to the Great Glen fault, but it is not precisely dated since "the available evidence, though fragmentary and inconclusive, suggests that the fault-movement occurred probably after the intrusion of the Lower Old Red Sandstone lamprophyres and almost certainly before the intrusion of the (supposedly Permian) camptonites"[2]. Dearnley regards the Great Glen, Minch and other minor faults as members of the same fault system.

Thirdly, displacements of tens of miles on fault zones hundreds of miles long must have been spread over considerable periods of time. Displacements may have been renewed after long intervals of quiescence as Holgate points out. Where a fault zone has several branches, movements may have occurred at different times on different branch faults.

Thus the whole situation is that the displacements which were found by Holgate to have occurred in Scotland before the deposition of the Middle Old Red Sandstone might have occurred in North America, without yet being detected; while the Lower Carboniferous movements found in North America could have their counterparts in the Minch fault without calling into question Holgate's observations along the Great Glen fault.

Thus, it is still possible to believe that the Cabot fault may be the same age as the Great Glen fault alone and even easier to maintain that it corresponds to the whole Great Glen–Minch fault zone.

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[1] Wilson, T., *Nature*, 195, 135 (1962).

[2] Dearnley, R., *Quart. J. Geol. Soc. Lond.*, 118, 143 (1962).

Devonian Conodonts in Stratigraphic Succession of Malaya

IN 1958, when correlating the pre-Tertiary stratigraphic successions then known in Malaya with those in Thailand, Alexander[1] indicated that no sediments of Devonian age had been proved in either country at that time, although the possibility existed of their occurrence. During the course of a short visit to Malaya in July–August 1962, at the invitation of the Geological Survey, one of us (K. J. M.)[2,3] was given and collected a number of rock specimens for conodont identification.

One random specimen picked up from the Government limestone quarry at Kanthan, Chemor, Perak, appears to have yielded three index fossils from the Upper Devonian[4], namely, *Polygnathus normalis* Miller and Youngquist, *Palmatolepis minuta* Branson and Mehl?, and *Ancyrodella nodosa* Ulrich and Bassler. However, further work will be necessary for systematic checking and confirmation before full descriptions can be prepared and published.

It is too early yet to assess the full implications of the discovery, and this preliminary announcement is being made to indicate the increased probability of an almost complete succession without major break of strata from Upper Cambrian to Triassic in Malaya.

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Sponge Spicule Pebbles at Hut Point Peninsula, Antarctica

PEBBLES that range up to 20 mm in diameter composed entirely of sponge spicules, originally found by Priestley[1], are sparingly distributed over the loessic gravelly soil of the two square miles of ice-free area at the southern tip of Hut Point Peninsula, lat. 77° 51′, long. 166° 48′ E.

The pebbles are extremely resilient and appear to have been eroded out of loess by the wind. By experiment it was found that the pebbles readily pick up sponge spicules and build them on to their surface in continuity with those below.

Sponge spicules are not uncommon in the loess of Hut Point Peninsula, and are common in marine sediments dredged from the Antarctic sea-bed. Sponges make up a large part of the bottom fauna of the sea-bed around the peninsula.

The sponge spicules which form the pebbles are considered to have been blown from sea-bed that was exposed during the last glaciation when the level of the sea was lower, to have been concentrated on ice surfaces because of their high albedo relative to rock fragments, and to have aggregated into pebbles on the ice by wind action. The pebbles were probably blown to Hut Point Peninsula when the loess was being deposited.

I shall be interested to learn of other occurrences of such pebbles, either fossil or recent.

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[1] David, T. W. E., and Priestley, R. E., *Brit. Ant. Exp. 1907–9, Geol.*, 1, 274 (W. Heinemann, 1914).

Transport of Pebbles over Smooth Ice in Antarctica

AT Hut Point Peninsula (lat. 77° 51′ S., long. 166° 48′ E.) pebbles of basalt up to 17 mm in diameter with some sand and silt have recently been blown half a mile up a snow-free ice slope to be stopped by snow cover 200 ft. higher than the foot of the slope. After being halted the pebbles melt their way down into the ice. The pebbles are probably transported during winter when southerly gusts reach a velocity of up to 120 knots.

At present only a small portion of the ice-covered part of the peninsula is free of snow, and the pebbles which are being wind transported now are entirely of local basalt. Widely distributed pebbles of granite and sandstone which are foreign to the peninsula are scattered through the gravelly loessic soil of the ice-free part. The pebbles have an unusual size distribution, already noted by David and Priestley[1], that is similar to that of the basalt pebbles, modal diameter being 16 mm, and maximum diameter, with the exception of a single pumice pebble, being not more than 18 mm. Because of their size distribution, it is considered that the pebbles were blown from the mainland some 30 miles to the west, probably during the last glaciation when there was more ice cover but less snow cover and the climate was colder, windier, and probably considerably drier than at present.

Pebbles with a similar shape, composition, and size distribution are abundant in dredgings from the nearby sea-bed, and wind transport of pebbles over smooth ice may have been of considerable importance in the past.

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CRYSTALLOGRAPHY

Crystal Structure of β-Tricaprin

THE triglycerides belong to a large class of compounds, the lipids, that are widely distributed in biological systems and frequently are structurally or functionally important. In spite of the interest in the part played by the triglycerides in such systems, no complete single crystal structural studies have appeared except that of Vand and Bell[1], who reported the chain structure and packing in β-trilaurin.

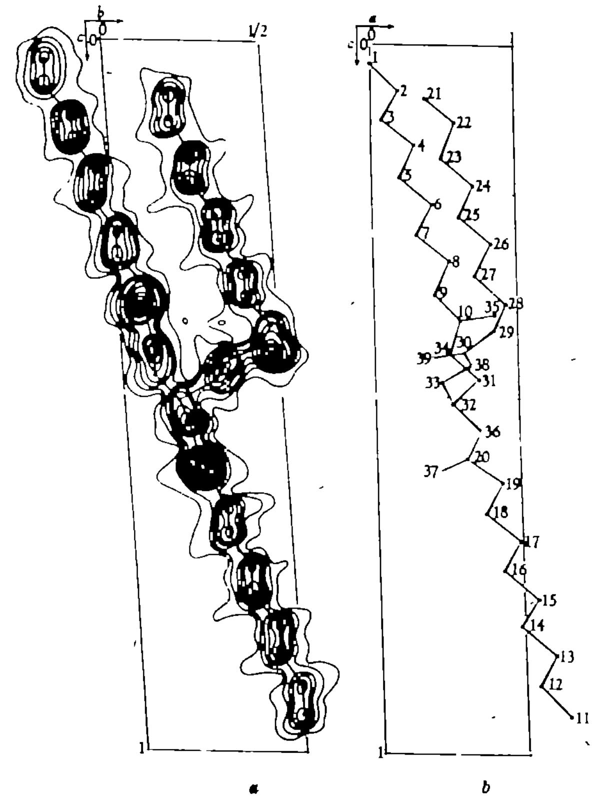

Fig. 1. *a*, F_o synthesis showing one molecule projected along [100]. Contours at integral values of electron density beginning at 1 e.Å^{-3}. *b*, Skeleton of molecule as viewed along [010]

The crystal structure of β-tricaprin has now been solved and refined in part from incomplete three-dimensional equi-inclination Weissenberg data collected with iron $K\alpha$-radiation (λ = 1·937 Å). Masses of plate-like crystals of the β-form (m.p. 31·9° C) were grown at 0° C by slow evaporation of hydrocarbon or hydrocarbon–ethanol solutions. The triclinic unit cell has parameters a = 5·50 Å, b = 12·10 Å, c = 27·03 Å, α = 85·25°, β = 87·30°, γ = 79·64° for the cell chosen according to Dirichlet[2]. Statistical treatment of the results of Ramachandran and Srinivasan's[3] modification of the method of Howells, Phillips and Rogers[4] indicated a centre of symmetry. Hence the space group was assumed to be $P\bar{1}$.

The structure was solved essentially from a Patterson projection along [100] and packing considerations, and the pronounced sub-cell lent evidence supporting the indicated orientation for the hydrocarbon chains. The position of the glyceryl group was initially chosen on the basis of steric considerations, and a Patterson projection along [100] based only on the non-sub-cell reflexions served as a validity check. Refinement by ΔF syntheses gave sufficiently good y, z co-ordinates to permit approximate evaluation of the x co-ordinates and to enable refinement to proceed with the general hkl data.

Fig. 1 *a* is an F_o synthesis showing the electron density of one molecule projected along [100]. The general configuration of the molecule shows well in this projection and may be described as an unsymmetrical 'tuning fork'. Two hydrocarbon chains with two ester oxygen atoms and two of the glyceryl carbon atoms form one long, nearly straight, chain. The third hydrocarbon chain branches off from the remaining glyceryl carbon atom, by way of the ester oxygen atom, bends and packs in parallel to and between the other two chains of adjacent molecules. The difference in configuration of this chain in the region of C$_{33}$ and C$_{36}$ shows to advantage when the molecule is viewed along [010] (Fig. 1*b*).

For approximately 600 observed reflexions, the present value of R is 0·179 with an over-all isotropic temperature factor parameter B = 3·0 Å^2 for the C and O atoms. The structure is being refined further and a more detailed account will appear elsewhere.

The computational part of this work was supported by a grant of computer time from the Research Computer Laboratory, University of Washington, and by grant *A*–3288 from the National Institutes of Health.

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Unusual Twinning in Graphite

ACCORDING to the Kennedy interpretation[1] of twinning in graphite, the twin boundary can be regarded as the result of insertion or removal of a full hexagon width on alternate planes in the hexagonal lattice. This means that the twins are regions of the crystal which are misoriented in relation to the matrix by an angle of tilt, tan^{-1} a/c, a and c being the unit cell dimensions, equal respectively to the width of a hexagon and the distance between alternate basal planes. This interpretation is corroborated by the recent work of Freise and Kelly[2], there being no doubt that the twin composition plane is $\{11\bar{2}1\}$ with respect to the morphological axes (conventional notation). In Kennedy's paper, theoretical reasons are adduced which favour the existence of $\{11\bar{2}1\}$ rather than $\{1\bar{1}01\}$ twins, which result from the insertion or removal of a hexagonal length, $2a/\sqrt{3}$, into every other plane. It is also stated that "No cases of the observation of a $\{1\bar{1}01\}$ twin composition plane appear to have been reported, although it may be possible to produce such twins in thin flakes of very perfect graphite". This statement forms the basis of this communication.

We have studied twins on the surfaces of over a thousand crystals of natural, Ticonderoga graphite[3-5]. The crystals are frequently not perfect single crystals, but are com-

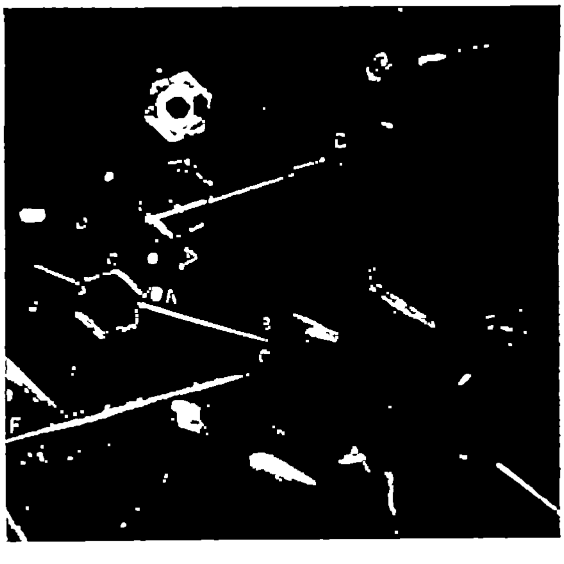

Fig. 1. (0001) surface of oxidized Ticonderoga graphite showing unusual twin (× c. 145)

posed of two or more flakes of highly perfect graphite twisted with respect to one another[4]. Almost invariably the twins were of the form $\{11\bar{2}1\}$. Very occasionally, however, twins which intersect the usual ones at 30° or 90° may be identified—they are therefore of the form $\{1\bar{1}0l\}$, l being unity if the angle of tilt is $\tan^{-1} 2a/\sqrt{3}c$. The twin AB (Fig. 1) which cuts the side of the pit C at 90° and the twins DE and FG at 30° is unusual and must clearly be composed of $\{1\bar{1}0l\}$ planes. Moreover, since this twin is not visible at the base of the pit C, and since the pit depth is about 1μ, we have evidence here which suggests that the perfect graphite flake on which the unusual twin appears is quite thin.

The views of Kennedy are therefore substantiated by our observations; but complete verification awaits the experimental determination of the angle of tilt which, if $l = 1$, $a = 2{\cdot}461$ and $c = 6{\cdot}708$ (refs. 4 and 6), should be 22° 57′.

This work was supported, in part, by the U.K. Atomic Energy Authority, Harwell. We are indebted to the Department of Scientific and Industrial Research for maintenance grants (to E. E. G. H. and B. R. W.) and Prof. A. J. Kennedy for his suggestions.

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CHEMISTRY

Spontaneous Phase Changes during Rapid Cooling of Beryl Melts; their Detection and Characterization

A FUSION-QUENCH sequence as a means to opening the very refractory cyclo-silicate mineral beryl, $Be_3Al_2Si_6O_{18}$, is the basis of a well-known extractive process[1] in beryllium metallurgy. Much of the fundamental chemistry involved has been obscured by uncertainties surrounding the constitution of beryl melts and the crystallization paths which they can follow during cooling at rates too swift to allow equilibrium to be attained. We have found non-equilibrium ordering processes, and their dependence on thermal conditions, to be of importance in determining the chemical reactivity of chilled beryl melts. The difficulty of systematically studying events occurring spontaneously during the course of rapid cooling has been overcome by a refinement to the technique of high-temperature microscopy[2]. In addition to being able to observe the melt directly, heat effects, accompanying phase generation during chilling, can be detected. A series of pseudo-liquidus temperatures associated with any crystallization can be defined, and any dependence that the ordering processes occurring in the melt may have on variation in the rate of cooling can be established.

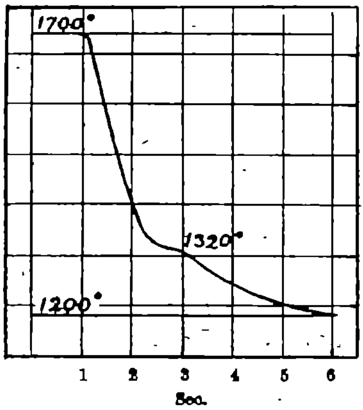

Fig. 1. Cooling-rate to 1,320°, 250°/sec

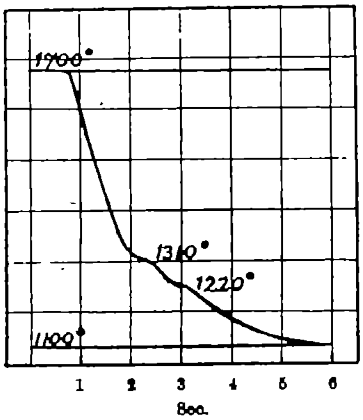

Fig. 2. Cooling-rate to 1,310°, 325°/sec

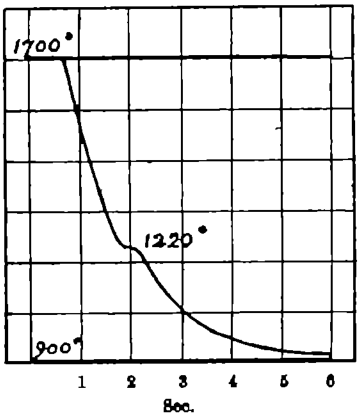

Fig. 3 Cooling-rate to 1,220°, 435°/sec

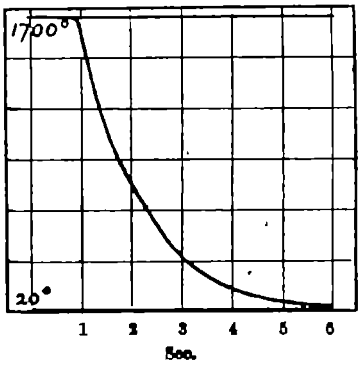

Fig. 4. Cooling-rate to 1,220°, 750°/sec

To achieve this the thermoelectric E.M.F. from the thermocouple microfurnace is monitored during cooling by a cathode-ray oscilloscope. The evolution of heat, signalling a phase change, is marked as an arrest on the E.M.F. (temperature) versus time trace. Initial cooling rates can be varied by altering the lower temperature to which the melt is quenched.

Provision is made for this by incorporating two parallel, interswitched, heating circuits into the instrument. Pre-heating of the system to any desired temperature by means of one channel can precede elevation to the desired temperature of the melt by the second channel.

The specimen is quenched in water immediately following the thermal arrest by impelling a water stream over the thermocouple with simultaneous cut-off of the heating current. Quenching rates of 10,000°/sec are thus achieved. The sample is removed and examined by X-ray methods to identify the crystalline phases.

Arrest temperatures and cooling rates are measured directly from the photographic record of the cooling curves. Four such curves are shown in Figs. 1–4. These are selected from many which trace an interesting relationship between the rate of cooling and the structures generated. The arrest at 1,320° (Fig. 1) was shown to arise from the simultaneous generation of beryllium oxide and mullite. The temperature at which they appear together is depressed to 1,310° when the cooling rate is increased and a second discontinuity marks the appearance at 1,220° of a new phase (Fig. 2). At a still higher rate of cooling (Fig. 3) ionic segregation into beryllium oxide and mullite was completely prevented and only one arrest, corresponding to the emergence of the new phase, noted. On quenching in air to room temperature the rate of cooling through these characteristic pseudo-liquidus temperatures was such as to prevent any crystallization and an X-ray-amorphous glass was produced (Fig. 4). This behaviour of beryl melts is fully reproducible.

The new phase is of uncertain composition, but wider investigations concerned with defining the conditions governing its formation and its mode of thermal decay strongly support the view that it is a metastable hybrid orthosilicate of beryllium and aluminium genetically related to both mullite and phenacite. The lines of its X-ray spectrum closely correspond to all those of natural phenacite for which l is either 0 or divisible by 3. It has a hexagonal unit cell; $a = 7 \cdot 38$ Å; $c = 2 \cdot 82$ Å.

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Micro-packed Columns for Gas Chromatography

As long ago as 1956, Dr. A. J. P. Martin pointed out[1] that operation of thermal conductivity detectors at reduced pressures should enable them to measure samples at the µg-level and he states that columns would be correspondingly smaller in diameter, perhaps 0·2 mm.

No work has been reported on such a system, and many workers have expressed the opinion that conventional packed columns of such small diameters could not be fabricated and would not be practical. I have confirmed that the system suggested by Martin is not only practical, but also gives good results and is surprisingly easy to fabricate.

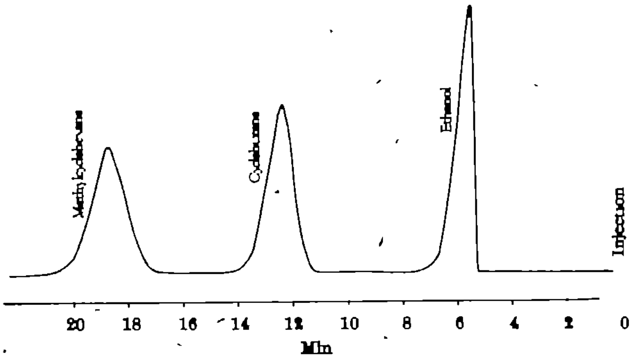

Fig. 1. Chromatogram with packed capillary. 2 m × 0·25 mm. Carrier, nitrogen; column, squalane; temp., 75°; sample, 1·3 × 10^{-6} g; flow-rate, c. 0·027 c.c./min (column conditions)

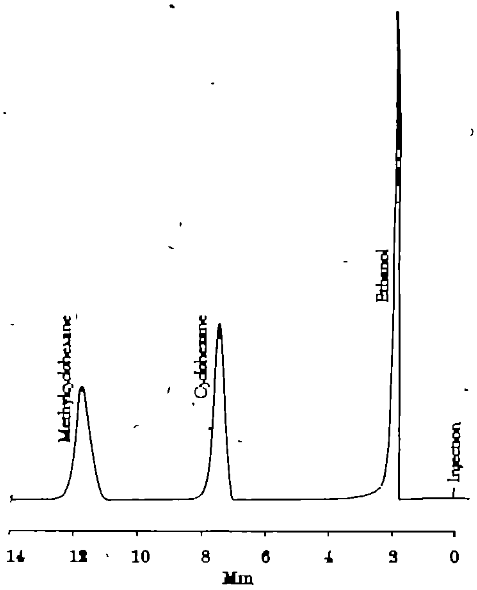

Fig. 2. Chromatogram with packed capillary. 2 m × 0·5 mm. Carrier, nitrogen, column, squalane; temp., 75°; sample, 1·3 × 10^{-6} g, flow-rate, c. 0·16 c.c./min (column conditions)

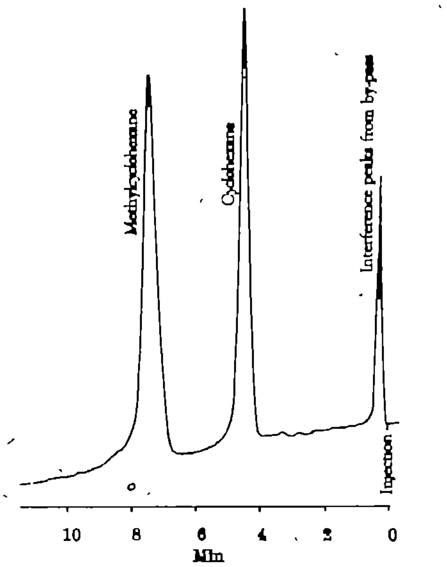

Fig. 3. Chromatogram with 0·5 mm packed capillary and thermistor detector. Carrier, hydrogen; column, squalane; temp., 75°; sample, 10^{-6} g; flow-rate, c. 0·25 c.c./min (column conditions)

Two columns were made, one of 0·25 mm internal diameter and one of 0·5 mm internal diameter. Each was 2 m long, made of stainless steel, and packed with dry glass beads of 0·04 mm diameter which were afterwards coated by passing a 10 per cent solution of squalane through the column. Packing was assisted by vibration and presented no difficulties. A wire insert prevented the beads from falling out.

The 0·25-mm column gave 1,000 theoretical plates for methyl*cyclo*hexane at 75° C and an inlet pressure of 130 cm (Fig. 1). The 0·5-mm column at 200 cm inlet pressure gave 2,800 theoretical plates for methyl*cyclo*hexane and a performance index[2,3] of 700 at a flow-rate of approximately 0·25 c.c./min at N.T.P. (Fig. 2).

The 0·5-mm column was later fitted into a Perkin-Elmer model 451 'Fractometer' and had a capillary restriction between the column exit and the thermistor detector (cell volume, 0·5 c.c.). The exit pipe of the instrument was connected to a vacuum pump so that the detector pressure was about 15 mm (estimated); the column outlet pressure was estimated at approximately

100 cm. A stream splitter was used to introduce smaller samples.

Peaks were 20 per cent wider (Fig. 3) than those for the flame detector; this is attributed primarily to the change of carrier gas from nitrogen to hydrogen, with an additional effect if the detector pressure were higher than estimated.

The detection limit (twice noise-level) for these particular conditions was 2×10^{-12} g/sec (which is about five times worse than a flame ionization detector yielding 0·4 coulombs/g), corresponding to a concentration of 5 p.p.m. in the detector.

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Mosaic Permselective Membranes

We have succeeded in preparing ion-permselective membranes from a sheet of commercial polyethylene which are cation-selective on part of their surface and anion-selective on the adjoining part with a sharp line of demarcation between them[1]. To this end we treated the original polyethylene film either with sulphuryl chloride in the liquid or vapour state or in a mixture of chlorine and sulphur dioxide gas, in the light of incandescent lamps. Swelling by addition of carbon tetrachloride enhanced the reaction. A film, 0·1 mm thick, reacted completely in 2–3 h at about 50° C. This sulphochloride film was washed in carbon tetrachloride, dried, and half of it was pressed between two sheets of rubber with two metal plates. The rest of the film projected from this device and was immersed in a bath of tetraethylene pentamine at 80° C for 2 h. It became yellow and was transformed into an anion-selective membrane: the pentamine molecules became linked by sulphamide bonds into the film. The device was then opened and the whole film immersed into a 5 per cent solution of sodium dihydroxide in 1 : 1 dioxane/water. In this bath all sulphochloride groups which had been protected from amination between the rubber sheets underwent hydrolysis to sulphonate groups whereas the aminated part of the film was not disturbed. The sulphonated portion of the membrane proved to be strongly cation-selective, having 95 per cent permselectivity and a resistance of 2 ohms/cm². The aminated portion was anion-selective with 87 per cent permselectivity, but improved after methylation with alcoholic methyl bromide to 96 per cent, its resistance being 1 ohm/cm².

Such mosaic membranes may be of technical interest since they provide the possibility of in effect joining two membranes to each other. They are also interesting from the point of view of electrochemistry.

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[1] Israeli Patent No. 14720.

Enhanced Salt Diffusion along the Border-line of Mosaic Permselective Membranes

An ideal permselective membrane between, for example, a salt solution and water only allows its counter-ions to pass at the moment of immersion. The ions which have passed are attracted to the surface by their partners of opposite charge which remained on the other side: both sides of the membrane become charged and under ideal conditions the Nernst potential is established. This prevents further counter-ions from passing the membrane so that no actual diffusion of salt takes place. The same holds true for a membrane of opposite charge, only the signs are reversed. However, at the border-line between two permselective membranes of opposite sign a new situation has to be expected. Anions and cations respectively are present on the two sides of the borderline. Here they eliminate, or in any event weaken, the potential difference in the neighbourhood of the border because they neutralize each other's charges. Thus it should be possible for further anions and cations to pass across their respective, adjacent, domains. Actually salt should diffuse across the membrane along the borderline, anions and cations passing through beside each other on both sides of the border.

We have succeeded in demonstrating this effect. The membrane was soaked in distilled water and pressed against a pad of filter paper which had been soaked in a 1 per cent sodium iodide solution. In order to immobilize all iodide ions which had diffused anywhere across this mosaic membrane (preceding communication) we prepared a sheet of 'Cellophane' impregnated with silver bromide by successive immersion in silver nitrate and potassium bromide solutions and rinsing with water. This 'reagent "Cellophane" ' was pressed against the free surface of the mosaic membrane. It acquired a bluish-grey colour in the daylight by photoreaction but changed over to yellow silver iodide wherever iodide ions reached it, demonstrating exactly where diffusion had been going on. This diffusion pattern may be fixed in the following manner: silver bromide dissolves in concentrated ammonia solution whereas silver iodide does not, hence bathing the reacted 'Cellophane' for 1–2 min in it removes all unreacted silver bromide. The pale yellow silver iodide, on the other hand, can be easily transformed into black silver by immersion in any photographic developer. Fig. 1 is the photograph of a mosaic membrane with the photograph of its diffusion pattern below it; it has been prepared in this manner. The black line on the diffusion pattern at the border between cationic and anionic parts of the membrane definitively proves that our assumptions concerning enhanced diffusion along the border are correct.

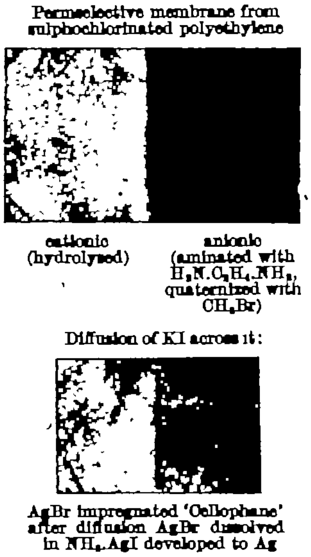
Permselective membrane from sulphochlorinated polyethylene
cationic (hydrolysed)
anionic (aminated with $H_2N.C_2H_4.NH_2$, quaternized with CH_3Br)
Diffusion of KI across it:
AgBr impregnated 'Cellophane' after diffusion AgBr dissolved in NH_3. AgI developed to Ag

Fig. 1

The aforementioned technique is not quite so simple. For one thing, membranes are never ideal and always allow salts to diffuse across them to some extent. It is important that this 'illegal' diffusion be small and not very different across the cationic and anionic parts of the membrane, otherwise borderline effects become swamped. Diffusion across the hydrolysed, cationic, sulphochloride sheet is appreciably stronger than across its counterpart if it has been aminated with tetraethylene-diamine. This is because the large attached amine molecule fills the pores in the membrane. Therefore, we changed over to ethylenediamine instead, quaternizing it with methylbromide after amination. Diffusion across this anionic membrane was slightly stronger than across the cationic part, but detection of the border effect became possible. Secondly, the diffusion experiment itself is very time-sensitive: under-exposure or over-exposure of the diffusion pattern ruins the experiment. In our case, with membranes 0·1 mm thick, 1–2 min proved to be the right time for diffusion.

Not much can be said about the breadth of the active surface along the border-line. However, it certainly is very narrow. We measured actual diffusion of potassium chloride from an N/10 solution against distilled water across the cationic, the anionic and the border-line part of the mosaic membranes using a 2-mm wide, 40-mm long slit for the experiment. Diffusion across the anionic part was about twice as quick as across the cationic part, but it was impossible to measure enhanced diffusion along the border-line: the phenomenon which is so easily demonstrated on the picture evidently proceeds along so narrow a strip that it is swamped in the diffusion of the adjacent normal membranes.

It seems possible that diffusion across border-lines between anionic and cationic membranes might play a part in nerve conduction[1]. The change-over of the nerve potential from inside negative to its opposite together with the enhanced diffusion of salts across the nerve membrane at the point at which the electric impulse is passing could be due to a wave of sign-inversion running along the nerve. This could be because acidic sites in the membrane become esterified to choline or this choline ester hydrolysed, thus changing the sign of fixed ions from negative to positive or vice versa.

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Crystal and Molecular Structure of Titanium (IV) Ethoxide

BOTH speculation and controversy on the stereochemistry of the trimeric titanium (IV) alkoxides[1–4] have followed the discovery[5] of their trimeric nature in solution. Opinions have differed on which structure is stereochemically reasonable and on which structure will account in a natural way for the hydrolysis products of titanium (IV) alkoxides, the so-called titanium oxide-alkoxides of composition $Ti_{3x+1}O_{4x}(OR)_{4x+4}$. These titanium oxide-alkoxides have been characterized, at least for $x = 1$ and $x = 3$, as solids[1]. The purpose of this communication is to indicate that titanium (IV) ethoxide is a tetramer in the solid, and hence that this long-awaited X-ray analysis of a titanium (IV) alkoxide not only fails to confirm one of the proposed structures, but also makes unreasonable the assumption, implicit in much of the recent discussions, that the species in solution are identical with those precipitated from solution.

R. L. Martin and G. Winter very kindly supplied several crystals of titanium (IV) ethoxide already mounted and sealed in soft-glass capillary tubes. Methods of preparation and analysis as well as techniques involved to maintain anhydrous conditions have been described previously[6,7]. Crystals from two different batches were examined and found to have identical unit cells. The white, prismatic crystals of titanium (IV) ethoxide belong to one of the space groups C^6_{2h} — $C2/c$ or C^4_s — Cc of the monoclinic system with $a = 19·50$, b (unique axis) $= 14·67$, $c = 17·14$ (all ± 0·03 Å), $\beta = 106·1 \pm 0·3°$. A density of 1·28 g/cm^3 calculated for 16 $Ti(OC_2H_5)_4$ in the unit cell is in satisfactory agreement with a density of 1·23 g/cm^3 found by Crowe and Caughlan[8] and with a density of 1·24 g/cm^3 measured by Martin and Winter by flotation of the crystals in benzene–ethylene dibromide solutions.

From these data alone it is probable that titanium (IV) ethoxide is dimeric or tetrameric. Nevertheless, a complete X-ray determination of the crystal structure was carried out. Three-dimensional X-ray data were collected at room temperature with copper radiation by the equi-inclination Weissenberg technique, and the intensities were estimated visually. The structure was solved by standard procedures: the space group $C2/c$ was assumed; the titanium positions were inferred from a three-dimensional Patterson function; these positions lead to only very slight contributions of the titanium atoms to the l-odd reflexions. As a result, a Fourier synthesis, based on the signs of the l-even reflexions from the titanium contributions, has, in addition to the titanium peaks, two sets of eight major peaks in the asymmetric unit that are related by a false mirror plane. From geometrical considerations one set of eight was chosen. Subsequent least-squares calculations and Fourier syntheses have led to the refinement of the titanium and oxygen positions and to the determination of approximate carbon positions. There is considerable thermal motion, and perhaps some thermal disorder. The temperature factors for titanium are about 6 Å^2, for oxygen about 8 Å^2, and for carbon somewhat higher. Work is in progress to resolve some of the overlapping carbon peaks in the difference Fourier. The usual R factors for the 572 observed reflexions are: 0·12 for all atoms; 0·23 if carbon atoms are omitted; 0·46 if carbon and oxygen atoms are omitted. None of the unobserved reflexions has a calculated intensity which exceeds the estimated minimum. The over-all good agreement is taken as justification for the assumption of the space group $C2/c$.

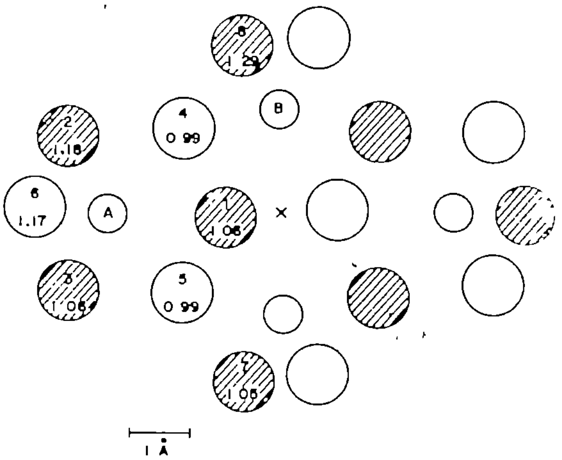

Fig. 1. The tetrameric titanium (IV) ethoxide molecule projected on to the *ab* plane. The four titanium atoms (smaller circles) are in the plane. The cross-hatched oxygen atoms are below the plane; the clear oxygen atoms are above the plane at distances (in Å) from the plane given beneath the atom number. The '×' marks the centre of symmetry of the molecule

The titanium–oxygen arrangement is shown projected on the *ab* plane in Fig. 1. The molecule is tetrameric, and possesses a centre of symmetry. The four titanium atoms lie at the corners of a planar rhomb. Above and below the titanium plane are nearly planar oxygen layers. The titanium atoms are octahedrally co-ordinated by oxygen atoms. There are three different oxygen–titanium interactions. Oxygen 1 is bonded to three titanium atoms at an average distance of 2·23 Å; oxygens 4 and 5 are each bonded to two titanium atoms at an average distance of 2·03 Å; the other five independent oxygen atoms are terminal, with an average distance of 1·77 Å. The estimated standard deviation of a Ti–O bond-length is 0·03 Å, and there are some significant variations among bond distances of a given type. The ethyl groups spread out from the oxygen atoms and lead to a molecule that is approximately cylindrical in shape. All intermolecular contacts are normal.

The structure depicted in Fig. 1 has several satisfying features, most prominent of which are the octahedral co-ordination of the titanium atoms and the readily visualized termination of polymerization. The relation of this structure to those of the titanium (IV) alkoxide species present in solution is as yet unknown. The structure is sufficiently different from any proposed for the trimeric species so that, at the least, reconsideration not only of the features of those structures but also of the proposed mechanisms of hydrolysis seems necessary. What is more important, however, is a re-examination of the evidence in favour of the trimeric species as the largest present. To this end Martin and Winter, as reported in the following communication, have carried out extensive cryoscopic molecular weight studies on titanium (IV) ethoxide and butoxide in benzene. They indeed find evidence for a tetrameric titanium (IV) ethoxide species in benzene solutions.

I thank Prof. R. L. Martin and G. Winter for their wholehearted co-operation.

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Association of Titanium (IV) Alkoxides in Benzene

The single crystal X-ray structure determination of titanium (IV) ethoxide described in the preceding communication establishes the fact that the molecule is a tetramer formed by the stacking of four TiO_6-octahedra so that edges are shared (Fig. 1). Dr. Ibers's analysis also confirms the long-accepted belief that intermolecular attachment between $Ti(OC_2H_5)_4$ molecules is through ethoxide bridges (two per titanium atom) and reveals that two types of intramolecular ethoxide bridges can be discerned in the molecular unit.

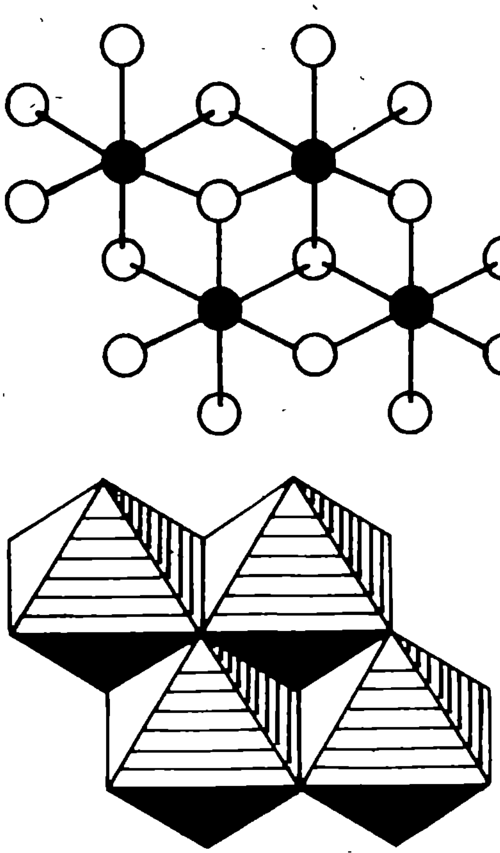

Fig. 1. Molecular structure of $Ti_4(OC_2H_5)_{16}$ showing two types of ethoxide bridging groups and the stacking of the TiO_6-octahedra

While it is not unknown for a structural entity in the solid state to differ from that present in solution, it now becomes important to extend previous cryoscopic measurements in benzene[1,2] to higher concentrations to establish whether the degree of association of titanium (IV) *n*-alkoxides can, in fact, exceed three.

For titanium (IV) *n*-butoxide we have now increased the range of concentration over which molecular weight measurements have been made from 15·1 × 10⁻² to 34·0 × 10⁻² molal, that is, approximately 11·5 wt. per cent (Fig. 2). The degree of association of the butoxide at the highest concentration is 2·6. We have also recently completed studies of the concentration dependence of the degree of association of titanium (IV) ethoxide in benzene using the techniques described elsewhere[2]. The data are presented graphically in Fig. 2 and it will be seen that although the curve rises more steeply than for the *n*-butoxide, even at the highest concentration the experimental degree of association has not exceeded three. Activity effects and problems associated with supercooling intervene in more concentrated solutions so that it cannot be resolved whether average degrees of association greater than three occur

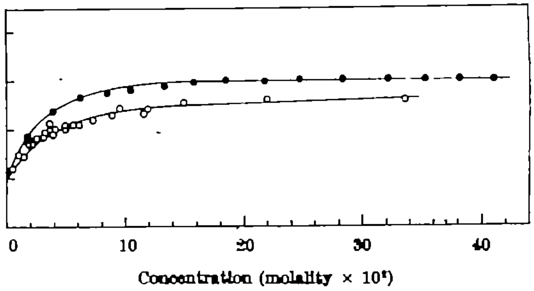

Fig. 2. Degree of association, *n*, of titanium (IV) *n*-alkoxides in benzene. ●, $R = C_2H_5$; ○, $R = n, C_4H_9$

It is significant, however, that while the new data for the butoxide can still be described by the curve previously derived[2] for an equilibrium involving monomeric, dimeric, and trimeric species in benzene, such a description is no longer adequate for the ethoxide. The introduction of at least one further equilibrium species, namely a tetramer, is required to fit the experimental variation of the average degree of association with concentration. Since the experimental values for the butoxide can also be fitted in terms of a similar set of equilibria, it is not possible to resolve cryoscopically whether for this compound also the limiting degree of association is greater than three.

Cryoscopic studies in solvents other than benzene are being made.

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Hydration of the Alite Phase in Portland Cement

CALORIMETRIC studies[1-3] indicate that the rate of hydration of the alite phase (tricalcium silicate) in neat Portland cement pastes reaches a maximum 6–11 h after mixing. Two heating cycles, or periods of high heat evolution, are found with all cements; some show a third. The first, occurring immediately on mixing, is most likely due to the dissolving of soluble species and to an initial rapid reaction of the aluminate phase. The second cycle, which reaches a maximum 6–11 h after mixing, is assigned largely to the alite phase. The third heating cycle shown by some cements is attributed to the exhaustion of sulphate ion (gypsum), the breaking down of the ettringite coating on the tricalcium aluminate particles, and the subsequent rapid hydration of the remaining aluminate to $Ca_4Al_2O_{18}\cdot XH_2O$. X-ray diffraction work in this laboratory offers direct evidence that the foregoing conclusion regarding the alite phase is correct.

Twenty grams of a Portland cement are made into a neat paste in a 50-ml. polyethylene beaker using a water-to-cement ratio of 0·25. Hydration is carried out in a carbon dioxide free atmosphere at 95 per cent relative humidity, 25° C. After a predetermined period, the sample is broken up, and the hydration stopped by thoroughly washing with acetone. It is then dried in a vacuum oven for 1 h at 70° C and 10 in. of mercury. A 10-g portion is ground with 10 per cent MgO (periclase) by weight. The 2·11 Å MgO line and the 1·76 Å cement line are used for the X-ray analysis. The 1·76 Å line in the diffraction pattern of a Portland cement is due mainly to the alite phase[4]. The small contribution that belite makes to this line is neglected.

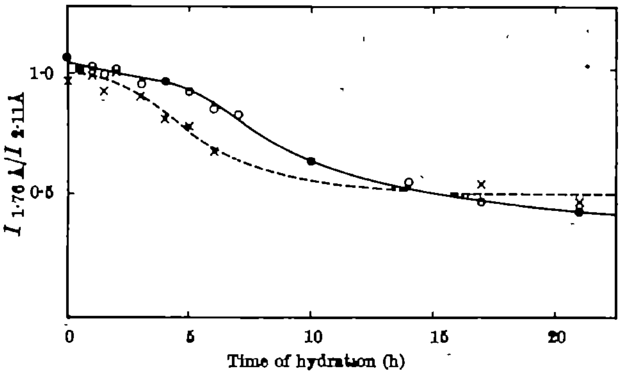

Fig. 1. Hydration of the alite phase in a Type I Portland cement. O, No additive present; ×, calcium chloride added 1·0 per cent by weight of solid material

Fig. 1 is a plot of the ratio of the intensity of the 1·76 Å line to the 2·11 Å MgO line versus the time of hydration for a Type I Portland cement. Each point is the average of three samples. Much of the alite hydration occurs during the period 5–15 h after initially mixing the cement with water. The hydration rate reaches a maximum about 7 h after mixing. When calcium chloride is added to the mix water at a concentration equivalent to 1·0 per cent by weight of calcium chloride to anhydrous cement, the maximum rate of hydration occurs about 2 h earlier. The hydration of the alite phase in the presence of calcium chloride is also shown in Fig. 1. The slight difference in the points at which the two curves intersect the y-axis is due to the fact that the two experiments were carried out on two different diffraction units with slightly different counting periods.

Hydration of neat pastes on the X-ray goniometer itself in a specially designed sample compartment confirms the general nature of the alite reaction. When hydrated in this manner, the alite reaction is most rapid 5–10 h after mixing and reaches a maximum about 6 h after mixing. This technique also shows that a commercial calcium lignosulphonate delays the occurrence of this period of rapid hydration. In Fig. 2 the y-axis is relative. A strict quantitative comparison is not possible when the reaction is followed in the paste itself. This is due to preferred orientation problems and the lack of an internal standard stable in the alkaline cement–water system. No reliable numbers can be obtained during the first few hours of hydration while the paste is still plastic.

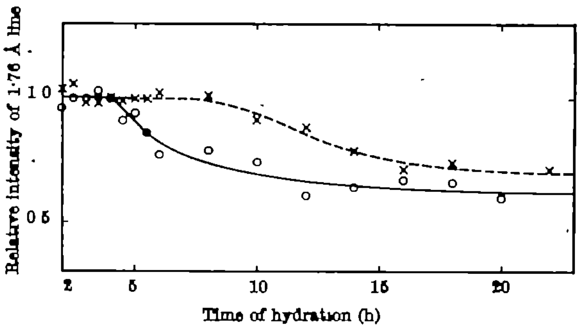

Fig. 2. Hydration of the alite phase in a Type I Portland cement. O, No additive present; ×, a commercial calcium lignosulphonate added, 0·30 per cent by weight of solid material

These results have an interesting relation to at least one physical property of cement pastes and mortars. Calcium chloride is commonly used to accelerate the set of Portland cements, calcium lignosulphonate to retard it.

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Preparation of Carcinogenic Polynuclear Hydrocarbons labelled with Tritium by Wilzbach's Method

THE need for radioactive labelled polynuclear hydrocarbon carcinogens, to be used in biological investigations, led to an investigation of the feasibility of a tritium exchange procedure for their preparation. Wilzbach's method[1] offers a simple method of labelling complex compounds which are comparatively difficult to synthesize. However, the procedure has its attendant problems in that, during exposure to tritium gas, concurrent with the exchange reaction, tritium will tend to be taken up by reducible compounds present. The purification of the product must, therefore, ensure removal of the high specific activity by-products.

Samples of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and 20-methylcholanthrene (500 mg of each) were exposed to tritium gas for 2 weeks. The specific activities of the products were, respectively, 0·80, 0·46 and 0·70 mc./mg. The distribution of radioactivity in the products was examined by chromatographing 15–100 μg samples on Whatman No. 1 paper in a dimethylformamide–isooctane system[2]; the chromatograms were cut into sections and eluted. The ultra-violet absorption spectrum of each fraction was taken in isooctane and the radioactivity assayed by liquid scintillation counting. Recovery of radioactivity was virtually 100 per cent and of the polynuclear hydrocarbons 90–100 per cent (these being in the single fluorescent zone on each chromatogram). Less than 10 per cent of the radioactivity was associated with the fluorescent zones, the remainder being mainly at the starting line and below the fluorescent zone. These

Table 1. RECOVERY OF TRITIUM IN REACTION PRODUCTS OF PURIFIED HYDROCARBONS

	7,12-Dimethylbenz(a)anthracene	Benzo(a)pyrene	20-Methylcholanthrene
Initial activity (d/s)	275,000	660,000	289,000
Treated with OsO_4, hydrolysed, dehydrated and allowed to oxidize, activity (d/s)	232,000	484,000	204,000
Tritium recovered (%)	84	73	71
Product	5,6-quinone (ref. 4)	4,5-quinone (ref. 4)	6,7-quinone (ref. 4)
Theoretical activity recovered for random labelling (%)	87	83	87
Mild oxidation with $Cr_2O_7^{--}$ activity (d/s)	168,000	376,000	160,000
Tritium recovered (%)	61	57	55
Product	7,12-benz(a)anthraquinone	1,6- and 3,6-quinones (ref. 5)	9-methyl-7,12-benz(a)anthraquinone-8-acetic acid (ref. 6)
Theoretical activity recovered for random labelling (%)	62	83	81
Vigorous oxidation with MnO_4^-, activity (d/s)	121,000	240,000	107,000
Tritium recovered (%)	44	31	37
Product	9,10-anthraquinone-1,2-dicarboxylic acid (ref. 7)	7H-benz(d,e)anthracene-7-one-3,4-dicarboxylic acid (ref. 5)	9,10-anthraquinonetetracarboxylic acid (ref. 6)
Theoretical activity recovered for random labelling (%)	38	67	25

fractions showed no ultra-violet absorption and must, therefore, contain material of very high specific activity.

Approximately 400 mg of each of the tritiated products was chromatographed on a 28 × 8 cm column of magnesia-'Celite'[8] and eluted with hexane–benzene, followed by mixtures of hexane–benzene–acetone. The non-fluorescent material preceding the fluorescent zone was collected in two portions, the beginning of the fluorescent zone next and the bulk of the fluorescent material in the fourth fraction. Each was evaporated to dryness and the material in the fourth fraction was crystallized from benzene–ethanol, this being the tritiated polynuclear hydrocarbon.

The yields of crystalline material were, respectively, benzo(a)pyrene 81 per cent, 7,12-dimethylbenz(a)-anthracene 92 per cent and 20-methylcholanthrene 92 per cent. All had sharp melting points, in agreement with those of the pure non-radioactive compounds. It is notable that the dimethylbenz(a)anthracene was colourless, whereas most samples of the non-radioactive compound are yellow, even after purification. The specific activities of the three crystalline hydrocarbons were—benzo(a)pyrene 0·045 mc./mg (11·3 mc./m.mole), 7,12-dimethylbenz(a)anthracene 0·018 mc./mg (4·5 mc./m.mole) and 20-methylcholanthrene 0·017 mc./mg (4·6 mc./m.mole). These values were almost unchanged after recrystallization. Paper chromatography of the compounds after two crystallizations, first on Whatman No. 1 paper in the dimethylformamide–isooctane system, followed by chromatography of the fluorescent zone on acetylated paper with toluene/methanol/water (1 : 10 : 1) as mobile phase[9], showed that more than 90 per cent of the radioactivity, in each case, was in the fluorescent zone containing the polynuclear hydrocarbon. Bearing in mind that some small amount of oxidation probably occurred on each paper chromatogram, this was evidence that the three tritiated hydrocarbons were sufficiently pure for biological experiments.

The distribution of tritium in the three polynuclear hydrocarbons was studied by oxidation to known products, the loss of radioactivity in the course of these reactions (tritium was removed as tritium oxide) being compared with that expected if the tritium were distributed randomly in the molecule. One series of reactions involved oxidation with osmium tetroxide to dihydrodiols, which were dehydrated to phenols and these allowed to oxidize to quinones[4]. The second series of reactions involved oxidation in glacial acetic acid with chromic acid to quinones and quinone acids, followed by further oxidation with acid permanganate to polycarboxylic acids[5–7]. The aromatic products of these reactions were extracted with benzene after dilution with water and the radioactivity assayed. The results are given in Table 1. The absorption spectra of the reaction products showed the absence of the hydrocarbons and the presence of quinones. It appears that tritium was distributed randomly in 7,12-dimethylbenz(a)anthracene, but not in benzo(a)pyrene or 20-methylcholanthrene.

Approximately half the radioactivity of the crude tritiated products was in strongly adsorbed material, probably phenols and other oxygen containing compounds, which could only be eluted by acidification of the absorbent.

The fractions eluted from the column chromatograms below the fluorescent hydrocarbon zone contained reduction products of the polynuclear hydrocarbon and constituted almost half the radioactivity of the crude tritiated products. Paper chromatography of these fractions in the dimethylformamide–isooctane system revealed the major portion of the radioactivity in material travelling near the solvent front, which probably consisted of perhydro compounds. There were fluorescent bands of smaller R_F on each of the chromatograms and these were eluted and examined spectroscopically. Those with indications of the presence of identifiable compounds were rechromatographed in the same system until background absorbing material was removed and spectra were obtained which could be compared with those of known compounds.

No identifiable reduction products were obtained from 7,12-dimethylbenz(a)anthracene. Two reduction products of 20-methylcholanthrene were identified spectroscopically as 6,7-dihydro and 17,20,22,23-tetrahydromethylcholanthrene[9], the yields and specific activities being, respectively, 760 and 180 μg, 6·6 and 15·9 mc./mg. Two reduction products of benzo(a)pyrene were identified as 4,5-dihydro and 7,8,9,10-tetrahydrobenzo(a)pyrene[9], the yields and specific activities of these being, respectively, 86 and 45 μg, 18 and 38 mc./mg. Two other reduction products of benzo(a)pyrene, both with high specific activity, had well-defined spectra but could not be identified with any known compound. One was spectroscopically identical with pyrene and the second had a spectrum similar to that of 7,8,9,10-tetrahydrobenzo(a)-pyrene, but with considerably more fine structure. These two compounds remained chromatographically homogeneous after chromatography in several different systems.

The spectrum of tritiated 7,8,9,10-tetrahydrobenzo(a)-pyrene was identical with that of the non-radioactive compound except that the wave-lengths of the absorption maxima were translated 4 mμ towards the visible. The chemical identity of the two compounds was established by crystallization from hexane of 50 mg of the non-radioactive compound with 40 μg (1·5 mc.) of the radioactive compound, which gave 31 mg of crystals of specific activity 0·028 mc./mg.

The exposures to tritium gas were conducted by the New England Nuclear Corporation. This investigation

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Observations on Potassium Hexacyanotitanate (III)

Schläffer and Götz[1] have recently prepared a complex cyanide of titanium by reacting a liquid ammonia solution of titanium (III) bromide with excess potassium cyanide. Chemical analysis of the dark green precipitate leads to the empirical formula $K_5Ti(CN)_8$. However, comparison of the absorption spectra with those of known trivalent titanium complexes[2,3] showed that the compound contained hexaco-ordinate titanium and should be assigned the formula $K_3Ti(CN)_6 \cdot 2KCN$. Although eight co-ordinate titanium (III) has been reported[4], and cyanide usually results in the highest co-ordination number of the central metal ion, the agreement of the absorption spectra with hexaco-ordinate complexes makes the higher co-ordination doubtful[3]. Carduck[5] has also reported the existence of a dark green complex cyanide of titanium having the formula $K_3Ti(CN)_6 \cdot 3KCl$ prepared by mixing stoichiometric solutions of titanium (III) chloride and potassium cyanide in liquid ammonia. The excess potassium chloride could not be removed from the precipitate by repeated washings with liquid ammonia. It is not clear how the excess potassium-cyanide or potassium chloride is bound to the parent complex[3], but the bond is apparently strong enough to resist removal when washed with excess liquid ammonia[1,5].

Work conducted in this laboratory has shown that a non-contaminated compound possessing the expected hexaco-ordination and the formula $K_3Ti(CN)_6$ may be prepared by mixing stoichiometric aqueous solutions of titanium (III) chloride and potassium cyanide. A typical preparation involved the following steps: titanium (III) chloride prepared by the Union Carbide Metals Co., with a minimum purity of 98 per cent, and potassium cyanide (J. T. Baker's 'Analyzed Reagent' grade), were used for each preparation. Entrained oxygen was removed from the solid reagents by successively applying vacuum and then bleeding the system with argon. This process was repeated five times in order to ensure the absence of oxygen. The distilled water used in the preparations was boiled to remove dissolved oxygen. Argon was bubbled through the water while it cooled and for 48 h afterward. A micro-Winkler oxygen determination[6] on a sample of the water treated in this manner showed that there was considerably less than 0·1 p.p.m. dissolved oxygen present. All glassware used in the preparation was rigorously dried and stored under argon in a dry-box equipped with a vacuum line. A dry-box was used, even though water was present, to ensure oxygen-free conditions. To start the actual preparation, an aqueous solution of 3·90 g (0·06 mole) of oxygen-free potassium cyanide in 250 ml. distilled water was stirred vigorously while a solution of 1·54 g (0·01 mole) anhydrous titanium (III) chloride in 100 ml. distilled water was slowly added. A deep blue curdy precipitate formed immediately and was filtered on a glass frit by a vacuum as soon as it settled. The precipitate was washed with six 25 ml. portions of the oxygen-free distilled water and dried under the argon atmosphere. (Anal., calculated for $K_3Ti(CN)_6$: K, 36·51; Ti, 14·91; CN, 48·58; found, K (as the tetraphenylboron salt), 36·46; Ti (as the oxide after igniting the cupferrate), 14·80; CN (by an argentometric titration after fusion with metallic sodium), 48·12.) When excess cyanide is used in the preparation the deep blue precipitate has the formula $K_3Ti(CN)_6 \cdot KCN$, an apparent seven co-ordination. The extra potassium cyanide was removed by successive washings with distilled water with considerable difficulty. Apparently, in both the aqueous and liquid ammonia systems the extra potassium cyanide and potassium chloride are rather strongly attached to the parent $K_3Ti(CN)_6$. The difference in colour observed between the compound prepared in the aqueous system (deep blue) and that prepared in liquid ammonia (dark green) could be due to a type of crystallization isomer similar to the solvation isomers obtained in the violet and green forms of hexhydrated titanium (III) chloride[7]. The absorption spectra of titanium (III) salts lead one to expect violet or blue colours due to the amounts of red and blue light transmitted[2,8].

Qualitative magnetic measurements by electron paramagnetic resonance indicate that the compound $K_3Ti(CN)_6$ is paramagnetic, quite typical of trivalent titanium compounds with one unpaired electron at the 3*d* level.

On prolonged contact with de-oxygenated water, or on immediate contact with air, the deep blue potassium hexacyanotitanate (III) becomes oxidized. The initial oxidation proceeds quite rapidly, resulting in a pale blue solid which approaches the composition $K_3Ti(CN)_6 \cdot K_2Ti(CN)_6$. Once a 1 : 1 ratio of Ti^{III} to Ti^{IV} is attained, oxidation proceeds more slowly until a snow white product is obtained which yields titanium dioxide, potassium hydroxide and potassium cyanide on contact with water. If the complex cyanide of tetravalent titanium—potassium hexacyanotitanate (IV), $K_2Ti(CN)_6$—does exist, it is quite different from other known complex transition metal cyanides which are hydrolytically stable[9].

When the deep blue solid was placed in a sealed tube under an argon atmosphere it was found to be stable for 10–12 days after which the colour slowly turned to pale blue. Possibly the titanium (III) was attacked by the oxygen in the glass.

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BIOCHEMISTRY

Glucose-6-phosphate Dehydrogenase and Aldolase in Lenses of Lactose-fed Rats

IT has been shown that in lenses of galactose-fed rats there is a decrease of glucose-6-phosphate dehydrogenase[1,2] and aldolase[3]. These findings support the statement that there is an interference of glucose metabolism in the lens during the development of galactose cataracts. In the previous experiments[1] glutathione reductase was also determined as a coupled system with the glucose-6-phosphate dehydrogenase through the utilization of the oxidized and reduced forms of nicotinamide-adenine dinucleotide phosphate (NADP). The activity of the glutathione reductase was reduced similarly to the glucose-6-phosphate dehydrogenase. Incubation of normal lens homogenate with galactose or galactose-1-phosphate showed no inhibitory effect on the glucose-6-phosphate dehydrogenase or aldolase activities[2,3]. Considering these results, and also that a lowered amount of soluble proteins per mg lens was found in the lactose-fed rats[3], we came to the conclusion that this was due to a decreased protein synthesis in lenses in this condition. However, aldolase was more affected than glucose 6-phosphate dehydrogenase, which may indicate a more direct action on the glycolytic pathway, which is, quantitatively, the most important pathway of glucose metabolism in lens[4]. The former experiments were performed independently with two different sets of rats. In order to know which of the two pathways is affected first, another group of animals was examined.

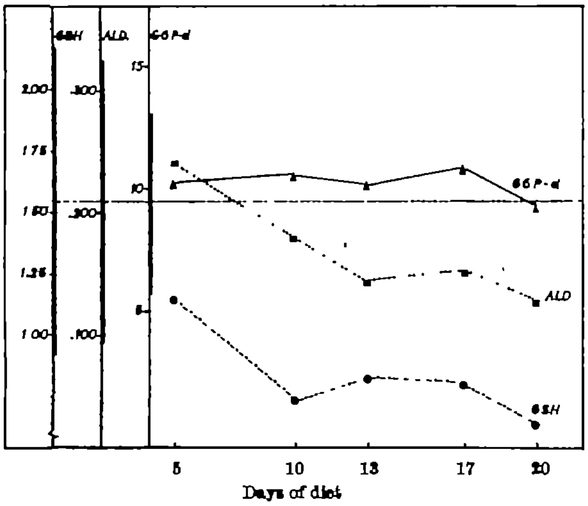

Fig. 1. On the ordinate, GSH (reduced glutathione)-level is given in µg/g lens; G6P-d (glucose-6-phosphate dehydrogenase) activity is expressed as the change in optical density at 340 mµ in 10 min/mg of lens; ALD (aldolase) activity is given in µmoles of substrate converted in 1 h/g lens. The horizontal broken line indicates the mean of paired lenses of eight normal animals. The thicker part of each ordinate shows the range between the mean ± 2 *S.D.* Two animals were killed on each day indicated; the values shown are the mean of both rats

Albino rats, weighing less than 100 g at the beginning of the experiment, were given a diet containing 40 per cent lactose and 60 per cent normal rat chow. Concurrently, another set of rats was maintained on a diet containing 40 per cent cane sugar and 60 per cent normal diet. This low galactose diet was chosen in order to obtain slower changes in the enzyme activities of the lenses. At appropriate intervals the rats were killed by decapitation, and the lenses removed immediately. Both lenses were homogenized with ice-cold distilled water in a glass tissue homogenizer in an ice-bath, and centrifuged at 4° C at 12,000*g*. The supernatant solutions were assayed for glucose-6-phosphate dehydrogenase by Zinkham's modification[5] of the method of Glock and McLean[6], and for aldolase according to Sibley and Lehninger[7] and following Fleisher[8]. In these experiments the level of reduced glutathione was also determined as an index of one of the earliest measurable changes in the lenses of galactose-fed rats[3], according to Beutler's modification[9] of the method of Grunert and Phillips[10].

Fig. 1 shows a very early fall in the glutathione content of the lens, even with this low galactose diet. It may also be seen that the aldolase activity decreases earlier than the glucose-6-phosphate dehydrogenase. After 20 days of feeding, aldolase fell to a lower value than the mean minus one standard deviation of the normals, while glucose-6-phosphate dehydrogenase was still normal. These experiments indicate that even though there is a decrease of the phosphogluconate pathway in the development of cataracts, the glycolytic pathway is affected perhaps more intensively than the other.

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Concentration of Nicotinamide Adenine Dinucleotide in Carrageenin-induced Proliferation

It has been shown[1] that the inhibitory effect of cortisone on the respiration of rheumatoid synovial tissue could be reversed by the addition of nicotinamide adenosine dinucleotide (NAD), and as there have been many reports[2,3] of lowered co-enzyme levels in rapidly proliferating tissues, the NAD concentration of normal and proliferating synovial tissue has been determined. Proliferation was stimulated in rabbit synovia by injection into the femorotibial joint of 2 ml. of a 0·2 per cent solution of carrageenin in saline[4]. The synovia were removed after 1, 2, 3 or 5 days. One knee of each rabbit was injected, the other being used as control. A fairly wide range in NAD values between rabbits was found but little difference between knees of the same rabbit. NAD and $NADH_2$ were extracted from the synovial tissue by the method of Jedeikin and Weinhouse[5] and estimated by the fluorescent method using methylethylketone and alkali[6].

Results obtained for NAD are given in Table 1. Values obtained for $NADH_2$ were one-tenth of those for the NAD.

The buffers used in the extraction of NAD did not affect the deoxyribonucleic acid phosphorus (DNA–P) content of the tissue so that it was possible to estimate the DNA–P content of the residue after centrifuging. The residue was washed and DNA–P extracted by the method of Schneider[7] and estimated by Burton's modification of the diphenylamine reaction[8]. The NAD results are thus expressed on a wet weight and DNA–P basis. It is seen that after the first day there is a rise in the co-enzyme concentration on a wet weight basis but when related to DNA–P, that is on a cellular basis, there is a fall in the concentration per cell until the fifth day. The lowest levels are seen on the first and second days after injection, corresponding to the day before and the day of greatest proliferation as shown by the mitotic index[4]. By the fifth day when the rate of proliferation slows down, the difference between injected and normal knees is not significant.

The effect of artificially increasing the concentration of NAD on carrageenin-stimulated growth has been investigated using the granulomata formed in rats by a dorsal subcutaneous injection of 0·2 ml. of a 2·5 per cent solution of carrageenin. 0·2 ml. of a 1·0 per cent aqueous solution of NAD was injected immediately after the carrageenin and a further 0·4 ml. after 2 and 5 days. The rats were killed after 9 days and the granulomata removed and weighed. An equal number of control rats received distilled water instead of the NAD solution. The average

Table 1. NAD Concentrations in Normal and Proliferating Rabbit Synovia

Days after injection	No. of rabbits	NAD concentration µg/g wet weight (µg/g ± *S.E.*)		Ratio of NAD to DNA-P $\frac{\mu g\ NAD}{\mu g\ DNA\text{-}P} \pm S.E.$		
		Left knee (Injected)	Right knee (Control)	Left knee (Injected)	Right knee (Control)	*P**
0 normals	9	19·1 ± 1·2	19·6 ± 1·5	0·42 ± 0·03	0·43 ± 0·045	>0·05
1	8	16·3 ± 2·1	19·8 ± 2·2	0·24 ± 0·03	0·37 ± 0·04	<0·001
2	12	23·6 ± 1·7	21·3 ± 1·3	0·30 ± 0·03	0·44 ± 0·03	<0·001
3	14	28·6 ± 2·4	19·9 ± 1·8	0·31 ± 0·02	0·39 ± 0·03	<0·01
5	6	27·0 ± 1·5	16·8 ± 1·5	0·22 ± 0·01	0·34 ± 0·02	>0·05

* Significance of variance between treated and control knees.

weight for granulomata from 14 rats treated with NAD was 0·544 ± 0·052 g while for control rats the value was 1·18 ± 0·10 g. The difference between these two results is significant ($P < 0{\cdot}01$). Rats which were injected with a 0·5 per cent solution of adenine instead of NAD had granulomata which were not significantly different from the control rats.

Thus, it has been shown that the NAD concentration per cell of carrageenin-stimulated proliferating tissue is lowered and increasing the NAD concentration exerts an inhibitory effect on cell division. A similar inhibition of growth by NAD has been shown in the case of methylcholanthrene sarcoma in rats[9].

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Nomenclature of Serum Proteins

HISTORICALLY, the first names and definitions of serum proteins were based on their solubilities. The classification established by Tiselius consists of five or six zones of electrophoretic mobility.

In order to be able to define the constituents of a mixture by at least two entirely independent criteria, we developed immuno-electrophoretic analysis[1]. This technique permits the definition of the constituents by their electrophoretic mobility and by their immunochemical specificity. Moreover, in certain cases, it is possible to introduce in the same experiments, by the use of special staining reactions, a third definition: either enzymatic activity or chemical composition. In the case of human serum, with the aid of convenient immune sera, 30–31 independent constituents can be distinguished[2]. In the sera of some other species similar, but generally smaller, numbers of constituents (due probably to the use of less-potent immune sera) have been described[3,4]. The multiplicity of components detected by immuno-electrophoretic analysis has obliged us, since our first investigation, to elaborate a simple nomenclature which would follow the general trends of the classification of Tiselius. Thus, we use the same Greek letters and indices to define the mobility zones; but we add Roman capital letters to designate the various components present in each zone.

The detection of some new constituents has necessitated our introducing the designation by the Greek letter ρ of those which migrate faster than the serum albumin, in order to stress their rapidity (this designation is preferable to 'prealbumin', because it is not a precursor of serum albumin) and by the letter α_0 those migrating in the zone of serum albumin and of distinctly greater electrophoretic mobility than the α_1 (ref. 2). The immuno-electrophoretic analysis has shown that the proteins which have been designated as γ-globulins form a 'family' of constituents[4], the members of which, although possessing the same antigenic specificity when analysed with hetero-immune sera, have mobilities which extend up to the zone of α-globulins. Therefore, when we detected a new component, the mobility of which was greater than the classical mean value accepted for the γ-globulins, we classified it as a β_2-globulin (ref. 6) and called it β_2A. Another globulin migrating in the same zone, but possessing a very high molecular weight, was called γ_1-macroglobulin by Kunkel *et al.*[7] and later β_2M by us[8].

In science, generally, one tries to preserve the name proposed by the author who first described a substance. Unfortunately, in recent years in different publications, designations have been used which may create confusion. The existence of common determinant groups on the proteins which we call γ, β_2A and β_2M and the existence of antibodies among them has led Heremans[8] to group them as immuno-globulins. More recently, several authors designate all of them by γ and divide them into γ_1 and γ_2 (some add even γ_3 for the slowest).

At first glance it would not seem important to call γ_1 those constituents which we classified as β_2, but, if one admits such a nomenclature, How can one designate those proteins which have the same antigenic specificity and the same sedimentation constant as the slowly migrating γ-globulins, but which possess mobilities of the β- or even of the α-globulins? If we give the name of γ_2 to the slowly migrating γ-globulins, it would be illogical and contrary to the general basis of the classification to designate also by γ_2 those which migrate in the β- or α-zones. On the other hand, if these latter are called γ_1, as some authors do, it would be necessary to find a criterion or a precise definition of the limits which would separate the 'family' into γ_1 and γ_2. So far, we have never observed experimentally, either by mobilities or by general antigenic specificity, any indication of the existence of such limits. The name of γ_{SS} has been proposed to designate the main γ-globulin family[9]; but this would unnecessarily introduce a complication in the usual classification.

Furthermore, it is known that with some immune sera one observes two extended and more or less parallel lines corresponding to γ-globulins and that sera of young children show two distinct γ-globulin precipitation lines, which would indicate some differences in antigenic determinants. Therefore, it seems to us logical to designate by γ without numerical indices the whole 'family', giving the elongated line of specific precipitation and possessing a 7_S sedimentation constant, but to use letters (γ_A; γ_B) for the parallel lines, and to continue to use β_2A, β_2M, β_2B, β_2C to indicate the distinct constituents which migrate in the β_2-zone and which do not necessarily belong to the immuno-globulins. Finally, in the case, for example, of mouse sera the number of relatively slow-migrating components seems larger than in human sera and an additional zone, β_3, has been envisaged to designate some of them[9,10].

It would certainly be desirable that the somewhat complicated nomenclature which we have been obliged to introduce, should be replaced by special names given to every detected component, as soon as its role and physical-chemical properties become known, as, for example, haptoglobin, caeruloplasmin, transferrin, etc.

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Isolation of Two Permeability Globulins from Human Serum

A PERMEABILITY factor, activated by dilution or surface contact, has been described in the β-globulin fraction of human serum[1]. This substance, designated *PF/dil*, seems to have much in common with the enzyme kallikrein first noted by Frey[2]. Whether the serum of any given species contains one, or more, permeability globulin has so far not been clear. Based on similarities of hypotensive and permeability properties, it has been suggested that *PF/dil* and serum kallikrein may be the same[3]. Indeed, di-isopropyl phosphofluoridate (DFP) and soybean trypsin inhibitor seem to inactivate both[4-6]. Permeability globulins from guinea pig serum have been reported with the electrophoretic mobility of α_2 (ref. 1) as well as γ-globulin[7]; whether this indicates two molecular species, or is an artefact of preparative procedures, is not certain. Early chromatographic work begun by one of us (J. P. L.) on certain lots of Cohn Fraction III, obtained commercially from human plasma, had suggested the presence of more than one non-dialysable permeability active substance. We wish to report the isolation of two permeability globulins, differing in their electrophoretic mobility and chromatographic behaviour, from sera of normal young men.

Permeability activity was assayed by its bluing action in the guinea pig skin, essentially as described by Miles and Wilhelm[8].

Samples of normal human serum, when subjected to preparative starch-block electrophoresis, demonstrated broad, merging peaks of activity in the γ- and β-globulin regions. Although these findings suggested the presence of two factors with differences in electrophoretic mobility, it was not possible to separate them completely from one another. We therefore turned to ion-exchange chromatography on DEAE cellulose.

As seen in Fig. 1, the proteins of whole serum were separated with the use of a variable ionic gradient, and two, non-dialysable, discrete peaks of permeability-inducing substances were found. The first of these (*A*) was eluted with a buffer conductivity of 0·0066 mhos, principally with the serum γ-globulins. The second peak (*B*) desorbed from the column at a higher ionic strength (between 0·0200 and 0·0351 mhos), along with a mixture of γ- and β-globulins. Because of the small amounts of active material present, attempts at starch-block electrophoresis of these fractions failed to recover activity, so that preliminary fractionation of larger amounts of serum was undertaken.

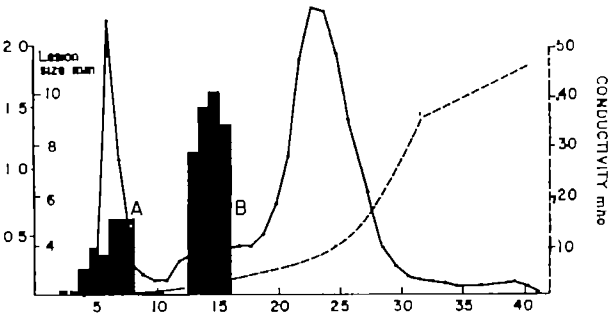

Fig. 1. DEAE cellulose chromatography of 4 c.c. of whole serum. Starting buffer: 0·005 M phosphate—conductivity 0 0069, pH 8·10, final buffer: 0 0125 M phosphate + 1 M sodium chloride—conductivity 0·459, pH 8 10. 10 c.c. collected in each tube. Column 20 cm × 2 cm packed with DEAE cellulose (approx. 9 g, 0·92 m.equiv./g) under 5 lb./in.² pressure. Bioassays were carried out in dilutions of 0·15 M saline. ——, Protein concentration; ---, conductivity; shaded, bioassay of 1 : 3 dilution of content of each tube

247 ml. of pooled human serum were fractionated with ethanol, according to the method 10 of Cohn *et al.*[9]. Permeability increasing activity was present mainly in fractions I + III-3, and III-1, 2, with trace amounts in fraction III-0. The first two of these fractions were separately chromatographed on DEAE cellulose, under the same conditions as for whole serum. From each fraction, two peaks of activity were again recovered in the same positions as shown in the elution diagram for whole serum (Fig. 1).

Samples of fraction *A* obtained from the chromatography of the two ethanol fractions were pooled, as were those of fraction *B*, and each was run in starch-block electrophoresis, with good recovery of activity. The permeability activity in fraction *A* migrated towards the cathode in the manner of the serum γ-globulins. Activity from *B* moved towards the anode with the mobility of β-globulin.

Active samples of *A* and *B* were recovered after electrophoresis and each was rechromatographed on DEAE cellulose, with no change in chromatographic behaviour.

The active fractions *A* and *B* were both inhibited by 50 μg/ml. of soy-bean trypsin inhibitor, or 5×10^{-4} M DFP after incubation at 4° C for 1 h.

Treatment of guinea pigs with the antihistamine diphenhydramine (20 mg/kg intraperitoneally, 2 h before testing) did not prevent the action of either permeability globulin.

In view of the report[10] of little affinity of serum kallikrein preparations for DEAE cellulose, the identity of the γ-globulin factor with serum kallikrein, and of the β-factor with *PF/dil* of Miles may be suggested.

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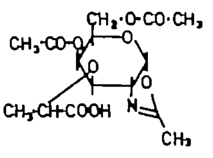
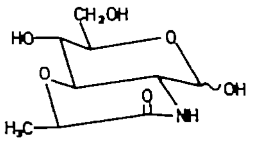
Acetyl Derivatives of Muramic Acid

ATTEMPTS to prepare the fully acetylated methyl ester of muramic acid for subsequent use in the Koenigs-Knorr condensation have led to some unexpected compounds. A lactam II ('muramo lactam') was obtained when muramic acid[1,2] was treated with diazomethane at 0° and the excess evaporated immediately to avoid methyl glycoside formation[3]. Lactam II, which can also be obtained by the action of di*cyclo*hexylcarbodiimide (DCC) in dimethyl sulphoxide for 48 h at room temperature, had negligible methoxyl content, negative ninhydrin and lactone[4] reactions and a broad absorption in the infra-red spectrum at 1,655 cm^{-1}, indicating its lactam character.



(I)



(II)

Acetylation of muramic acid with pyridine and acetic anhydride (24 h at 40° C) and crystallization of the product from ethanol gave a mixture of two isomeric compounds III and IV in 26 per cent yield. Chromatography of the residue from the ethanolic mother liquor on silicic acid gave more of crystalline IV and a further crystalline isomeric compound V. Compound IV appears to be sensitive to even mildly alkaline agents. Similar acetylation of lactam II and crystallization from ethanol gave compound III in 37 per cent yield.

The infra-red spectra of III and IV do not show amide II bands; the spectra are not changed by treating the compounds with diazomethane so it is unlikely that either compound could be an isomeric acid oxazoline I. It is therefore assumed that they are anomers of tri-*O*-acetylmuramo lactam and, if this is so, the optical rotations indicate that III has the β- and IV the α-configuration. The infra-red spectrum of V shows amide I and II absorptions and with the analytical figures this indicates that V is a fully acetylated 6- or 8-membered lactone of *N*-acetylmuramic acid. Another lactone of muramic acid has been reported[5].

For the preparation of *N*-acetylmuramic acid VI, the use of acetic anhydride, which might cause intramolecular cyclization, was avoided and *N*-acetoxyphthalimide[6] (prepared in 84 per cent yield from acetyl chloride and finely ground sodium *N*-hydroxyphthalimide[7] in benzene) was used. Triethylamine (1 equiv.) in methanol was added to muramic acid (1 equiv.) in about 90 per cent methanol at 0°, followed immediately by *N*-acetoxyphthalimide (1·4 equiv.) in ethyl acetate. After 0·5 h at room temperature the solution was evaporated, the residue dissolved in water and filtered. Treatment with 'Amberlite 1*R* 120' (H$^+$ form) until the filtrate was acidic, extraction with portions of ethyl acetate (until these no longer coloured sodium bicarbonate solution) and evaporation of the aqueous solution gave VI as a crisp foam in nearly quantitative yield.

Acetylation of VI and crystallization from ethanol gave the lactone V (32 per cent yield). Methyl *N*-acetylmuramate VII (found —OCH$_3$, 10·8 per cent, calculated 10·1 per cent) was prepared from VI with diazomethane and could not be crystallized. Acetylation of VII gave a product which was separated by chromatography on silicic acid into two isomeric fractions, VIII (crystallized from benzene) and IX (crystallized from ethanol). The overall yield of crystalline VIII and IX from muramic acid was 69 per cent in the ratio 9 : 1. From the analyses, these are assumed to be the anomers of methyl tri-*O*-acetyl-*N*-acetylmuramate, and the optical rotations indicate that VIII has the α- and IX the β-configuration.

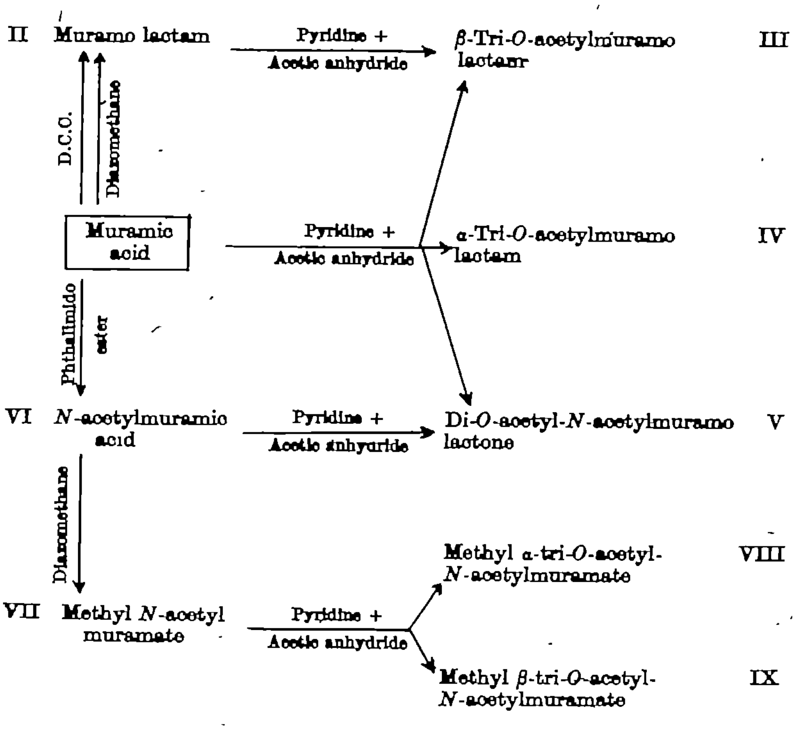

II Muramo lactam
Pyridine +
Acetic anhydride
β-Tri-O-acetylmuramo lactam III
D.C.C.
Diazomethane
Muramic acid
Pyridine +
Acetic anhydride
α-Tri-O-acetylmuramo lactam IV
Phthalimido ester
VI N-acetylmuramic acid
Pyridine +
Acetic anhydride
Di-O-acetyl-N-acetylmuramo lactone V
Diazomethane
Methyl α-tri-O-acetyl-N-acetylmuramate VIII
VII Methyl N-acetyl muramate
Pyridine +
Acetic anhydride
Methyl β-tri-O-acetyl-N-acetylmuramate IX

Fig. 1

Table 1

	Melting point	$[\alpha]_D^{22}$ c, 2 CHCl$_3$	C (%)	H (%)	N (%)	$-$CO.CH$_3$ (%)	$-$O.CH$_3$ (%)
$C_{15}H_{21}NO_9$ (III, IV and V) requires			50·13	5·89	3·9	35·9	0
III	192° change of form; liquefying c. 210°	+ 57°	50·3	5·94	3·9	37·4	0·83
			50·1	5·83	4·1		
IV	200–205°	+ 118°*	50·19	5·89	4·17	33·08	
V	210–212°	+ 154°	50·14	5·64	3·87 4·09	31·5	
$C_{18}H_{27}NO_{11}$ (VIII and IX) requires			49·9	6·28	3·23	39·7	7·16
VIII	103–104°	+ 103°	49·90	6·17	3·22	38·30	7·52
IX	173–174°	+ 28°†	50·06	6·24	3·34	38·32	7·56

* c. 1·56 at 20° C. † c. 1·45.

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Combination Analysis of Rabbit Serum Proteins, using High-resolution Porath Zone Electrophoresis and Starch-gel Electrophoresis

Two-dimensional electrophoresis by the method of Poulik and Smithies[1] provides a powerful tool for the identification of serum protein bands separated in unidimensional starch-gel electrophoresis. It has in some cases revealed the presence of unsuspected components which are masked by others in the unidimensional run[2]. The method is, however, severely limited by small loading of protein sample possible by means of filter-paper insertion. Some improvement may be gained by making the preliminary run in agar where not only is the loading higher but also the resolution is more powerful than on paper. Thus two α_1 and two α_2 components are to be distinguished in agar runs of rabbit serum[3].

In order to start the starch-gel electrophoresis of the second phase of a two-dimensional analysis with an adequate loading of protein, it was decided to concentrate fractions from a Porath zone electrophoresis experiment. Protein concentrations were brought back to 1 per cent or somewhat more, with the exception of the fractions from the small γ_2 peaks.

The analysis obtained by this combination of methods is presented in qualitative form in Figs. 1 and 2. It is immediately apparent that there exists a much greater complexity of serum protein components than has previously been recognized. Albumin has been run off the Porath column and is not retained in the analysis. Four α components are recognized in the Porath scan, and three of these, α_{1a}, α_{1b} and α_{2b}, have been concentrated and run on starch. α_{2b} appears to be resolved scarcely from the β_1, yet the gel analysis shows a marked difference of composition between even these two closely adjacent fractions. Little slow α_{2b} macroglobulin, for example, is present in the β_1, and the heavily staining major β_1 component is only slightly represented in the α_{2b}.

Perhaps the most interesting finding lies in the demonstration that the leading slow α band is an α_{1b} and to a lesser extent an α_{1a} component. Here is a first example of an α_1 slow component, possibly macroglobulin, normally present in rabbit sera in our experience, though apparently not demonstrated by the older horizontal starch-gel technique. A component similar to the slow α_2 has been demonstrated to appear during pregnancy and certain tumour-bearing conditions in the rat[4]. We are investigating the presence of this component in other species.

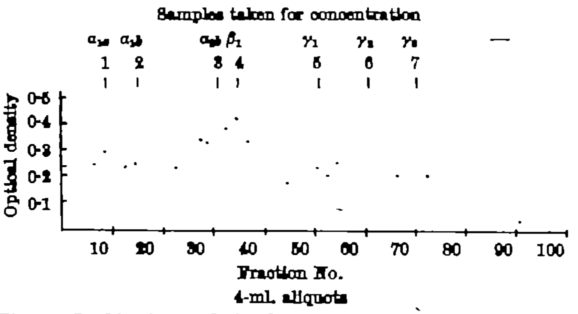

Fig. 1. Combination analysis of normal rabbit serum protein. First phase, Porath column electrophoresis showing fractions cut for reconcentration to 10 mg/ml. protein content

A further point of interest is that the γ_1 (or β_2) fraction gives a single rather diffuse zone which has in fact greater mobility than the slow α_1 and thus lies ahead of it in the unidimensional pattern. Most accounts in the literature fail to mention the presence of γ material so far ahead of the well. The resolution of the γ-globulins would thus appear to be much greater on starch than is generally supposed, although they do not form sharp bands. It is open to question whether this γ_1 zone includes the γ_1 macroglobulin, and work is in progress to investigate this point.

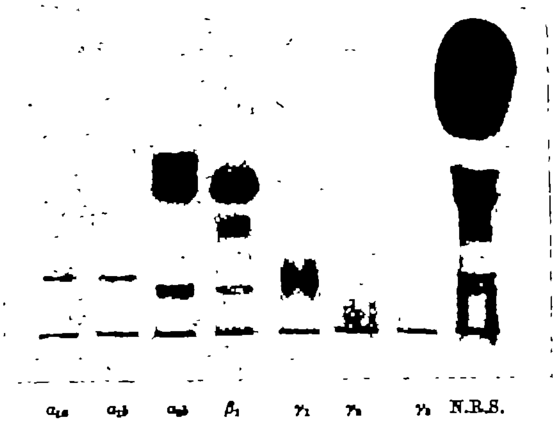

Fig. 2. Second phase. Vertical starch-gel electrophoresis of the series of concentrates derived from Fig. 1. N.R.S., Normal rabbit serum

The total number of components recognized as distinct by visual inspection of the original gel, and not shared by adjacent fractions, we record as 25 in this analysis. This compares with the previous 10–11 distinguished in this species by unidimensional starch-gel electrophoresis. Since several of the major zones, such as $f\alpha_{1a}$, $f\alpha_{1b}$ and γ_1, are diffuse, it may be that further refinements of technique will lead to sub-fractionation of some of these also. No attempts have yet been made to vary buffer conditions from those established by Smithies.

Zone electrophoresis was carried out by Porath's method[5] on a column kept at about 5° C, of dimensions 90 cm long and 2·5 cm diameter, on Munktell prepared cellulose, using veronal buffer pH 8·5, $i = 0{\cdot}05$.

The run was for 120 h at 1,000 V, 20 m.amp.: elution set at a flow-rate of about 16 ml./h. Aliquots of approximately 4 ml. were collected on a time-controlled 'Beaumaris' fraction collector working overnight. Albumin was run off the column, being collected in a dialysis sack tied over the outlet. Protein determinations were made on alternate samples, using the method of Lowry *et al.*[6]. Starch-gel electrophoresis was carried out by the vertical method of Smithies[7] using his buffer solutions.

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Essential —SH Groups in Liver Ketohexokinase

PREPARATIONS of liver ketohexokinase (ATP: D-fructose 1-phosphotransferase) phosphorylating fructose at C-1 have been obtained mainly by Leuthardt and Testa[1,2], Staub and Vestling[3], Hers[4], Kuyper[5] and Parks[6]. Recently, Cadenas and Sols[7] obtained a preparation from intestinal mucosa. The importance of the —SH groups of the enzyme has not been investigated, except by Kuyper, whose results were rather negative[5].

Our ketohexokinase preparation was obtained from beef liver, according to Parks's method, omitting the second fractionation with methanol, introducing small modifications and lyophilizing for better conservation. This preparation phosphorylates 11 μmoles of fructose/mg protein nitrogen in 20 min, under the conditions set up by Hers[4]. It also phosphorylates sorbose at a velocity of 57 per cent of that of fructose. The Michaelis constant for fructose is $4{\cdot}6 \times 10^{-4}$ M.

With this preparation we have tested for —SH groups with four reagents.

The *p*-chloromercuribenzoate is a strong inhibitor of the ketohexokinase. At 8×10^{-5} M concentration, the whole enzymatic activity disappears. This inhibition is partially reverted by cysteine 2×10^{-4} M and the recuperation reaches up to 90 per cent with cysteine 2×10^{-3} M. If the unbound inhibitor is removed by dialysis, the same effect is observed.

N-ethylmaleimide, another effective —SH reagent, is also a powerful inhibitor of the enzyme, as is shown in Fig. 1.

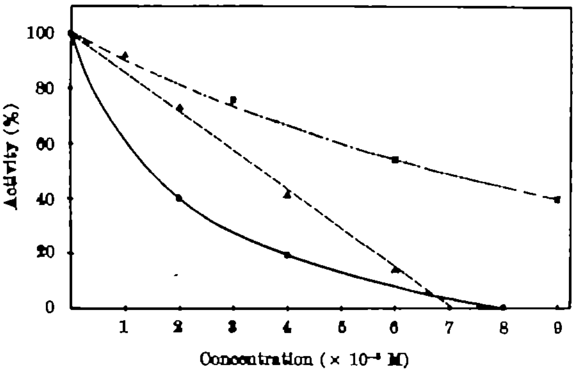

Fig. 1. Inhibition of ketohexokinase by *N*-ethylmaleimide, *o*-iodosobenzoate and *p*-chloromercuribenzoate. Reaction mixtures: 0·5 ml. enzyme solution with or without inhibitor, 0·1 ml. fructose 0·4 per cent, 0·1 ml. ATP 0·05 M, 0·1 ml. $MgAc_2$ 0·05 M, 0·3 ml. KAc 4 M, 0·1 ml. NaF 0·5 M, at 37° C, 20 min. ■, *o*-Iodosobenzoate; ▲, *N*-ethylmaleimide; ●, *p*-chloromercuribenzoate

o-Iodosobenzoate as well inhibits the enzyme. The inhibition is about 50 per cent at 6×10^{-5} M and reaches 100 per cent at $2{\cdot}5 \times 10^{-4}$ M. Cysteine does not produce reactivation even at a concentration of 10^{-3} M.

It has been reported[5] that iodoacetate does not inhibit ketohexokinase. However, if the enzyme solution is previously maintained in the presence of 0·02 M iodoacetate at 37° C for some time, the enzymatic activity becomes clearly inhibited. This inhibition increases with the time of previous incubation.

If iodoacetic acid is recrystallized several times, some diminution of the inhibitory effect is observed. However, the monoiodoacetate action cannot be attributed to the possible presence of iodine. In fact, iodine is an inhibitor of the enzyme, but only at concentrations higher than 10^{-5} N, which cannot be found when the recrystallized monoiodoacetic acid is used. The inhibitory effect of iodoacetic acid is not due to the possible decomposition products formed from it during the time of previous incubation, nor can it be attributed to a thermal inactivation of the enzyme. Neither is the *p*H appreciably modified during the previous incubation.

These results seem to offer sufficient evidence for the presence of essential —SH groups in the ketohexokinase. The behaviour with monoiodoacetate can be explained by a lower reactivity between the sulphidryl groups and this inhibitor.

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Isolation of a Polar Aldosterone-like Substance from the Urine and Adrenal Incubates of Patients with Arterial Hypertension

A CONJECTURED role of disordered sodium-retaining function of the adrenal cortex in the genesis of essential hypertension has been largely founded on indirect evidence[1,2], including the rationale of low-sodium intake[3,4] and of naturetic agents[5,6] in therapy; and on direct steroid measurements[7,8]. These latter have shown hyper-excretion of the mineralocorticoid, aldosterone (11β,21-dihydroxy-20-oxo-pregn-4-ene-18-al), and its major metabolite, tetrahydroaldosterone (3α,11β,21-trihydroxy-20-oxo-5β-pregnane-18-al), in uncomplicated as well as complicated forms of this disease. These observations have favoured the hypothesis that essential hypertension may represent, in effect, a special form of hyperadrenocorticism. Correlation of normal aldosterone regulation with the renin-angiotensin mechanism in the kidney[9,10] has further focused attention on possible regulatory abnormalities of aldosterone in the pathogenesis of arterial hypertension.

Investigations here of mineralocorticoid function in essential hypertensives have led to the isolation from urine and adrenal incubates of an uncharacterized substance, designated as X_2, which may be chemically and physiologically related to aldosterone but differing from aldosterone in that it is chromatographically more highly polar (? hydroxylated) in character. The isolation of this substance, and of three other highly polar metabolites (X_1, THX_1 and THX_2), occurred fortuitously as a result of the application of reversed-phase partition chromatography[11] to the concurrent measurement of aldosterone and tetrahydroaldosterone in urine. This chromatographic modality has been considered of particular value for the separation of relatively polar C_{19} and C_{21} steroids which, because of retarded mobilities, are poorly resolved in conventional liquid–liquid systems. Our interest in this unknown substance was intensified by its absence in the urine of normal subjects under basal conditions. The results of our biosynthetic studies have also stimulated this research. An adrenal site of biogenesis of X_2 has been suggested by the incorporation of ^{14}C-acetate into this fraction during *in vitro* incubations of surviving adrenals surgically removed from one patient with essential hypertension (3·0 μg/g tissue/3 h) and from another patient with Cushing's syndrome due to adrenocortical hyperplasia (2·1 μg/g tissue/3 h). A relationship of the excretion of this metabolite to stress and to cardiovascular disease was early seen on its discovery in the urine of a subject who, while being studied by us during the course of intense psychological stress, developed sinus tachycardia, mild hypertension, and retinal hæmorrhages.

The mobilities of this metabolite isolated from the urine of hypertensives by reversed-phase partition chromatography, eluted and rechromatographed in two different systems as the acetylated derivative are shown in Table 1. Aldosterone and tetrahydroaldosterone were used as reference compounds. Inferences drawn from these studies were that X_2 is more polar than either of the foregoing and that it probably possesses four or five acetylable hydroxyl groups. From its solubility in organic solvents and its exhibition of a 239 mμ spectroscopic absorption maximum, we have assumed X_2 to be a Δ^4,3-ketonic steroid. Further semi-qualitative information derived from chromagen formation has shown a similarity of this substance to aldosterone with respect to the presence of an α-ketolic function at carbon 21 and has confirmed the absence of a 17α-hydroxyl substituent.

Table 1. CHROMATOGRAPHIC MOBILITIES

Steroid	Chromatographic system		
	RPPC* (R_{DOC})	Modified Bush 'A' (12) (R_F)‡	GLPC† (retention time (min))
Aldosterone	4·61	0 187	4 0
Tetrahydroaldosterone	5·80	0·625	3·8
X_2	6·22	0·629	0 4 (Major) 1 0 0·5

* Reversed phase partition chromatography. mobilities compared with DOC (11-deoxycorticosterone).

† Gas-liquid partition chromatography; 5·50 per cent '*SE-30*' column. Steroids and unknown fraction acetylated.

‡ Steroids and unknown fraction acetylated.

Table 2. NORMAL, PHYSIOLOGICAL AND PSYCHO-PHYSIOLOGICAL STUDIES (URINARY EXCRETION, μG/24 H)

Type of stimulus; type of subject	No. of subjects	Aldosterone*	Tetrahydro-aldosterone	X_2
None Normal ♂ baseline	5	21 8 ± 8 2	48·7 ± 26·7	0
Normal ♂	1			
Baseline	Same	12·7	28 0	0
Corticotrophin	Same	16·2	124·1	0
Normal ♂	1			
Baseline	Same	19·2	21·7	0
Sodium deprivation	Same	78·2	59·8	12·6
Anxious ♂	1			
Baseline	Same	17·8	68·0	5·4
Stress; schol. exam.	Same	56·2	158·6	104·8
Hypomania †	1	14·6	609·0	109 2

* Standard recovery, 91 ± 8 per cent.

† ? Sodium intake; ? excessive sweating.

Investigations designed to demonstrate the possible physiological implications of X_2 have shown that its excretion could be induced by sodium deprivation and psychological stress, while exogenous corticotrophin appeared to be an ineffective stimulus (Table 2). Its maximal excretion during psychiatric hyperactivity will be noted.

A systematic study of mineralocorticoid excretion, including X_2 (measured as α-ketolic material), was undertaken in arterial hypertension. These results are shown in Table 3. It would seem that the excretion of X_2 described a provocative, if unknown, feature of essential hypertension and of hypertension associated with two hyperadrenocortical disease entities. None of this substance was found in the urine of patients with hypertension and hypokalæmic alkalosis due to unilateral renal disease. Of significance, it is believed, was the discernment of X_2 during each of the stages in the evolution of essential hypertension, including its earliest detectable expression in two genetically predisposed subjects.

Table 3. ARTERIAL HYPERTENSION* (URINARY EXCRETION, μG/24 H)

Ætiology of hypertension	No. of patients	Aldosterone	Tetrahydro-aldosterone	X
Essential, early	2	8·6	24·0	16 0
Essential, advanced	5	79·5	108·3	86·5
Essential, advanced with hypokalæmic alkalosis and mild azotæmia	2	321·3	328·0	64·1
Conn's syndrome	3	66·0	859·1	25·2
Cushing's syndrome	1	45·6	24·1	10·8
Unilateral renal disease	2	47·5	47·3	0

* Does not include patients with œdema, congestive heart disease, ascites, etc , or those on sodium restriction.

The implications of this work are that the unknown substance X_2, conceived at present as a Δ^4,3-ketonic steroid physiologically and chemically related to aldosterone. is a stress-directed biosynthetic product of the adrenal which may play a part in the pathogenesis of essential hypertension and in the hypertension of hyperadrenocorticism. Chemical and physiological characterization studies of this substance are now in progress.

We thank Dr. Horace Martin, of Monsanto Research Corporation, Everett, Mass., for his co-operation in the characterization studies and for doing the gas-chromatographic analyses. We also acknowledge the support of research grants Nos. *CA* 06745 and *HE* 07041 from the National Institutes of Health, Bethesda.

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Solubility of Bean Leaf Protein in Ethanol

IT has been generally held that leaf proteins are insoluble in 80 per cent ethanol or similar solvents[1]. This belief has furnished a useful means of distinguishing two forms of nitrogen, 'soluble' (that is, amino-acids and peptides) and 'insoluble' (that is, proteins and nucleic acids). We became aware of a possible discrepancy in this logic when an alcohol-soluble 'peptide' fraction of bean leaf displayed an amino-acid composition indistinguishable from the bulk protein. Further tests, which included the biuret[2] and Folin[3] assays, trichloroacetic acid precipitability, and stainability with light green, indicated the protein-like nature of this 'peptide'. The recovery of such presumed protein from fresh bean leaves was about 1 per cent of

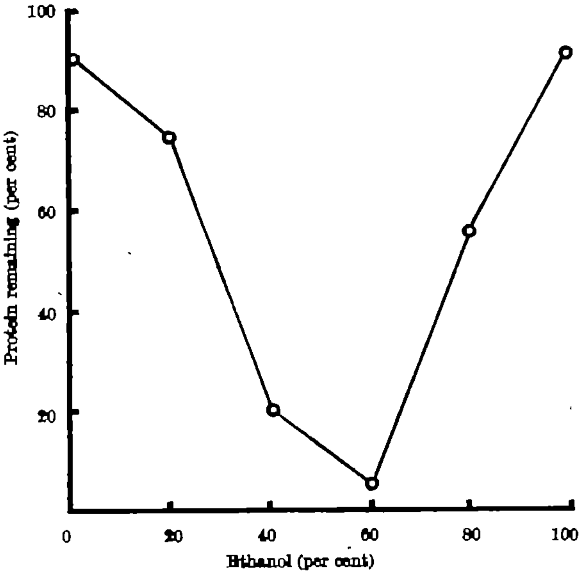

Fig. 1. Solubility of bean leaf protein, precipitated by trichloroacetic acid, in ethanol–water mixtures. The vertical axis shows the percentage protein which did not dissolve

the total protein. When examining peptide metabolism, this error would be more or less serious depending on the amount of peptide present. The amount usually present in bean leaves was quite low judging by paper chromatographic methods[4], hence the presence of ethanol-soluble protein was an unfortunate complication.

To establish the limits of bean leaf protein solubility in ethanol, freshly prepared protein solutions in buffer were treated with trichloroacetic acid and the resulting precipitates digested for 5 min with 4 ml. of varying concentrations of hot ethanol–water. The remaining precipitates were dissolved in 0·5 N sodium hydroxide and assayed with modified Folin reagent[3]. The results (Fig. 1) show that the protein was maximally soluble in 60 per cent ethanol. Experiments with protein precipitated by hydrochloric acid showed similar results. Likewise, other solvents such as acetone, methanol, and isopropanol had an equally great solvent action on the protein precipitates.

This anomalous behaviour had earlier been considered unique for insulin and serum albumin[5]. Whatever the cause of such solubility, we must re-evaluate the proposition that 80 per cent ethanol-extractable nitrogen does not contain protein. It seems possible that some leaf protein may be in the acid-denatured state either naturally (as a result of cellular senescence), or as an artefact of grinding which brings low *p*H materials into contact with native proteins.

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Solubilization and Proteolytic Activation of Glucose-6-phosphatase from Microsomes of Bovine Livers

IN earlier publications[1,2] we described the preparation of soluble glucose-6-phosphatase (G6Pase) from microsomes of bovine liver. We always found that a combined treatment of the microsomes with sodium deoxycholate and digitonin leads to a reproducible solubilization of this enzyme. However, it was noted that G6Pase is very sensitive towards sodium deoxycholate and under the latter's influence activity is markedly diminished.

For this reason we looked for another possibility of treating microsomes with sodium deoxycholate in order to elucidate their structure. Heise and Kleitke[3] found that the fructose-1,6-diphosphatase is activated by trypsin in a proteolytic manner. Therefore, expecting a similar attack on the microsomes by trypsin we examined the influence of variable concentrations of trypsin and times of incubation on the disintegration of the structure of the resuspended microsomes. After discovering the best conditions of treatment with trypsin we examined the influence of the time of incubation with digitonin on the pretreated particles.

In order to prepare microsomes, bovine livers were minced mechanically and this was followed by homogenization of the liver in saline in Potter–Elvehjem-homogenizer with a 'Teflon' pestle for 90 sec. Undisrupted cells, nuclei and mitochondria were removed by centrifugation at 18,000*g*. By mixing the homogenate with 0·1 M citrate buffer *p*H 5·4 the microsomes were agglutinated and separated by centrifugation at 18,000*g* for 20 min.

Determination of activity was carried out by incubation of the samples with glucose-6-phosphate at 38° C (ref. 4). The liberated phosphate was measured by the method of Fiske and Subbarow[5]. Absolute activity was defined as µmoles liberated phosphate/h in 1-ml. sample; the specific activity as µmoles liberated phosphate/mg protein. Determinations of the protein were carried out by the biuret method and by the method of Lowry *et al.*[6] to reveal very small amounts of proteins.

We incubated the suspension of microsomes with trypsin at 40° C, the optimal temperature of this enzyme. Using 'Trasylol', a specific inhibitor of trypsin, we were able to prevent the action of the proteinase. 50 Kallikrein inactivator units of 'Trasylol' inhibit 100γ of crystalline trypsin ('Trasylol,' Trypsin-Kallikrein-Inaktivator: Bayer-Leverkusen). However, the amounts of 'Trasylol' necessary for the complete inhibition of trypsin needed for a successful treatment of the microsomes were too large for economic use. Therefore we prevented the action of trypsin by a rapid refrigeration of the samples in an ice-bath, washing the residue obtained by centrifugation.

To 1·0 ml. of suspended microsomes with 150 mg proteins, 2·5 ml. of solutions of trypsin of variable concen-

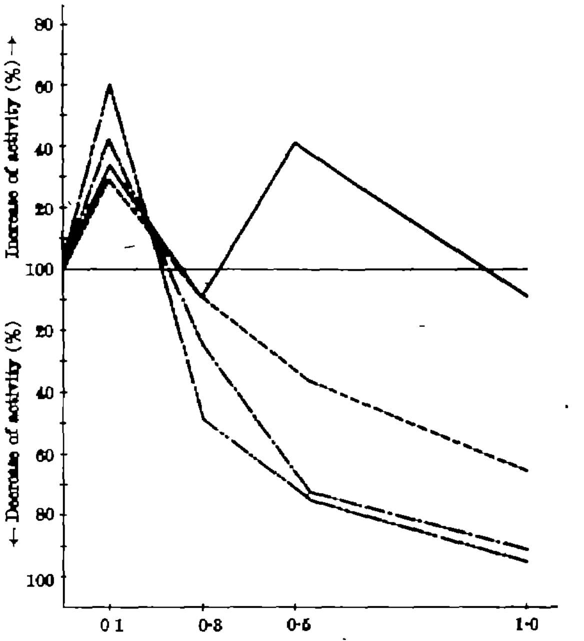

Fig. 1. Proteolytic activation of glucose-6-phosphatase inhibited by 'Trasylol'. Time of incubation: ——, 5 min.; – – –, 15 min; —·—, 30 min; —··—, 50 min

Table 1. INFLUENCE OF DIGITONIN OF TRYPSIN PRE-TREATED MICROSOMES. TEMPERATURE, 0 °C

	Suspension of microsomes	Trypsin supernatant	Trypsin residue	Digitonin supernatant (5 min)	Digitonin residue (5 min)	Digitonin supernatant (10 min)	Digitonin residue (10 min)	Digitonin supernatant (30 min)	Digitonin residue (30 min)	Digitonin supernatant (45 min)	Digitonin residue (45 min)	Digitonin supernatant (120 min)	Digitonin residue (120 min)
Absolute activity	152·5	8·0	62·5	25·5	61·0	28·5	67·0	27·5	70·0	57·0	57·5	36·0	47·5
Specific activity	8·18	1·71	12·5	10·4	8·33	16·0	13·4	12·2	30·04	21·5	24·68	11·07	9·5

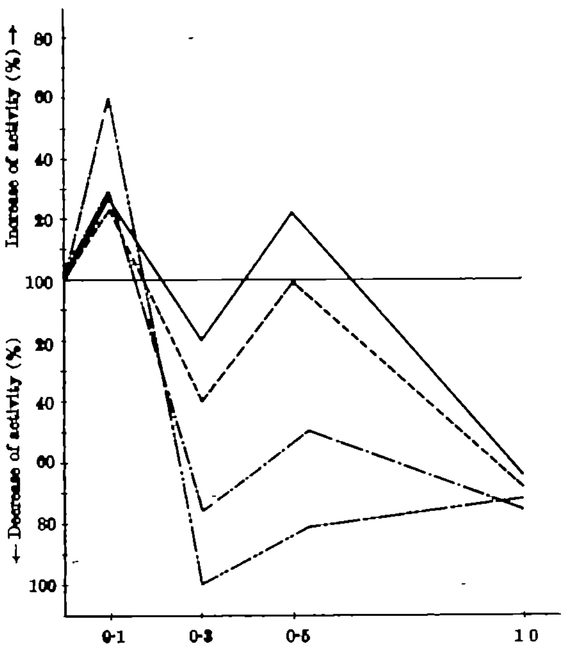

Fig. 2. Proteolytic activation of glucose-6-phosphatase inhibited by cooling. Times of incubation: same as in Fig. 1

trations and 0·5 ml. 'Trasylol' or 0·5 ml. distilled water respectively were added to obtain a final volume of 4·0 ml. for incubation.

Four concentrations of trypsin (0·1, 0·3, 0·5 and 1·0 mg trypsin/ml. suspension) were applied. The influence of the time of incubation on the activity of the G6Pase was also examined. Alterations of enzymatic activity were expressed in per cent, taking the activity of the untreated microsomes as 100 per cent. According to Swanson[7] the activity of G6Pase is not proportional to dilution. However, our experiment showed that good proportionality exists up to a dilution 1 : 3.

The results obtained are illustrated in Figs. 1 and 2. Fig. 1 shows the influence of trypsin inhibited by 'Trasylol' on the activity of G6Pase; Fig. 2 shows the influence of trypsin without 'Trasylol' on the activity of the G6Pase. It can be seen that both methods alter the activity of G6Pase in the same manner. It was surprising that there were two peaks of enzymatic activity at 0·1 mg and at 0·5 mg trypsin. This fact can be explained either by assumption of two isoenzymes of the G6Pase or by the existence of two different activable centres in the same molecule of the enzyme. The lowered altitude of the second peak can be interpreted by assumption of a proteolytic activation of a G6Pase molecule, which is already damaged by trypsin.

It can be seen that incubation for 50 min at a concentration of 0·1 mg trypsin appeared to be the most favourable pretreatment of the microsomes for the preparation of soluble G6Pase.

Examining the influence of digitonin, the washed and centrifuged residue of the trypsin-treatment was mixed with the same amount of 2 per cent solution of digitonin and stirred at 0° C. After different times (5–120 min) aliquots of the mixture were removed, mixed with the same amount of 0·1 M citrate buffer *p*H 5·4 and centrifuged at 18,000*g*. Thereafter we determined the enzymatic activity in the supernatant and in the resuspended residue.

The results of these experiments are summarized in Table 1. It can be seen that the time of digitonin-action is without influence on the solubilization of the enzyme. For this reason we suppose that a 30-min action of digitonin is sufficient for loosening the G6Pase from trypsin-pretreated microsomes.

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PHYSIOLOGY

A Linear Transfer Function underlying Impulse Frequency Modulation in a Cockroach Mechanoreceptor

Pringle and Wilson[1] have indicated the potential usefulness in neurophysiology of the linear transfer function concept of the engineering sciences[2], in their investigation of the dynamic behaviour of the mechanoreceptor of the large tactile spine on the femur of the cockroach *Periplaneta americana*. They have pointed out in particular that adaptation of impulse frequency following a step function stimulus (the step function transient response) and the responses to be expected from stimulation with sinusoidal forcing functions (the steady-state frequency response) are related through the transfer function, and that independent observations made with these two stimulus forms can therefore be compared to test the validity of proposed models of dynamic behaviour. In their work[1], these authors proposed that the present receptor adapts according to a time function containing three exponential terms. However, only a small range of forcing frequencies was available for their frequency response study, and their conclusion was based largely on an interpretation of the adaptation curve. Moreover, the close agreement of their sinusoidal data with this model is evidently due to a miscalculation of three coefficients in their equation (2).

In the investigation reported here we have extended this work, following essentially the same theoretical and experimental approach except in details of apparatus and methods of data analysis. In our preparation the isolated femur was mounted by insect pins, which also served as recording electrodes, on the armature of a strain gauge dynamometer (Statham *G*1-4-250), which recorded the tension applied to the spine by a servogalvanometer (Sanborn 171) driven with square or sine waves by an electronic function generator (Hewlett-Packard 202*A*).

In a study of more than 20 receptors, the discharge patterns (Fig. 1) were in general agreement with those of Pringle and Wilson[1]. With sinusoidal forcing, impulse frequency modulation usually occurred over a tension-dependent range of forcing frequencies lying between 0·01 and 100 c.p.s., with modulated impulse frequency approximating a clipped sine wave at the forcing frequency (Fig. 1, inset). Continuous firing throughout the forcing cycle was found on only one occasion. Usually with forcing frequencies below the frequency modulating range no discharge occurred, and above this range there was a single impulse per cycle, as with other mechanoreceptors under vibrational stimuli[1,3,4]. In analysing the clipped sinusoidal discharges we have computed the mean and fundamental terms of Fourier series of the impulse frequencies, using a digital computer. The result is a sine wave at the forcing frequency, fitting the data in the least squares sense, and filling in the silent part of the response cycle with fictitious negative impulse frequencies (Fig. 1, inset). These sine

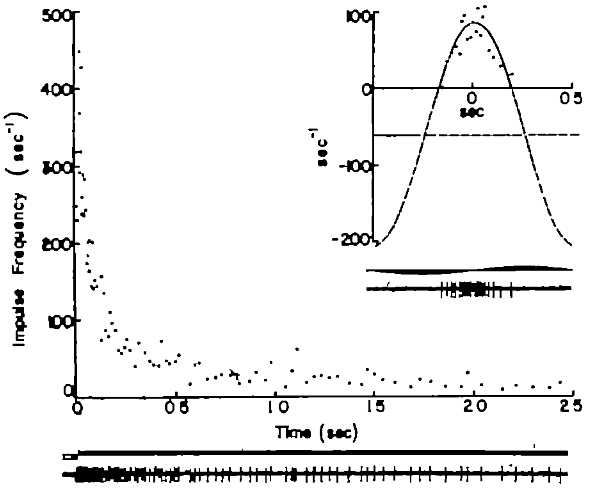

Fig. 1. Transient response to step function of applied tension (main graph) and steady state response to 1 c/s sinusoidal forcing function (inset). Tension applied 0·65 mm from base of spine; tension records above impulse discharge traces indicate range 0·8 – 2·4 kdynes increasing upward, and mean tension 1·6 kdynes in both cases. Spike amplitudes 100 μV. Sine wave through impulse frequency plot in inset is fundamental term of Fourier series of the discharge

waves were taken as the response in evaluating the frequency response function from sinusoidal stimulation data.

The step function transient responses of most of the receptors could be adequately described by a power relation of the form:

$$y = B\,t^{-k} \tag{1}$$

in which y = impulse frequency, t = time, and B and k are linear regression coefficients of log (y) on log (t) (Fig. 2). Thus B is related to sensitivity of the receptor and k to adaptation rate. Landgren[5] has reported a similar relationship for carotid sinus baroreceptors in the cat. The corresponding linear transfer function is given by the Laplace transform of equation (1) divided by that of the step function stimulus, as:

$$F(s) = B\ \Gamma(1-k)\ s^{k} \tag{2}$$

in which s is a complex variable (the 'complex frequency', p in the notation of ref. 1), $\Gamma(1-k)$ denotes the Γ function, and B and k are as in equation (1). The imaginary values of s correspond to the forcing frequencies of sinusoidal stimulation; substituting these into equation (1) yields the frequency response function:

$$F(j2\pi f) = B\ \Gamma(1-k)\ (2\pi f)^{k}\ \exp(jk\,\pi/2) \tag{3}$$

in which $j = (-1)^{1/2}$ and f = cyclic forcing frequency. Hence in this model the expected sinusoidal modulation leads the stimulus by a constant phase angle, $k\,\pi/2$ radians, and modulation sensitivity (response/stimulus amplitude ratio) increases as the k power of the forcing frequency. By contrast, a triple exponential adaptation model predicts in-phase modulation at low forcing frequencies, with increasing sensitivity and leading phase angles passing through one or more maxima at intermediate frequencies, terminating asymptotically in a greater in-phase sensitivity at higher frequencies[1].

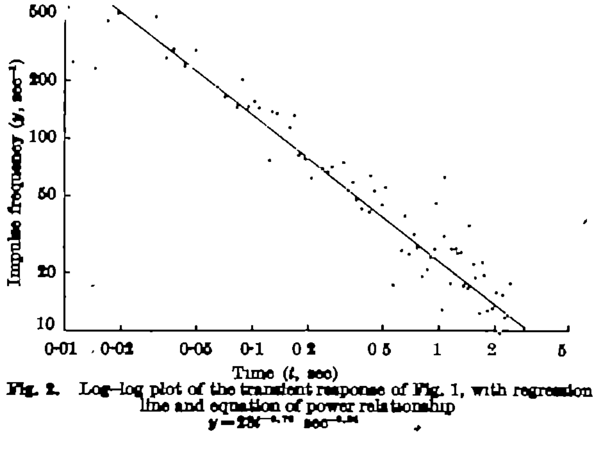

Fig. 2. Log–log plot of the transient response of Fig. 1, with regression line and equation of power relationship $y = 23t^{-0.76}$ sec$^{-0.24}$

In many but not in all of the receptors studied, frequency response functions determined with sinusoidal forcing agreed well with equation (3), with B and k evaluated from the transient response data (Fig. 3). We have not succeeded in fitting these data with exponentially adapting models, but the possibility that some of the underlying mechanisms are exponential in nature is not necessarily excluded.

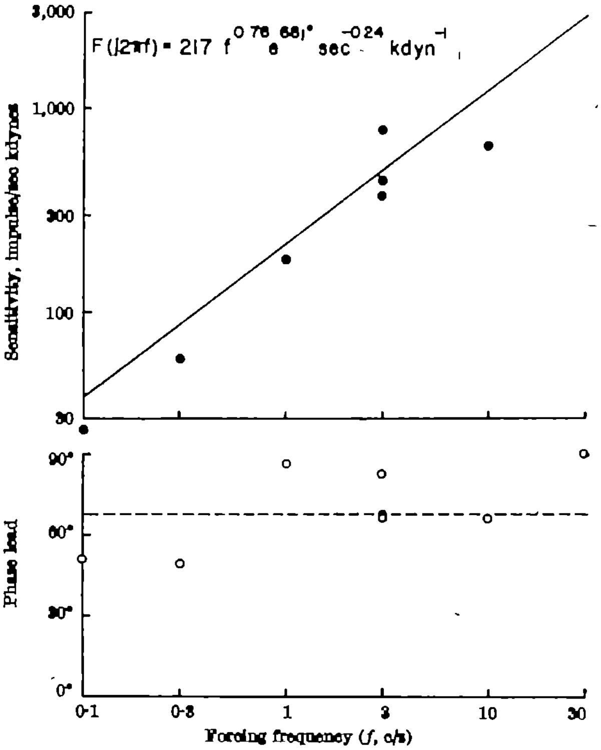

Fig. 3. Modulation sensitivity (solid circles and line) and phase lead (open circles, dashed line) plots of the frequency response function of the receptor of Fig. 1. Points were determined with sinusoidal stimulation, tensions as in Fig. 1, forcing frequencies over entire range for which impulse frequency modulation occurred (phase angle at 30 c.p.s. from single impulse per cycle). Lines and equation computed from transient response of Fig. 1, using equation (3), with B and k taken from Fig. 2

We conclude, therefore, that the time dependence of impulse frequency modulation in this cockroach mechanoreceptor arises in a dynamic biological process or set of processes for which a power law transfer function (equation 2) represents an important mode of behaviour. In view of the s^k factor in the transfer function, it is convenient to summarize the net mathematical behaviour of these processes as fractional (kth) order differentiation of the stimulus with respect to time. The relative contributions to these processes from tissue mechanics, generator mechanisms, impulse initiation phenomena, and all attendant non-linearities, remain to be demonstrated.

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Evidence for 'Electrogenic' Active Sodium Transport in an Epithelial Membrane

In the course of active transport across the skin of a frog and the bladder of a toad, sodium moves against an electrical potential gradient both on entering and leaving the functional epithelial cell layer[1,2]. Transport in both tissues is dependent on the presence of potassium in the serosal medium[3-5].

To explain these findings in frog skin, Koefoed-Johnsen and Ussing have proposed a model which, as applied to the toad bladder, presumes passive entry of sodium through a selective permeability barrier at the mucosal surface, followed by active transport by a 'sodium pump' at the serosal surface[6]. Extrusion of sodium is thought to be coupled with active uptake of potassium, the latter leaking back out through the serosal surface, which is selectively permeable to potassium. Thus, according to this hypothesis, the sodium pump is not 'electrogenic', that is, it is not directly responsible for the separation of charge. Instead the potential steps are attributed to sodium and potassium diffusion potentials at the mucosal and serosal surfaces, respectively.

The characteristics of the mucosal surface of the toad bladder are consistent with this hypothesis[2,7]. The experiments recorded here examine the nature of the active transport process at the serosal boundary of the epithelial cell layer.

Ling-Gerard glass micropipettes were used to determine the polarity and magnitude of the serosal potential step. In 34 measurements in bladders exposed on the serosal surface to solutions of high potassium and low chloride concentration ($K^+ = 116$ m.equiv./l., $Cl^- = 3$ m.equiv./l.) the median value was 13 mV, with serosal surface positive to cell interior. Since the potassium concentration of whole bladder averages 111 m.equiv./l. of non-inulin space water[8], a potential of the observed magnitude and polarity is unlikely to be a potassium diffusion potential. With constant water content, and control cellular chloride concentration greater than 3 m.equiv./l., a chloride diffusion potential also is unlikely.

In order to examine the possibility of an anionic diffusion potential at the serosal surface due to the movement of any anion, net cation transport from mucosal to serosal surfaces was measured in the open-circuited bladder, exposed on the serosal side to sodium-free potassium Ringer's solution. Demonstration of net cation transport from mucosal to serosal surfaces in the absence of significant tissue swelling ruled out net movement of any anion from serosal medium into cell interior, and hence an anion diffusion potential, if anions move only passively across this boundary. The alternative to bidirectional passive anion movement across the serosal surface, an ion-pair transport mechanism, seems improbable[9].

A 150-fold change of serosal hydrogen ion concentration (pH 9·25–7·02) did not affect the spontaneous trans-bladder potential or net sodium transport. A coupling of hydrogen ion uptake with active extrusion of sodium, or a hydrogen ion diffusion potential, therefore seems unlikely.

Additional studies were performed to explain the dependence of sodium transport on the presence of potassium in the serosal medium. No direct coupling of sodium and potassium transport was demonstrable; uptake of potassium-42 from the serosal medium was independent of the rate of net sodium transport[10]. Instead, the influence of serosal potassium appeared to be mediated at the mucosal surface, by regulation of the permeability of this barrier to passive entry of sodium. With sodium Ringer's solution as serosal medium, removal of potassium resulted in a decrease of net sodium transport to 5 per cent of the control value, in association with significant alteration of tissue electrolyte content and diminution of tissue labelling with sodium-22 from the mucosal medium.

When choline replaced sodium in the serosal medium removal of potassium resulted in only slight alterations of electrolyte content and mucosal permeability, and net transport fell only to 56 per cent of the rate in control tissues. Although sodium moved down a chemical potential gradient in this situation, in separate experiments net transport of sodium was shown to occur even against an imposed electro-chemical potential gradient. Thus, active sodium transport persisted despite absence of potassium from the serosal medium, when removal of serosal sodium prevented marked depression of mucosal permeability.

The depression of active sodium transport resulting from removal of potassium from the bathing medium, a property common to many tissues, has been demonstrated here to be an indirect effect, a consequence of decreased availability of sodium at the site of active transport. The results indicate the necessity for critical evaluation of evidence for coupling in tissues having experimentally inaccessible permeability barriers. The results suggest also that the active transport mechanism in toad bladder involves movement of sodium across the serosal boundary without simultaneous and obligate movement of a diffusible anion in the same direction or a cation in the opposite direction. The transport of sodium gives rise to the serosal potential step directly, that is, the sodium pump is 'electrogenic'.

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Formation of Histamine in Rat Tissues

It has recently been suggested by Kahlson *et al.* that the capacity of tissues to form histamine is related to the general process of growth, regeneration and repair[1-4]. Other workers have cast doubt on this theory, finding little or no histidine decarboxylase activity in various normal and malignant rapidly growing tissues[5-8]. Telford and West, finding that the optimal conditions for the determination of enzyme activity in the rat vary from tissue to tissue[8,9], and that the enzyme in different tissues behaves differently from drugs[10], suggested that in this species there may be several enzymes capable of forming histamine[8].

Recent experiments show that the methods at present being used for the determination of histidine decarboxylase activity do not give a true estimate of histamine-forming capacity. Using the optimal conditions for determination of enzyme activity as previously described[8,9], tissues were incubated with histidine for periods varying from 5 min to 5 h, and the amounts of histamine formed (μg/g) determined by biological assay. The results for adult liver are plotted in Fig. 1; similar curves were obtained for other tissues. In all tissues, maximum formation of

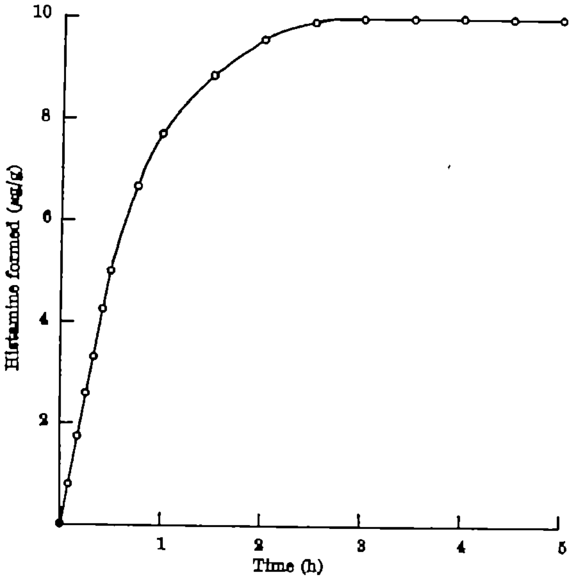

Fig. 1. Rate of histamine formation by adult rat liver *in vitro*. Incubation at 37° C under constant agitation, at pH 8·0, in presence of 20 mg/g benzene; 15 mg/g L-histidine as substrate

histamine occurred within an incubation period of 3 h. However, the rate of histamine formation was greatest during the first 30 min, up to 60 per cent of the total histamine production being formed within this time. During this early stage of the incubation period the relationship between histamine formed and time was linear, and it was thus possible to determine for each tissue the initial velocity of the reaction (µg histamine formed/g/min) by drawing a tangent to the origin of the curve.

Calculated in this way, the histamine-forming capacity of rat tissues *in vitro* is approximately three times higher than when calculated, as in previous work, from the total amount of amine formed after incubation for 3 h. For example, the adult liver forms 0·17 µg histamine/g/min. This represents 30·6 µg/g in 3 h (1·7 mg/tissue/day), as compared with only 10·0 µg/g in 3 h (0·6 mg/tissue/day) when estimated as total histamine production. The histamine-forming capacities of various rat tissues using the two methods of estimation are compared in Table 1; considerable quantities of histamine may also be formed by other tissues in which enzyme activity cannot be detected by the present method.

Table 1. Comparison of the histamine-forming capacities of rat tissues when estimated as the initial velocity of the enzyme reaction (µg histamine formed/g/min) and when estimated as total histamine production (µg histamine formed/g/3 h)

Tissue	Histamine-forming capacity: Estimated as initial velocity			Estimated as total production	
	µg/g/min	µg/g/3 h	µg/tissue/day	µg/g/3 h	µg/tissue/day
Fœtal liver (day 14)	46·1	8,298·0	331·9	2,534·6	101·5
Fœtal liver (day 21)	7·7	1,386·0	2,106·7	452·4	687·7
Whole fœtus (day 21)	0·13	23·4	1,609·9	8·0	550·4
Adult liver	0·17	30·6	1,713·6	10·0	560·0
Adult pyloric stomach	0·24	43·2	138·2	18·0	57·6
Adult duodenum	0·08	14·4	34·6	5·2	12·5
Adult kidneys	0·04	7·2	69·1	3·0	28·8

The progress curves of most enzymatic reactions are of the general form shown for rat liver histidine decarboxylase in Fig. 1, in which the velocity falls with time. Various factors may contribute to this fall in velocity, and they result in the curves not fitting the standard equations of homogeneous chemical reactions. It is only at the initial point that the conditions of the experiment are accurately known, and once the factors mentioned above have begun to operate the amount of histamine formed after a given time ceases to be a true measure of the rate of histamine formation. It is important, therefore, that histidine decarboxylase activity be measured from the initial velocity of the reaction. Measurements of changes in the histamine-forming capacities of tissues should include determination both of the initial velocity of the reaction and total histamine production, since it is possible that while the former may alter, the latter may not, and vice versa.

The initial velocity is similar in all tissues (range 0·15–0·19 µg histamine formed/g/min) when the amount of enzyme used for incubation is the same. Thus, different amounts of enzyme and different optimal conditions for enzyme activity do not reflect differences in the rate of the enzymatic reaction.

Summation of the amounts of histamine capable of being formed by the adult liver, pyloric stomach, duodenum and kidney shows that the adult rat is capable of forming as much as 2 mg of histamine a day. This is more than is capable of being formed by the rapidly growing fœtal rat, which on the 21st day of gestation when enzyme activity is highest is able to form 1·6 mg/day. However, it is worthy of note that at term a pregnant rat together with a litter of seven fœtuses has, by deduction, the ability to form more than 13 mg of histamine in 24 h. In comparison, the fœtal and adult guinea pig are probably capable of producing even larger amounts, histamine-forming capacities of a high order being recently reported in this species[8,11].

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HISTOLOGY

Demonstration of Perineural Epithelium in Whale and Shark Peripheral Nerves

IT has been shown previously by us[1,2] that the peripheral nerve fasciculus in rat, cat, guinea pig and frog is surrounded by flat, stratified squamous epithelial layers, five in number in the sciatic nerves of the former three animals and only two-layered in the frog. The squamous cell layer surrounding the peripheral nerve fibre has also been shown by Key and Retzius[3], Krenjevic[4], Lehmann[5,6], and Rohlich and Weiss[7]. The cells of this squamous epithelium are uninucleated, the nucleus being very flat, and sex chromatin has been demonstrated in female cats and rats in these cells. In serial isolation of this epithelium we have demonstrated that the perineural epithelium represents a continuation of the pia-arachnoid membrane from the central nervous system. In a further investigation, we have been able to show[2] that the lamellated sheath of the Pacinian corpuscle is made up of the extended perineural epithelium, indicating that the pia-arachnoid not only covers the central nervous system but also covers the peripheral nervous system as a perineural epithelium extending up to and over the motor and sensory end organs.

In continuation of this work and in an attempt to demonstrate the universality of this membrane among vertebrates our work was extended to include marine vertebrates. The two animals discussed here represent

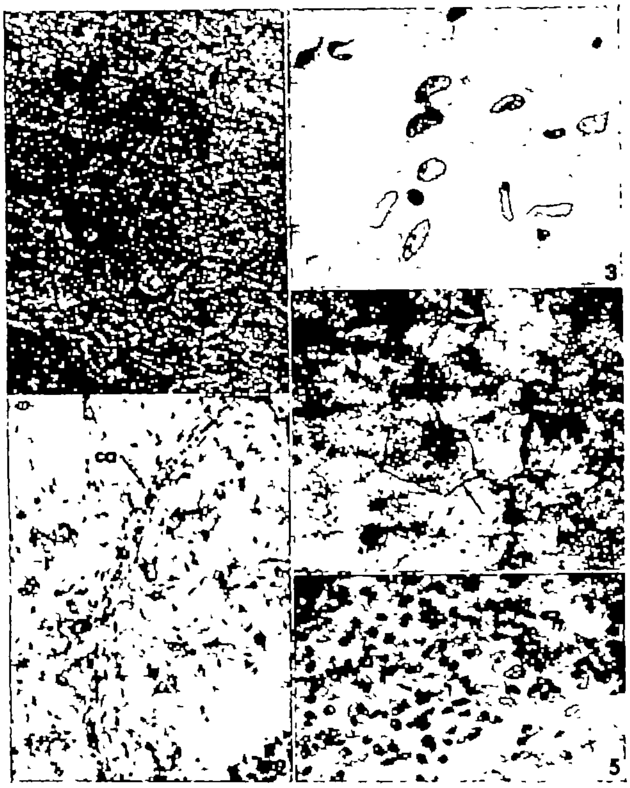

Fig. 1. Phase-contrast micrograph of formalin-fixed segmental nerve of shark, showing perineural epithelium, isolated under binocular dissection microscope, mounted in glycerine. Note clear-cut squamous epithelial layer with nuclei. Cell borders of some deeper layers are seen crossing underneath the superficial layer. (× 255)

Fig. 2. Formalin-fixed peripheral nerve of whale showing perineural epithelium, isolated under binocular dissection microscope, and four layers of the epithelium removed. Stained with cresyl violet. Note the capillary network (arrow *cs*) in the perineural epithelium. (× 68)

Fig. 3. Formalin-fixed shark segmental nerve showing isolated perineural epithelium stained by hæmatoxylin and eosin. Note cellular borders are not demonstrated by this method but nuclei are well stained. (× 660)

Fig. 4. Formalin-fixed whale peripheral nerve showing isolated perineural epithelium treated with 1 per cent aqueous silver nitrate (after repeated washing to remove formalin from tissue). Note cellular borders are clearly demonstrated (arrow), indicating the squamous nature of the cells. (× 255)

Fig. 5. Formalin-fixed whale peripheral nerve showing isolated perineural epithelium. Method same as Fig. 3. Multiplicity of nuclei overlapping each other indicates many layers of epithelial cells. (× 170)

groups low on the evolutionary scale (sharks) and those high on the scale (whales).

The material was collected from the waters around North Bimini in the Bahama Islands. Facilities were provided in Bimini by the Lerner Marine Biological Laboratories of the American Museum of Natural History.

A female nurse shark (*Ginglymostoma cirratum*), an elasmobranch, was anæsthetized and segmental nerves were removed and fixed in formalin. A female false killer whale (*Orca* sp.) was killed in the sea by a shot gun and the segmental and sympathetic chain nerves were removed and fixed in formalin. The formalin-fixed material was processed in our laboratory.

Isolation of the perineural epithelium was carried out under a stereo-binocular dissecting microscope as described previously[1]. Standard hæmatoxylin and eosin staining was applied to the isolated perineural epithelium. 0·5 per cent aqueous cresyl violet staining was used to demonstrate sex chromatin material. The cellular borders were visualized by washing the isolated epithelium repeatedly in distilled water to remove formalin from the tissues, and then treating with 1 per cent silver nitrate for 24 h in the dark, mounting in glycerine and then exposing to sunlight to demonstrate the cell borders. Some isolated tissue was mounted in glycerine directly for observation with the phase-contrast microscope.

During isolation of the perineural epithelium it was observed that the arrangement of the connective tissue components in both species of animals was the same as that of the peripheral nerves of rat, cat, guinea pig and frog. After removal of the epineurium, perineural connective tissue was seen. Meticulous clearing of incidental connective tissue components revealed a thin, glistening semi-transparent membrane under the perineurium which completely covered the entire nerve fasciculus. On silver nitrate impregnation it was seen that this semi-transparent membrane was made up of flat, stratified squamous epithelium arranged as a continuous sheet (Fig. 4). The formalin-fixed, glycerine-mounted tissue also demonstrated the presence of a squamous epithelium quite clearly under the phase-contrast microscope (Fig. 1). The epithelial layers were greater in number in the whale and it was possible, as in other mammals, to peel off up to five individual layers. The perineural epithelium of shark nerves, on the other hand, appeared to have three layers. The standard hæmatoxylin and eosin method does not demonstrate cell borders, but the nuclei were well stained and the cytoplasm pale (Figs. 3 and 5). Cresyl violet staining showed possible sex chromatin in the whale, planoconvex in shape, lying at the edge of the nuclear membrane. In shark nerves the perineural epithelium showed no evidence of sex chromatin. A capillary network was also identified in this epithelium (Fig. 2).

With these findings it becomes clear that, as in the other mammals and one amphibian examined, the pia-arachnoid membrane extends over the peripheral nerves as a perineural epithelium, following the nerves to their termination, in both shark and whale. The only difference between the perineural epithelium in peripheral nerves and the pia-arachnoid in the central nervous system is the absence of the sub-arachnoid space. According to Harvey and Burr[9] and Harvey *et al.*[10] the pia and arachnoid membranes are ectodermal in origin and it is highly probable that in whale, as in other mammals, they have the same origin. The origin of the membranes in fish, however, is uncertain but we feel they, too, are likely to be ectodermal in origin.

This communication thus demonstrates the peripheral nerves of whale and shark to be encased in an epithelial sheath which isolates them from the surrounding tissue fluids, maintaining them in an environment of their own which is akin to that of the central nervous system.

Further histological, histochemical and electron microscopical studies of peripheral nerves of various types of marine animals, vertebrate and invertebrate, are in progress in this laboratory.

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IMMUNOLOGY

Electrophoretic Analysis of Serum and Pleuroperitoneal Fluid Proteins and Antibody from Guinea Pigs

INTRAPERITONEAL injection of Freund's adjuvant (with or without antigen) into mice induces the production of ascitic fluid[1-3]. Ascitic fluid contains γ-, β_2-, β_1-, α_2-, α_1-globulin and albumin determined by moving boundary electrophoresis[4,5]. Anti-bovine serum albumin precipitins in mouse ascitic fluid were identified as β_2-globulins[4,5]. This work has been extended to include the guinea pig. In many of the animals ascites formation was also associated with fluid accumulation in the thoracic cavity. Serum, pleural and peritoneal fluids were characterized by moving boundary electrophoresis. Preliminary evidence indicated little or no electrophoretic difference between pleural and peritoneal fluids. Since it is possible that pleural fluid accumulates as a result of ascites formation, these fluids collectively are referred to as pleuroperitoneal fluid.

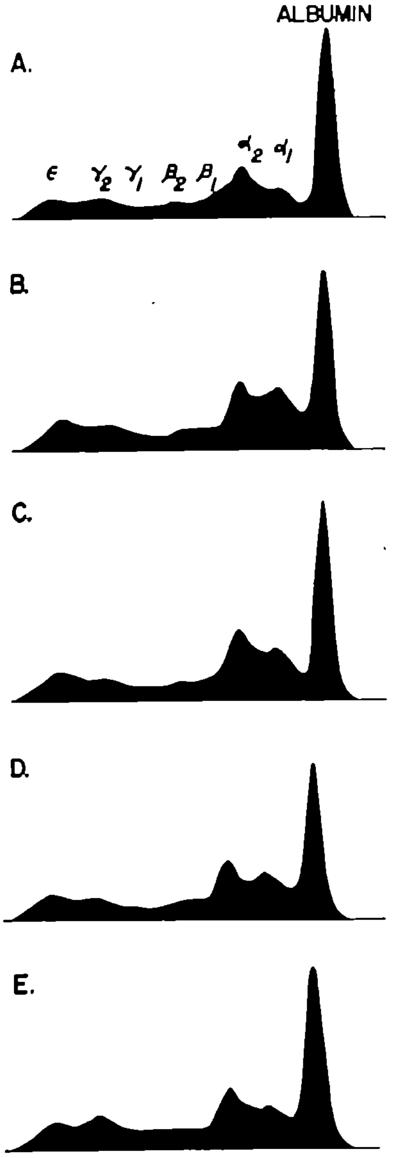

Fig. 1. Electrophoretic patterns of guinea pig sera and ascitic fluids. *A*, Serum from uninjected guinea pigs; *B*, serum from guinea pigs injected with Freund's adjuvant; *C*, serum from guinea pigs injected with Freund's adjuvant containing bovine serum albumin; *D*, ascitic fluid from guinea pigs injected with Freund's adjuvant; *E*, ascitic fluid from guinea pigs injected with Freund's adjuvant containing bovine serum albumin

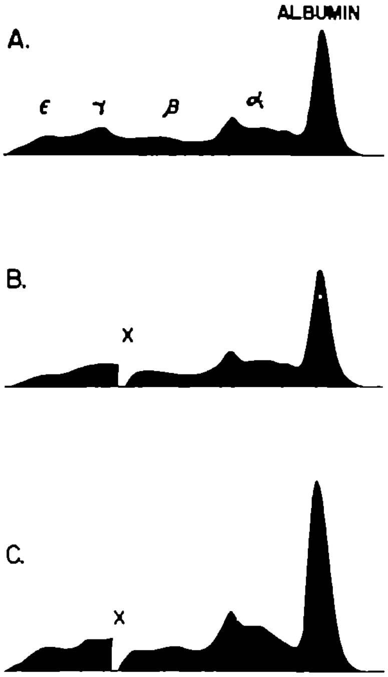

Fig. 2. Detection of anti-bovine serum albumin precipitins in guinea pig serum and ascitic fluid. *A*, Ascitic fluid from animals injected with Freund's adjuvant containing bovine serum albumin; *B*, a sample of the same ascitic fluid used in *A* with bovine serum albumin in the buffer; *C*, serum from animals injected with Freund's adjuvant containing bovine serum albumin with bovine serum albumin in the buffer

Random bred guinea pigs of both sexes were injected by the intraperitoneal route with Freund's adjuvant alone (85 parts 'Drakeol' mineral oil, 15 parts 'Arlacel *A*', 1 mg of *Mycobacterium butyricum*/ml.) or with Freund's adjuvant containing bovine serum albumin (4 mg/ml.). Each guinea pig received six 0·5 ml. injections of adjuvant over a period of three weeks. One week after the last injection pleural and peritoneal fluids were obtained by paracentesis and blood by cardiac puncture. Serum and pleuroperitoneal fluids were clarified by centrifugation. Moving boundary electrophoretic analyses were made in a Spinco model *H* instrument (barbital buffer: pH 8·6, $\mu = 0·1$; or glycine sodium hydroxide buffer: pH 10, $\mu = 0·1$). The electrophoretic component containing antibody was determined by the boundary disturbance test[6]. In this procedure antigen added to the buffer in the electrophoresis cell migrates into the antiserum and forms a specific precipitate with antibody. Location of the antigen–antibody reaction in the photographic record of the electrophoretic pattern is determined by a clear zone caused by interruption of light by the precipitate.

Electrophoretic patterns of serum from uninjected guinea pigs and serum and pleuroperitoneal fluid from animals injected with Freund's adjuvant alone or Freund's adjuvant containing bovine serum albumin are shown in Fig. 1. Seven components, γ_2-, γ_1-, β_2-, β_1-, α_2-, α_1-globulin and albumin, were resolved. In some samples only 5 components were resolved since γ_1-, β_2- and β_1-globulin could not be distinguished. Except for α_1-globulin, which is higher in the sera and pleuroperitoneal fluids of immunized animals, the relative concentrations of electrophoretic components of serum and pleuroperitoneal fluid were similar to the values determined for serum from uninjected animals.

To study the effect of pH on the electrophoretic resolution of guinea pig proteins experiments were also done at pH 10. All components migrated faster than at pH 8·6; however, the resolution of γ_1-, β_2-, and β_1-globulin components was not greatly improved.

Serum and pleuroperitoneal fluid from guinea pigs injected with bovine serum albumin in Freund's adjuvant contained anti-bovine serum albumin precipitins which were located in the γ_1-β_2-globulin region (*x*, Fig. 2) of serum and pleuroperitoneal fluid.

Although 7 components, γ_2, γ_1-, β_2-, β_1-, α_2-, α_1-globulin and albumin, could be resolved in guinea pig serum and pleuroperitoneal fluid, in some samples only 5 components were distinguished due to incomplete resolution of the γ_1-, β_2-, and β_1-globulins. Electrophoretic analysis at *p*H 10 did not significantly improve the separation of these proteins. The γ_1- β_2- β_1-globulin region probably consists of protein molecules differing progressively only slightly in charge.

Anti-bovine serum albumin precipitins of guinea pig serum and pleuroperitoneal fluid have the electrophoretic properties of γ_1- β_2-globulin. On the basis of these studies it is difficult to determine whether the precipitins are γ_1- or β_2-globulins or a mixture of both. Experiments to determine the properties of guinea pig precipitins in serum and pleuroperitoneal fluid are now in progress.

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A New Allotypic Specificity in the Mouse (MuA2)

IMMUNOLOGICAL differences between γ-globulins of the same species have been demonstrated in man, rabbit, guinea pig, mouse and possibly baboon. In man and rabbit, these differences have been examined in great detail[1]. In the mouse, only one allotypic specificity has so far been described in a report by Kelus and Moor-Jankowski[2]. These authors elicited, in *C57BL* mice, an antibody to the γ-globulin of *BALB/c* mice. This antibody reacted with the γ-globulin of *BALB/c*, *C3H/He* and 'Champagne Glaxo' individuals. We have immunized *BALB/cJ* mice with the antigen obtained from *C3H/HeN* × *C57BL/6J* mice in two ways: (1) by injection with sera incorporated in Freund's adjuvant; (2) by injection with bacteria coated with antibody incorporated in Freund's adjuvant or suspended in 0·15 M sodium chloride[3].

In the first of these procedures, each mouse was given 12 subcutaneous injections with 0·1 ml. of emulsion; the total period of immunization was 8 months. On the 7th day after the last injection, animals were bled and the sera were pooled. A second group of *BALB/cJ* mice were immunized with *Proteus vulgaris* (*X*19) coated with antibody to the organism which had been raised in hybrid mice *C3H/HeN* × *C57BL/6J*. The mice were injected subcutaneously at weekly intervals; at first with the antigen in Freund's complete adjuvant, and later with the antigen suspended in 0·15 M sodium chloride. Seven days after the last injection, the animals were bled and the sera were pooled.

Both pools of immune sera reacted in an identical manner, giving one zone of precipitation in double diffusion with *C57BL/6J*, *C57BL/10J* and *SJL/J*. The antibody was shown by immunoelectrophoresis to react with γ-globulin. The allotype was found to be absent in *A/HeJ*, *A/J*, *AKR/J*, *BALB/cJ*, *CBA/J*, *C3H/HeJ*, *C57BR/cdJ*, *C57L/J*, *C58/J*, *DBA/1J*, *DBA/2J*, *RF/J*, *SWR/J*, 129/*J*, 2*BC3H* and 2*C3H*. A number of hybrid strains were also tested (*C3H/HeN* × *C57BL/6J*, *AKR/J* × *DBA/2J*, *C57BL/6J* × *A/J*, *C57BL/6J* × *DBA/2J*, *BALB/cJ* × *A/J*, *C57L/J* × *A/HeJ*, *C3H/HeJ* × *DBA/2J*) and it was found that all hybrids having in their ancestry one of the strains containing the allotypic specificity also possessed the allotypic specificity. In all cases, 5 or more males and 5 or more females were tested for the presence of the allotypic specificity, and in all cases, the distribution of the allotypic specificity in male and female animals was identical.

We also examined one strain of non-inbred 'Swiss' mice maintained at the Connaught Laboratories and found the allotypic character in 10 of the 21 animals tested.

In accordance with the agreed notation for rabbit allotypy[4], and after discussion with Dr. A. Kelus[5], we propose to call the allotypic specificity described here, MuA2. The allotypic specificity described by Kelus and Moor-Jankowski (γ^{BA}) will now, following Dr. Kelus's suggestion, be called MuA1.

Our allotypic specificity, MuA2, has been detected in three inbred strains of mice: *C57BL/6*, *C57BL/10* and *SJL*. The presence of the same allotype in *C57BL/6* and *C57BL/10* is not surprising since these two strains are very closely related[6]. It is, however, interesting to note that strain *SJL*, which does not have any known relation to *C57BL/6* and *C57BL/10* (refs. 6, 7) contains the same allotype. This observation could result from the fact that the mutation which led to the formation of the MuA2 allotypic specificity occurred before the aforementioned strains were bred. If this were the case, the allotype MuA2 should be present in some non-inbred strains. This assumption was supported by the finding of 10 MuA(2+) individuals among the 21 non-inbred 'Swiss' mice tested; this would indicate that this allotype may be present among non-inbred populations from which inbred strains have been derived.

Generally, allotypic antisera have been obtained by immunization with antigen–antibody complexes. Dray and Young[8] reported the successful production of allotypic antibodies in the rabbit by immunization with serum incorporated in Freund's adjuvant. In the hands of some other investigators, immunization with serum incorporated in Freund's adjuvant has not been equally successful. In our hands, and using the mouse strains described, immunization with serum in Freund's adjuvant has led to the production of an antibody of satisfactory potency and would seem to be, in this species, the method of choice.

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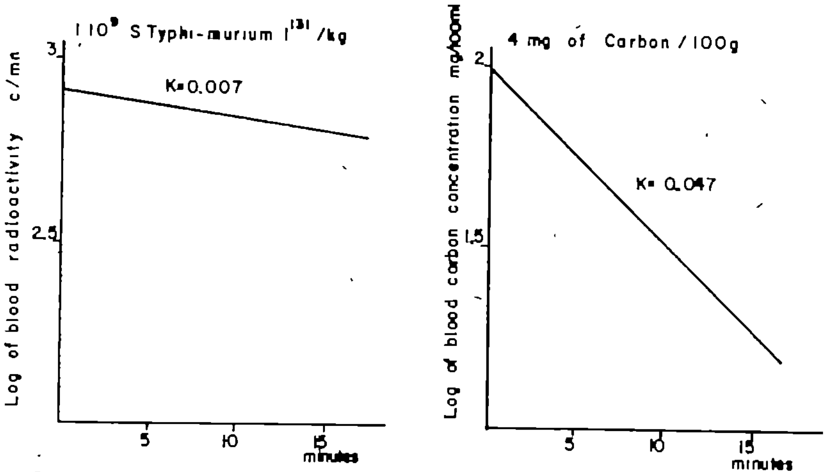
Phagocytosis of *Salmonellae* by Reticulo-endothelial Cells of New-born Piglets lacking Natural Antibody

It is a well-established fact that the uptake of bacteria by phagocytes is enhanced by serum factors (opsonins) acting on bacterial surface. The importance of serum opsonins in the phagocytosis of bacteria by fixed macrophages of the reticulo-endothelial system has been demonstrated by organ perfusion technique. It has been shown that serum factors playing a part in bacterial opsonization include immune or natural antibody, complement and possibly a third unidentified factor[1,2].



Organ distribution of Salmonellae I^{131}

	c/min/g	per cent of injected radioactivity
Liver	1,000	17
Spleen	80,000	44
Lungs	1,200	10
Kidneys	150	0.7
Blood		10
		81.7 (total)

Organ distribution of Carbon

	mg/g	per cent of the injected dose
Liver	1.1	75
Spleen	4.6	12
Lungs	–	traces
Kidneys	–	traces
		87 (total)

Fig. 1. Rate of blood clearance (*K*) and organs distribution of *S. typhimurium* labelled with iodine-131 and colloidal carbon in new-born piglets. The figures represent the mean value of three animals. The *K* value is determined by the formula: $K = \frac{\log C_t - \log C_{t'}}{t' - t}$. *C*, blood concentration; *t*, time expressed in min

The use of radiolabelled bacteria has made possible quantitative measurement of opsonins in the phagocytosis of bacteria by the reticulo-endothelial system *in vivo*[3-5]. The importance of antibody and complement in the phenomenon of bacterial opsonization has therefore been demonstrated in the intact animal[6,7]. It has also been suggested that the rate of phagocytosis of bacteria in different animal species was related to the amount of natural antibodies present in their serum[5]. In germ-free mice which are supposed to contain a lower amount of natural antibodies, the blood clearance of *E. coli* is slightly decreased. Nevertheless the bacterial repartition in the phagocytes of liver and spleen is similar in germ-free and control mice[8].

Recently, Šterzl *et al.* have shown that serum from new-born piglets deprived of colostrum was totally lacking in natural antibodies but contained 1–3 units of complement per ml.[9]. New-born piglets offer, therefore, an exceptional opportunity to investigate whether phagocytosis of bacteria by reticulo-endothelial cells *in vivo* occurs even in the absence of natural antibodies.

Investigation of phagocytosis of *S. typhimurium* by the reticulo-endothelial system in new-born piglets deprived of colostrum are reported here. These experiments were performed in fasting piglets 3–5 h after birth. The smooth phase of *S. typhimurium* 34/59 trace labelled with iodine-131 (ref. 4) was injected in an ear vein. The rate of blood clearance of labelled bacteria was established by measuring the radioactivity of blood samples removed from the opposite ear vein. When about 90 per cent of the radioactive bacteria were cleared from the blood the animals were killed and the radioactivity of different organs determined.

In another group of piglets the phagocytic activity of the reticulo-endothelial system was established by measuring the blood clearance and organ distribution of intravenously injected colloidal carbon using a technique previously described[10].

The results obtained in these experiments are summarized in Fig. 1.

It appears that *Salmonellae* are removed very slowly from the blood with a *K* value of 0·007. The distribution of the radioactivity in the organs shows that the majority of the bacteria are fixed in the spleen. The spleen, which weighed only about 1 g, accumulated 44 per cent of the injected bacteria while the whole liver (about 33 g) contained only 17 per cent. The number of bacteria per g of tissue was 80 times higher in the spleen than in the liver and this latter organ contained about the same amount of radioactivity as the lungs. It is important to note that the organ distribution of *Salmonellae* was very different in adult animals containing natural or immune antibodies. In adult animals the liver fixed 50–80 per cent and the spleen 5–25 per cent of the injected number of bacteria[3,4,7].

Examination of the phagocytic activity of the reticulo-endothelial cells with colloidal carbon in new-born piglets shows that the lack of bacterial fixation in the liver is not due to the inefficiency of the phagocytic function of the Kupffer's cells in the early stage of life, since these cells phagocytize 75 per cent of the injected amount of carbon. The phagocytic index *K* and the carbon distribution in the organs of new-born piglets are similar to those of adult animals[11]. Šterzl *et al.* have also shown that the reticulo-endothelial cells of new-born piglets deprived of colostrum phagocytize carbon particles at the same rate as piglets fed with colostrum[12].

These results indicate that, in new-born piglets, the absence of natural antibody inhibits the uptake of bacteria by Kupffer's cells, while the phagocytes of the spleen are capable of vigorous phagocytosis. It is interesting to emphasize the striking difference in the part played by opsonins in phagocytosis of bacteria by the Kupffer's cells and by the reticulo-endothelial cells of the spleen.

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PATHOLOGY

Glycoprotein Excretion following the Injection of Papain into Rabbits

UNACTIVATED papain, injected intravenously into rabbits, passes from the blood to cartilage tissues where it is activated locally, causing ear collapse due to depolymerization of chondromucoprotein (chondroitin sulphate–protein complexes). The enzymatic products (for which the term 'chondromucopeptides' is proposed) leach out of the cartilage into the blood and are excreted in the urine[1].

In view, however, of the broad substrate specificity of papain[2], it would be surprising if papain attack *in vivo* was restricted to chondromucoprotein. Several glycoproteins (which contain sialic acid) act as papain substrates, while papain has been found to liberate glycoproteins from reticulum isolated from spleen[3]. Following injection of papain into rabbits increased levels of serum glycoproteins (seromucoid, protein-bound sialic acid) have been observed which were presumed to be liberated from ground substance components other than chondromucoprotein[4].

It therefore seemed of interest to know whether the increased mucopolysaccharide excretion observed following papain injection into rabbits was accompanied by an increase in urine glycoprotein components. The fraction investigated represents, in human beings, the major urinary glycoprotein component[5–7], the excretion of which is closely correlated with serum glycoprotein-levels[8].

The following method was adopted. Five rabbits were injected intravenously with crude papain (Messrs. A. M. Zimmerman, London) and the urines collected in 24-h periods. Their volumes were made up to 200 ml. with water and the samples stored at −10° until assayed. Aliquots (about 25 ml.) of each urine were incubated at 37° to re-dissolve any material that may have come out of solution during storage. After filtration, 20 ml. were taken, 20 ml. of water added and the mixture (at *p*H 8·5 ± 1·0) adjusted to *p*H 7 with hydrochloric acid using a *p*H meter. 2 M sodium benzoate (5 ml.) was added, followed by 2 N hydrochloric acid (4 ml.). The resulting *p*H was 4·1. After standing for 30 min at room temperature the precipitate of benzoic acid, containing adsorbed glycoprotein, was filtered off under suction and transferred quantitatively to a centrifuge tube (50 ml. capacity) with acetone. After the benzoic acid had dissolved, the glycoprotein residue was centrifuged down, washed once with acetone, suspended in water (2 ml.) and dissolved by the addition of 0·1 N sodium hydroxide (0·1 ml.). Glycoprotein was precipitated with acetone (18 ml.) followed by saturated aqueous sodium chloride (0·02 ml.). After the precipitate had flocculated it was centrifuged down, drained thoroughly, suspended in 0·1 N sulphuric acid (2 ml.) and heated (80°, 1 h) to liberate free sialic acid. Aliquots (0·2 ml.) of the hydrolysate were taken and sialic acid estimated by the thiobarbituric acid method of Warren[9]. The adsorption curve of the chromophore was identical to that produced by *N*-acetylneuraminic acid, indicating the absence of 2-deoxy-D-ribose and other interfering chromogens in the glycoprotein fraction. Results were expressed as mg sialic acid/24 h. Urine chondromucopeptide-levels, expressed as mg hexuronic acid/24 h, were determined by a method already described[10]. The results are summarized in Table 1.

Table 1. URINARY CHONDROMUCOPEPTIDE AND GLYCOPROTEIN EXCRETION FOLLOWING THE INJECTION OF RABBITS WITH PAPAIN

No.	Weight of rabbit (kg)	Amount of papain injected (mg) contained in 2·5 ml. physiological saline	Glycoprotein Excretion: mg Sialic acid/24 h Pre-injection level	Glycoprotein Excretion: mg Sialic acid/24 h Post-injection level	Glycoprotein Excretion: No. of days after injection to reach excretion peak	Glycoprotein Excretion: No. of days to reach pre-injection level	Chondromucopeptide Excretion: mg Hexuronic acid/24 h Pre-injection level	Chondromucopeptide Excretion: mg Hexuronic acid/24 h Post-injection level	Chondromucopeptide Excretion: No. of days after injection to reach excretion peak	Chondromucopeptide Excretion: No. of days to reach pre-injection level
1	1·0	40	0·76	1·48	1	7	1·6	5·0	1	2
2	1·5	60	0·85	1·88	3	8	2·2	4·4	2	3
3	2·1	100	1·30	2·30	2	4	1·1	5·8	2	3
4	1·1	40	0·88	2·05	1	4	5·1	9·7	1	2
5	2·2	100	1·70	2·25	4	6	1·5	7·1	1	2

The expected rise in chondromucopeptide was accompanied by increased excretion of glycoprotein material, the raised glycoprotein-level being maintained for longer periods than the chondromucopeptide-level. This finding correlates with the changes observed in serum[4], where glycoprotein and mucopolysaccharide-levels were maximal 2–5 days and 9–18 h respectively after papain injection.

Although some of the urine glycoprotein fraction measured here is derived from the urinary tract[11], plasma components entering the urine by glomerular filtration are also present. Part of the rise in urine glycoprotein following papain injection can be presumed due to release of cartilage chondromucoprotein, the protein moiety of which may be a sialoprotein[12], and also to the release of glycoprotein components from cartilage and perhaps other connective tissues.

Plasmin injected into rabbits causes a similar, although less dramatic, sequence of events so far as mucopolysaccharide liberation is concerned[10]. Whether or not plasmin liberates other tissue components leading to raised serum and urine glycoprotein-levels is under investigation, although the problem is rendered difficult since plasminogen, the precursor of plasmin, is itself a glycoprotein[13], preparations of human plasminogen used for injection, prepared from plasma fraction III by Kline's method[14], containing 75 μg sialic acid/ml.

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Synthesis of Plasma Antihæmophilic Factor

IN *Nature* of May 12, 1962, L. I. Woolf offered an explanation of 'severe' and 'mild' *X*-linked hæmophilia *A* while discussing gene expression in heterozygotes. He proposed that the plasma-level of antihæmophilic globulin (*AHG*, *AHF*, Factor VIII) regulated its rate of synthesis, that is, that 'end-product (feedback) inhibition' controlled the synthetic rate. 'Feedback inhibition' is known to be a very important regulatory mechanism in biosynthetic pathways, producing small anabolites such as amino-acids, vitamins, etc.[1]. We can find no documented evidence, however, that 'feedback inhibition' is a controlling mechanism in protein synthesis. 'Feedback inhibition' should be sharply distinguished in this respect from 'feedback repression', which is recognized to be a highly important genetic mechanism concerned with regulating the synthesis of enzymes (proteins) in bacteria[1–4].

Woolf apparently has not considered the implications of the existence of another disorder in which there is *AHF* deficiency. This condition, von Willebrand's disease (*v.W.D.*), clearly results from a dominant mutation occurring on an autosomal chromosome, and its main features have been confirmed by many investigators[5–10]. The bleeding time is prolonged in *v.W.D.* but is normal in hæmophilia *A*. Thus, another plasma component, tentatively designated 'vascular factor' (*V.F.*), apparently controls the bleeding time and is reduced in *v.W.D.* along with *AHF*.

One of us pointed out several years ago that the occurrence of these two disorders of *AHF* synthesis clearly distinguishable on genetic grounds, one of which is dominantly inherited, makes it difficult to understand the regulation of the *AHF*-level[11]. It would appear that an explanation of *AHF* regulation must take into account that: (*a*) genes on at least 2 chromosomes are concerned; (*b*) at least two plasma factors are involved; (*c*) 'feedback inhibition' is almost certainly not the controlling mechanism.

Recently, French workers[8] have clearly documented an important earlier observation by a Swedish group[10]. When fresh plasma from persons with hæmophilia *A* (low *AHF*, normal *V.F.*) is transfused into persons with *v.W.D.* (low *AHF*, low *V.F.*), a remarkable result is obtained. Within a few minutes the bleeding time becomes normal as might have been expected; but there is also a progressive and completely unexpected increase in circulating *AHF*. The rising *AHF*-level may reach a peak as much as 24 h later and only slowly returns to the low, pre-transfusion level. This 'paradoxical' effect is observed neither when the plasmas are mixed *in vitro* nor when *v.W.D.* plasma is transfused into persons with hæmophilia *A*. The observations suggest that the low *AHF*-levels of hæmophilia *A* and *v.W.D.* are achieved by quite different biochemical mechanisms.

One explanatory model which comes to mind is one which might be called the 'activator' model. In this model an activator or co-factor of *AHF* is produced from information carried on the autosomal chromosome, while the structural information for a pro-*AHF* is coded on the *X*-chromosome. In *v.W.D.* the amount of activator is reduced and only a small quantity of the structurally and qualitatively normal *AHF* precursor becomes activated. When hæmophilia *A* plasma, rich in activator, is infused into a patient with *v.W.D.* the activator reaches the site where pro-*AHF* is activated. This is followed by a rising plasma *AHF*-level, until the activator becomes exhausted. When *v.W.D.* plasma, presumably containing normal pro-*AHF* molecules, is transfused into persons with hæmophilia *A*, only the expected rise in plasma *AHF* is observed, probably because the pro-*AHF* cannot reach the site where activation occurs.

To explain the dominance of the mutation this model assumes either that the genes normally producing activator are working at maximum speed and that one normal allele does not produce enough of the competent activator, or that the mutant activator substance acts as an inhibitor. It also assumes that the activator is a molecule which can enter and leave cells with ease, while pro-*AHF* is a molecule which is expelled from cells but does not enter them readily. The simplest situation would be one in which the *V.F.*, which is responsible for the normal bleeding time, is also the activator of the *AHF* system, but this is not essential. *V.F.* might be a product of an associated reaction.

The model described here is not without precedent. It has recently been observed that the activity of xanthine dehydrogenase in *Drosophila melanogaster* is controlled by two loci on different chromosomes (I and III). Mutation at either locus results in decreased xanthine dehydrogenase activity; however, *in vitro* mixture of extracts from the mutant flies results in return of enzymatic activity[12].

Another model can be imagined which operates in accordance with the system proposed by Monod and colleagues for regulating protein synthesis in bacteria[13]. In this 'inducer' model a dominant regulator gene mutation of the i^s type would explain *v.W.D.* The altered repressor substance produced by this autosomal gene would not be inactivated by its inducer, and its operon would always be repressed. Since this operon, which would ordinarily synthesize the inducer for the *X*-chromosomal *AHF* operon, is repressed, the plasma-level of *AHF* is reduced. This model also would be most elegant if the *V.F.* were also the inducer; but this is not essential. Here again the inducer would be required to be a small molecule because known inducers are small, and it is required to pass from blood of hæmophilia *A* into nuclei of persons with *v.W.D.* if *de novo* *AHF* synthesis is to occur.

The chemical nature of *V.F.* is unknown. It is interesting to note, however, that both models require a highly mobile molecule which probably implies small size. Coincidentally, several small molecular species are known to have striking physiological effects on blood vessel walls (serotonin, pitressin, adrenaline, acetylcholine). It is also of interest that *AHF*-levels in normal persons increase temporarily following adrenalin injections[14] or heavy exercise[15]. The presence of the inducer in Fraction I-0[10] may be explained by the occurrence of a protein carrier or by its particular solubility characteristics.

Sex-linked hæmophilia could be due to a structural mutation under either model. Severe sex-linked hæmophilia might result either from an operator mutation (0^c type) or a major change in the structural gene in the 'inducer' model. 'Mild' hæmophilia, on the other hand, might be a 'mis-sense' structural alteration with mild but not complete loss of the activity of the molecule.

It is difficult to imagine tests which could be carried out and lead to a decisive choice between these models. It is also possible that neither model is entirely correct; but we believe their heuristic value justifies their description.

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RADIOBIOLOGY

Experimental Oxidation of Hæmoglobin: Effect of Repeated Whole-body γ-Radiation in Rats

WHEN washed suspensions of mammalian red cells are treated with isotonic solutions of nitrites, the iron of their contained hæmoglobin is oxidized to the ferric state with the formation of brown methæmoglobin. Using standard strength red cell suspensions and weak solutions of nitrite, the resistance (reaction time, Figs. 1–4) of the hæmoglobin to this change can readily be measured[1].

The increased sensitivity of cord and infant blood to oxidizing agents has long been known, but a similar increase in sensitivity of hæmoglobin to oxidation in the growing mammal, in the pregnant and in those bearing malignant tumours has only recently been reported[1,2]. The resistance of the hæmoglobin of rats to oxidation can also be decreased by starvation[1], by certain dyes and drugs[3] and by ionizing radiations. The exact effect of such radiation is dependent on the age of the animal at the time of irradiation[4] and on the dose administered.

In rats, 500 rads produce a rapid increase in the hæmoglobin sensitivity which persists for at least three months. With smaller doses (1/30–250 rads) in adult animals, the onset of the effect is somewhat delayed and recovery occurs in from 4–7 weeks (Figs. 1, 2 and 3). The hæmoglobin sensitivity of sham irradiated controls did not deviate from the normal value. Re-irradiation of the animals within one month of the return of their hæmoglobin sensitivity to normal produces an earlier change in their nitrite sensitivity and an earlier reversion. Subsequent doses of radiation at the same intervals produce a progressive acceleration of the phenomenon (Figs. 1, 2 and 3).

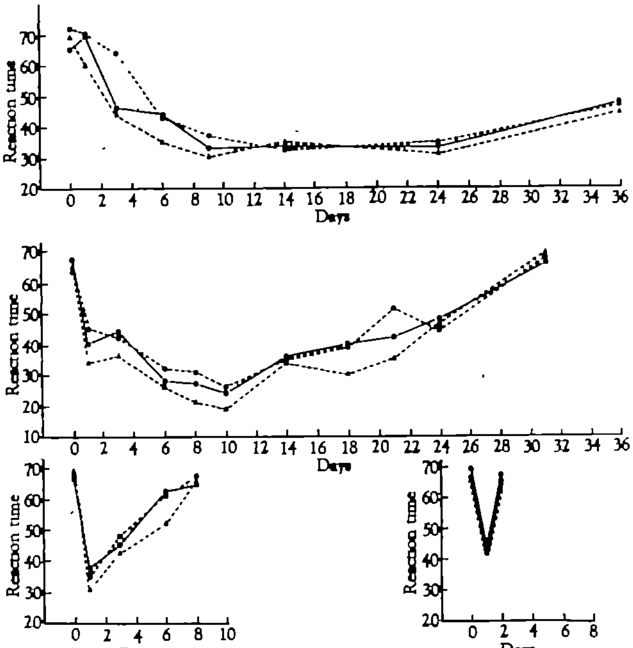

Fig. 1. Change in the reaction time (the time taken to convert the hæmoglobin of a standard saline red cell suspension to methæmoglobin using 0·1 per cent sodium nitrite *in vitro*) with differing and repeated doses of cobalt radiation. (The *S.D.* of any point is approximately 5 sec.) Upper, first dose; middle, second dose; lower left, third dose; lower right, fourth dose. ▲, 250 rads; ○, 125 rads; ●, 62·5 rads

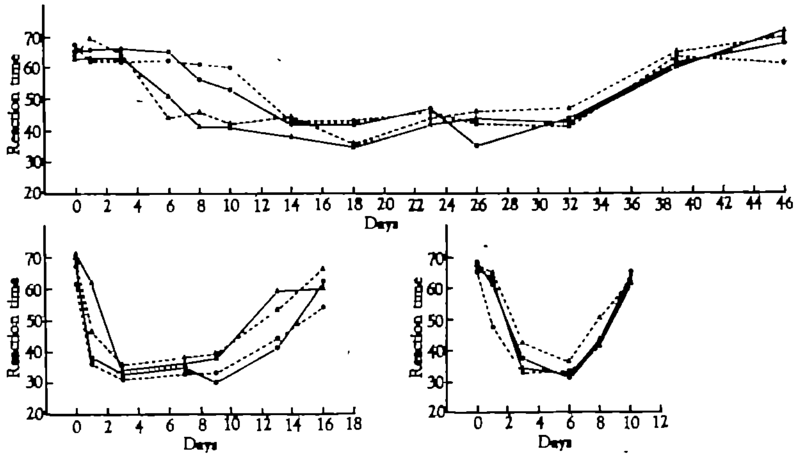

Fig. 2. As Fig. 1; the effect of much smaller doses. Upper, first dose; lower left, second dose; lower right; third dose. ●, 30 rads; ○, 15 rads; △, 6 rads; ▲, 3 rads

The mechanism of the change in sensitivity of the hæmoglobin is, as yet, completely unknown. However, since the increased sensitivity to nitrites of the young pregnant or starving animal is at least partially reversible by the administration of riboflavin and the increased sensitivity following 500 rads of γ-radiation is temporarily reversible by riboflavin plus adenosine triphosphate (although not by riboflavin alone)[1,4], it would appear that the flavoproteins may be involved in these phenomena. The latter observations would fit in with the increased ATPase activity and the disturbance in oxidative phosphorylation following radiation reported by several authors[5,6].

The possibility that the change in response with repeated doses of radiation is of an immunological nature is perhaps suggested by the finding that, while an initial series of T.A.B. injections caused a marked increase in the sensitivity of the hæmoglobin to oxidation, a second series of T.A.B. injections in the same animals 7 weeks later was completely without effect (Fig. 4). In the case of radiation, the antigenic stimulus would presumably be the products of tissue breakdown.

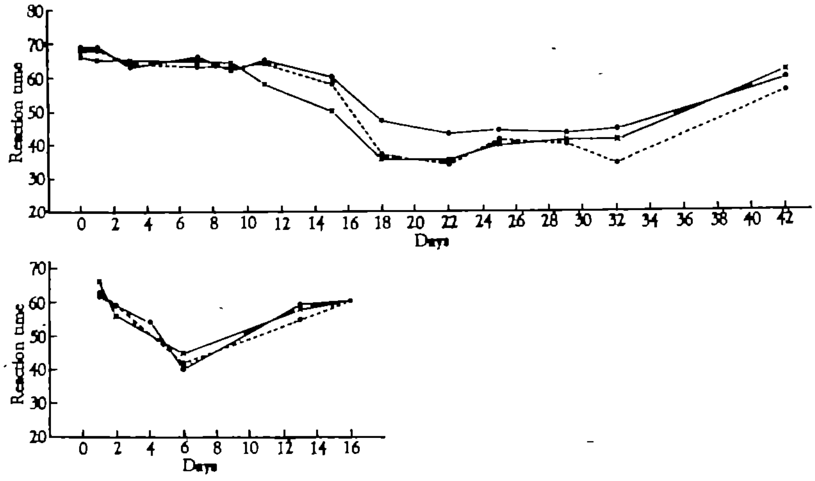

Fig. 3. As Fig. 1; the effect of doses in the maximum permissible dose range. Upper, first dose; lower, second dose. ○, 1/30 rad; ●, 1/10 rad; ×, 1/3 rad

A fuller discussion of the possible mechanism involved and of the experiments completed, in progress and planned, to elucidate the findings is beyond the scope of this communic-

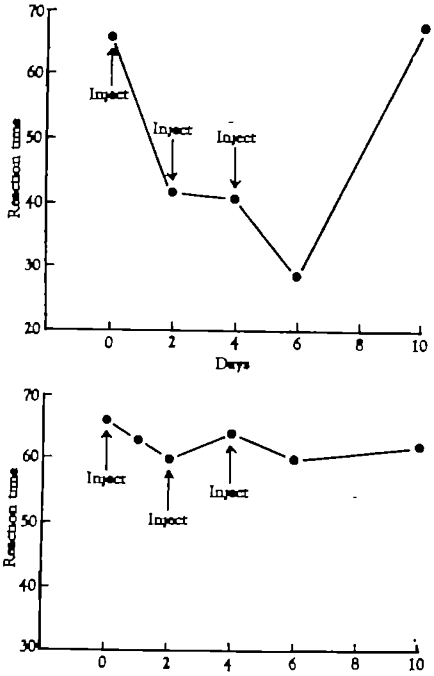

Fig. 4. Changes in the reaction time following two series of T.A.B. injections (250 × 10⁶ organisms) (separated by a 7-week interval). Upper, first series of injections; lower, second series of injections

ation, but the bulk of the evidence so far strongly implicates the involvement of the intracellular electron transfer systems and hence supports the views of Tahmisian[7] and many others that these systems are of paramount importance in the reaction of a tissue to radiation.

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Binding of Strontium in Blood

WE have reported[1] that, following the ingestion of radioactive strontium by normal subjects, the specific activity in urine was appreciably greater than that in contemporary plasma. Consequently, we postulated that strontium existed in blood in two different chemical forms. We were unable, however, to find any difference in the specific activity of strontium in plasma ultrafiltrate compared with that of the non-dialysable fraction. This result prompted further experiments which have now revealed an artefact in the original measurements. Heparin, which is known to be a source of adventitious stable strontium[2], was used in the collection of the blood. An incorrect allowance for this gave us falsely low values for the specific activity of plasma. A complete re-estimation of the relative specific activities of strontium in urine and plasma has therefore been made. The results of these measurements are given in Table 1. The probability of the ratio being greater than unity is 32 per cent, the 95 per cent confidence limits 0·84 and 1·40.

Table 1. SPECIFIC ACTIVITY OF STRONTIUM IN URINE AND PLASMA

Subject	Specific activity of strontium (counts/min/μg)		Ratio specific activity urine/plasma
	Urine	Plasma	
R. H. M.	176	170	1·03
G. E. H.	208	145	1·43
A. J. P.	135	158	0·85
T. E. F. C.	240	192	1·25
P. W. B.	222	218	1·02
		Mean	1 1 ± 0 1

In some experiments already reported[1], and in others unreported, heparin (batch No. 64373) was added to the blood samples. This addition was 40 mg (equivalent to 4,800 units) to all blood samples of 100 ml. or more. As the strontium content of this amount of heparin is known, it was possible to correct these earlier determinations. The observed and corrected values for the specific activity of strontium in urine relative to that in plasma are given in Table 2. The mean corrected value of the ratio 0·98 ± 0·05 confirms the more recent values in Table 1.

Table 2. EFFECT OF HEPARIN ON THE OBSERVED RATIO OF SPECIFIC ACTIVITY OF STRONTIUM IN URINE RELATIVE TO PLASMA

Subject	Specific activity of strontium in urine relative to plasma	
	Observed	Corrected
A. J. P.	2·68	1·38
P. W. B.	1·70	0·67
,,	1·18	0·98
R. H. M.	1·70	0·81
,,	1·69	0·85
,,	1·80	1·10
,,	1·17	1·02
,,	1·01	0·80
,,	1·45	1·14
,,	1·49	1·02
F. S. W.	2·20	1·23
	Mean	0·98 ± 0·05

We conclude that the specific activities of strontium in plasma and urine are probably equal. It should be added that the same result has also been obtained for calcium by others[3,4]. There is, therefore, no experimental evidence to support the view that there are two different forms of strontium (or calcium) in blood which the kidney is able to differentiate.

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A Multicomponent X-ray Survival Curve for Mouse Lymphosarcoma Cells Irradiated *in vivo*

NEARLY all investigations hitherto reported of the survival of animal cell populations exposed to single doses of ionizing radiations have yielded data which have been fitted by curves of the same general shape: a shoulder of varying size (which may be absent) followed by a region of exponential decrease. Only a single exponential component has been found even in those investigations in which survival was studied to fairly low levels (less than 10^{-3}), irrespective of whether survival was measured by colony formation *in vitro*[1-5], or *in vivo* by tumour induction[6,7]. These results have been obtained despite the fact: (*a*) that a given cell strain may be heterogeneous with respect to innate sensitivity[8]; (*b*) that cells may undergo marked fluctuations in radiation sensitivity during the division cycle[9]; (*c*) that inhomogeneities in degree of oxygenation might exist, particularly in a tumour cell population *in situ*[10]. It may be presumed, therefore, that one or more of the following has been pertinent in each study: (1) A particular population may be homogeneous with respect to inherent sensitivity, degree of oxygenation, and presence of protective agents or other modifiers of radiation response. (2) The overall sensitivity of cell populations heterogeneous with respect to radiation

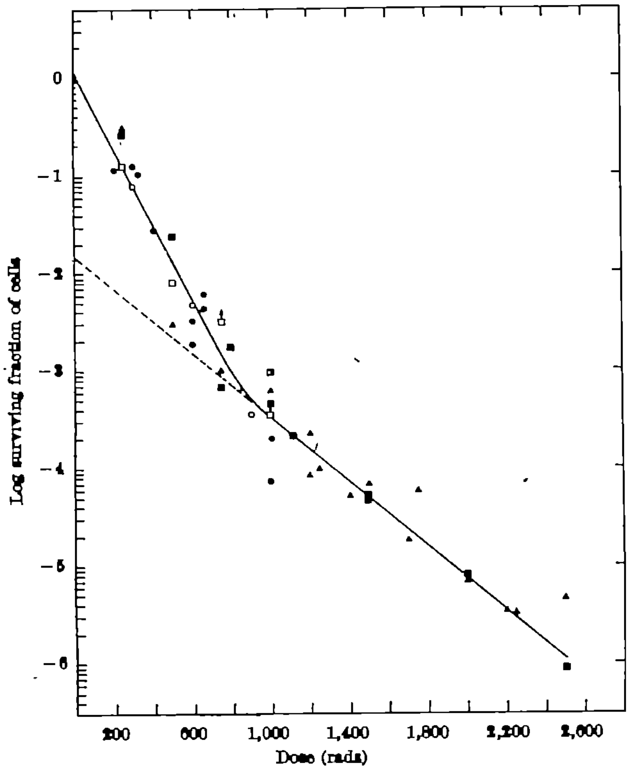

Fig. 1. Survival of *6C3HED* mouse lymphosarcoma cells irradiated *in vivo*. The curve has been fitted by eye. Each symbol refers to a separate experiment. Each point involved determination of the number of cells required to produce tumours in 50 per cent of 30 mice, using the end-point dilution method of Hewitt (ref. 14). This number for unirradiated cells was generally in the range 2–5 cells. The arrows indicate minimum values

response may, under certain conditions, exhibit relatively simple survival kinetics[9,11,12]. (3) A less-sensitive cell type may be present in amounts too small to be detected unless many decades of inactivation are effected[13]. (4) The precision of cell survival measurements, particularly those carried out *in vivo*, is limited.

We have observed a very different radiation response in the cell populations which constitute solid, subcutaneous *6C3HED* mouse lymphosarcomas, 1–2 cm in diameter, in *C3H* mice. When the viability of such cells is measured by Hewitt's method[14] immediately after total body exposures to graded doses of X-rays (220 kV C.P.; 0·5 mm copper + 1·0 mm aluminium added filtration; half-value layer 1·9 mm copper; 70–90 rads per min), survival is found to follow a multicomponent exponential curve, preceded by a small shoulder (Fig. 1). The results suggest that the survival kinetics are determined mainly by cells of only two sensitivities. The relative proportions of these remained essentially constant in four experiments. The initial exponential slope corresponds to a mean lethal dose (D_0) of 110 rads; its extrapolation number is about 1·2. The terminal slope indicates a D_0 of 260 rads, and extrapolates to about 0·01 on the ordinate. Neither the precision of the data nor the size of the resistant fraction warrants resolution of the measured survival curve into components for the sensitive and resistant fractions at doses less than 1,000 rads.

When tumours containing 10^8–10^9 cells (about 1 g) are irradiated with 3,000 rads, the surviving cells, which should be of the resistant type exclusively, give rise, on injection into new mice, to populations which exhibit a survival curve identical to that shown in Fig. 1. Hence, the resistant cells do not appear to constitute a sub-group the low sensitivity of which is inheritable.

The ratio of the exponential slopes, 2·3, is typical for oxygen enhancement of radiation sensitivity as observed in many studies[15], including those concerned with cytological damage[16] and survival[7,17,18] in tumour cells. It is proposed, therefore, that about 1 per cent of the cells of these tumours are anoxic and that the terminal slope characterizes their radiation sensitivity. The following preliminary results tend to support this hypothesis: (1) Irradiation in air of cells suspended in chilled foetal calf serum yields a survival curve which has only one exponential slope; the value of D_0 is close to that for the majority population irradiated *in vivo*. (2) Irradiation of cells *in situ* in mice killed by either cervical fracture[18] or asphyxiation in nitrogen, all of the tissues of which are presumably anoxic, yields a survival curve from which it is estimated that the fraction of radiation-resistant cells is about 10 times larger than in live mice. Further tests, both *in vitro* and *in vivo*, of the effects of oxygen and of anoxia on the radiation sensitivity of these tumour cell populations are under way.

The possibility that a given tumour cell population may be heterogeneous with respect to oxygenation has been considered previously[15,16], and the implications of the foregoing type of cell population response for the radiation therapy of tumours has been discussed theoretically by several authors[10,19,20]. Such discussions would now appear to rest on a much firmer foundation.

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Carbohydrate Metabolism in the Sapwood of *Pinus radiata*

THE use of carbon-14 produced in nuclear bomb testing in biological investigation has been discussed in an earlier communication[1]. Since 1954 the specific activity of the carbon dioxide in the atmosphere over New Zealand has been increasing, as is shown in Fig. 1 [2]. We have used this effect to examine the carbohydrate metabolism of *Pinus radiata*.

Annual rings of a *Pinus radiata* tree felled in January 1960 in the Kaingaroa Forest were ground up and acetone extracted in a Soxhlet extractor. The ground rings were treated with steam in an autoclave and then exhaustively extracted with water. Cellulose was separated from the residue. The water-extracted material and the cellulose were converted to carbon dioxide and counted in the Institute of Nuclear Science low-level carbon-14 counter. The $^{13}C/^{12}C$ ratio of each sample was measured so that the results could be corrected for any isotope effect in

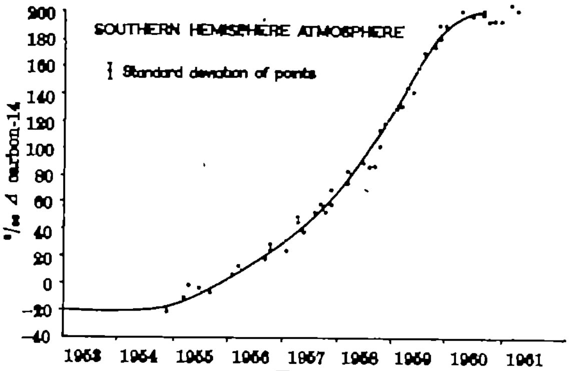

Fig. 1. Specific activity of southern hemisphere atmosphere, after Rafter (ref. 2). $\Delta^{14}C$ expressed as parts per thousand enrichment above 0·95 of National Bureau of Standards oxalic acid standard and normalized for isotopic fractionation to 0·975 of the P.B.D. Chicago belemnite standard

the metabolism of the tree or any possible fractionation in the laboratory procedure. The results are given in Table 1.

If the specific activity of the cellulose (Table 1) is compared with that of the atmosphere (Fig. 1) it is seen that the cellulose activity corresponds to that of the atmosphere when its ring was the outside of the tree. Thus the cellulose is not in dynamic equilibrium with the metabolism of the tree and, once synthesized, remains fixed. This result is not altogether unexpected and has long been assumed by those engaged in the carbon-14 age dating. The cellulose was included in these experiments as an internal standard.

The activity of the water-soluble carbohydrate (Table 1) is considerably lower than that of the atmosphere at the time of felling the tree. Thus the soluble carbohydrates in the inside rings of sapwood of *Pinus radiata* are not in dynamic equilibrium with the metabolism of the tree. These carbohydrates, therefore, are not analogous to the fat reserves of an animal.

Table 1. PARTS PER THOUSAND Δ CARBON-14 VALUES FOR CHEMICAL FRACTIONS OF ANNUAL RINGS OF *Pinus radiata*

Annual ring	Cellulose	Water-soluble (soluble carbohydrate)
1953/54 + 1954/55	− 20 0	+ 8·0
	σ = 4·0	σ = 4·9
1955/56 + 1956/57	− 14 8	+ 7·8
	σ = 2·3	σ = 4·4

Atmosphere at time tree felled (January 1960) = + 200.

* Δ Carbon-14 expressed as parts per thousand enrichment above 0 95 of National Bureau of Standards oxalic acid standard and normalized for isotope fractionation to 0 975 of the P.B.D. Chicago Belemnite Standard. σ = standard deviation of counting.

It should be pointed out, however, that a small amount of 'bomb carbon-14' has been incorporated into the water extractives since they are significantly higher than the cellulose. This might be due to the metabolism of some of the parenchyma cells in the medullary rays. This casts doubt on the practice of attempting to determine the specific activity of the atmospheric carbon dioxide at times in the past by accurate counting of the wood in the rings of trees[3], and suggests that a better procedure might be to do a chemical separation and to count only the cellulose.

We thank the Forest Research Institute, Rotorua, who kindly felled the tree and separated the rings, the Institute of Nuclear Science for counting the samples, and the New Zealand University Grants Committee for financial support.

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BIOLOGY

Use of Spleen-slice Homografts in the Study of Graft-versus-Host Reactions

PRESENT knowledge concerning the graft-versus-host reaction has been derived chiefly from examination of the consequences of injecting homologous adult lymphoid cell suspensions into tolerant hosts[1–4]. We have attempted further investigation of this phenomenon in so far as it may occur when morphologically intact lymphoid tissue is transplanted.

In earlier work[5], a technique was developed for transplanting slices of renal tissue from parental strain mice on to the kidney of F_1 hybrid recipients. Homografts between such strain combinations are of particular interest since genetically they are capable of inducing a graft-versus-host response without a concomitant host-versus-graft response. These kidney slice grafts failed to exhibit any evidence of a graft-versus-host response, presumably because the kidney lacks cells capable of proliferating in response to homologous antigens. In the experiments recorded here, slices of an immunologically competent organ, the spleen, were transplanted in a similar fashion. On microscopic examination of these spleen slice grafts, changes indicative of a graft-versus-host reaction were observed.

Adult male mice weighing 20–24 g were used. The parental strain was $C_{57}Bl_6$, and the F_1 hybrid strain was $B_6AF_1/J(C_{57}Bl_6$ ♀ × A/J ♂). The mice were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine.

Mice were anaesthetized by the intraperitoneal injection of pentobarbital and the left kidney was exposed through a flank incision. A small atraumatic vascular clamp (bulldog) was placed across the renal pedicle to prevent subsequent bleeding. A thin slice of renal cortex was shaved off the convex surface of the kidney with a sharp scalpel in order to provide a bed for the new spleen graft. The graft was taken from the spleen of the donor with fine

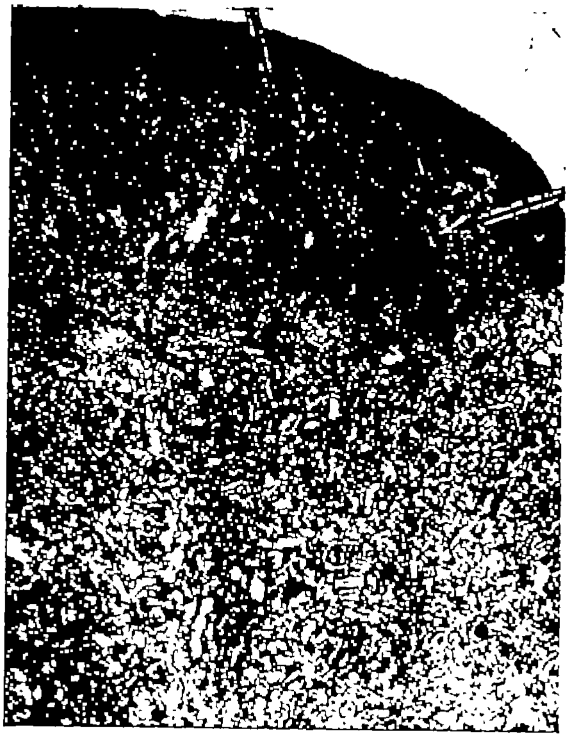

Fig. 1 Parental line spleen slice on F_1 hybrid kidney seven days after grafting. Arrows point to one of the hyperplastic follicles present in the graft. The preservation of the follicular component and the red pulp are evidence of the integrity of the splenic architecture (× c. 93, haematoxylin and eosin)

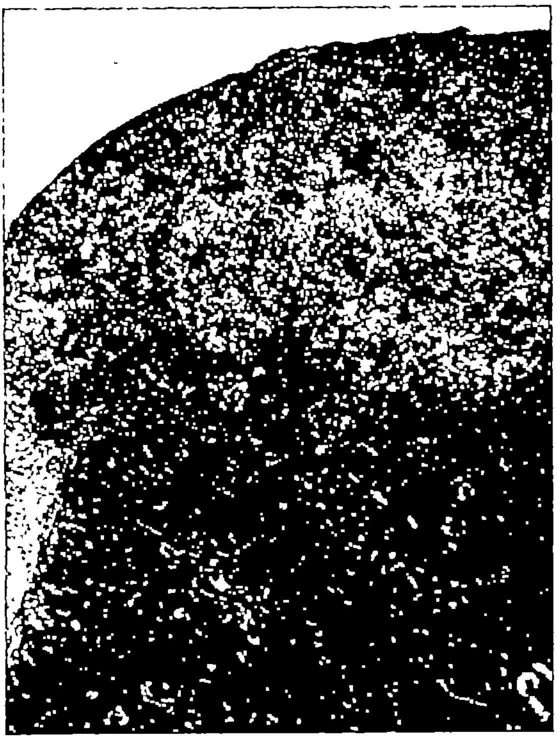

Fig. 2. F_1 hybrid spleen slice on parental line kidney seven days after grafting. Follicular architecture has been destroyed. Not evident at this magnification are the focal areas of necrosis in the graft. The mononuclear cells at the base of the graft infiltrate the interstitium of the kidney (× c. 98, hæmatoxylin and eosin)

scissors, excising from the convex surface a disk of tissue measuring about 4 by 3 by 1 mm. The cut surface of the graft was then placed on the renal bed and pressed down gently. The vascular clamp was released and the abdominal wound closed. Seven days later the kidney with its splenic graft was excised and sectioned transversely. Blocks were fixed in 10 per cent formalin and sections stained with hæmatoxylin and eosin. Adjacent blocks were fixed in absolute methyl alcohol and stained with methyl green-pyronin and with hæmatoxylin and eosin.

Each experimental group was composed of at least 20 animals. During the week following transplantation there was no mortality, and no systemic manifestations of runt disease were observed.

On gross examination at seven days, the control parental isografts ($P \rightarrow P$) and F_1 hybrid isografts ($F_1 \rightarrow F_1$) were reddish-purple, firm disks resembling normal spleen and measuring 1·0–3·5 mm in greatest dimension. Follicles were frequently apparent on the cut surface. Homografts of $P \rightarrow F_1$ were also reddish-purple, but were larger than the isografts and on sectioning were seen to contain larger, grey follicles. Homografts of $F_1 \rightarrow P$ were swollen, soft, and reddish-brown. Greyish-yellow foci of necrosis were present on cut section. No follicles were seen.

In histological sections, characteristic splenic architecture, including lymphoid follicles, was observed in the isografts. Homografts of $P \rightarrow F_1$ were distinguishable from the isografts by the presence of larger follicles (Fig. 1) and of numerous large immature cells of the lympho-reticular series. These cells had prominent pyroninophilic cytoplasm and were most numerous in the lymphoid follicles. They appeared similar to those cells described by Fagraeus as transitional cells[6] and by Scothorne[7] as large lymphoid cells. Grafts of $F_1 \rightarrow P$ were enlarged to nearly twice the size of the $F_1 \rightarrow F_1$ controls and were heavily infiltrated by macrophages, small lymphocytes and immature cells of the lymphocytic and/or plasmacytic series. The graft bed was heavily infiltrated by similar immature cells and by small lymphocytes. Areas of necrosis were observed in the grafts and follicular architecture was completely obliterated (Fig. 2).

This technique of spleen-slice transplantation permits direct observation of the histological changes occurring after transplantation of immunologically competent tissue. The hyperplasia of immature lymphoid cells with enlargement of the splenic follicle in the $P \rightarrow F_1$ graft is interpreted as a proliferative response of the surviving cells in the graft to host antigens. In the $F_1 \rightarrow P$ grafts the focal necrosis, loss of lymphoid follicles, and cellular infiltration of graft bed are interpreted as manifestations of the reaction of the host against the graft. The proportion of donor and recipient lymphoid cells surviving in such grafts and the functional capabilities of the cells of the graft are under investigation.

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Use of *Cyclo*phosphamide in an Attempt to modify the Canine Renal Homograft Response

In view of the prolongation of skin homografts in mice as a result of administering cyclophosphamide as reported by Sutton, Griffith and Preston[1], with the finding that the same drug used as a preconditioning agent permitted an increased survival of tumour transplants in rats by Henegar, Jackson and Preston[2], it was felt that this drug might be of use in prolonging canine renal homografts in line with previous investigations using other metabolites such as those of Calne[3]; Pierce, Varco and Good[4]; and Zukoski, Lee and Hume[5].

A comparative examination was made to evaluate this possibility. Twelve mongrel dogs were used in the control series. They underwent bilateral nephrectomies and were recipients of a renal homograft from a similar type unrelated dog of the same sex. The renal artery and vein of the donor kidney were anastomosed end-to-end to the iliac artery and vein of the recipient and the ureter was anastomosed to the bladder. Eleven dogs survived the operative procedure and lived for a period averaging twelve days with a range between seven and fifteen days. The blood urea nitrogen (BUN) increased precipitously about the seventh day and serial needle biopsies showed an intense cellular infiltration of lymphocytes, plasma cells and macrophages beginning at this time. The white blood count and hæmatocrit remained at almost the pre-operative level until this time and then fell.

In the *cyclo*phosphamide-treated group, 5 mg/kg of the drug was given intravenously at the time of surgery and 10 mg/kg of body-weight was given orally every other day beginning on the first post-operative day and continuing until their death. In this group there was one immediate post-operative death with the other animals living an average of twelve days but ranging from 4–23 days. There were three animals who exceeded the range of the control group, living 17, 21 and 23 days with two animals expiring before the control group at four days showing signs of drug toxicity. Outside of the last two, autopsy findings were similar in all cases with the kidneys markedly swollen and hæmorrhagic. Microscopically, they showed the intense cellular infiltration mentioned above. The onset of this cellular infiltration and rising BUN appeared approximately four days prior to the animal's death. The white counts post-operatively were depressed to about one-third of the pre-operative level with the hæmoglobin showing an approximate 30 per cent depression. Both these findings occurred during the first few days and remained generally at this level.

In conclusion, it was felt that *cyclo*phosphamide in the dosage and method used for administration in this series had little over-all effect in prolonging the survival time although further study will be necessary to evaluate the response in the two that did survive beyond twenty days.

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Effect of Light on the Oxidation of Reduced Nicotinamide-Adenine Dinucleotide Phosphate in Animal Tissues

DURING the course of experiments on glucose-6-phosphate : NADP and phosphogluconate : NADP dehydrogenases it was noted that the reduction of methylene blue occurred much faster in the light than in the dark. Subsequent experiments showed that this effect also occurred with isocitrate : NADP dehydrogenase but only to a slight extent with malate : NAD and succinate dehydrogenases.

The rate of reduction of methylene blue was determined by the Thunberg method. The following reagents were used: dye, 0·267 mM methylene blue; buffer, M *tris*-hydrochloric acid, *p*H 7·5; substrates mM, glucose-6-phosphate (disodium salt); 0·02 M (±)-isocitrate trisodium salt; 0·01 M sodium malate; 0·01 M sodium succinate; coenzymes, 0·05 mM NADP (sodium salt); 0·2 mM NAD; enzyme preparations. (*a*) Hæmolysate of human red blood corpuscles. 5 ml. of heparinized venous blood was centrifuged for 10 min at 2,500 r.p.m., the supernatant plasma withdrawn, the corpuscles washed twice with 0·9 per cent sodium chloride, and the volume finally made up to 5 ml. with distilled water. A 1-in-20 dilution of this hæmolysate in distilled water was used for the experiments on glucose-6-phosphate and phosphogluconate dehydrogenases. (*b*) Homogenate of mouse heart. A mouse heart was homogenized in 2 ml. of distilled water and the homogenate diluted with distilled water to contain 10 mg heart tissue/ml. for the experiments on isocitrate dehydrogenase and 20 mg/ml. for the experiments on malate and succinate dehydrogenases.

For the experiments on the red blood cell dehydrogenases 0·5 ml. each of the dye, buffer and diluted hæmolysate and a drop of octanol were placed in the Thunberg tube and 0·5 ml. each of glucose-6-phosphate and NADP in the hollow stopper. The tube was evacuated for 5 min to ensure the complete deoxygenation of oxyhæmoglobin. For the experiments with heart homogenate 0·5 ml. each of the dye, buffer, substrate and coenzyme were placed in the tube and 0·5 ml. of the diluted homogenate and a drop of octanol in the hollow stopper. The tube was evacuated for 3 min.

After evacuation, the tube was placed in a beaker of 1 l. capacity about three-quarters filled with water at 37° and allowed to equilibrate for 5–7 min, after which the reaction was started by mixing the tube and stopper contents. The tube was then either replaced in the beaker of water and illuminated with a 150-W frosted bulb or placed in the dark in a beaker of water at 37° in an incubator. In the former case, the tube was placed in the water-bath in a sloping position with the reaction mixture nearest the observer and 12 cm in a horizontal line from the rounded end of the bulb.

The time taken for the methylene blue to become reduced by 70 per cent, as estimated by comparison with a standard tube, was recorded as the reduction time. For the experiments on red blood corpuscles the standard tube contained 1·5 ml. buffer, 0·5 ml. diluted hæmolysate and 0·5 ml. methylene blue 30 per cent the concentration used in the reaction mixture. The tube was evacuated for 5 min. The standard tube for the experiments with heart homogenate contained 1·5 ml. buffer, 0·5 ml. diluted homogenate and 0·5 ml. methylene blue 30 per cent the concentration used in the reaction mixture.

At a certain time, when it was learnt by experience that the end-point had almost been reached, the tube in the dark was removed from the incubator, examined in daylight, and immediately replaced in the incubator. This procedure was repeated at 1–2 min intervals until the end-point was reached.

The concentration of the enzyme preparations used was chosen so as to give reduction times of 3–6 min in the light.

Typical results are shown in Table 1.

Table 1. METHYLENE BLUE REDUCTION TIMES AND DARK/LIGHT RATIOS

Dehydrogenase system	Dark (min)	Light (min)	Dark/light ratio
Glucose-6-phosphate : NADP and phosphogluconate : NADP (human red blood corpuscles)	41·0	4·5	9·1
Isocitrate : NADP (mouse heart)	84·0	8·3	10·2
Malate : NAD (mouse heart)	6·0	4·2	1·4
Succinate (mouse heart)	8·8	5·9	1·5

In a further experiment with isocitrate : NADP dehydrogenase a reaction mixture incubated in the dark for 10 min and then exposed to the light (150-W bulb) took 50 sec to reach the end-point.

The results show that light has a striking effect on the reduction time of methylene blue in systems in which NADP is the coenzyme. Since the reduction of methylene blue involves the oxidation of reduced coenzymes it can be concluded that light exerts a major stimulating effect on the rate of oxidation of $NADPH_2$.

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Size and Feeding of Different Types of Fishes

THE pelagic, predatory, marine fishes are a remarkable group, containing many species of great importance for both commercial and game fisheries. Some examples are the mackerel (*Scomber scomber*), the Thunnidae, the spear-fishes (*Makaira* sp.) and *Tarpon atlanticus*.

As these fishes are both fast swimmers and pursue their prey persistently, they may be defined as 'stayers', that is, fish capable of maintaining a high speed over a period of time. The 'stayers' are characterized by a body-musculature with a comparatively high amount of red muscle tissue, consisting of fibres of the thin red type. Thin red muscle fibres are characterized by the presence of myohæmoglobin, coupled with a good vascularization[1,2] and an aerobic metabolism, in which not only glycogen but also probably fat may be used for the production of energy. The alternative, broad white fibres have a completely different complex of metabolic enzymes[2-4]. They have an anaerobic action, using glycogen exclusively for energy production, and have a faster contraction than the narrow red fibres with a greater number of sarcomeres. The slower narrow red fibres are suited for prolonged action. Consequently, a large amount of red muscle tissue in the body musculature enables the fish to cover long distances at a relatively high cruising speed. It is, however, an expensive ability, which demands a good supply of both calories and oxygen to function efficiently. A 'stayer' like the trout has a higher basal metabolism and food-coefficient than other types of fish[6,7].

The drag on the surface of moving fish is correlated with the surface areas of its body, which is in turn proportional to the cross-section of the musculature. This cross-section estimates the power of the muscle. As the fish grows, the increase of the swimming speed is only slight[8]; but the possibilities for fuel storage increase with the contents in the third degree of the proportions, much faster than the drag on the surface.

In this connexion it is interesting that the biggest representatives of this group of typical 'stayers' occur in oligotrophic seas. Representatives of this group in the eutrophic North Sea are: *Scomber scomber* (mackerel), *Trachurus trachurus* (horse mackerel) and *Belone belone* (garfish), all small fishes with a maximum weight of 1–3 lb.[9].

On the contrary, in oligotrophic tropical seas, a considerable number of pelagic predators with a length of more than 50 cm are found, especially in the family Carangidae, and also a number of real giants with a maximum weight of 100–1,500 lb., with a maximum length varying from 1·5 to 5 m. The latter belong to the families Histiophoridae, Xiphiidae, Scomberomoridae, Thunnidae, Coryphaenidae, Rachycentridae, Elophidae and Carangidae[10,14].

The more favourable relation between fuel-storage and drag on the surface gives the bigger fish a larger action radius. The migration of big pelagic fish-eating species is often remarkable. For the albacore (*Germo alalunga*) migrations of 4,724 miles in 324 days and 3,166 miles in 176 days have been recorded[11]. These big species can thus search huge areas for food, which is an obvious advantage if food is scarce.

An additional advantage the larger predatory fish has over a smaller species is the greater variation in the size of the potential prey. The huge swordfish and spearfishes also feed on very small prey[12,13]. If food is scarce and heterogeneously dispersed, we may conclude that an increase of size is a favourable factor in the struggle for life of this type of fish.

If food is abundant and more or less homogeneously dispersed, the greater possibilities for fuel storage of the bigger species are not important.

In flat-fishes, range and normal swimming speed are not important, but time is the main factor which determines the quantity of food captured by the fish. Flat-fishes obtain the biggest part of their food (worms, lamellibranchs, crustaceans) by careful search of the bottom, which has to be done at a low speed: so that these fishes do not need a large amount of red muscle tissue which makes a high main speed possible. In order to escape from their enemies and to catch a moving prey, the ability to sprint fast is very favourable. As one might expect the body-musculature of flat-fishes consists mainly of broad white muscle fibres with low sarcomeres. This is well suited for the sprint.

In eutrophic boreal seas flat-fishes are, in the main, much bigger than their relatives in oligotrophic tropical seas. The maximum length of flat-fishes from Ceylon varies from 9 to 62 cm with an average of 20 cm. In the Norwegian and North Sea the corresponding variation is 12–200 cm with an average of 55 cm[9,10].

The Gadoid fishes form another group of bottom-dwelling 'sprinters' which mainly live in the open sea. They are very common in eutrophic boreal seas but even there concentrated on spots abounding with food. They are almost absent from tropical waters.

In tropical seas, fish-eating 'sprinters' are concentrated on coral reefs. Here they find quiet water, where their lack of stamina is of no importance, and hiding places, which increase their chances of seizing prey.

A relatively large stock of small fishes feeding on the corals and other invertebrates, and extremely clear water, are advantages to fishes who have to find prey by the eye. Fishes of this type are the baracuda (*Sphyraena baracuda*), which reaches a weight of 100 lb., and the groupers (Serranidae), some of them reaching a weight of 1,000 lb.[14].

On the shores of boreal seas, conditions are not so well suited for 'sprinters'. There is not such a concentration of small fish, the water is much less clear and, in most cases, moving strongly. The genera *Gobius* (gobies) and *Cottus* (sea-scorpions) are good representatives of the small coast-dwelling 'sprinters' in the North Sea and the Atlantic.

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Endocrinology of Reproduction in the River Lamprey

EXISTING literature on endocrine aspects of reproduction in cyclostomes has recently been reviewed[1,2] and we have already reported the results of a preliminary investigation on controlling mechanisms in the reproductive physiology of the river lamprey, *Lampetra fluviatilis*[3]. The present communication records the results of more extensive work.

The experimental animals were obtained from the River Severn and kept in well-aerated running tap-water. Hypophysectomy was carried out as previously described[3] on a large number of individually marked lampreys, biopsy specimens of gonad from both hypophysectomized and sham-operated control animals being examined at the time of operation and at intervals afterwards. Ten lampreys were gonadectomized. Survival of all operated animals was excellent.

When male lampreys of this particular population begin their anadromous migration in late September, the testis contains only spermatogonia, and mitotic divisions are numerous. Between October and December the germ cells enter the prophase of meiosis, and this stage lasts until mid-February when metaphase plates first become evident. From the beginning of March the testis contains an increasing number of spermatozoa and progressively fewer earlier stages. Maturation is complete and spawning occurs in early April. The development of the germ cells is approximately synchronous within an individual testis and throughout a particular population, though this is not absolute. Secondary sexual characters become evident during March and are usually fully developed by early or mid-April.

Eighty lampreys were hypophysectomized between early October and early March, and in all cases of complete hypophysectomy secondary sexual characters failed to develop.

Examination in April of biopsy samples from the testes of lampreys hypophysectomized in October revealed the presence of primary spermatocytes in late prophase, together with meiotic metaphases, secondary spermatocytes, spermatids and, rarely, spermatozoa. Several of these animals survived until the end of June when their testes were again examined by biopsy. In every case the testis contained spermatozoa, occasionally accompanied by late spermatids. These spermatozoa were never whorled within the lobule, with their heads grouped together, as in the normal mature testis, and their appearance in sectioned testicular tissue suggested that they were incompletely developed.

When examined in April, the testes of lampreys hypophysectomized in November and December contained all stages of spermiogenesis from early spermatids to spermatozoa, the latter being loosely scattered, similar to the June configuration of the October-hypophysectomized testis. The testes of lampreys hypophysectomized in January and later, when examined in April, contained only spermatozoa, the degree of whorling being greater in the later-operated animals.

From the foregoing results it appears that removal of the pituitary, even when the germ-cells are only at the spermatogonial stage, is not an insuperable barrier to the progress of spermatogenesis and spermiogenesis, though under these conditions spermatozoa are formed approximately two months later than in normal animals. Hypophysectomy performed when the germ-cells are in early prophase of the first meiotic division (in November and December) causes less delay in sperm formation, and when performed during the later stages of prophase (January and February) there is little, if any, disturbance of the timing of sperm maturation and the nuclear events which precede it.

These results suggest that spermatogenesis and spermiogenesis in lampreys, unlike other vertebrates, are autonomous processes though they are appreciably catalysed by the pituitary, the absence of which from October onwards delays the appearance of sperm by some ten weeks. Moreover, in animals maintained for this length of time after the normal population has spawned, the sperms are loosely distributed in the lobules and appear incompletely developed though, because no normal females are available at this time, their capacity for fertilization has not been tested. It may be noted in this context that a virtually normal testis picture could be obtained if hypophysectomy is carried out at such a time that it does not unduly delay the development of the spermatozoa. This would appear to suggest that it is a time factor, rather than absence of the pituitary, which is responsible for the somewhat abnormal appearance of the testis of animals hypophysectomized in October and examined late in the following June.

In female lampreys, hypophysectomy abolishes the normal increase in size and dry-weight of the eggs which occurs between January and April. After hypophysectomy the ovary remains small and compact and the eggs are not released into the body cavity, whereas in the control animals they are ovulated by the beginning of April. However, no signs of atresia were observed, and this is a point of considerable interest since, so far as is known, atresia of yolk-containing eggs invariably follows hypophysectomy in other female vertebrates.

The effects of replacement therapy using lamprey pituitary extracts, mammalian gonadotrophins of extra-pituitary origin, and steroids, have also been investigated. Of two groups of hypophysectomized lampreys, one was injected with a suspension of lamprey pituitary material at a dose level of three glands per animal in 0·1 ml. saline, and the other with a mixture of 200 I.U. pregnant mares' serum and 300 I.U. chorionic gonadotrophin in 0·1 ml. saline, at approximately fortnightly intervals between December and April. The animals displayed secondary sexual characters in April and sperm was present in the testes, in most cases whorled within the lobules as in intact mature lampreys. Ovulation occurred in the females. These changes were more marked in the case of the pregnant mares' serum—chorionic gonadotrophin injections, in which the dose was evidently more suitable. Control animals injected with saline retained the immature status of hypophysectomized lampreys.

Intact lampreys of both sexes were also injected with pregnant mares' serum and chorionic gonadotrophin at the same dose-level and at similar intervals between November and early March. Stimulation of both primary and secondary sexual characters was obtained. Secondary sexual characters first appeared during February, though the injections started in November. This suggests that the tissues concerned are insensitive to hormonal stimulation until shortly before the time at which the secondary sexual characters normally develop.

Intraperitoneal implantation of a 25-mg pellet of testosterone into hypophysectomized male lampreys in December or February caused the development of secondary sexual characters in early May. Implantation of a 12·5-mg pellet had only a slight effect. Intact and hypophysectomized female lampreys implanted each with a 25-mg pellet of testosterone failed to develop the characteristic female post-anal fin, but developed an enlarged urinogenital papilla similar to that of a mature male. Thus, the nature of the secondary sexual characters appears to depend on the type of steroid hormone administered.

Three lampreys gonadectomized in November and seven gonadectomized in February survived until mid-April. None showed any sign of secondary sexual characters. It therefore appears likely that the relationship between gonad and secondary sexual characters, well established in other vertebrates, holds also in cyclostomes.

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Unequal Weighting in Numerical Taxonomy

IN the Fungi Imperfecti, the system of classification which has evolved over the past 150 years involves the recognition of 'form species' and 'form genera' which lack any established genetic basis. Thus it is possible to speak of 'similarity' but not of 'relationship'. This system, though admittedly artificial, is at present essential to sorting and identification procedures.

As no more natural scheme is yet in prospect, any consistent and reasonably objective way of placing an unknown or undescribed fungus within the existing framework would be useful both to the non-taxonomist attempting identifications, and to the scientific historian trying to explain the rationale of the present system.

We have accordingly attempted to apply the numerical approach to taxonomy within the existing scheme, as a first step toward making mathematics an operational tool in the taxonomy of the Fungi Imperfecti.

The method originally tested was that described by Sneath[1] in which coefficients of similarity are formed between pairs of organisms or taxa by the comparison of large numbers (at least 40 and preferably more than 60) of equally weighted qualitative and quantitative characters. Our initial work used a small group of Fungi Imperfecti comprising the genera *Haplobasidion*, *Lacellin-*

opsis and *Lacellina*, each with three species. The data were derived from a taxonomic treatment by Ellis[4], a list of 70 features being compiled for use in the numerical scheme.

When Sneath's method was followed, it was found that similarity coefficients between 'form species' from different existing 'form genera' were in some cases higher than those between 'form species' now considered congeneric (Table 1), and no acceptable alternative classification could be visualized. Consequently, it was felt that the method in its original form was unsuitable for this application.

In most discussions of numerical taxonomy[1–3] equal weighting of all characters has been a prerequisite, because of the difficulty of devising any rational criteria for differential weighting.

Table 1. SIMILARITY COEFFICIENTS WITH EQUAL WEIGHTING

Species	1*b*	1*c*	2*a*	2*b*	2*c*	3*a*	3*b*	3*c*
1*a*	60	59	71	67	61	44	54	61
1*b*		68	58	59	67	50	50	54
1*c*			73	68	62	58	54	58
2*a*				74	72	70	73	71
2*b*					70	64	62	66
2*c*						63	57	64
3*a*							90	78
3*b*								79

Table 2. SIMILARITY COEFFICIENTS WITH DIFFERENTIAL WEIGHTING

Species	1*b*	1*c*	2*a*	2*b*	2*c*	3*a*	3*b*	3*c*
1*a*	78	78	73	71	69	50	54	57
1*b*		83	67	68	71	53	53	54
1*c*			75	72	69	56	54	56
2*a*				86	85	74	76	75
2*b*					84	71	70	72
2*c*						71	68	71
3*a*							96	88
3*b*								88

Key to species: 1*a*, *b*, *c*, *Haplobasidion thalictri*, *H. lelebae*, *H. musae*. 2*a*, *b*, *c*, *Lacellinopsis sacchari*, *L. levispora*, *L. spiralis*. 3*a*, *b*, *c*, *Lacellina graminicola*, *L. macrospora*, *L. leonensis*.

The principal differences between *Haplobasidion*, *Lacellinopsis* and *Lacellina* are that only the first two possess swollen sporiferous vesicles at the apex of the conidiophore, and only the last two possess setae. In organisms like the Fungi Imperfecti, which are described largely in morphological terms, those terms may conveniently be divided into primary and secondary characters. Primary characters are those organs (setae, vesicles, conidia) which require further characterization or description. This is supplied by a variable number of secondary descriptive features (width, septation, pigmentation). Differences in secondary characters will naturally lower the similarity coefficient, and since secondary characters considerably outnumber primary characters, such differences can sometimes outweigh similarities in primary characters. Where a species lacks a primary character, the associated secondary characters are not considered when making comparisons with other species and hence do not affect the similarity coefficients. It seems unsatisfactory that it is possible for two species, both possessing a given primary character, to appear less similar to each other than to a third species lacking the primary character entirely, because of differences in secondary features of that character. This anomalous situation seems, in fact, to have arisen in the Fungi Imperfecti, although it has not apparently been encountered in other groups to which the method has been applied.

To obtain more realistic coefficients of similarity in the Fungi Imperfecti, an amended scoring system was devised. To each primary character was added a weighting of unity for each secondary character needed to describe it. The primary character 'setae present' is described by a maximum of eight secondary characters in any individual. In *Lacellinopsis sacchari*, for example, these are as follows: (1) simple, (2) straight, (3) subulate, (4) thick-walled, (5) pigmented, (6) septate, (7) 400–700μ long (max.), (8) 7–9μ thick (max.).

Under the modified scheme, the presence of setae in *Lacellinopsis* and their absence from *Haplobasidion* was now scored as nine character differences.

When this weighting system was used, the classification obtained was in accordance with present-day practice, the 'form genera' being clearly delimited (Table 2). While this was to be expected, since the weighting system was devised for this particular group of organisms, a more recent and larger analysis (which will be discussed in detail elsewhere), involving intraspecific as well as interspecific and intergeneric comparisons, has confirmed the usefulness of the modified method in the Fungi Imperfecti.

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Germination of Basidiospores and Production of Fructifications of Members of the Agaric Family Bolbitiaceae using Herbarium Material

DURING the taxonomic examination of the Bolbitiaceae it has been necessary, in order to carry out mating determinations, to germinate the basidiospores of numerous collections. The source of the spores has included not only fresh spore prints but also spores from dried fruit bodies and from spore prints accompanying herbarium material.

Fresh turgid spores, collected from a mature sporophore and immediately plated out on 5 per cent malt extract agar, have germinated after a 48-h period in the dark at 25° C. On dung agar the germination vesicles, typical apparently of this family, and especially pronounced in the genus *Conocybe*, have been produced within a similar period of time. Spores of dried material, however, have not germinated satisfactorily, even during a 28-day period. Out of almost eighty collections, only in three have one or two spores been observed to develop vesicles and germ tubes. The subsequent growth of these germ tubes has been poor, and the vesicles have soon collapsed. No increase in germination has been noted if the spores from the herbarium were agitated in sterile distilled water for ½, 1, 2, 3, 4 h or longer periods of time. This suggests that the lack of germination is not due to the activity of a simple water soluble and leachable inhibitor. Sterile dilute aqueous solutions of organic acid and/or mineral nutrients have had no stimulatory effects.

Spores from herbarium material, however, germinate if they are incubated in a saturated atmosphere overnight, transferred to a phial of sterile distilled water, dispersed by agitation in a micro-shaker and plated out.

The incubation was carried out by placing the dry spores in a welled slide mounted on glass disks in a sterile Petri dish and surrounded by 5 mm of sterile water. This was left overnight in a darkened incubator at 27° C, the latter being the most favourable temperature.

By this simple technique basidiospores of the type material of *Conocybe percincta*, after two years in the dry conditions of a herbarium, have been induced to germinate. The viability of collections varies according to the way the herbarium material has been dried. Herbarium material treated with insecticides has not germinated by this method.

A few collections, approximately three years old, of *Bolbitius vitellinus*, *Conocybe subpubescens* and *Agrocybe semi-orbicularis*, have germinated after the foregoing treatment, but normally after two years the spores have completely dried out and the protoplast has become disorganized.

Scarth[1] has shown that after freezing plant tissue in micro-cold chambers and suddenly thawing under constant observation there is a flooding of the intercellular spaces, rupturing of the protoplast, and consequently a low survival of the cells constituting the tissue. If gradual thawing is allowed there is no such tearing of the cell wall away from the protoplast and the organization seems to be retained or not drastically disorganized as with a violent deplasmolysis.

A similar mechanism may explain the results with agaric basidiospores. If water molecules gradually diffuse into the internal parts of the spore the survival rate is considerably higher than if the spores are swamped with excess free water. Using the dried herbarium material and incubating the spores in a saturated atmosphere, water is gradually imbibed by the viable spores present in the spore deposit. The colloidal components are allowed slowly to absorb sufficient water for reorientation over a twelve-hour period and are not disorganized by flooding.

The spores so treated are in a suitable state to be agitated in either sterile distilled water or aqueous mineral solutions without further injury to the protoplast. A suspension of them in sterile distilled water was sprayed on nutrient agar, supplemented with 10 per cent dung extract. Agar disks with germination vesicles and germ tubes were cut out with a flamed cork borer and transferred to sterile half-pint milk bottles. In the bottles was placed a pad of shredded block paper pulp, 10 cm in depth, soaked in water, partially squeezed dry and covered with nutrient agar. The bottles were kept on the laboratory window sill at 20° C.

Sufficient of the nutrient agar, made up according to Lange's formula[2] plus 0·5 per cent malt extract, was added to form a 1–2 cm-layer at the bottom of each bottle and a thin coat over the paper pulp. The alveolate nature of the pulp presents a larger aerated surface for fungal colonization.

After about 50 days under the foregoing conditions primordia appear and within 60 days mature fruit bodies bearing normal basidia, basidiospores, cheilocystidia and dermatocystidia are formed. The spores produced by these fructifications are quite viable and fresh inoculations have been made from them. Some cultures, however, take longer to produce primordia or once they are formed do not develop further. Favourable growth and the production of sporophores in these cultures can be induced by covering the paper pulp pad with a layer of a sterile sand and soil mixture.

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Incorporation of Hydrocyanic Acid labelled with Carbon-14 into Asparagine in Seedlings

In the course of work on the biosynthesis of cyanoglucosides in plants, it was observed in feeding experiments that young seedlings of a number of plant species incorporate radioactivity from $H^{14}CN$ into asparagine to a considerable extent.

Sorghum vulgare, var. Honey Drip, was used in most of the experiments. Seeds were germinated and grown in the dark for 4 days at 29 ± 1° C. Twenty seedlings were selected for uniformity and placed in a 10-ml. beaker containing 5 ml. distilled water. This beaker and a vessel containing 1 μM $Na^{14}CN$ (3·48 μc.) in 0·17 ml. 0·1 N sodium hydroxide were placed in a 2·5-l. desiccator which was closed immediately after addition of a slight excess of mineral acid to release $H^{14}CN$. The seedlings were maintained in continuous light under incandescent lamps for 40 h, during which period they appeared to grow normally. The plants were then collected, weighed, frozen in liquid nitrogen, ground and extracted with 100 volumes of boiling 80 per cent (v/v) aqueous ethanol for 5 min. The solvent was evaporated *in vacuo* and the residue extracted with 2 ml. of 10 per cent (v/v) aqueous isopropanol. The extract was clarified by centrifugation if necessary and suitable aliquots were used for paper chromatography. Similar feeding experiments were also carried out with barley (*Hordeum vulgare*, var. California Mariout), pea (*Pisum sativum*, var. Alaska), flax (*Linum usitatissimum*, var. Imperial), red clover (*Trifolium pratense*, var. Medium) and white clover (*Trifolium repens*, varieties Ladino and New Zealand Certified).

Two-dimensional chromatograms were prepared on Whatman No. 1 paper in phenol/water (80 : 20 w/v) followed by butanol/acetic acid/water (120 : 30 : 50 v/v), using the descending technique[1]. Autoradiographs were made using Kodak 'No-screen' X-ray film with exposure times of 1–2 weeks. In all cases, a very intense spot was observed on the autoradiographs, which superimposed exactly with the characteristic brown spot for asparagine observed on spraying the chromatograms with ninhydrin reagent and developing at room temperature. Percentage recoveries of carbon-14 in the suspected asparagine were determined by eluting the radioactive zones from one-dimensional chromatograms run in butanol/acetic acid/water and counting suitable aliquots at infinite thinness in a gas-flow counter (Nuclear-Chicago model 181*B*). Recoveries in two experiments with sorghum were 14 and 17 per cent.

Some less-intense spots were also observed on the autoradiographs, the pattern varying with different species. In several instances a spot was observed in the position where sucrose would be expected, corresponding exactly with a positive reaction to aniline phthallate reagent[1] on the chromatograms. A tracing of a typical

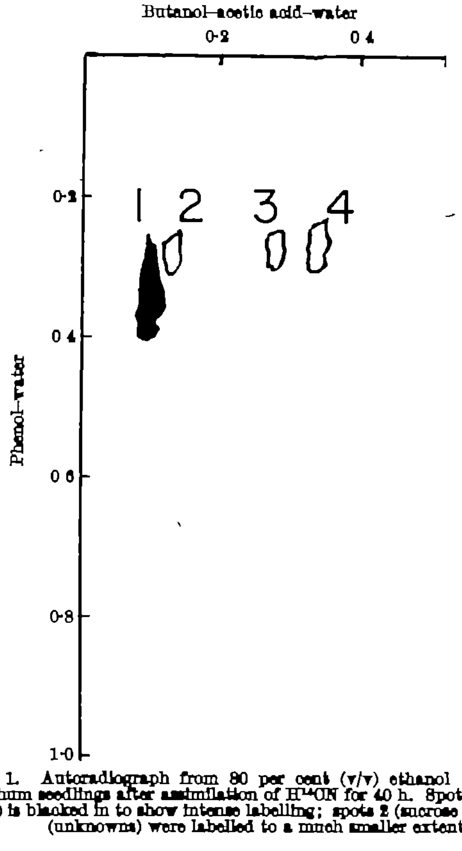

Fig. 1. Autoradiograph from 80 per cent (v/v) ethanol extract of sorghum seedlings after assimilation of $H^{14}CN$ for 40 h. Spot 1 (asparagine) is blacked in to show intense labelling; spots 2 (sucrose ?), 3 and 4 (unknowns) were labelled to a much smaller extent

autoradiograph from the experiments with sorghum is shown in Fig. 1.

The identity of the most heavily labelled compound as asparagine was established conclusively for all the species studied. Aliquots of the extracts were chromatographed in one dimension on Whatman No. 1 paper in each of the 4 following solvent systems: methanol/water/pyridine (160 : 40 : 8 v/v), ethanol/water/ammonia (180 : 10 : 10 v/v), methyl ethyl ketone/acetone/water (30 : 10 : 6 v/v), ethanol/*tert*-butanol/water/ammonia (60 : 20 : 15 : 5 v/v). The distribution of radioactivity was determined using a chromatogram strip scanner (Nuclear Chicago model *R*1000). In all cases the major radioactive peak was found to correspond with DL-asparagine marker. A superposition test with authentic L-asparagine using methyl ethyl ketone–acetone–water as solvent was positive. Exact correspondence was also obtained when an aliquot was run on DEAE paper in 0·02 M phosphate buffer, *p*H 4·7 (ref. 3).

Formation of aspartic acid was demonstrated by enzymatic hydrolysis with a *Pseudomonas* enzyme which exhibits both glutaminase and asparaginase activity[4]. Prof. D. M. Greenberg and Dr. M. E. Ramadan, University of California Medical School, San Francisco, kindly supplied an enzyme preparation of activity 200 enzyme units per ml. (1 unit is defined as liberation of 1 μM ammonia per 15 min). Radioactive asparagine in the extracts was separated from glutamine by paper chromatography in butanol–acetic acid–water. Suitable aliquots of the eluates were incubated for 15 min at 37° with constant shaking with 0·1 ml. enzyme in the presence of 0·09 M sodium veronal buffer, *p*H 8·2, and 0·023 M potassium cyanide in a total volume of 1·1 ml. Chromatograms were then run in butanol–acetic acid–water, scanned for radioactivity and sprayed with ninhydrin. A single radioactive peak was observed to correspond with aspartic acid in marker strips and no radioactivity was detected at the asparagine position.

In a preliminary assessment of the distribution of carbon-14 in the asparagine, chromatograms of the partially purified asparagine-^{14}C and of aspartic acid-^{14}C derived by enzymatic hydrolysis were sprayed with ninhydrin, dried for 5 min at 100° and scanned a second time in the strip counter. Whereas there was no reduction in radioactivity in the case of asparagine, all the radioactivity was lost from the aspartic acid by decarboxylation after spraying twice with ninhydrin. This suggested that the label was present solely in the β-carboxyl carbon atom of aspartic acid, or the amide carbon atom of asparagine[5].

The distribution of carbon-14 in the carboxyl- and amide-carbon atoms of asparagine was determined more precisely by the decarboxylation of asparagine-^{14}C and aspartic acid-^{14}C with *N*-bromosuccinimide[6]. The $^{14}CO_2$ evolved was collected in 1 ml. *M* potassium hydroxide in the centre-well of Warburg vessels and determined by a modification[7] of the scintillation counting method of Gordon and Wolfe[8]. Whereas 100 per cent of the carbon-14 of aspartic acid was recovered as $^{14}CO_2$, only 1·6 per cent of the carbon-14 of asparagine was thus recovered.

These experiments show that the carbon atom of hydrocyanic acid is incorporated in relatively large amounts into the amide-carbon atom of asparagine in seedlings of 6 species. It appears likely that asparagine can be synthesized in these species by 3-carbon plus 1-carbon condensation, where the 1-carbon fragment is hydrocyanic acid or a related compound such as formamide. In similar feeding experiments with $^{14}CO_2$ a much wider distribution of the label was found, showing that the observed results with $H^{14}CN$ were not due to conversion to $^{14}CO_2$. Preliminary experiments with serine-^{14}C support the hypothesis that serine or a related metabolite could be the 3-carbon unit.

It will be noted that hydrocyanic acid was incorporated into asparagine both in seedlings of species which contain relatively large amounts of cyano-glucosides (flax, sorghum and white clover)[9], and in seedlings which contain very small amounts, if any, of organic nitriles.

These results may also be of interest with respect to the use of cyanide as an inhibitor in experiments with plant tissues, since the level of inhibitor might change during an experiment due to metabolism of hydrocyanic acid in the manner described here.

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Efficiency of Infective Propagules of *Rhizoctonia solani* in reducing Vigour of Strawberry Plants

WE have found that the number of strawberry plants produced by the end of the season is inversely related to the number of mycelial fragments of *Rhizoctonia solani* adhering to small roots of strawberries at mid-season.

On May 2, 1961, four different soil treatments were disked into the upper 3–5 in. of 7 × 10 ft. plots. The treatments, plus untreated control plots, were replicated four times. Six days later, Midway variety strawberry plants, naturally infested with *R. solani*, were set out. There were ten plants per plot. On August 28, samples of roots, 1 mm in diameter or less, were taken from the plants. Soil particles were shaken from the roots. The latter were dipped in lactophenol, drained, and mounted in lactophenol for examination under the microscope. The average number of mycelial fragments of *R. solani* adhering to a 5-cm length of root was determined. The total number of strawberry plants—original plus all runner plants—were counted at the end of the season.

Fig. 1 shows the highly significant negative correlation between root infestation by *R. solani* and the final numbers of plants. The data fit both an arithmetic and a logarithmic regression equally well. Any essential differences between the two regressions is evident only above or below our range of observations as shown in Table 1. If the relation between cause and effect is arithmetic, then reducing the number of mycelial fragments from 10 to 1 per 5 cm of root could increase the expected number of plants by less than 10 per cent. A logarithmic relation, however, implies that such a reduction in mycelial fragments would about double the number of expected plants, if there were no other limiting factors. At the other end of the scale, an arithmetic relation would predict a complete loss of plants if there were more than 70 fragments

Table 1. EFFECT OF NUMBER OF *R. solani* MYCELIAL FRAGMENTS ON THE FINAL NUMBER OF STRAWBERRY PLANTS. COMPARISON OF AN ARITHMETIC WITH A LOGARITHMIC RELATION

Mycelial fragments per 5 cm of root	Expected No. of plants at end of season	
	Arithmetic	Logarithmic
1	49	87
5	46	58
10	43	40
25	33	30
50	16	17
7	0	10
100	0	5

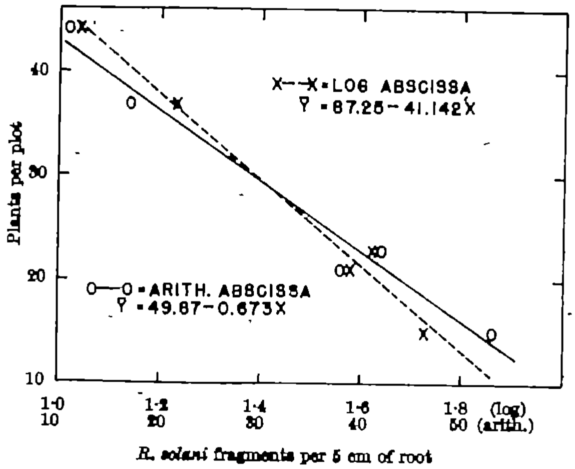

Fig. 1. Relation between number of *R. solani* mycelial fragments per 5 cm of root and final number of strawberry plants per plot. The regression coefficients obtained with either the logarithmic or arithmetic abscissae are both significant well beyond the 1 per cent level

per 5 cm of root. The logarithmic relationship would predict that more than 100 fragments would be needed to reduce the number of plants to one.

Regardless of which relation applies, *R. solani* must be reducing the vigour of strawberries in Connecticut where this fungus is omnipresent. As biological cause–effect relations are usually logarithmic, the reduction in vigour must be appreciable. In addition, the logarithmic relation accounts for why *R. solani* of itself rarely causes complete failure of plantings.

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Correlated Seed and Tuber Dormancy in Potatoes

The seeds of temperate-zone wild plants are usually dormant at maturity and germinate only after a lapse of time and/or the application of certain more or less specific dormancy-breaking conditions. By contrast, the seeds of cultivated plants are commonly non-dormant, and this must usually represent a response to human selection; loss of dormancy has thus been a significant episode in the evolution of many cultivated plants. Since few genetical investigations of dormancy in crop plants have been reported, an investigation of the cultivated potatoes was begun in 1959; the finding that, in this group, seed and tuber dormancy are correlated, an observation of some general and practical interest, prompts a brief description of the leading results so far obtained.

An inverse measure of seed dormancy may be obtained by counting germination of freshly gathered seed four weeks after being set out in the Petri dish and this method has been adopted as routine[1]. Tuber dormancy is estimated by the mean time to appearance of shoots from tubers buried in moist peat in the glasshouse.

One set of experiments started from highly heterogeneous (open pollinated) bulks of seed[1] derived from field plantings of cultivated diploid potatoes ($2n = 24$) and Andigena Group tetraploids ($2n = 48$) (ref. 2). The material all came from the Commonwealth Potato Collection. Fresh seed was laid out in trays on moist filter paper and selection for early and late germination was carried out: this was, effectively, selection for low and high dormancy. Of the four resulting lines, the two diploid (which were self-incompatible[2]) were carried on by sib-crossing, the two tetraploid by sib-crossing and selfing. Responses were striking: the two low-dormancy lines both gave more than 90 per cent germination in three generations; the high-dormancy lines dropped to 40 per cent germination in two generations for the diploids and to about 1 per cent in three generations for the tetraploids Thus selection for and against seed dormancy was effective Moreover, tuber dormancy showed a parallel response to the same selection: thus the low-dormant lines bear tubers that sprout in about 2–3 weeks (diploids) or 4–5 weeks (tetraploids); and the high-dormant lines, by contrast, bear dormant tubers, sprouting in about 8–9 weeks (diploids) and in over 12 weeks (tetraploids).

Change in tuber behaviour therefore occurs as a correlated response to seed selection. The converse is also true: seed derived from plants selected for different levels of tuber dormancy responds with a parallel shift in seed dormancy. Among the edible diploids, the Stenotomum Group (*stn*) have, in general, dormant tubers while the Phureja Group (*phu*) have non-dormant ones and this difference represents a response to human selection for different agronomic conditions in South America[2]. Fig. 1 shows a comparison of mixed lots of Phureja and Stenotomum seed. It was six months old when tested, so some natural dormancy decline had certainly occurred[1]; nevertheless, the difference is striking. The subject has been further investigated by means of diallel crossings of *stn* and *phu* clones, and the results reveal significant clonal and reciprocal effects. The work is yet incomplete, but one experiment gave a mean seed germination of 74 per cent for *phu* crosses, 39 per cent for *phu*/*stn* hybrids and 27 per cent for *stn* crosses. The corresponding figures for tuber dormancy are 2–3 weeks, 4–8 weeks and 10–12 weeks or more. Once again, therefore, the two dormancies are correlated.

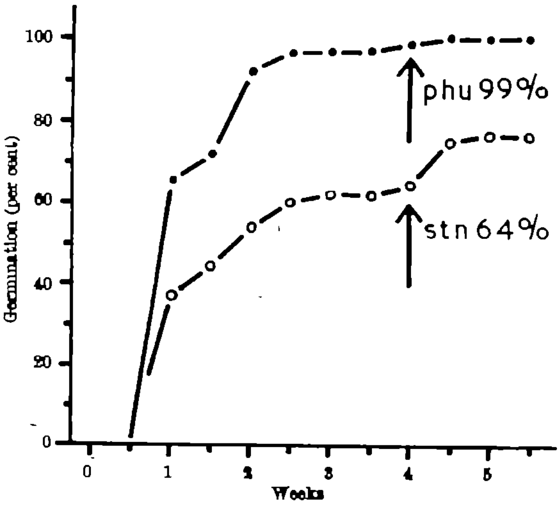

Fig. 1. Seed germination curves of diploid potatoes, Groups Phureja (*phu*) and Stenotomum (*stn*). Five replicate plates of 50 seeds each

The implications of these results are: first, that seed and tuber dormancy are under a common biochemical control; and, secondly, that selection at the seed-level is of potential practical importance. Thus the potato breeder could select for early sprouting tubers by picking the first germinators in dormant seed lots; and the breeder who needs slow sprouting ('good keeping quality') could advance his selection by picking the slowest germinators. Emilsson[3] has shown that European selection generally favours a rest period of 9–11 weeks (roughly equivalent to the same period of dormancy in my test though measured quite differently), so the lower extreme displayed by the South American cultivars is largely eliminated. But other cultivations, in warmer countries, need lower dormancy to permit frequent replanting, just as low dormancy has been selected in the Phureja Group in South America[2]. Examples are Israel[4] and Japan[5]. Seedling selection could be a useful adjunct to potato breeding in these circumstances.

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ENTOMOLOGY

Influence of Relative Humidity on the Uptake of Insecticides from Residual Films

It is now well known that the effectiveness of insecticides absorbed by certain soils is much greater at high than at low humidity. Analytical results[1–3] have shown that this is not due to an increase in concentration of the toxicant in the superficial layers of the soil.

It has been suggested[1,2] that water is adsorbed preferentially on the soil particles, with consequent displacement of the insecticide molecules and increase in their mobility. Under these conditions the toxicant was thought to be more readily available to the insect.

The increased availability of dieldrin in humid conditions has now been confirmed by the direct determination of toxicant pick-up by insects from residual films on glass.

Insects were exposed in glass tubes, the inner surface of which (200 cm^2) had been treated with dieldrin labelled with carbon-14. A gentle flow of air was led through the tubes during exposure to prevent accumulation of insecticide vapour and condensation of insecticide directly on to the insects[4]. Pick-up of toxicant by the insects was thus achieved mainly by contact with the treated surface. The relative humidity was varied by feeding either dried or humidified air into the exposure tubes (relative humidity < 10 or > 90 per cent respectively). The exposure tests were carried out using dieldrin-resistant strains of *Musca domestica* and *Anopheles gambiae*, and a normal susceptible strain of *Aedes aegypti*.

After exposure, extracts of both the insects and the remaining insecticide residue on the glass were examined by a liquid scintillation counting technique. The total recovery of radioactive material, in terms of dosage applied, amounted to 95 per cent.

Table 1 shows that uptake of insecticide was always higher under humid than under dry conditions, and provides a quantitative confirmation of previous findings, indicating a greater availability of toxicant at higher relative humidity.

Table 1. UPTAKE OF ^{14}C-DIELDRIN UNDER DRY AND HUMID CONDITIONS

Species	No. of insects	Residue (µg)	Exposure time (h)	Insecticide recovered from insects (µg)		Ratio of uptake humid/dry
				Dry	Humid	
Musca domestica	10	1	24	0·480	0·769	1·6
Aedes aegypti	20	0·1	17	0·022	0·069	3·1
Anopheles gambiae	30	1	15	0·067	0·606	9·0

It is interesting to note that the influence of humidity on the uptake of dieldrin is least with *Musca domestica*, which walks the most actively during exposure, and most pronounced with *Anopheles gambiae*, which seldom walks at all. *Aedes aegypti* is intermediate in both respects.

The most likely explanation for this inverse relationship is that insects walking over the treated surface pick up the insecticide mechanically from a large area compared with that contacted by the stationary insects. The rate of pick-up by the stationary insects would depend largely on the mobility of the insecticide molecules in the residual film, and under these conditions the greater influence of relative humidity would be expected.

The results obtained with residual films on glass were confirmed in further exposure tests on dieldrin-impregnated filter paper, which showed that the uptake of toxicant by *Anopheles gambiae* was 2·2 times higher under humid than under dry conditions. It seems likely, therefore, that the effect of humidity is not restricted to specific substrates. Furthermore, similar observations have been made with γ-BHC and with DDT on dried soils, and it would appear that we are dealing with a fairly general phenomenon. The influence of relative humidity should, therefore, receive careful consideration in the interpretation of bioassay tests of the kind described here.

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MICROBIOLOGY

Endotoxoid Preparations

The methods which are used for the preparation of non-toxic but highly antigenic toxoids from Gram-positive bacterial toxins could not be applied to the detoxification of toxic *O*-antigens of Gram-negative micro-organisms.

During investigation of the relationship between chemical structure and various biological properties of Gram-negative bacterial *O*-antigens, it was found possible to abolish selectively one or more biological activities by a variety of chemical treatments[1]. Endotoxic *O*-antigens soluble in trichloroacetic acid were isolated from *Serratia marcescens* cultivated on beef infusion broth. The lyophilized preparations were purified by extraction with methanol, a procedure which removes inert impurities but does not influence the biological properties of the endotoxins[2]. The purified materials were then subjected to chemical procedures. Those treatments which diminished the mouse lethal toxicity while retaining the reactivity with homologous rabbit *O*-antiserum were further investigated. Three methods were found to be especially effective.

(1) 100 mg of the foregoing endotoxin refluxed for 60 min with 10 ml. of 2 per cent boron trifluoride in anhydrous methanol resulted in the almost complete dissolution of the material in this solvent. When the reaction mixture was diluted with 4 volumes of water and dialysed against distilled water for 48 h, the material showed less than one-tenth of the original lethal toxicity.

(2) 100 mg toxic *O*-antigen preparation was refluxed for 60 min with 20 ml. of 0·02 M potassium methylate in anhydrous methanol. The insoluble residue was filtered on a glass filter, washed with methanol and dried in a vacuum desiccator. This preparation was practically free from any toxic properties.

(3) Our earlier observation showed that the use of a powerful dissociating agent formed by concentrated pyridine and formic acid in a 2 : 1 ratio dissolves bacterial glycolipids at boiling water bath temperature without altering their mouse lethal toxicity. A 10 per cent aqueous solution of the same reagent under identical conditions in 60 min not only dissolved the *O*-antigen preparation but detoxified it as well. 100 mg endotoxin and 10 ml. of concentrated pyridine and formic acid in a 2 : 1 ratio were first stirred in a boiling water bath. After 15 min 90 ml.

Table 1. TOXICITY AND ANTIGENICITY OF THE PREPARATIONS

Preparation	LD_{50} (µg)	Precipitated (µg) Antibody N/µg preparation*	Rabbit serum titre after immunization †
Parent *O*-antigen	160	5·4	1 : 1,280
Endotoxoid 1	2,300	1·2	1 : 320
Endotoxoid 2	4,000	9·4	1 : 1,280
Endotoxoid 3	2,700	11·0	1 : 1,280

* Measured by the ultramicro-bromsulphthalein test.
† Determined by passive hemagglutination.

Table 2. QUANTITATIVE CHEMICAL ANALYSES OF THE PREPARATIONS

Preparation	Nitrogen	Phosphor	Carbohydrate (anthrone)	Hexosamine	Amino-acids	Long-chain carboxylic acids*	Volatile carboxylic acids†
	Analytical data (per cent)						
Parent *O*-antigen	2·8	1·1	31·1	29·0	11·4	11·4	1·73
Endotoxoid 1	3·0	1·1	23·5	14·2	10·9	14·1	1·62
Endotoxoid 2	4·0	0·8	32·0	30·2	12·5	5·5	1·83
Endotoxoid 3	3·9	1·0	29·6	20·3	11·3	9·8	1·96

* Expressed as palmitic acid. † Expressed as acetic acid.

water was added and stirring was continued at the same bath temperature for 60 more minutes. The *O*-antigen was then precipitated in the cold with 4 volumes of methanol, washed with 80 per cent methanol, dissolved in 50 volumes of water and lyophilized.

Some immunobiological properties of the preparations were investigated. The results are summarized in Table 1. The mouse LD_{50} of the materials was determined following intraperitoneal injections. The results of quantitative precipitin tests revealed that while the treatment with boron trifluoride diminished the reactivity with homologous *O*-antiserum, the second and third detoxification procedures approximately doubled the amount of protein nitrogen precipitable by these modified *O*-antigens. The *O*-antiserum was obtained by injecting rabbits with a heat-killed suspension of *Serratia marcescens* cells. Immunization of rabbits with the detoxified preparations produced in all cases a potent antiserum; however, the first procedure reduced the antigenic character of the endotoxin.

It was found, in co-operation with other groups, that the endotoxoids greatly increased the non-specific resistance of mice and rabbits against experimental infections[1]. Preparation obtained by the second procedure causes marked hyperplasia of granulocytes in the bone marrow 7 days after injection[2]. The same preparation gave 100 per cent protection against total-body 700-r. irradiation in mice when injected 24–72 h before irradiation[4]. Detailed reports of these results will be published separately.

Quantitative analyses revealed certain changes in the composition of the preparation. Table 2 presents some of the results of chemical analyses. Evaluation of these results and clarification of the changes in the structure due to detoxification are under further investigation.

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Accumulation of Free Proline during Yeast Sporogenesis

ALTHOUGH the composition of the pool of free amino-acids in the yeast cell has been much investigated, this has been done using commercially supplied yeast[1,2] or vegetative cells from growth cultures[3,4]. No work has been reported on the amino-acid content of sporulated cells. We have undertaken to compare vegetative, sporulating and sporulated cells in respect of their content of free amino-acids.

Culture methods were essentially those of an earlier investigation[5]. 100-ml. volumes of pre-sporulation growth medium in 200-ml. round-bottomed flasks were inoculated from 2-day cultures on stock culture medium. The cells from a given pre-sporulation flask were collected by centrifugation, washed twice in distilled water, and suspended in 100-ml. of sporulation medium (0·1 M *p*H 5·0 phthalate buffer with addition of 1 per cent sodium acetate), and aerated in a 200-ml. flask at 27° C. For determination of amino-acid content the cells in a given flask were separated from the medium by centrifugation, washed twice in distilled water, and suspended in 80 per cent ethanol for at least 12 h. They were then removed by filtration and dried for weight determination. The filtrate was evaporated to dryness in a hot water bath. The residue was added to 1 ml. of distilled water and centrifuged to remove water-insoluble material. The supernatant, preserved by addition of a small crystal of thymol, was subjected to a quantitative chromatographic procedure[6-8].

Fig. 1 summarizes in part the results obtained with a strain of *Saccharomyces cerevisiae* used in earlier sporulation work[5]. Spores were not present in the vegetative cultures and began to appear after about 24 h in the sporulation environment. Frequency of cells containing spores at successive times were: 24 h, 3 per cent: 48 h, 40 per cent; 72 h, 55 per cent; 96 h, 60 per cent; 120 h, 70 per cent; 144 h, 80 per cent. Twelve amino-acids and glutathione were detected. After six days in sporulation medium the total content of these substances was less than in vegetative cells, largely in consequence of a marked diminution in amount of glutathione, glutamic acid, lysine and glycine, which were initially the most abundant (at least 1,000 μg/g). It is of interest that proline increased from minute amounts in vegetative cells to become the most abundant free amino-acid in 6-day sporulated cells. The increase began, moreover, long before spores could be seen. It also preceded the meiotic nuclear divisions, which commence in cells of this yeast

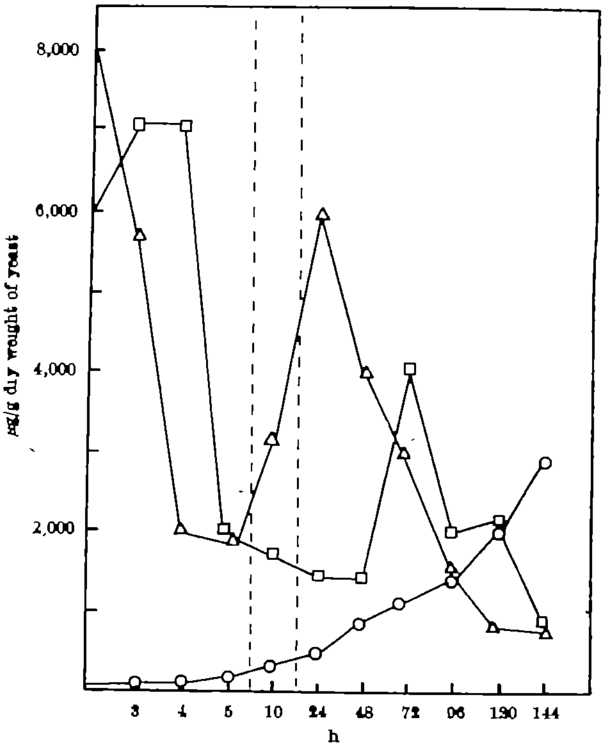

Fig. 1 Changes in content of proline, glutamic acid, and glutathione in cells of *Saccharomyces cerevisiae* during six days of incubation in sporulation medium. △, Glutathione; □, glutamic acid; ○, proline

strain under equivalent cultural conditions about 15 h after transfer to sporulation medium[8].

The procedures were done twice with both vegetative and 6-day sporulated cultures of *Saccharomyces oleaceus* Santa Maria and *S. mellis* (Fabian and Quinet) Lodder and Kreger-van Rij. Proline was not detected in the vegetative cells of either yeast; but it was prominent among the amino-acids in sporulated cells. However, no proline was found in four experiments using vegetative and sporulated cells of *Schizosaccharomyces pombe* Lindner, nor in an experiment with *Torulopsis famata* (Harrison) Lodder.

Changes in amino-acid content after transfer of 6-day sporulated cells of *S. cerevisiae* to fresh growth medium were also followed. In about 4 h the spores were distinctly swollen. A day later most had germinated, and growth was in progress. Whereas most amino-acids gave evidence of increase during two days in growth medium, proline diminished to 200 μg/g.

It would have been preferable to use for these experiments large volumes of sporulation medium, taking successive samples during six days, instead of running each chromatogram with an individual 100-ml. culture. But as sporulation was poor in large sporulation cultures, the less-accurate method had to be used, and this may account in part for the fluctuations in amount of glutamic acid and glutathione indicated by successive determinations. But as each point is based on at least four, and in many cases ten, experiments it is possible that marked changes occur in the content of these substances during active sporogenesis.

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Stimulation of Sporulation in *Penicillium* by Anhydroglucose

In submerged shaken cultivation in a synthetic medium containing potassium nitrate, potassium dihydrogen phosphate, and magnesium sulphate, trace elements and pure (analytical reagent) glucose, *Penicillium griseofulvum* Dierckx (Strain *ARL*. 375) grows vegetatively but does not sporulate even when it has used up all the available nitrogen. Abundant sporulation at a relatively early, exponential, stage of growth was observed when pure glucose was replaced by a crude commercial glucose derived from maize starch by acid hydrolysis[1]. Crude glucose was also found to stimulate submerged sporulation in 5 other species of *Penicillium* out of a further 21 species which were tested. The nature of the sporing factors in crude glucose was therefore investigated.

It was found that the sporing stimulus depended on the simultaneous presence of two substances, of which the first proved to be calcium (Ca^{++}), occurring in amounts sufficient to give a level of approximately 5×10^{-4} M in a medium containing 5 g/l. crude glucose. Calcium was already known to induce submerged sporulation of *Penicillium griseofulvum*, but only at levels above 5×10^{-3} M, and then only at a late, post-exponential stage of growth when the nitrogen in the medium is either exhausted or nearing exhaustion. The amount of calcium present in the crude glucose added to our standard medium does not, however, normally induce sporulation, except in the presence of a second substance occurring in crude glucose. This was found to be a neutral organic heat-stable compound which appeared to be an acid reversion product of glucose. In fact variable amounts of a similarly active substance were found to be produced by autoclaving pure glucose with acid. The substance was separated from crude glucose by adsorption on charcoal and elution with ethanol, and the active fraction was identified as a 1,6-anhydroglucose by preparative paper chromatography. When this fraction, together with 1×10^{-4} M Ca^{++}, was added to a submerged culture of *P. griseofulvum* in a medium containing pure glucose, abundant sporulation was induced early in the exponential phase of growth, although neither Ca^{++} nor the anhydroglucose fraction produced this sporing effect alone.

The nature of the organic sporing factor was confirmed by synthesizing 1,6-anhydro-β-D-glucopyranose by alkali treatment of β-phenylglucoside[2]. This substance gave the characteristic induction of sporulation in *P. griseofulvum* when added, with 1×10^{-4} M Ca^{++}, to a culture growing in medium containing pure glucose. Maximal sporulation occurred when the anhydroglucose concentration was between 2×10^{-4} and 6×10^{-4} M and was not increased at higher concentrations. No sporulation occurred in the absence of added Ca^{++} even with very high concentrations of anhydroglucose in the usual growing medium. When the potassium nitrate was replaced by ammonium succinate, however, very high concentrations of anhydroglucose did induce (late) sporing without added Ca^{++}. This does not seem to be due to traces of Ca^{++} already present, and it indicates, as do certain accompanying morphological changes, that anhydroglucose may have specific biological effects apart from its interaction with Ca^{++}. A mixture of the pyranose and furanose forms of 1,6-anhydro-D-glucose, produced by the pyrolytic distillation of starch[3], proved to have the same sporulation-inducing effect as the pure pyranose form, when tested (with Ca^{++}) in the same conditions. Smaller but significant inductions of sporulation were also given in the presence of Ca^{++} by 1,6-anhydro-β-D-galactopyranose and by 1,6-anhydro-α-D-galactofuranose.

These observations show that the stimulus to sporulation of *P. griseofulvum* (and probably of other species of *Penicillium*) caused by crude glucose is due to the presence of anhydroglucose and traces of Ca^{++}. Further confirmation is provided by the absence of any sporing effect from crude glucose produced by enzyme hydrolysis instead of by acid hydrolysis of starch. Studies of the effects of anhydroglucose and related compounds on metabolism and sporulation of fungi are now in progress. The ability of 1,6-anhydro-β-D-glucopyranose to increase so powerfully the spore-inductive effect of Ca^{++} provides another example of a biological effect elicited by carbohydrate reversion products[4,5]. Interactions between Ca^{++} and hexose phosphates have been observed in the stimulation of perithecia formation in *Chaetomium*[6].

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VIROLOGY

Two-sided Effect of Steroids on Interferon in Tissue Culture

In the course of an investigation of interferon production in a line of rat tumour cells (*DE7R* cells), infected with Sindbis virus Δ′,17α-methyltestosterone ('Dianabol', Ciba), an androgenic steroid known to increase protein synthesis in rats[1] was added to the virus-cell system, in the hope of obtaining higher interferon yields. The effect of 'Cortisol' was evaluated at the same time in order to compare the action of a glucocorticoid known to decrease protein anabolism *in vivo*[2] with that of an androgen. The methods used for preparing the interferon and to test for its activity have been previously described in detail[3].

'Cortisol' and 'Dianabol' were obtained in pure crystalline form. Each steroid was dissolved in ethanol at a high concentration and kept at −10° C for a maximum of 30 days. Final dilutions in culture medium were carried out before each experiment. The final ethanol concentration varied between 2×10^{-4} and 5×10^{-5}. Care was taken to have the same amount of ethanol in the control fluids. Two concentrations of each steroid were tested: 'Dianabol' was used at 2 γ/ml. and 0·2 γ/ml. (7×10^{-6} M and 7×10^{-7} M respectively), and 'Cortisol' was used at 0·25 γ/ml. and 0·025 γ/ml. (7×10^{-7} M and 7×10^{-8} M respectively). These concentrations had previously been shown to have no toxic effect on rat cells *in vitro* by comparing growth curves of rat embryo fibroblasts with and without the steroids.

Cultures of *DE7R* cells were infected with Sindbis virus at a multiplicity of 0·1. The steroids tested were added to the culture after virus adsorption had occurred. Culture fluids were collected 3 days after onset of infection for determination of virus and interferon production. At this time the cytopathological effect (CPE) of the virus was 75–100 per cent; there was no difference in degree of cytopathological effect between control and steroid-treated cultures. The infectious virus was destroyed by overnight dialysis against Sörensen glycine buffer at *p*H 2, followed by two consecutive 4 h dialyses against phosphate-buffered saline at *p*H 7. Preliminary studies with tritiated 'Cortisol' had shown that about 10 per cent of the steroid was not removed after this procedure. The dialysed fluids were examined for interferon activity in secondary cultures of rat embryonic cells, using Sindbis virus as a challenge. The results of such an experiment are shown in Table 1.

Table 1. Influence of 'Dianabol' and 'Cortisol' on Interferon Production by Sindbis Virus in *DE7R* Cells

	Interferon titre	Infectious virus produced
Control medium	1/80	$10^{4.75}$ $TCID_{50}$/ml.
'Dianabol' 0·2 γ/ml.	1/40	$10^{3.75}$
2 γ/ml.	0	$10^{4.75}$
'Cortisol' 0·025 γ/ml.	1/20	$10^{4.50}$
0·25 γ/ml.	1/10	$10^{4.75}$

It can be seen that, at concentrations which alter neither cellular nor virus growth, both 'Cortisol' and Δ′,17α-methyltestosterone strongly decrease final yields of interferon in tissue culture. A similar effect has been previously described by Kilbourne with the diethylaminoacetate hydrochloride of 'Cortisol' and 17α-ethyl-19-nortestosterone ('Nilevar' Searle) in chick embryo membranes[4]. This inhibitory effect on interferon synthesis could possibly be related to the capacity of steroids to release latent ATPase activity of rat tissue mitochondria, as shown by White *et al.*[5]. It is interesting to speculate whether a similar mechanism might explain the partial inhibitory effect of bicarbonate on interferon synthesis in rat cells, earlier described by us[3]. Wright *et al.*[6] have indeed shown that bicarbonate has an inhibitory effect on ATP-levels in rat liver homogenates. Experiments are planned to investigate this possibility.

The influence of 'Dianabol' and 'Cortisol' on the action of interferon was examined using the same steroid concentrations as in the previous experiments. Under certain conditions the presence of the steroid enhanced the viral inhibitory activity of the interferon, the effect being most pronounced with 'Dianabol'. This increase of interferon activity was observed either after overnight incubation of the cells with the steroid and interferon, followed by addition of the challenge virus in the morning, or after overnight incubation with the steroid alone, and removal of the steroid containing culture medium before subsequent incubation with interferon. No increase in interferon activity could be obtained when the cultures were incubated with the interferon first, and the steroid added after removal of the interferon. Similarly, overnight incubation with the steroids and interferon gave no enhancement of interferon activity if the cultures were washed and fresh medium added before addition of the challenge virus. Thus the continuous presence of interferon in the culture fluid seemed to be essential in order to obtain the enhancing effect of the steroids. No enhancement, but no clear cut decrease of activity either, was found when the experiments were performed in the conditions reported by Kilbourne[4], that is, short incubation of 2 h with the steroid, and removal of the steroid before addition of interferon. Table 2 summarizes the result of an experiment in which secondary rat embryo cell cultures were incubated with two-fold interferon dilutions in the presence of 'Dianabol' at 2 γ/ml. Sixteen hours later, 1,000 $TCID_{50}$ of Sindbis virus were added to each culture. The cytopathological effect was recorded 72 h after addition of the challenge virus, and the cultures were then put at −20° C for determination of virus development at each interferon dilution with and without the steroid.

The enhancement of activity of interferon under the conditions described here could be explained by an alteration of the cell membrane permeability, which would facilitate the uptake of new interferon after inactivation of the intra-cellular one.

It is of interest that 'Cortisol', a glucocorticoid, and Δ′,17α-methyltestosterone, a synthetic androgen, had similar actions at cellular levels. The inhibition of interferon synthesis, on one hand, and the enhancement of interferon activity, on the other hand, are suggestive for a double mechanism of steroidal action in the cell. Interferon may reveal itself to be an interesting tool for dissociating steroidal effect on specific protein synthesis and on cell membrane permeability.

We thank Dr. P. Osinski for providing us with tritiated 'Cortisol'. 'Dianabol' was generously supplied by Ciba,

Table 2

Interferon dilution		1/20	1/40	1/80	1/160	1/320	1/640	1/1,280	Control fluid
Interferon only	CPE* Virus produced†	+ ± N.D.	± + N.D.	+ + + $10^{4.50}$	+ + + + + $10^{4.75}$	+ + + + + $10^{4.75}$	+ + + + + + + $10^{4.75}$	+ + + + + + + + $10^{4.50}$	+ + + + + + + $10^{4.50}$
Interferon + 'Dianabol'	CPE Virus produced	0 0 N.D.	0 0 N.D.	± ± $10^{3.50}$	± + $10^{3.75}$	+ + $10^{4.00}$	+ + + + $10^{4.50}$	+ + + + + + $10^{4.50}$	+ + + + + + +/+ + + + $10^{4.50}$

* ±, a few foci of CPE, +, 25 per cent of cell sheet destroyed; + +, 50 per cent of cell sheet destroyed; + + +, 75 per cent of cell sheet destroyed, + + + +, 100 per cent of cell sheet destroyed. Two cultures were followed at each dilution.

† The amount of virus is recorded as $TCID_{50}$/ml. The fluids of the two cultures were pooled before virus titration.

Basle, Switzerland. 'Cortisol' was purchased at Fluka, A. G. Buchs, S. G., Switzerland.

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GENETICS

Interchromosome Effects of Chiasmata and Crossing-over

The significance of chiasmata in relation to crossing-over inferred by Janssens was more fully recognized with the development of the partial chiasmatypy theory by Darlington[1]. Although in recent years mechanisms of crossing-over, very different from that based on the breakage and reunion hypothesis, have been proposed, a direct relationship between chiasmata and genetic recombination of the reciprocal type characteristic of most organisms has continued to be valid. Thus, as indicated by Pritchard[2], chiasma formation would be expected to occur regularly even on the copying-choice model of Lederberg[3] so long as the two copies are synchronized in their switching from one template to another, as they are expected to be, in the case of reciprocal exchanges. Whatever may be the mechanism of crossing-over—and it is widely recognized that the copy choice model as well as the mechanism proposed by Belling, though attractive, present considerable difficulties with regard to their wider applicability[4,5]—further evidence, briefly described here, has become available in recent years which either shows a direct correlation between chiasma formation and chromatid exchange or indicates in other ways a parallelism between chiasmata and crossing-over.

A one-to-one relation between chiasma formation and chromatid exchange has been demonstrated by Brown and Zohary[6] following their observations on heteromorphic bivalents and their anaphase separation in the pollen mother cells of *Lilium*. Further evidence which indicates a correspondence between chiasmata and genetic recombination is provided by studies on interchromosome effects in relation to these cytological and genetical events although the two types of observations have not so far been made on the same organism.

Interchromosome correlations for genetic recombination in *Drosophila* have been reported by a number of authors, more particularly by Schultz and Redfield[7], who have made a detailed analysis of this effect. The main findings, based primarily on the work of these authors, are that in structural homozygotes negative correlation of recombination in given regions of non-homologous chromosomes may occur, giving evidence of interchromosome interference. In structural heterozygotes the presence of inversions increases crossing-over in other chromosomes. Numerical changes in chromosomes such as those in triploids have effects on crossing-over identical with those of inversions and provide similar evidence of interchromosome influences. In the case of plants, compensatory increase in genetic recombination has mostly been demonstrated at intrachromosomal level[8].

Interchromosome effects in relation to chiasma distribution have been described by several authors[9], and more recently evidence has become available which indicates the factors controlling this distribution[10]. A particularly detailed analysis has been carried out on *Delphinium*, a favourable material for investigations of this type. Of the eight chromosome pairs in *Delphinium ajacis* var. Comet. two are considerably larger than the other six, and two groups of chromosomes can, thus, be easily recognized. There is good evidence to show that chiasma frequencies in these two groups of chromosomes are negatively correlated in most plants. The evidence for such a correlation was first obtained from observation on five plants[10]. This analysis has now been extended to another group of 25 plants all of which were found to show similar negative correlations.

The *Delphinium* material also offers an opportunity to examine interchromosome effects for chiasma distribution under conditions of structural heterozygosity corresponding to those in the inversion heterozygotes of *Drosophila*. This can be done if a large population of plants is raised from X-irradiated seeds. Several of these X_1 plants carry randomly induced structural changes in a number of their chromosomes as a result of which their meiotic pairing is affected and chiasma formation suppressed partly or completely. There was evidence that experimental reduction of chiasmata in some of the chromosomes in this way leads to increased chiasma formation in others[11]. This interchromosome effect in structurally heterozygous X_1 plants has now been systematically analysed, using for the purpose of comparison univalent carrying and normal pollen mother cells of the same plant, as shown in Table 1.

The foregoing observations make it clear that following the failure of one or two chromosome pairs to form chiasmata the other chromosome pairs, particularly the large ones, show a distinct tendency for increased chiasma formation. A statistical analysis of these observations confirmed that the mean chiasmata per bivalent in all the plants with one exception is significantly more ($P < 0.05$) in those groups of cells which carry univalents.

A further correspondence between interchromosome effects for genetic recombination and chiasma distribution is indicated by the observation that both are rather variable and not always manifested. In the case of

Table 1

Plant No.	Mean chiasma frequency per bivalent							
	Cells with no univalents		Cells with 2 large univalents		Cells with 2 small univalents		Cells with 4 small univalents	
	*L**	*S*†	*L*	*S*	*L*	*S*	*L*	*S*
R_4P_{21}	1·625	1·208	2·00	1·333	1·800	1·280		–
41b	1·740	1·133			1·900	1·200		
75a	1·780	1·137	1·666	1·111	1·975	1·150		
33b	1·851	1·124			1·968	1·161	2·187	1·281
R_4P_{12}	1·875	1·225	2·142	1·309	1·666	1·333		
R_4P_4	2·109	1·182			2·250	1·200		
R_4P_{21}	2·001	1·185			2·194	1·300		
$R_{11}P_4$	1·500	1·195	2·000	1·261				
$R_{12}P_4$	1·791	1·125	2·000	1·3000	2·071	1·171	2·166	1·250
R_4P_{11}	2·060	1·152			2·218	1·250		

**L*, per large bivalent
†*S*, per small bivalent.

chiasma distribution, some of the factors accounting for this variability have been indicated by the analysis of *Delphinium*[10] and other plants[9]. An interesting observation in this connexion is that not always the presence of univalents elicits a response from the bivalents in the pollen mother cells. Inbreeding was found to be an effective method for inducing univalent formation in the pollen mother cells of *Delphinium*; when such plants were analysed, there was no evidence of compensatory increase in chiasma frequency as in the material from X-ray-treated seeds. Obviously a genotypically determined decline in chiasma frequency operates at the level of the nucleus as a whole, leaving little scope for interchromosome effects. Finally, while no triploids of *Delphinium* were available for observation, it is well known that chiasma frequency in such organisms registers an increase over the corresponding diploid as shown by Darlington[12].

The observations described above thus show that the 'Schultz–Redfield' effect operates for interchromosome distribution of chiasmata also. The observations reported here in a way supplement the earlier analysis of White and Morley[13], who found that pericentric arrangements of chromosomes in grasshoppers lead to compensatory formation of chiasmata at intrachromosome-level. These findings and the observations of Brown and Zohary referred to earlier provide additional support for interpreting chiasmata as being associated with genetic recombination.

We thank Dr. B. P. Pal and Dr. M. S. Swaminathan for their interest in this work.

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PSYCHOLOGY

Information Load and Discriminability

It is clear that subjects take longer to differentiate between two stimuli if they are closely similar than if they are widely separated along some continuum of difference[1,2]. Conditions such as poor contrast or dim illumination which tend to obscure differences between complex stimuli may be expected to have the same effect. Recent models for discrimination and recognition[2,3] entail the assumption of some interaction between a factor of discriminability and the number of items to be differentiated. While data on reading-speed for various English vocabulary-sizes under dim illumination[4] may be taken as support for this, a situation in which subjects make responses to complex stimuli presented one at a time has not been manipulated to demonstrate directly such an interaction. The two experiments summarized here show such interactions between size of stimuli, illumination-level, and the number of items between which discriminations are made.

(1) *Size and information load.* Four packs of forty white playing card blanks were made up. Each card bore a single letter of the alphabet stencilled in indian ink as follows: pack *2S*, the letters *A* or *Z* appeared in equal numbers in a face 3 mm high; pack *2L*, the same, in letters 22 mm high; pack *8S*, the letters *A*, *B*, *C*, *D*, *E*, *F*, *G* and *H* appeared in equal numbers in a face 3 mm high; pack *8L*, the same, in letters 22 mm high.

Twenty subjects with 20/20 uncorrected vision (Royal Navy ratings aged 18–32 years, mean 23·7) were trained to sort these packs into piles according to the letters stencilled on the cards. Packs *2S* and *2L* were sorted into two piles and packs *8S* and *8L* into eight piles. Subjects held the packs face-downward in their non-dominant hands and turned cards over singly, dealing them on to the appropriate piles as quickly as possible. The overall sorting time for each pack was taken with a stopwatch. Subjects sorted the packs in random order, each man sorting each pack fifteen times over three experimental sessions. Results presented in Table 1 are derived from the mean sorting times for the last five trials on each pack.

Table 1. Mean Sorting Times (sec) for Two-Choice and Eight-Choice Packs with Large or Small Type-Face

Two-choice large type	38·7
Two-choice small type	39·5
Eight-choice large type	56·0
Eight-choice small type	60·6

An analysis of variance shows the interaction between type size and information load to be significant ($P < 0{\cdot}001$). It is thus apparent that a reduction in size of the symbols used affects performance more in the eight-choice than in the two-choice situation.

(2) *Level of illumination and information load.* The *2S* and *8S* packs described here were sorted by a second group of subjects also having 20/20 uncorrected vision (undergraduates aged 19–23, mean 21·3) under two conditions of illumination. Cards were sorted either in normal daylight illumination, or in dim illumination. In the latter condition subjects sorted cards after forty minutes' adaptation in a lightproof cubical illuminated by a dimmed tungsten lamp allowing 0·1 ft.-lamberts to be reflected from the surface of each card. This measurement was carried out with an *S.E.I.* photometer in the absence of a subject.

Ten trials were given on each pack under each of the conditions described, nine subjects beginning in the dim, and nine in the normal condition. Conditions were tested in separate experimental sessions. The results described in Table 2 are again based on the means for the last five trials on each pack.

Table 2. Mean Sorting-Times (sec) for Two-Choice and Eight-Choice Packs in Normal and Dim Illumination

Two-choice in normal illumination	37·4
Two-choice in dim illumination	40·5
Eight-choice in normal illumination	58·9
Eight-choice in dim illumination	65·3

An analysis of variance performed on the data shows that both two-choice and eight-choice packs take longer to sort in dim than in normal illumination ($P < 0{\cdot}001$). The interaction between illumination and information load is significant ($P < 0{\cdot}01$).

It must be concluded that dim illumination reduces efficiency more in the eight-choice than in the two-choice situation.

The extremes of choice between theoretical models for decision processes of this type are taciturnity on problems of discriminability[5] and an attribution of the entire effects of information load to problems of discrimination as the number of items to be distinguished increases[2]. The present results may further a more decent eclecticism. It is at least satisfactory that their practical implications are important and obvious.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 18

Society of Chemical Industry, Pesticide Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. K. S. Hocking: "The Work of the Tropical Pesticides Research Institute, Arusha".

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should an Undergraduate Mechanical Engineering Course Include Production Engineering ?"

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. R. W. Beattie and Mr. N. Fairweather: "Electroplating and Inspection of Electrical Contacts".

Tuesday, February 19

British Computer Society (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 4 p.m.—Mr. P. G. Lowe: "The Matrix Exponential Function with an Application to Structural Engineering".

University of London (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. C. W. Jones: "Applied Mathematics in the Context of Engineering" (Inaugural Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. Oswald: "Physiology of Sleep Accompanying Dreaming".* (Last of twelve lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Some Problems in Metal Box Production".

Royal Institute of Chemistry, London Section (at Norwood Technical College, Knight's Hill, London, S.E.27), at 7 p.m.—Dr. S. A. Miller: "The Liquefaction of Gases".

Wednesday, February 20

Royal Statistical Society (at the Piccadilly Hotel, London, W.1), from 10 a.m. to 4.30 p.m.—Productivity Conference. Morning Session, Opening address by Sir Robert Shone. Papers by Mr. W. B. Reddaway, Mr. C. T. Saunders, Prof. J. Johnston and Prof. H. D. Dickinson. Afternoon Session, Introduction by Dr. J. O. Irwin. Papers by Prof. G. A. Barnard, Mr. P. A. Dainley, Mr. L. H. C. Tippett and Mr. G. W. Sears.

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Prof. L. Doniach: "Pathology of Thyroid Carcinoma".*

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. A. J. Dyer: "Measurements of Evaporation and Heat Transfer in the Lower Atmosphere by an Automatic Eddy-Correlation Technique"; Mr. J. L. Monteith and Mr. G. Szeicz: "The Radiative Balance of Bare Soil and Vegetation"; and "Radiative Temperature in the Heat Balance of Natural Surfaces".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. H. Page: "U.H.F. Television Broadcasting: Planning the Transmitting Equipment".

Royal Microscopical Society (at Tavistock House, South, Tavistock Square, London, W.C.1), at 5.30 p.m.—Dr. F. J. Jackson: "Problems of Extra-Terrestrial Microbiology".

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. S. Young: "Nuclear Power".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. H. Chaddock: "The Development of High-Speed Automatic Loading Mechanisms for Heavy Anti-Aircraft Artillery".

Oil and Colour Chemists' Association (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mrs. S. M. Rybicka: "The Versatility of Chromatographic Methods in Paint Research".

Thursday, February 21

Parliamentary and Scientific Committee (in the River Room, Savoy Hotel, London, W.C.2), at 11.30 a.m.—Annual General Meeting.

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. R. P. Miller, Miss Elizabeth Napier, Mr. R. A. Wells, Dr. A. Audsley and Mr. G. R. Daborn: "Natural Leaching of Uranium Ores"; Dr. D. A. O. Morgan: "Some Economic Aspects of Mineral Exploration".

Linnean Society of London (at Burlington House, Piccadilly, London W.1), at 5 p.m.—Scientific Papers.

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. D. R. Hughes: "Geometry and Division Rings".

Institution of Electrical Engineers, Science and General Division P.G.S2 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Ferromagnetic Films" opened by Mr. C. E. Fuller.

University of London (at the Institute of Archæology, 31–34 Gordon Square, London, W.C.1), at 5.30 p.m.—Prof. J. M. C. Toynbee: "Early Christian Art and Græco-Roman Symbols".*

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Performance Testing of Modern Large Steam Turbo-Alternators".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Meeting on "Chemicals for the Control of Vegetation on Roads and Airfields".

Royal Institute of Chemistry, London Section (joint meeting with the Borough Polytechnic Chemical Society, at the Borough Polytechnic, Borough Road, London, S.E.1), at 7 p.m.—Prof. R. S. Nyholm, F.R.S.: "Higher Co-ordination Numbers of the Transition Metals".

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. D. C. Gajdusek: "Kuru".

Thursday, February 21—Friday, February 22

Royal Society (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m. daily—Discussion Meeting on "Recent European Contributions to the Development of the Physics of Elementary Particles", organized by Prof. C. F. Powell, F.R.S.

Friday, February 22

Royal Astronomical Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "The Upper Mantle". Chairman: Sir Edward Bullard, F.R.S.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. J. Harrison. "Bones, Teeth and Identification".

Saturday, February 23

Association of Clinical Biochemists, Southern Region (at Lewisham Hospital, London, S.E.13), at 2.45 p.m.—Scientific Papers and Demonstrations.

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Anthony Christie: "Looking at Oriental Art".*

Monday, February 25

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Stress and Psychosomatic Disorders".*

Royal Institution (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. F. N. L. Poynter: "On Collecting Books for a Library of the History of Medicine".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. George Pendle: "Latin American Culture". (Second of three Cantor Lectures on "Latin America—World of Tomorrow".)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.15 p.m.—Prof. D. B. DeLoach (California): "Agricultural Technology and the Management of Surpluses".* (Fifth of eight lectures on "The United States of America".)

Institut Français du Royaume-Uni (at Queensberry Place, London, S.W.7), at 8.15 p.m.—French Scientific Films.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. W. W. Herbert: "In the Footsteps of Roald Amundsen".

Monday, February 25—Tuesday, February 26

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2)—Symposium on "Transient, Fluctuating and Distorting Loads and their Effect on Power Systems and Communications".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer in Geology—The Registrar, The University, Leicester (February 23).

Assistant Lecturer in Zoology—The Registrar, The University, Leicester (February 23).

Assistant Lecturer (with special interests in palæontology and stratigraphy) in Geology—The Registrar, The University, Sheffield (February 23).

Chair of Theoretical Physics—The Secretary, The University, Exeter (February 23).

Lecturer or Assistant Lecturer in Pure Mathematics—The Registrar, University College, Singleton Park, Swansea (February 23).

Hospital Biochemist (Senior Grade), to work in the Department of Clinical Pathology—The Superintendent, Guy's Hospital, London Bridge, S.E.1 (February 25).

Lecturer in Biochemistry in the Department of Physiology and Pharmacology—Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (February 25).

Lecturer or Assistant Lecturer in Philosophy; and an Assistant Lecturer in Moral and Political Philosophy—The Registrar, The University, Keele, Staffs (February 25).

Postgraduate Research Student in Land Use Studies in the Department of Geography—The Registrar, University College of Swansea, Singleton Park, Swansea (February 28).

Research Assistant in Plant Biochemistry in the Department of Botany, to work on enzymes in isolated chloroplasts under the direction of Dr. J. Friend—The Registrar, The University, Hull (February 28).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Chemical Oceanography, Engineering or Physics—The Secretary and Registrar, The University, Southampton (February 28).

Research Assistant (with good qualifications in mathematics, and preferably a knowledge of numerical analysis and computing methods) to Prof. A. T. Price, to work on the analysis of magnetic observations made during the I.G.Y.—The Secretary, The University, Northcote House, Exeter, Devon (February 28).

Research Fellow (graduate in animal husbandry, science, veterinary science or agricultural science) in the Department of Physiology, University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 28).

COLLEGE LECTURER IN PURE MATHEMATICS—The Master, Emmanuel College, Cambridge (March 1).

READER or SENIOR LECTURER IN CLINICAL BACTERIOLOGY, for duties mainly concerned with the clinical bacteriological services of the Manchester Royal Infirmary—The Registrar, The University, Manchester 13 (March 1).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Sheffield (March 2).

ASSISTANT LECTURER (with a good honours degree and some special knowledge of soils or practical experience in soil survey) IN GEOGRAPHY; and an ASSISTANT LECTURER or DEMONSTRATOR (with a good honours degree and some research experience in one of the branches of the subject) IN GEOGRAPHY—The Registrar, The University, Hull (March 4).

SENIOR LECTURER/LECTURER (graduate in anatomy or physiology or psychology, and some research and/or teaching experience relevant to ergonomics) IN ERGONOMICS—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 3/G (March 7).

LECTURER (with an interest in experimental taxonomy and cytology) IN THE DEPARTMENT OF BOTANY—The Secretary, University College, Gower Street, London, W.C.1 (March 8).

UNIVERSITY DEMONSTRATOR IN BIOCHEMISTRY—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford (March 8).

DEMONSTRATORS (with good honours degrees and approximately one year's research experience) IN CHEMISTRY—The Registrar, University College of Wales, Aberystwyth (March 9).

LECTURER IN ZOOLOGY—The Secretary, The University, Aberdeen (March 9).

PURDIE CHAIR OF CHEMISTRY (preferably organic chemist)—The Secretary, The University, College Gate, St. Andrews (March 9).

CHAIR OF METALLURGY in the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, March 15).

CHAIR OF BIOCHEMISTRY—The Registrar, The University, Sheffield (March 16).

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY, to carry out researches into the behaviour of olefinic acids in strong acid media in collaboration with Dr. M. F. Ansell—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 18).

LIBRARIAN—The Secretary of the Executive Council for the University of Lancaster, P.O. Box 78, County Hall, Preston, Lancashire (March 22).

LECTURER (with special interests and qualifications in mineralogy and petrology) IN GEOLOGY in the Faculty of Science—The Registrar, The University, Manchester 13 (March 23).

SENIOR LECTURER or LECTURER IN ZOOLOGY at the Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 25).

TECHNICAL OFFICER (with a degree or comparable qualifications in physics or electrical engineering, and preferably experience in the field of high frequency techniques) IN THE ELECTRONICS SECTION OF THE DEPARTMENT OF PHYSICS—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge (March 25).

CHAIR OF AGRICULTURE in the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, March 28).

READER IN PHYSICAL ANTHROPOLOGY—The Registrar, University Registry, Oxford (March 28).

PRINCIPAL LECTURER and a SENIOR LECTURER (with good academic qualifications and research experience, and preferably teaching experience) IN THE DEPARTMENT OF PURE AND APPLIED MATHEMATICS—The Secretary, Royal College of Advanced Technology, Salford 5, quoting Ref. No. P/11 (March 29).

CHAIR OF PATHOLOGY in the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, March 31).

LECTURER IN MICROBIOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W.1 (Australia and London, March 31).

ASSISTANT CONSERVATOR OF FORESTS (under 40, with a degree in forestry) in the Ministry of Tourism, Forests and Wild Life, Kenya, for work in connexion with the administration and technical direction of a Forest Division—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. T/R.C.324/80/02.

ASSISTANT (with a degree in physics, with some medical physics or other appropriate experience) IN THE PHYSICS DEPARTMENT—The Deputy Secretary, St. George's Hospital, Hyde Park Corner, London, S.W.1.

LECTURER or SENIOR LECTURER (with good academic qualifications and preferably research experience to doctorate level) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—Dr. J. H. Calderwood, Royal College of Advanced Technology, Salford, Lancs

MASTER TO TEACH PHYSICS to "A" level—The Headmaster, Berkhamsted School, Herts.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Science, State, Society. By Prof. B. H. Flowers. (Old Dy'vorians Annual Lecture delivered Thursday, 18th October, 1962.) Pp. 16. (Swansea: Dynevor School, 1962). 1s. 6d. [2712

Privy Council. Medical Research Council Monitoring Report Series, No. 5: Assay of Strontium-90 in Human Bone in the United Kingdom—Results for 1961, Part II. Pp. ii+17. (London: H.M. Stationery Office, 1962.) 2s. net. [2712

Ministry of Housing and Local Government; and Scottish Office. The Surface Water Year-Book of Great Britain, 1960–61: Hydrometric Statistics for British Rivers, together with related Rainfalls, for the year ended 30th September, 1961. Pp. xiv+121. (London: H.M. Stationery Office, 1962.) 25s. net. [2712

British Drug Houses, Ltd., Poole. The Colorimetric Determination of Oxidation-Reduction Balance. Sixth edition. Pp. 20. Flash Points. Pp. 40. 3s. 6d. (Poole: British Drug Houses, Ltd., 1962.) [2812

Controlled Heat and Air, Ltd. Bulletin No. 1A: CHAL Tinprinting Ovens. Pp. 12. (Smethwick, Birmingham: Controlled Heat and Air, Ltd., 1962.) [2812

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 723, Vol. 245 (20 December 1962): The Evolution of the Labyrinthodonts. By D. M. S. Watson. Pp. 219–265. 16s. No. 724, Vol. 245 (20 December 1962): The Geology of the Western Approaches of the English Channel. 1: Chalky Rocks from the Upper Reaches of the Continental Slope. By D. Curry, E. Martini, A. J. Smith and W. F. Whittard. Pp. 267–290. 7s. 6d. No. 725, Vol. 245 (20 December 1962): The Fine Structure of Crustacean Proprioceptors. 1: The Chordotonal Organs in the Legs of the Shore Crab, *Carcinus Maenas*. By Mary Whitear. Pp. 291–325+plates 56–62. 24s. (London: The Royal Society, 1962.) [2812

Other Countries

The Walter and Eliza Hall Institute of Medical Research. The Director's Forty-third Annual Report, 1961–62. Pp. 52. (Melbourne: The Walter and Eliza Hall Institute of Medical Research, 1962.) [2812

Western Australia: Fisheries Department. Fauna Bulletin No. 2: Bernier and Dorre Islands, Shark Bay, Western Australia—The Results of an Expedition, July, 1959. By W. D. L. Ride, G. F. Mees, A.M. Douglas, R. D. Royce and C. H. Tyndale-Biscoe. Edited by A. J. Fraser. Pp. 131 (34 plates). (Perth: Government Printer, 1962.) [2812

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 99: l'Acariose à *Hemitarsonemus*, Affection Foliaire du Cotonnier. Par G. Schmitz. Pp. 50. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1962.) 50 francs. [2812

Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wool Research Laboratories for the year ending June 30, 1962. Pp. i+41. (Melbourne: Wool Research Laboratories, C.S.I.R.O., 1962.) [2812

New Zealand. Department of Scientific and Industrial Research. Information Series No. 34: Soil Bureau—Handbook. Pp. 51. Information Series No. 35: Plant Chemistry Division—Triennial Report, 1958–61. Pp. 56+15 plates. (Wellington: Government Printer, 1962.) [2812

Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Pastures for the year ending June 30, 1962. Pp. i+35. (Brisbane: Division of Tropical Pastures, C.S.I.R.O., 1962.) [2812

Records of the Dominion Museum, Wellington, New Zealand. Vol. 4, No. 6 (30 August, 1962): Additional Archibenthal Mollusca from New Zealand. By R. K. Dell. Pp. 67–76. Vol. 4, No. 7 (14 September, 1962): Wear to the Incisors of the New Zealand Short Tailed Bat. By P. D. Dwyer. Pp. 77–78. Vol. 4, No. 8 (14 September, 1962): *Melanitis leda* (Linn.) and other Migrant Butterflies in New Zealand during 1962. By Beverley A. Holloway. Pp. 79–82. Vol. 4, No. 9 (21 September, 1962): The Taxonomic Status of the Beaked-Whale, *Mesoplodon hectori* (Gray)—Cetaceae. By Charles McCann. Pp. 83–94 (5 plates). Vol. 4, No. 10 (21 September, 1962): The Taxonomic Status of the Beaked Whale, *Mesoplodon pacificus* Longman Cetacea. By Charles McCann. Pp. 96–100. Vol. 4, No. 11 (26 October, 1962): Hepatics from the Subantarctic Islands of New Zealand, including "Cape Expedition" Collections from the Auckland and Campbell Islands. By E. A. Hodgson. Pp. 101–132. Vol. 4, No. 12 (12 October, 1962): Mosses from the Auckland Islands and Campbell Island. By G. O. K. Sainsbury and K. W. Allison. Pp. 133–142. Vol. 4, No. 13 (26 October, 1962): New Zealand Occurrences of the Sydney Mud Cockle, *Anadara trapezia* (Deshayes). By Margaret Crozier. Pp. 143–148. (Wellington: Dominion Museum, 1962.) [2812

Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Fisheries and Oceanography for the year ending June 30, 1962. Pp. ii+82. (Cronulla: Division of Fisheries and Oceanography, C.S.I.R.O., 1962.) [2812

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 18, No. 8 (December 1962): Population Growth and Immigration Policy—2: Asia. Pp. 161–180. (Washington, D.C.: Population Reference Bureau, Inc., 1962.) 50 cents. [2812

The Australian Atomic Energy Commission. Uranium in Australia: a Collection of Articles on the History and Development of the Uranium Mining Industry in Australia. Pp. 64. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1962.) [2812

National Research Council and Medical Research Council, Ottawa. Report on University Support, 1961–62. Pp. vii+138. (N.R.C. No. 6862). (Ottawa: National Research Council and Medical Research Council, 1962.) 50 cents. [2812

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SCIENTIFIC RESEARCH AND THE INDEPENDENT INSTITUTION

SIR HENRY TIZARD often viewed with some concern any tendency to look to Government institutions to undertake the risks which properly belong to private enterprise, and was wont to quote with approval a remark of Lord Rutherford: "We haven't any money; we've got to think". Dr. Caryl Haskins's latest presidential address to the Carnegie Institution of Washington* brings to mind that remark if in the context of bounty rather than of scarcity. Looking back over the sixty years in which the Institution has now been operating—the first of its kind in America to be devoted entirely to the ideal of research scholarship over wide fronts of science in its broadest, most unfettered, most completely uncommitted aspect—Dr. Haskins looks afresh at the place of the independent research institution in the world of to-day. This he does in the light of present experience of different forms of organization, of the present scale of research and the disposition of the resources made available for research, and of some of the results achieved.

His survey is wide, his analysis searching and cogent. He looks at those problems of Government support of science which arise inevitably out of the dependence of some 65 per cent of research in the United States on Federal funds. This is natural enough when the National Science Foundation alone in 1963 has a budget of 165 million dollars for scientific research, when the Department of Defence is spending about 7,000 million dollars on research and development, the National Aeronautics and Space Administration another 2,500 millions, and the Atomic Energy Commission some 1,500 millions—compared with a total Federal research programme of 100 million dollars twenty years ago. Without following Prof. J. S. Dupré and S. A. Lakoff into the examination, in their recent book, of *Science and the Nation: Policy and Politics*, he touches on the fundamental issues involved in deciding on forms of organization and administration. In a report which in cogency, insight and distinction fully maintains the high standard he has already established for himself, Dr. Haskins provides a brilliant and stimulating vindication of the place in this technological society of the gifted, unorthodox and unfettered individual.

Dr. Haskins begins by noting that within four years of its foundation the Institution had been organized into some fourteen departments, representing as many subjects. However, during the next five years of experiment and trial it was decided to concentrate the resources of the Institution primarily on its own research, making it an operating, rather than a grant-making, institution. By 1911 its departments were firmly established and reduced to ten, and the Institution had assumed essentially the form of organization that characterizes it to-day. The number of departments has indeed been further reduced to five, and whole fields included in 1911 have been discarded as the resources of the nation in these areas have strengthened, while others on the frontiers of research, like embryology, molecular and cellular biology, the study of the mechanism of photosynthesis, have been included. Through the years the major philosophies of the Institution have stood constant, and with them the decision made at the outset that flexibility and effectiveness in the kind of research to which the Institution is dedicated can best be achieved through a series of rather small unit laboratories. Each of these units, being mobile and relatively independent, is able to seize the initiative in new and appropriate fields as they appear, yet as a whole remain sufficiently connected so that they may be of mutual assistance as the needs arise.

That decision was remarkable at the time for its uniqueness and vision, but naturally over the decades many other experiments in organization have been tried. In the meantime, it is undoubtedly true that some of the most modern thought and experiment in this field, both in the United States and elsewhere, has returned to this pattern as one of the most effective in exploring the dynamic frontiers of scientific knowledge. Dr. Haskins's report owes some of its value and inspiration as a contribution to thought on the organization and administration of research from the fact that he is prepared to re-examine impartially the question of the place and value of the small and mobile group. May it not be true to-day that major advances in new knowledge, the setting of radically new scientific directions, can be achieved in the environment of great and highly organized research teams, that in practice such environments are essential, or at least the most favourable to the process? Is it possible that we are witnessing a profound revolution in the very character of research itself? Is it possible that small and mobile groups, which in the past characteristically had an influence on scientific progress out of all proportion to their numbers or their social cost, can no longer provide such significant approaches to the unknown?

To ask such questions is to challenge the underlying philosophy—developed in the days of scarcity in science—that envisages the environment of brilliant original unfettered individual research as the *milieu* in which the great new directions of scientific thought were born and nourished. Now we live and work in a world committed to an unparalleled rate of growth in the material resources for research, in a world in which perhaps 80 per cent of all the scientists who have ever lived are our contemporaries. Is it indeed now true that research is, after all, essentially a matter of adequate resources and organization, that quantity rather than quality comes first?

Rightly and wisely, Dr. Haskins goes back to the philosophy that inaugurated the Carnegie Institution and has remained unchanged through the years: the philosophy that all its resources and all its deepest purposes are centred in the creative individual whatever his field, that in the truest sense he is the uncommitted investigator, suitably endowed and protected, whose time, quite literally, is bought by the Institution, and then returned as unconstrained endowment. Equally deep-seated and important is the philosophy that this freedom from fixed commitment applies to fields of endeavour as well as to men. High mobility within specific fields, the unfettered crossing of fields, and the fashioning of unconventionally wide-ranging programmes, are subject only to the limitations imposed by Nature and by the judgment of gifted and discriminating investigators. Making this mobility and this flexibility possible has been, and is, a principal objective of the Institution.

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* Report of the President of the Carnegie Institution of Washington, 1961–1962. Pp. 112+xi. (Washington, D.C.: Carnegie Institution of Washington 1962.)

The practical success that has attended the pursuit of programmes inspired by this philosophy does not inhibit Dr. Haskins from re-examining its basis, or from recognizing that new and important problems of organization now confront the Institution in the context of the changed situation in national scientific and technical development. It is because of this and because of the recognition also of the temptations to-day to imagine and to expect: (1) that cost and magnitude themselves may provide an important index of scientific significance; (2) that the speed and effectiveness with which new scientific frontiers are breached may be a simple function of numbers of men and rates of expenditure; (3) that the attainment of new scientific vision in an area of basic research may be accelerated in direct proportion to the size of teams and the amounts of money committed, that Dr. Haskins's survey is so stimulating and suggestive in the present discussion of the organization and administration of research and development in Britain.

It is not only in the United States that this pragmatic philosophy finds support, and the distinction between the contribution of the gifted individual and of the massive research team with its powerful new tools is made less clear than is desirable. It is as easy as it is dangerous to assume that great teams and high costs are prerequisites for setting new directions in scientific thought, rather than the means by which intelligent planning and concerted effort achieve the possibilities disclosed by the search of gifted individuals in new fields, seeking knowledge for its own sake and yielding unexpected and radically new ideas about Nature. The confusion is evident, for example, in some of the present criticism of support of research into kidney graft surgery in Britain; wise decisions as to the magnitude or administration of grants for research cannot be taken when such a philosophy persists.

The Carnegie Institution of Washington is deeply committed to the belief that the distinguished, unfettered individual can bring unique gifts to his society, and to belief also in the uniqueness and the importance of the influence which a community of independent scholars can exercise on scientific progress. Dr. Haskins examines three outstanding advances marking the technical and scientific progress of the past three years, all three of which he finds justify the belief that the basic contributions of small and mobile groups retain their old significance. Moreover, he points out that recent great advances in our knowledge of the universe and of our own more immediate environment—the original ideas of scientific stature achieved in the past few years, which promise to open truly novel avenues of thought—have not been necessarily, or even primarily, associated with the massive programmes of great teams. His first example is the immense complex of research and development dedicated to the placing of man in outer space; the second, an achievement in astronomy of the past year, is the identification of what has proved to be by far the most remote celestial object ever discovered in the heavens and the measurement of the red shift of its spectrum; and the third, the establishment beyond reasonable doubt that the information governing the inheritance of all the qualities of living things is structurally graven on the chromosomes within their germ cells in the form of a genuine code.

All these advances have won important and striking new knowledge. All have involved a variety of scientific disciplines apparently far removed from the main concern. All three, and especially the third, have resulted in profoundly new directions of thought. All three, Dr. Haskins observes, are characteristic of their age and could not have been achieved with the tools of any other era. Equally, he maintains, they demonstrate that the magnitude and organization of a research effort may be the least significant of parameters in any fundamental and enduring sense. The essential qualities and requirements of inquiry at the very frontiers of man's knowledge of his universe do not now, and in all probability will not in the foreseeable future, differ significantly from those of our classical scientific past.

Scientific inquiry will then continue to bear the unmistakable stamp of the gifted and untrammelled individual, whatever the scale of resources, in knowledge, in tools, in human and material support, which he may require. We may regard the large and the small research enterprises in our society as essentially symbiotic, each fulfilling its specific role. But on the small and mobile group rests, above all, the heavy responsibility of maintaining the vision of the creating individual—a responsibility that may well become wider and more challenging. Dr. Haskins, discussing the implications of the needs for the integration of knowledge and the demands made by the processes of research, points out that there can be no more urgent imperative than the creation of opportunity for individuals to address themselves once again to wholly new fields of inquiry. This, he feels, lies peculiarly in the domain of the small and mobile and basically highly uncommitted research groups.

Dr. Haskins concludes his survey with a notable tribute to the value and intrinsic importance of the individual in the society of to-day. The future of that society depends above all on the continued integrity and freedom of small and mobile enclaves of thoughtful and imaginative men and women. Their persistence alone can effectively preserve the conditions which have nurtured the rich sources of greatness, for it is the gifted unorthodox individual who has always brought, and will continue to bring, all the basic resources by which we live.

Dr. Haskins's address is fundamentally a summons to guard and honour and implement the position of the gifted individual with every resource that we can muster, because he is irreplaceable. However, beyond this his address is full of implications for all who are considering the scale and organization of research in Britain, the relations between research institutions and Government. Lord Hailsham has repeatedly shown that he is well aware of the importance of these fundamental aspects of effective research which Dr. Haskins emphasizes, and that he appreciates that if money spent on research is not to be wasted it must go to the support of first-class men with first-class ideas. The problem lies, of course, in the assessment of the men and of the ideas. More than this, it is important to ensure that the men supported are given the freedom and the conditions in which they can give of their creative best, and that so far as possible no first-class men with first-class ideas are hampered by lack of support.

Taking into account all that is involved in respect of organization as well as of manpower and resources, including that of securing effective use of the large and costly equipment often needed, all this would seem to be a full-time occupation for the Minister for Science. Quite apart from recent suggestions that his Office requires a considerably stronger staff of high competence, the appointment of Lord Hailsham to fresh responsibilities for unemployment in the North East can scarcely fail to raise misgivings as to whether these responsibilities as Minister for Science will receive adequate attention. It is not, indeed, as if the present administrative arrangements for the control and stimulation of research could be regarded as static or ideal. The Zuckerman Report alone has shown how much room there is for further critical inquiry and constructive thinking. Nor should it be thought that this is solely the responsibility of the Minister for Science and his Advisory Council for Scientific Policy. There is far too much shallow, if not politically biased, criticism of the scale of research expenditure as well as of its organization. The Minister for Science is entitled to expect from scientists and technologists generally a level of informed and constructive criticism which should materially facilitate the development of a pattern of organization appropriate to the nation's needs and resources for research. He can also expect their co-operation in the vitally important task of educating public opinion as to the necessary scale of that effort and its implications, whether in economies elsewhere or in the training or deployment of manpower.

TECHNICAL CO-OPERATION AND SERVICES OVERSEAS

THE Queen's speech at the opening of the present session of Parliament referred specifically to the Government's citation to encourage men and women from Britain to offer their services for work in developing countries overseas, whether in the service of the other Governments concerned, as specialist advisers, or under schemes of voluntary recruitment. This matter was discussed in the House of Commons on December 12, when a motion urging the Government to give every assistance to ensure the expansion and development of voluntary service in the under-developed countries was agreed without a division, although the attendance at the debate was disappointingly small. The public interest in this field, however, is far from being represented by this debate. It was no less manifest in the debate on the Commonwealth Scholarships (Amendment) Bill which received its second reading in the House of Commons on November 19, and it is equally apparent in the recently published second annual report of the Commonwealth Scholarship and Fellowship Plan for the period ending March 31, 1962[*].

This latter report shows that the Plan is fast becoming an established feature in the educational life of the Commonwealth, and during the period 729 scholars and Fellows held awards in thirteen different countries. During the year, Cyprus accepted an invitation to participate and is instituting three scholarships tenable at the Cyprus Forestry College. Sierra Leone has also instituted awards, and others were taken up for the first time in India, Pakistan, Ceylon, Nigeria, the Federation of Rhodesia and Nyasaland, and Malta. The target of a thousand current awards was expected to be achieved during 1962. The most important difficulty which arose in operating the Plan was that experienced by the nominating agencies in submitting nominations at different times of the year. This was fully discussed by a Working Party of the second Commonwealth Education Conference at New Delhi in January 1962, in the light of differences in the academic year in the northern and southern hemispheres; it was recommended that two common closing dates should be established, on December 31 and July 31 each year. It was also agreed that while each awarding country would continue to issue its own prospectus or information sheet describing its awards, applications should be invited on a form printed in each nominating country. The Working Party's report is annexed to the report of the Second Commonwealth Education Conference, published last March (see p. 752 of this issue of *Nature*).

A major problem confronting the Commonwealth Education Liaison Committee was the extension of the tenure of scholarships into a third year. The 1,000 awards current at any one time contemplated in the original scheme were mainly intended for two-years postgraduate study, and it was assumed accordingly that about half the awards would be available each year as new awards. In Britain and Canada it has been found that most successful candidates wish to be admitted to courses of research leading ultimately to the Ph.D. degree, for which more than two years study is often required. Some nominating countries are already concerned at the prospect of a decline in the number of new awards made annually. It was to deal with this difficulty that the Commonwealth Scholarships (Amendment) Bill was introduced.

In moving its second reading on November 19, the Under-Secretary of State for Commonwealth Relations, Mr. J. Tilney, explained that the Bill was intended to remove the restriction limiting the number of Commonwealth scholars in the United Kingdom at any one time to 500 under Section 2 (*a*) of the 1959 Act. More than half the Scholars wished to read for their Ph.D., and so long as the total number of awards was limited to 500 (although a substantial number of scholars did not require a full third year) these extensions block a scholarship unit for the full academic year. In consequence, the quota dropped to 450 between April and October, and the average current for the full year was less than the 500 visualized when the 1959 Act was passed and which would be possible under the new Bill. Between 225 and 230 new awards would then be available at present costs instead of 200. The overall limit of £6 million over the five years imposed by the Commonwealth Teachers Act, 1960, for expenditure under both Acts would still apply. Clause 1 would bring the Act into line with that Act by covering the Channel Islands and the Isle of Man.

Reviewing the working of the Plan in Britain, where the Commonwealth Scholarship Commission continued to act as the scholarship agency, it is noted in the report that during the year 394 Commonwealth scholars held awards at universities and colleges in Britain, of whom 229 took up their awards in October 1961 or afterwards. Of those who took up awards in October 1960, 89 were granted extensions into a third year, beginning in October 1962. In the 1961 competition, 232 scholarships were accepted, but these extensions into a third year meant that new awards in 1962 would be fewer than in 1961. Commonwealth visiting fellowships, tenable for up to one year, have also been instituted, and three were held in Britain during the period; it is hoped that up to five fellowships can be awarded.

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[*] Second Annual Report of the Commonwealth Scholarship Plan for the period ending 31 March, 1962. Prepared by the Association of Universities of the British Commonwealth for the Commonwealth Education Liaison Committee. Pp. 59. (London: Commonwealth Education Liaison Committee, Marlborough House, Pall Mall, 1962.) 5s. (See also p. 749 of this issue of *Nature*.)

Canada comes next to Britain in the number of scholarships awarded and held (with 199 during the year), with Australia next (75), followed by India (22) and New Zealand (18).

This report was freely quoted in the debate when the Bill received an unopposed second reading and was generally welcomed as providing the cross-fertilization of contacts which gave the Commonwealth more reality as a world-wide community. Mr. G. M. Thompson, however, thought that the Government might have used the opportunity for something more imaginative and generous than an increase of some £20,000 a year on the Commonwealth scholarship side of Commonwealth education, and he urged the need to give particular attention to technical and technological awards under the scheme. He was also critical of the concentration on postgraduate awards, which could limit the value of the scheme to a number of emergent territories, some of which regard undergraduate, professional and technical awards as at least as important as awards for postgraduate study. He wanted a larger proportion of one-year scholars and made an incidental reference to the Peace Corps of the United States.

Mr. R. P. Hornby suggested that both educational aid and co-operation should be considered in the wider context of technical co-operation, and that it was important to see that the courses to which students or graduates were nominated were suitable to their needs and that on completion the graduates did, in fact, return to help the countries from which they came. Mr. T. Dalyell stressed the need, as recommended at Delhi, to direct adequate attention to induction courses if overseas students were to obtain the full benefit of their scholarships. He also referred to a number of other ways in which the development of ancillary aids, like audio-visual aids and books, could promote the efficiency of such schemes, particularly for teachers. Constructive suggestions also came from Miss Vickers, who regarded the Bill as one of the most important to come before the House this session, stressing also the value of informal education. Mr. J. Boyden called for more imagination, for greater provision for social education scholarships and stressed the part which voluntary bodies could play, while Mrs. Eirene White touched on the need for more hostel accommodation and on the bearing of developments in Commonwealth scholarships on the expansion of Britain's universities, particularly in respect of staffing.

In replying on the debate, Mr. Tilney said that expenditure on the scheme in the current year was about £1 million and would rise to £1·25 million next year and £1·5 million in 1964–65. He defended the concentration at present on postgraduates; informal education was to some extent a matter for local education authorities in the countries concerned, and in welcoming the appeal for more teachers to work in the Commonwealth, he appealed to local authorities to deal generously on their return with teachers who did so. Two scholarships in adult education were awarded in 1962. In 1961–62 there were 4,017 applicants for about 250 awards available, and the British Council had recently recruited the first ten of the thirty experts in teaching English as a second language under the British scheme announced at Delhi; these would be available for secondment in key posts in Commonwealth countries in the autumn of 1963. There were now 1,225 Commonwealth teacher training students in Britain and about 9,000 Commonwealth students at the technical colleges, and up to 45 bursaries had been offered for a two-year technical teacher training course beginning in September 1963.

The contribution of Britain in training teachers and particularly in technical education is well brought out in two further annexes to the report of the Second Commonwealth Education Conference. The report of the Committee on the training and supply of teachers, which forms Annex 3, stresses the need for more detailed information both about needs and resources, and recommends that special note should be taken of the requirements of developing countries on the training of technical teachers, and priority given to strengthening existing departments or institutions which train technical teachers. Once again stress is laid on the need for assurances that help will be given to those teachers who require it in finding suitable posts on return from overseas. However, the Committee also recommended a strong appeal to the idealism of teachers and to the honour of service abroad in a Commonwealth country.

The report of the Committee on Technical Education and Co-operation in the provision of text-books and other books, which forms Annex 4, reviews the report of the Commonwealth Education Liaison Committee, *Co-operation in Technical Education*, in the light of the recommendation of the Oxford conference in 1959. It warmly welcomes the British offer of special training for six months at a technical college in Britain and six months of planned industrial experience before proceeding to a one-year course of technical teacher training, and of 40–50 bursaries annually to enable teachers from developing countries to attend this special course. It reaffirms the recommendation of the Oxford conference regarding the interchange of scientists and technologists. It suggests that more awards in this field are desirable under the training of teachers scheme, that the share of assistance devoted to technical education should at least be maintained and preferably increased, and that professional bodies should be asked to consider expansion of their advisory services in relation to the recognition of qualifications.

It is thus apparent that there is a considerable and growing volume of organized and official co-operation in education as a background to any voluntary effort. The Commonwealth Education Conference itself fully recognized that the balancing of new needs against available resources is fundamental to the consideration of extensions to the Commonwealth Scholarship and Fellowship Plan. While its outlook on finance is essentially realistic, education is regarded as a productive agency, and expenditure on education as an investment in human resources. The Commonwealth Scholarship and Fellowship Plan, however, could not have been operated without the help and encouragement which the universities of the Commonwealth have so willingly given. The magnitude of co-operation of this kind cannot be expressed in concrete terms.

Such co-operation, however, must be fully considered by the Department of Technical Co-operation or in any appraisal of the total effort of the United Kingdom in service overseas, for example, in the discussion of the problems of staffing universities overseas arranged by the

Overseas Development Institute in December. Important as may be official arrangements to deal with difficulties such as loss of seniority or security, a change in atmosphere or in the climate of opinion could be of decisive importance. While devices such as the dormant contract offer prospects of overcoming such difficulties and a small Working Group was set up by the meeting to look into this and other suggestions, there can be no doubt as to the vital importance of a much deeper public understanding of the whole question of technical co-operation and service overseas. An informed public opinion could do much to remedy the startling neglect which the Government has shown over recent years in such fields as overseas research as well as encourage young men and women to come forward. However, professional associations have also a major part to play, and a responsibility which cannot be ignored to do their utmost to see that those who serve overseas on their return lose neither prospects nor security. Public debate and decision in and out of Parliament are to be warmly welcomed as assisting the formation of an enlightened opinion needed to support effectively the Department of Technical Co-operation. Moreover, such would ensure that neither the Departments of State nor professional associations disregard their opportunities and responsibilities in what is now one of the most vital ways in which Britain can play her part in world affairs. A recent issue of *Impact of Science on Society* (No. 4, 12; 1962) was devoted by Unesco to some problems of interest for developing countries, and directs attention to the dependence of the scientist on a host of skilled technicians and assistants if science is to make its full contribution to the development of natural resources in these countries. The training of such personnel and the provision of scientific and technological staff for teaching and research until a competent indigenous staff is available is one of the key problems in technical assistance. It calls for the particular attention of all scientific and technological associations.

IAN CLUNIES ROSS: A GREAT AUSTRALIAN

Ian Clunies Ross

Memoirs and Papers, with some Fragments of Autobiography. Pp. xxiii + 240. (London and Melbourne: Oxford University Press, 1961.) 40s. net.

The death of Sir Ian Clunies Ross at the age of sixty deprived Australia of one of her best-known public figures as well as a leading scientific administrator. To say that for the last ten years of his life he was chairman of the Commonwealth Scientific and Industrial Research Organization gives only a small indication of his contributions to the intellectual life of Australia. This book is not so much an account of a notable career as an attempt to capture in print a personality that left a deep and lasting impression, not only on friends and associates, but also on the Australian public.

Lady Clunies Ross tells us in the preface that her husband had cherished the hope of writing a personal history of Australia as he had known it over a remarkable half-century of growth and change. Evidently the present volume was conceived and compiled partly as a memorial and partly in an effort to preserve some of the ideas and ideals and the spirit that Ian Clunies Ross might have put into the book that was never written.

There are an introduction followed by two chapters of autobiography, a diary kept during the last two months of his life, eleven memoirs written by public figures and colleagues, a short story by Ross himself and ten of his papers, lectures and broadcasts.

It is the introduction by Frank Eyre that will convey most to those not well acquainted with Ross. It outlines briefly his career and introduces the reader to his personality and character. This is an intimate account told with discernment and understanding. Here we first meet the theme reiterated by several other contributors: Ross's most memorable characteristic was his immense charm, which was completely natural and sprang from a sincere and intelligent interest in all with whom he came in contact, from chauffeur to prime minister. He developed to an extraordinary degree the knack of bringing out the best in people. Little wonder that he was rewarded with their affection. Was it not Lord Chesterfield who said that if a man makes people like themselves a little more they will like him very well? Ross had earned the friendship of so many, even among those who had no opportunity of knowing him personally but only through his talks and broadcasts, that when he died Lord Casey said in a broadcast that a wave of sadness swept over the country.

But Ross's natural liking for people was not without discernment. The one thing he could not tolerate was insincerity—and he took a puckish delight in uncovering it.

Ross graduated in veterinary science at Sydney in 1921. After postgraduate study in Japan and at London and Cambridge he became lecturer in parasitology at the Veterinary School in Sydney. Afterwards, he was officer-in-charge of the McMaster Animal Health Laboratory, Sydney, chairman of the International Wool Secretariat in London, professor of veterinary science at Sydney and finally chairman of the C.S.I.R.O.

Ross made valuable contributions to knowledge on hydatid disease, on toxic paralysis of dogs and man caused by the tick *Ixodes holocyclus* and on helminth diseases of sheep. But his main contributions to science were as an organizer and administrator. One hesitates to measure research in terms of financial expenditure, but it is informative to mention that under Ross's chairmanship the annual budget of the C.S.I.R.O. grew to more than £9 million. The remarkable achievements of the Organization, in fundamental as well as applied science, even considering the large sum at its disposal, testify to the efficiency and wisdom of the administration.

Ross was a truly gifted speaker and hence was called on to talk on all manner of occasions. His facility with the English language, his aptness with analogy and felicity of phrase are evident in the parts of this volume written by himself. The words flow effortlessly and with a clarity and continuity of thought that hold the listener's or reader's interest. But the written speeches convey an incomplete portrait of the speaker, for here again it was his lively personality and obvious sincerity that won his audiences.

Some idea of the wide range of Ian Clunies Ross's interests and activities is given by the list of people who have contributed to this volume. These include the Right Hon. the Lord Casey, the Right Hon. Sir Owen Dixon, chief justice of the High Court of Australia, R. J. F. Boyer, chairman of the Australian Broadcasting Commission, Brian Jones, master of International House, Melbourne, in addition to several colleagues in the scientific field, and his son.

Lord Casey wrote: "Clunies Ross believed passionately in Australia and had a very clear vision of its future greatness". He was in every sense a great Australian: born and educated in Australia but with an enlightened interest of other lands—he learnt to speak Japanese the better to understand that people—his influence in many aspects of the intellectual life of his own young country will be a lasting one.

W. I. B. Beveridge

THE NEPTUNE STORY

The Discovery of Neptune

By Dr. Morton Grosser. Pp. 172. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1962.) 40s. net.

We conduct our lives by means of theories, none more so than those that delude themselves by thinking they never rely on theory, and the difficulties occasioned by misconception as to its nature are perennially with us. The great impact of the discovery of Neptune lies in that perhaps never before or since has it eventually seemed so inescapably demonstrated how wrong the anti-theorists can be: an 'actual object' was found, something that could be 'seen', and therefore a 'real fact'.

Dr. Morton Grosser, the American historian of science, has done us the honour of presenting a beautifully expounded analysis of this classic scandal. A scandal, by the way, is an episode that occurs when the official theory breaks down publicly. From the vantage point of the United States and the passage of a century, he sees the whole affair with crystal clarity and scholarly judgment. The species-Airy, not entirely extinct, is described with fearless accuracy: ". . . he divided people round him into two groups, those that had succeeded and were worthy of cultivation, and those that had not succeeded and were beneath consideration . . . a man strongly opposed to theoretical investigations and sceptical of the abilities of younger scientists . . . expressive of that intellectual emasculation that he encouraged in his corps of drudges".

These few excerpts indicate Dr. Grosser's considered opinion of the leading man of the play-acting farce that was carried through in all seriousness and effectually prevented Adams obtaining there and then the due credit for his brilliant work. A similar atmosphere prevailed in France, where the astute Leverrier soon withdrew reliance on his unenthusiastic compatriots, though the successful outcome of his resorting to Berlin (after even trying Airy) rested heavily on good luck: first, there were two young astronomers, Galle and d'Arrest, prepared to oppose the reluctant Encke, and, secondly, they chanced to have a star-map of the vital zone. The planet was found at once, and the game began: official theory was in perilous danger of being exposed as bogus. In such circumstances, nothing must be admitted, and the order of the day is "the truth and nothing but the truth, but never the whole truth". In retrospect, the devious manœuvres are comically funny, but it was not funny for Adams or for the reputation of British science. A superlative opportunity was culpably thrown away, and followed with a flood of prevarication and recrimination. And nothing has happened since to add to the intellectual credit of British observational astronomy. When the discovery became known, minds were made up with negligible appreciation of what was involved, and excesses of adulation lavished freely in many directions except that of the luckless Adams—a mere theorist.

Without more study than has yet been given to the processes involved, all that can certainly be claimed is that a planet was found near a place that emerged from theoretical procedures. It may be that all the steps can be shown to have complete validity, but this remains to be demonstrated even to this day. Consider just two of many queries: the use of Bode's law meant a period about three times that of Uranus, so the principal perturbations would arise from terms in $v-3v'$, whereas since the true period is about 160 years they actually arise from terms in $v-2v'$, and these have quite different periods and coefficients. Then again, there is difficulty in justifying the curious conversion to 'errors of mean longitude' that was Adams's initial step, and there are other unanswered questions.

The passage over from data to conclusions is sometimes very easily made, especially when strong emotion is involved, indeed so easily that we mainly fail to perceive that an intricate subjective psychological process of a not-necessary character must be concerned, and one that almost defies analysis. But if we are to get to the rights of complex matters, it is no use rushing to agreeable conclusions; instead, every step must be brought to the surface and its validity demonstrated. This has yet to be done even for the present century-old case, and so the true meaning of this sensational piece of scientific history remains very much an open question. Dr. Grosser's erudite work can be thoroughly recommended from every point of view, not least that it may help in directing attention to some of the mysteries that still surround the celebrated discovery of Neptune. R. A. Lyttleton

LINGUISTICS

A Functional View of Language

Being the Waynflete Lectures Delivered in the College of St. Mary Magdalen Oxford, 1961. By Prof. André Martinet. Pp. viii + 163. (Oxford: Clarendon Press, 1962), 21s.

Prof. Martinet is one of the most distinguished of contemporary linguists. He is no doubt most widely known for his phonological work, both theoretical and practical (and more especially for his theory of 'economy' in phonological changes and his application of this theory to the study of the innovations that have taken place in the sound-systems of various Indo-European languages); there are few branches of linguistics, however, to which he has not contributed in the course of the past twenty-five years. In 1961 he was invited to deliver the Wayneflete Lectures in Oxford and in them to develop his views on the nature of language and the principles of linguistic analysis. The present volume represents an expanded version of these Lectures.

Martinet's general theme, as his title suggests and as he explains in his preface, is the role of 'function' in language. He argues that recognition of the communicative function of language and of its constitutive elements will necessarily lead to a 'realist' view of language and show that it is far more varied and far less homogeneous than many 'formalists' or 'structuralists' would admit. Although I have much sympathy with this point of view, I find that Martinet's expression of it is unnecessarily polemical. Moreover, he greatly oversimplifies the issues and seems to use the crucial term 'function', which is constantly invoked and never precisely defined, in a number of different senses in different places in the book. The 'functional' criteria for syntax are far from being as clearly applicable as he suggests.

There are two other points which should be criticized. The first is the definition of minimal grammatical units ('monemes' in Martinet's terminology) as the "smallest segments of speech that have some meaning attached to them". Many linguists would hold that one of the most significant developments of recent years has been the emancipation of grammar from semantics. To say that a form like the Latin word *homini* "conveys three elements of experience: 'man', 'dative' and 'singular' " is to confuse very different kinds of relations and to do so in intolerably vague terms. The second point is the author's tendency to give unverifiable, or at least unverified, causal explanations of linguistic developments. One wonders, for example, whether there is any evidence to support the statement that "an increasing complexity of social relations will be accompanied by an increasing complexity of syntax". The Latin example used by Martinet does not seem very convincing.

Provided that he is not looking for a balanced and comprehensive statement of present-day linguistic theory, the general reader will find much to interest him in the treatment of particular questions. There is a good discussion of dialects in the chapter on "Linguistic

Variety", and of alternative ways of classifying and grouping languages by 'types' in the chapter devoted to this topic. Martinet's account of 'liaison' and part of the verbal system of modern spoken French (what he calls 'unguarded' French—a language which is very different from what we, and the French themselves, learn as 'French' in school and university) is particularly valuable. So too is his outline of the hypothesis that the formally marked nominative case of the Indo-European languages has arisen from an earlier 'ergative' construction such as one finds, for example, in Basque. J. LYONS

ECHINODERMS

Starfishes and their Relations

By Ailsa M. Clark. Pp. v+119 (16 plates). (London: British Museum (Natural History), 1962.) 11*s.* net.

Echinoderms

By Dr. David Nichols. (Hutchinson University Library.) Pp. 200 (26 figures.) (London: Hutchinson and Co. (Publishers) Ltd., 1962.) 12*s.* 6*d.*

MONOGRAPHS by Dr. L. H. Hyman (*The Invertebrates*, 4, *Echinodermata*, New York, 1955) and the late Prof. L. Cuénot ("Echinodermes" in *Traité de Zoologie*, edit. by P–P Grassé, Paris, 1948) have provided, in recent years, admirable comprehensive treatises on the phylum Echinodermata. But to the general reader, the two small volumes that are reviewed here will furnish succinct, clearly written and well-informed guides which are in large measure complementary in their coverage and treatment of the echinoderms, both living and fossil.

Dr. Clark's book, following the tradition of the British Museum (Natural History) hand-books, is amply illustrated with an excellent series of photographs and figures which not only enable the reader to identify the more commonly occurring of the starfishes, brittle-stars, sea-urchins, sea-cucumber and feather-stars that are to be found in British seas, but also give a clear impression of the wide range of form and ecological adaptations of animals that are represented in all the seas of the world and which number within the five classes of the Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea and Crinoidea, some 6,000 species.

Dr. Nichols's volume, though less copiously illustrated, includes a number of good figures in explanation of the internal structure of animals the interior arrangements of which are not always easy to understand; and, drawing in part from the results of his own published researches, he surveys the variations in form of their different organ systems, notably the tube feet, and relates them to their services in locomotion, burrowing, feeding and other organismal activities. Perhaps the most valuable parts of the book are, however, those in which he describes the extinct classes and discusses, in the light of their structure, their probable habits. Echinoderms, after a modest beginning in the Cambrian period with eocrinoid representatives, experimented, in the Ordovician and later periods of the Palæozoic, with a number of designs characterized by the classes of the Heterostelea, Cystoidea, Blastoidea, Edrioasteroidea, Ophiocystioidea, and with other groups such as the Machaeridea, which are less assuredly to be included within the echinoderms. Dr. Nichols discusses their probable methods of feeding, respiration and general mode of life and gives a well-argued account of their supposed phylogenetic radiations. Although there may not be general agreement with some of his views, for example, the origin of post-Palæozoic echinoids from edrioasteroid ancestors, it is refreshing to find the brittle-stars and asteroids once again brought into close relationship through common somasteroid ancestors; and among some novel suggestions, the possibility that the Machaeridea represent the plating systems of the tube feet of ophiocistioids is certainly worthy of further consideration.

Both Dr. Clark's and Dr. Nichols's books are to be commended as authoritative, highly interesting accounts of the structure, classification, development, mode of functioning, natural history and interrelationships of the echinoderms: and in both books there are numerous observations and comments which suggest problems in need of further investigation and research.

J. E. SMITH

ENZYME TESTS IN CLINICAL DIAGNOSIS

Clinical Enzymology

Enzymes in Pathogenesis, Diagnosis and Therapy. By Rudolph Abderhalden. Translated by Prof. Peter Oesper. Pp. xvi+448. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 9.75 dollars; 76*s.*

An Introduction to Diagnostic Enzymology

By Dr. J. H. Wilkinson. Pp. viii+288. (London: Edward Arnold (Publishers), Ltd., 1962.) 45*s.* net.

ONE of the extraordinary aspects of to-day's boom in enzyme tests for the diagnosis of disease is that there is no reason why it should not have come ten or even twenty years earlier. Most of the reactions have been known for an even longer time, and there is no spectacular 'break through' in technique to account for the present vogue. The concept that tissue enzymes might escape on injury and then be found in the serum or urine at an abnormally high concentration is as old as measuring the pancreatic enzyme amylase in the urine as an aid in the diagnosis of pancreatic disease. This was introduced by Wohlgemuth in 1910 and is still widely used, virtually as it was first described. It has recently been recognized that what was thought to be one serum enzyme, as, for example, serum lactic acid dehydrogenase, is in fact a mixture of isoenzymes. These have the same effect on the substrate, in this case lactic acid, but come from different tissues—heart muscle, liver, etc.—and can be separated by electrophoresis. Yet except for the name 'isoenzyme' this concept has long been accepted for the serum phosphatase. There are at least two serum phosphatases which can be separated according to their *p*H optimum into 'acid' and 'alkaline' phosphatase. Even these have long been known not to be single enzymes—and acid serum phosphatase was separated into that derived from the prostate, those from the liver, the spleen and kidney or from lysed red cells. Special techniques were devised using the unique sensitivity of the red cell enzyme to small amounts of formaldehyde for assessing the true share of the prostatic enzyme in the overall level of serum acid phosphatase.

Perhaps the most important new development, and that which started off the intensive search for new tests, has been the demonstration by Wroblewski *et al.* that after cardiac infarction a rise follows in the level of serum glutamic–oxalacetic acid transaminase. This has turned out to be an invaluable test, almost rivalling the electrocardiogram in the diagnosis of recent cardiac infarct and necrosis. Undoubtedly this discovery has been the stimulus which has triggered off so much new research on serum enzymes in the diagnosis of disease. The time has come to collect the numerous scientific articles in this field into manuals for the non-specialist. The two volumes by Abderhalden and by Wilkinson will both allow the general biochemist to read up much of the past and more recent work. Both are written clearly and tidily subdivided. Thus in Abderhalden's book for each enzyme is neatly presented its physiology, methods of assay, and clinical significance. One-fifth of its pages is given over to a copious index and to more than 1,500 full bibliographical references. This makes it an excellent work of reference. However, though it appeared in 1961, it appears that it was finished several years before then. Wilkinson's book seems to be written more with the view of bench work and is much more up to date. It has excellent brief résumés on subjects such as "enzyme tests in myocardial infarction", "enzyme tests in liver diseases", "diagnostic applications of isoenzyme separation". If the purse will allow it, many laboratory workers will find it well worth their while to acquire both.

H. Lehmann

BIOCHEMISTRY AT THE MOLECULAR LEVEL

Comprehensive Biochemistry

Edited by Prof. Marcel Florkin and Prof. Elmer H. Stotz. Vol. 2: Organic and Physical Chemistry. Pp. xii + 328. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 80s.

There is a wind of change blowing through biochemistry. Those who welcome it as a refreshing breeze will appreciate the appearance of the first volume (actually Volume 2) of this advanced treatise on biochemistry. In the past, biochemists had been concerned with determining what reactions occur in living organisms; but more recently, interest has been focused on the mechanism of biological processes at the molecular level. The present volume reflects this type of approach to biochemistry.

A novel feature is the decision to publish the treatise in five sections, each of which will comprise several small volumes. It is claimed that this will have several advantages. The specialist will be able to purchase only those sections which are of particular interest to him; future revision of volumes will be simpler; individual volumes will not be badly out of date when they appear. It may be noted that, in support of the last point, there are numerous references to papers published in 1960.

The present volume is devoted to organic and physical chemistry and opens with a chapter of some 200 pages by M. L. Bender and R. Breslow on the mechanisms of organic reactions. Both authors are well known for their investigations of the mechanisms of enzyme catalysis and both write in a clear, concise style. They describe the kinetic and mechanistic aspects of all the main types of reactions of organic compounds which are relevant to biochemistry, including nucleophilic substitution in aliphatic systems, elimination and addition reactions involving carbon–carbon double bonds, nucleophilic reactions of carboxylic and phosphoric acid derivatives, addition to carbonyl groups, enols and their relevance to various types of condensation reactions, carboxylation and decarboxylation, aromatic substitution, and various types of oxidation-reduction reactions. Where possible, the authors illustrate the several reactions with examples of biochemical import. In addition, particular attention is paid to the mechanism of action of simple catalysts and their possible relevance as models for the more complex catalysis by enzymes.

The remaining two chapters are written by W. D. Stein. The first of these deals with the equilibria of solutions, while the second treats problems such as diffusion and osmosis, which involve movement of molecules and ions within solutions and across membranes. The author presents his subject logically and attractively by starting with ideal solutions of non-electrolytes and developing the treatment progressively for non-ideal behaviour, solutions of electrolytes, and finally for macromolecules. No attempt is made to give rigorous mathematical proofs of equations, but there are adequate references for the more determined reader. The pattern of the final chapter on diffusion and osmosis is similar. After dealing with Fick's laws of diffusion, the relationships between diffusion coefficients on one hand and molecular weight and viscosity coefficient on the other are discussed. Consideration of more complex systems involving large solutes, electrolytes, concentrated solutions, and the presence of membranes follows naturally.

I myself should have liked to see this book open with a short chapter on classical thermodynamics and the thermodynamics of irreversible processes. The latter topic has been neglected in biochemical text-books, and it would have formed a bridge between the static and dynamic aspects of biochemical processes. It is to be hoped that the editors will find room later in this treatise to rectify this deficiency.

D. T. Elmore

RESIN CHEMISTRY

Alkyd Resin Technology

Formulating Techniques and Allied Calculations. By T. C. Patton. (Interscience Manuals No. 8.) Pp. ix + 197. (London and New York: Interscience Publishers. Division of John Wiley Inc., 1962.) 74s.

This book is specifically designed to cover a quantitative approach to the formulation and design of alkyd resins. It does so in a manner such that some theoretical background is stated and, from this, formulæ are derived and illustrations worked out to make perfectly clear the steps which are taken in the development of a comprehensive scheme to cover the very wide field of alkyd resins. In putting forward his main thesis, namely the application of an alkyd constant based on Carother's classical work, the author has displayed a wide experience and his book has been written with an aptitude to explain in simple language what is, in effect, an exceedingly complex subject. The use of this constant permits the formulator to design a satisfactory alkyd or to effect adjustments where necessary.

Perhaps it is inevitable in a mathematical treatise of this type that simplifications have to be made. In the technological field this may be permitted even if, from a purist point of view, there are a number of postulates which are less acceptable. Examples of these are, the differentiation of acid and monoglyceride alkyds based on attaching the fatty chain at the beta- and alpha-positions respectively (p. 20), or the postulates (p. 74) arising from the reaction of the alpha and beta glycerol groups which assume that the alpha groups react completely before the beta groups, giving linear polymers.

Again, of the four formulæ systems discussed in detail is one concerning M_{AV}, the average molecular weight of the alkyd at gelation. No distinction is made of the big difference between the weight average and the number average molecular weight. When dealing with physical concepts such as gelation, it is the latter which dominates the system. Nevertheless, from a practical point of view, the validity of the foregoing concept is shown to hold for many alkyds of known behaviour. It covers also urethane oils, water-soluble alkyds and modifications with other polymers such as styrene.

No major attempt is made, or has been intended, to describe process work or the influence of temperature on many of these results, but such factors will be familiar to the plant chemist.

A comprehensive bibliography is given at the end of the book drawn largely from American sources. It is gratifying to note several references to British work.

The resin chemist will find stimulus and much to help him in his calculations from this volume. It should find a place in the library and on the bench of many laboratories.

A. I. Escolme

SCIENTIFIC RESEARCH AS A CAREER*

By Dr. I. W. WARK

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THOUGH scientific research is as old as civilization itself, it was not graced with that name until a hundred years ago. Men had to try experiments to survive; they abandoned procedures that led to worse results; they adopted those that gave better. So, by a series of small advances, with many a false start, they evolved methods of tanning and brewing and of constructing shelters; they introduced agriculture, they went from the hazards of the dug-out to the safety—and the incredible beauty—of the Viking ship. False starts had to be eliminated, for they meant hardship, starvation or death.

Nowadays, the scientist strives to keep all but one variable constant, or by statistical methods to make allowance for those he cannot control. Had early man realized the desirability of these things, it would have availed him little, for in general the controlled experiment was impossible. *En masse* he did adopt the statistical approach, though he had no multi-million dollar computers to help him. It was the individual who did the experiments, or had them forced on him—it was he who observed the effects of variations and made the innovations and the inventions. His successes spread because his neighbours looked across the boundary fence; he encouraged their use without payment of royalties.

As time went on, a privileged class was established which had the opportunity to think about matters other than immediate survival. Some members of this group painted or sculptured, some turned to literature and music. Others, the earliest scientists, merely pondered why certain natural phenomena occurred and how the universe worked in all its ramifications. For centuries, however, there was no organized science. The individual was still the unit. He conducted such experiments as could be performed by himself; if he was affluent he may have had hired a single assistant. Old paintings of the alchemists' dens seem to be faithful representations of the laboratories of the day.

Nowadays in many fields the single investigator has given place to the team, with all that this implies. Dependent on the outlook of the leader, the work of the assisting scientists can be made intolerably drab or intensely interesting. There are two sorts of leader, and it is a pity that the first seems to be the more numerous type. He is exemplified by a chemist coming into prominence to-day. One of our C.S.I.R.O. senior studentship holders was working with this man for a Ph.D. degree, and being nearby on a short visit myself, I inquired about his progress. The professor gave me an excellent report; the student was all he could wish for, was a fine experimentalist and had followed up every suggestion with enthusiasm and alacrity. When asked whether the student had shown any originality or initiative, the professor looked very pained and replied: "Look here, Wark, no student of mine is expected to show originality; he does what he is told". Fortunately, our man was so good that even this treatment did not ruin him.

The other type of leader is typified by those whom I, myself, had the good fortune to work with. On my final evening in London, Prof. F. G. Donnan invited me to dinner and, over the brandy, expounded his philosophy: "Half the people trying to do research", he said, "should find something they can do better. They need for ever to be tied to their mentor's apron-strings. A student who requires detailed consultation more than once in two or three months should be encouraged to try some other calling than research". Donnan literally meant a discussion—an exhaustive discussion—once in two or three months, though, of course, one saw him much more frequently. You then presented a short summary. When he had studied it he sent for you, and made suggestions—often brilliant—as to how you might proceed. You decided whether you would take them, for you were master of your own research problem, and by no means all his suggestions were practicable: some, in fact, you had already tried and abandoned. But you certainly got help rather than direction.

In the atomic energy establishments where men work as teams, that director is ablest who encourages every officer to accept the maximum degree of responsibility and independence that he can carry, increasing the load as the officer develops. With all this talk of teamwork, it is well to remember that no team ever made a brilliant discovery. A discovery is made in the mind of a single person. If the time is ripe it may arise independently in the minds of more than one person. But the discoveries for which Nobel prizes are awarded are those that one man alone senses well ahead of his contemporaries, the sort that started rushes into a field of science similar, in a way, to the gold rushes of New Zealand, the Klondike and Kalgoorlie†.

In an extended trip round the world in 1948, and on a shorter one in 1954, I was amazed to find how little true research was in progress. I numbered the really exciting chemical laboratories on the fingers of one hand. On the whole, the celebrated schools were dull, though there were bright patches. The young men were not being given their heads.

I am convinced that far more superior research workers are born than are revealed under present-day conditions. In support of this assertion, perhaps I should reveal my theories as to why many do not do justice to themselves or to the expensive preparations over 8–10 years which were made to launch them.

It is generally agreed that it is the drive to satisfy his own curiosity that characterizes the successful research man. Everybody is born curious. The young child asks question after question, and as often as not gets a reprimand for his inquisitiveness. When he gets to school, let him beware if he seeks knowledge outside the ken of his teacher; as likely as not he will be ridiculed for wanting to know 'more than the text-book'. Not all university lecturers are blameless in this regard. However, the parental attitude may be the most important in encouraging or discouraging an inquiring attitude of mind.

It is in the early postgraduate years that the main good or the main damage is done. At this stage, the student has survived all the other pitfalls and discouragements. As he undertakes his first research assignment he is a precious responsibility to his supervisor. How many professors at this stage are willing to admit that there are some students—albeit few in number—who are inherently better than themselves and who should be encouraged to explore new paths rather than to follow the well-worn tracks of the 'school'? How many professors are willing to send a best graduate to another university—as they do in the United States and elsewhere—to experience the excitement of a new environment and to widen his interests while he is still young? How many are willing to grant a Ph.D. when the man is ready for it, rather than to conform to the present ridiculous trend that nothing short of three years is adequate? Is it not time that the students received more consideration than the 'school'? Professors should arrange suitable exchanges: admittedly there are difficulties, but they can be overcome if the will is there.

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* Substance of an address delivered on October 10, 1962, at the University of Otago, New Zealand, under the auspices of the University Grants Committee.

† This article was prepared before the Nobel Prizes for 1962 were announced [EDITOR].

There is another very rapidly expanding and insidious influence at work to take the initiative out of the man in his 'twenties just when he should be blossoming into an independent thinker and research worker. Having obtained his Ph.D. degree with an already one-year-too-long course behind him, the young postgraduate often proceeds to some overseas laboratory for further postgraduate studies. He is usually enticed to some laboratory with similar interests, and for two years he now devotes his time to getting what is fittingly described as a 'bar' to his Ph.D. With a paper or two to his credit he is now becoming known in his field and is invited to go from Europe to North America to earn his second bar. Slipping across the border into Canada he acquires the third. By this time he feels he cannot afford to move into any other field. His life has been determined by his first research problem, by his first research supervisor. Along the way three later supervisors have cashed in for their 'schools' on his knowledge and his ingenuousness. He will now have acquired the outlook of what I have heard called 'the professional fellowship holder'. Tied to three or four sets of apron-strings over a period of eight years, he will be fast approaching thirty years of age. He has never made a major scientific decision for himself, never selected his own research project, never tried his own wings. What he now does, in nine cases out of ten, is look for a position which will enable him to go on for the next ten years—and maybe for his whole life—playing minor variations on a theme—not even his own theme, but someone else's.

Yet our young man has done much that is right. To have lived in three or four countries while one is still young is a magnificent cultural start for life, and one which I thoroughly commend. He has made but one mistake, namely, that he has clung too tenaciously to his first scientific love. Had he changed his subject and acquired a new technique—experimental or mental—with each move, he would be in a far better position now. In addition, if instead of a fellowship he had obtained a lectureship or a job with responsibilities attached—in industry or in a research institute—one could not fault his decisions. In particular, the man who is not going to follow the academic life, but is wise enough to do some research work, should make the break with the universities a year or two earlier than is now customary. It is perfectly true that further experience before leaving the university would be valuable. However, the point at issue is whether the experience to be gained in a totally different environment while still young would not be more valuable still.

Prof. W. E. Garner, whose Department of Chemistry in Bristol I so greatly admired in 1948, told me that he encouraged his students and his lecturers to acquire three different techniques before they were thirty-five. This allows four or five years for each technique—long enough for a good grounding. By technique he meant, on the experimental side, such things as X-ray crystallography, vacuum techniques in inorganic chemistry, or distillation methods, and on the theoretical side, subjects like thermodynamics, spectroscopy or quantum mechanics. With any three of these at his command, the research worker can bring to bear on problems at the heart of his subject, big guns that most of his contemporaries lack; he will also be able, from his broader outlook, to recognize what are those very problems.

I should like to register certain reservations with respect to overseas experience and study at the start of a research career. Fifty years ago Australasia was almost a nonentity in the scientific world. To get any appreciation of the excitement of science or even of the method of scientific research it was imperative to travel. Except in a few brave cases of hope and confidence, there was a feeling that it was almost hopeless to try to compete with the world: we were miles behind scratch and would never catch up. Yet we have caught up in some fields, and can lead the world in a few, and there is a justifiable feeling of optimism about the future. I have even found a few kindred spirits from Europe and America who agree with me that one has a far greater chance of being original in the southern hemisphere, for one is not for ever being told there what is impossible by the 'authorities' of the northern hemisphere. (This is a point which might be noted by the student of Europe or North America. There are schools in Australia and New Zealand where a Ph.D. degree could mean more to him than one from his own country.) Too many of them are so surrounded by research students—some try to look after a dozen—that they have no time to think, and thus never escape from the variations of their theme of last year. Beware of any supervisor with more than five research students or you may find yourself in the position of a young man I met in Holland, part of a team of ten. The professor was out of town, and when I asked the student how his research fitted into the main topic of the laboratory, he had no idea. I met him later, when I myself had seen the connexion. Tears of gratitude came to his eyes as I explained it to him: he had been just another pair of hands. Obviously, not all the fault lay with the professor: the student should have had more initiative.

Lest the professional research worker should become too self-satisfied, he should recall that research is not the prerogative of the select few. Every member of the community conducts his private research about a host of matters that interest him. Who, for example, has not done some experimenting with the view of chopping the week's firewood with less effort. Nevertheless, the efforts of the amateur, important though they are, leave much to be desired. Thus, the novice at golf, suspecting his grip is wrong, may change it along right lines. Too anxious, his head follows his club and his shot becomes even worse. So he reverts to the incorrect grip. We all assume the amateur status at times; when fishing for trout, I have a new theory each day but with so many variables it is impossible to confirm or condemn it: that is the charm of something that will for long remain an art.

One might think that having embarked on a career in research the young scientist would have a mind so open that he would welcome new ideas and new generalizations. He must, of course, be critical but not too much so. Thus, Bernard Barber (*Science*, 134, 596; 1961) is convinced that as a class he is resistant to scientific discovery. He quotes Helmholtz as saying that the new ideas need the more time for gaining general assent the more really original they are. Helmholtz, however, resisted Planck's theories: and Lister warned students against blindness to new ideas in science. Others whose ideas met with belated acceptance were Heaviside, and Mendel, whose ideas, expounded in 1865, met with resistance until the turn of the century. Barber says that as late as 1901 the Royal Society asked Karl Pearson in future papers to keep his mathematics apart from biological applications. I myself once ran into trouble because I wished to publish a paper in the *Kolloid Zeitschrift* criticizing the views of the editor, Wolfgang Ostwald. It was published in the *Zeitschrift für Physikalishe Chemie*, but only after a committee had been set up to adjudicate between our rival views. Maybe if one has difficulty in gaining acceptance of one's views, one should be flattered, for one would certainly be in good company.

The term 'research' is used in many different ways. I suppose it is valid to speak of historical and sociological research, but the methods are so different that it is impossible to bracket them with research in physics and chemistry—at least for the present. In the physical sciences the hypotheses can be verified experimentally and should be verified before action is based on them. Verification, while it may be costly, places no human lives in jeopardy. In sociological and economic investigations so many factors are unrecognized or uncontrolled that conclusions are less certain. If the community decides to accept them, the results may be very uncomfortable. Thus, adjustments in the economy in Australia in recent years had more far-reaching results than their architects expected. I would be the last to decry an experimental approach to sociological problems; but the limitations must be recognized. Arising out of these observations, I now refer to a trend that is bound to continue and intensify and which a young man might take into account in deciding on his future career.

The mathematician of to-day is a much more powerful figure in research than his predecessor of fifty years ago. Indeed, it is probably fair to say that the mathematician is gradually taking over much of applied physics. This need not worry the physicist, who is moving into chemistry with growing momentum. Nor the chemist, who, with the physicist, is now a key figure in biological research: and what of the biologist? Does he need to worry? The answer is surely no, for he will become more and more a leader in the sociological fields, ergonomics being a case in point.

There are, of course, fashions in research. Whether one would be well advised to follow one of them is a question each man must decide for himself. Radiophysics and chemical physics are glamorous subjects which, in recent years, have been able to compete for staffing with atomic physics, but classical physics has been neglected. Yet atomic physics may have had its heyday for it is essentially a field for team work, and it is a field, moreover, where the costs are so high that the director must direct rather than inspire. Those physicists whom we have recruited in C.S.I.R.O. for work in classical physics seem to have had a rather more interesting scientific life—at least they have made quite original advances. Fashionable fields might be best for the near first-class, the other branches for the first-class. However, the rewards come later to the most original: the fashionables have a stranglehold on the committees which allot the prizes. In the long run it must be one's own assessment of one's life-work that is most important: public acclaim is nice when it comes, but those who seek it may sacrifice their self-respect.

The universities necessarily set up departments for each of the main branches of science. These often inspire a loyalty towards a particular science. Thus one tends to regard oneself as a chemist or a physicist, or, if one is particularly daring, as a physical chemist. But Nature recognizes no barriers; a natural phenomenon may require a knowledge of many different disciplines for its understanding. Three such phenomena come to my mind: ore genesis, plant growth, and genetics, respectively. So I would propose that, once one has left the university, it would be wise to subordinate one's original loyalty to the wider banner of science. Many of the most exciting fields of research lie in the indistinct regions between the sciences. They give rise to new hybrid disciplines such as biochemistry, biophysics, biological chemistry, geochemistry and geophysics. However, there is no need for a new name every time the fields overlap. The need is for courage to stray into the unexplored border regions. I was a contemporary of, and have had a lifelong association with, Sir Macfarlane Burnet, and so know something of the manner in which he has continued to 'grow' throughout his scientific career. At one stage, with some small amount of help from his friends, he taught himself enough of the calculus to handle the problem then uppermost in his mind. Burnet is far more than a virologist and an immunologist: he is a scientist in the widest sense.

It needs both courage and humility to enter a new field successfully. If one is too humble one does not take the plunge, whereas if one is too adventurous one enters new fields too often and, therefore, too superficially. Coming into an old field with a different background may present some striking advantages. I can illustrate this by referring to a small group of C.S.I.R.O. chemists and petrologists who entered the field of cement manufacture and utilization. They knew nothing of cement at the time, but they brought to its study some techniques hitherto lacking. They revolutionized some of the thinking, and in a short time became one of the leading laboratories of the world.

Perhaps I have dwelt too long on how a man or a woman may fit himself or herself for a career in research. There is the career itself. How satisfying it is, and how does it pay off? It is forty years since I made known my intention to adopt research as a career. I then received much the same sort of reception as a butcher's son of to-day might expect on deciding to become a composer in the style of Bartok. The gloomiest views were taken—not by my parents, I am glad to say—but by the family friends in industry and commerce. Overseas, the career research worker had just been born: he was no longer regarded by statesmen as a luxury, but as a necessity. Different countries have made provision for him in different ways, usually determined on a basis of expediency, sometimes backing a laboratory where good research was being done, sometimes setting up new facilities. Britain set up the Department of Scientific and Industrial Research; the United States established research institutes such as the Batelle, the Mellon, Stanford and Southwest. Continental countries tended to sponsor additional work in the universities, their professors acting as consultants to industry. No country has remained true to any of these ways: all have adopted a mixed approach.

Meanwhile, industry itself, commencing with Europe and rapidly expanding elsewhere, had discovered that the research scientist earns for the company more—far more—than his salary. Large private research laboratories were founded, and particularly in the electrical and chemical industries, help to pile up profits. In the United States, and partly for a very curious reason, the Government, through the Office of Naval Research, found itself placing contracts for quite fundamental research projects with industry. By the adoption of this subterfuge the best scientist became available for governmental work; the maximum salaries fixed by Congress for Government laboratories were too low to attract them.

Thus a person on the threshold of a research career has a wide choice of employing agencies: industry, Government and semi-government laboratories, the universities, and, in certain countries, the private research foundations. I have tried the first three of these types. In the university one gets the maximum freedom, but facilities are often inadequate; in industry the facilities are usually good but there is less freedom; in a Government laboratory both the degree of freedom and the facilities are intermediate. Industry gives one the broadest outlook; the universities, particularly those as isolated as Cambridge and Princeton, are extraordinarily narrow and self-satisfied. Salaries vary; first one group gets ahead and then another. Industry pays the highest top salaries, and the universities the best medium-level salaries. All these are broad statements; there are wide variations with time and with country.

One will find a higher proportion of fundamental research in the universities than elsewhere. By fundamental research I mean that which, if successful, will lead to a change of outlook of major importance to the development of a particular field of science. It is not, as I implied earlier, doing with strontium what somebody has already done with calcium.

Traditions are being forsaken, and fundamental research is no longer the exclusive prerogative of the universities. (I have the feeling that some few university men, not willing to regard teaching as their major function, have resented the spread of research outside university walls. My view—for what it is worth—is that strong support for university research is essential, since the university teaching function would languish without the stimulus from it. Nevertheless, company research, Government research and private institute research will continue to flourish because they pay exceedingly high dividends. There is room for all.) Excellent work of this class is done in industry, in research institutes on the American plan, and in such bodies as the Australian C.S.I.R. and its counterparts elsewhere. However, we have gone further in this respect in Australia than any other similar body and have found it pays handsome dividends. For this the credit must go to the first executive of the C.S.I.R.O., and particularly to the chief executive officer, Sir David Rivett, who was able to convince the Government of the day that the C.S.I.R. must be free to tackle its scientific problems on as fundamental a basis as was necessary for their solution (C.S.I.R.O. was formerly known as C.S.I.R.).

Perhaps you cannot yet decide whether you are more interested in fundamental or applied research ? Whatever you do choose, please try not to be snobbish about it, for there is a tendency for each group to air a superiority (inferiority ?) complex. Since progress in research is quite unpredictable—if one knows the answer it is not research—one can never be certain that a basic discovery will not be exploited, or that the attempt to solve a specific problem will not unearth quite new and profound knowledge. I believe that scientists with these two different outlooks contribute greatly to the successes of one another and do best when sharing a common environment. I myself have tried to 'back it both ways' by working in fields which I hoped would lead to useful applications, but which, at the very worst, would almost certainly provide new scientific knowledge. Thus there is a nice interplay between surface chemistry on one hand and such useful matters as flotation of minerals and hindrance to evaporation on the other.

The Monsanto Chemical Co. of America, believing that it must encourage the most talented recruits to its Research Department to remain there, rather than to chase the dollar elsewhere, has established for its top-flight research workers a classification which enables them to receive salaries to match those in other departments. This is a far-sighted step which one hopes will be copied widely.

Society does not always do the right thing by sections of the community, and in Australia we have some curious survivals in wage-fixing methods. Research workers and university lecturers are not strong pressure groups, but they have one protective characteristic which ensures for them the rudiments of justice. A degree in science is an international passport. So unless a nation pays its scientists reasonably, it may lose all except those who are tied by such strong bonds that even penury cannot weaken their allegiance. Actually, Australia loses more scientists to other countries because of better facilities for research than for higher salaries: most scientists are not mercenary. There is a second protective mechanism at work, for nowadays the community greatly values the scientist, and most members realize that it is his work in the background rather than the very earnest efforts of the trades union secretary or the politician that is responsible for increasing standards of living.

Some of my colleagues chide me for saying that the research man has tangible rewards besides his salary; I refer to his great freedom of action and to his satisfaction in his work. "Have not the business man and the artisan", they say, "similar rewards ?" Be that as it may, I count myself exceptionally fortunate in that I have always found a backer willing to pay me reasonably for what I most wanted to do. I have been permitted, like Tennyson's Ulysses, to travel far, and have enjoyed the company and respect of my fellow men. Perhaps I may be permitted to say that while scientists lack the perquisites of the salesman, they do have some 'fringe' benefits.

I have little patience with the scientist who maintains that because he is a scientist and has shown sufficient talent to get a Ph.D. the community have an obligation to provide him with a job to his liking and a comfortable existence. It is present performance that should count. Research men are justified in claiming some special privileges, but only those that enable them to do their work better. With these privileges they must accept responsibilities, and the vast majority are willing to do so. For example, most work exceptionally hard. Indeed, the scientist who finds he must force himself to work should give serious thought to changing his job—or at least his research project.

From very long experience, the Commissioners of the Exhibition of 1851 state that the only way to find out if one is good at research is to try it. Academic successes as an undergraduate, however spectacular, are nothing more than a good recommendation, and not an infallible guide.

A false start in a scientific life is not all that important, for a year spent in research will provide a good training of mind and hand, which will stand one in good stead later, in any walk of life. A strong group of industrialists in Britain, however, have come out emphatically against a prolonged period of research for those not contemplating a research career.

I would put two criteria above all others for the research man. First, he must have an overpowering curiosity about natural phenomena. When wandering through the bush he must wonder why ants never attain the size of lions, why some trees have darker foliage than others, why some rocks persist while others disintegrate, and where the robins go in winter. Still more, he must have the desire to experiment to find out. Without this he may be a good philosopher, but he is no scientist. He needs patience and diligence, the willingness to work for days and nights on end, at times (but only at times) when the job demands it. He needs a Micawber temperament to see him through the bad periods, and I hope, for his own sake, he is able to exult at successes.

My second major criterion is a strong educational background, leading, among other things, to an easy facility with one's own language. If a man cannot write clearly he cannot think clearly, and there is no place for muddled thinking in research. All whom I have known who have achieved outstanding success in science have written well; almost all have the capacity to describe their most intricate work to the layman. I do not think they all began life with that facility; but they certainly had acquired it before they became recognized as leaders.

I should hate to think that there would be many who would look on research merely as a career. To most of us it is more than that; it is our—perhaps minute but sincere—contribution to the development of science, that man may be better able to control his environment and to live a longer and fuller life. It is more than this; it is our contribution to the culture of the human race. For we must never be fooled by those who talk of the two cultures. Culture is as indivisible as is life itself, and the scientists give as much to it as does any other group.

A career in science opens up far horizons. At the end, one will be able to say with Ulysses:

> "I am a part of all that I have met ;
> Yet all experience is an arch wherethro'
> Gleams that untravell'd world, whose margin fades
> For ever and for ever when I move."

CHEMICAL TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM*

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THE evidence that nerves commonly produce their effects by releasing chemical substances at their endings is mostly based on experiments on peripheral tissues. This evidence is summarized in Table 1. The theory of chemical transmission had been discussed for many years before Loewi provided the first experimental evidence of it in 1921. He must have suspected from the first that the substance liberated by the vagus was acetylcholine; but he was careful not to say so and cautiously spoke of "*vagusstoff*". It was not until 1930 that acetylcholine came to be generally accepted as the substance released, and this was mainly due to two factors. Dale and Dudley[1] isolated acetylcholine from horse's spleen, and Chang and Gaddum[2] showed that it was possible to distinguish acetylcholine from other esters, such as propionylcholine, by making parallel assays by different pharmacological methods. At one time it seemed that it would not be easy to distinguish acetylcholine from pyruvylcholine; but Gill and Parsons[3] have now shown that this is not the case. The sample tested in 1933 was probably not pyruvylcholine, but an impure sample of acetylcholine. So far as we know, parallel assays will distinguish between acetylcholine and any other substance. This method was used to show that acetylcholine is liberated in the superior cervical ganglion[4] and elsewhere. Since then other esters, including propionylcholine, have been found in animal tissues, but the only one which has been shown to be liberated after stimulation of nerves is acetylcholine.

The second item in the Tables is that the administration of the transmitter reproduces the effect of stimulating the nerve; the effects of acetylcholine on peripheral tissues are well known[5]. Tissue extracts provide much indirect evidence. An extract of a nerve which liberates acetylcholine (a cholinergic nerve) contains acetylcholine itself, and the enzymes cholineacetylase and cholinesterase, which make it and destroy it. Table 2 shows the drugs which act on the various factors controlling acetylcholine. The release is inhibited by botulinum toxin[6] and morphine[7]. These drugs block transmission, but do not block injected acetylcholine. Drugs such as atropine, which block acetylcholine, do not always block transmission. Some people would say that this shows that acetylcholine is not the transmitter liberated; but Dale and Gaddum[10] came to the conclusion that this phenomenon might be "due to the liberation of a cholinester, but in a relation of so much greater intimacy with the receptor mechanism that atropine cannot prevent its access thereto". This explanation may appear unlikely, but similar explanations are still put forward[11].

The Central Nervous System

It seems likely that some of the mechanisms found in peripheral structures are also present in the central nervous system. Acetylcholine may be excitor or inhibitor, since it causes depolarization and excitation in voluntary muscle and hyperpolarization and inhibition in the heart. The same is true of noradrenaline, which excites the heart and inhibits the intestine. In both these tissues acetylcholine and noradrenaline are opposed; but their roles are reversed. It was therefore not surprising when Bradley and Wolstencroft[12] applied these drugs to single cells in the medulla and found that different cells were stimulated or inhibited, by either drug or both, in almost all possible combinations.

Table 1. EVIDENCE FOR CHEMICAL TRANSMISSION OF NERVOUS IMPULSES

Direct evidence

(1) Stimulation of a nerve releases an identifiable substance (the transmitter).
(2) The administration of the transmitter reproduces the effect of stimulation of the nerve.

Evidence from tissue extracts of

(3) Storage of the transmitter
(4) Synthesis
(5) Destruction

Evidence from the effects of drugs which may block any of these 5 actions.

Tissue extracts have given an indication of the probable distribution of cholinergic fibres in the central nervous system. The distribution of choline acetylase is similar to that of acetylcholine[13], but it can be detected in smaller amounts of tissue[14,15]. The concentration is high in motor roots, which are known to be cholinergic, and high in the caudate nucleus and the thalamus, but negligible in the pyramidal tracts and the optic nerves. It seems probable that these tracts liberate some other substance. The intracellular distribution of acetylcholine or cholineacetylase can be determined by homogenizing brain in sucrose and centrifuging. Acetylcholine and various other interesting substances are found in a fraction which consists largely of pinched-off nerve endings[16].

Until recently there was little direct evidence for the release of acetylcholine in the central nervous system. Bülbring and Burn[17] detected some release in the dog's spinal cord perfused with Ringer, when the whole sciatic nerve was stimulated. MacIntosh and Oborin[18] detected the diffusion of acetylcholine from the surface of the cerebrum into eserinized Ringer, contained in plastic cups. Mitchell and others[19,20] have been using these plastic cups and also push-pull cannulae[21]. In this apparatus Ringer runs down a fine needle into a tissue and is then collected through a plastic tubing surrounding the needle. The acetylcholine released is detected by its action on leech muscle in a microbath[22]. In these ways it has been shown that acetylcholine is constantly released from the cerebral cortex at a rate which is directly related to the local electric activity. The rate is increased by systemic injections of atropine or of the convulsant drug 'Leptazol'. It is increased by local stimulation of the cortex or by transcallosal stimulation, or by stimulation of the contralateral forepaw. When the cannula was in the middle of the caudate nucleus the rate of release was increased by stimulation of a small region of cortex in front of the motor area, but not by stimulation elsewhere. The largest yield of acetylcholine was obtained with stimulation at a rate of 1 per sec; faster rates produced less or none. There is clearly much more to be done with these techniques, which might be used to detect the release of other substances besides acetylcholine. Ramwell and Mitchell[23] have, in fact, detected the release of something else with an action on rat uterus.

Table 2 ACETYLCHOLINE

Direct evidence	Increase	Decrease
(1) Release	Stimulation of nerves	Botulin[6] Morphine[7]
(2) Action	Administration	Atropine Curare Ganglion blockers
Evidence from tissue extracts		
(3) Store[8]	Eserine Anaesthesia	Atropine Convulsions
(4) Formation	Cholinacetylase	Hemicholinium[9]
(5) Destruction	Cholinesterase	Eserine, etc.

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* Substance of a lecture delivered at University College, London, on December 6.

Another technique for detecting the release of substances in the central nervous system involves the use of the isolated spinal cord of a frog, or toad. Mitchell and Phillis[24] have found that in this preparation acetylcholine is liberated on stimulating motor roots, but not on stimulating sensory roots. This surprising observation suggests that the only cholinergic nerves in the spinal cord are recurrent nerves from motoneurones running to Renshaw cells. This is interesting because of the evidence discussed here that Renshaw cells are the only cells in the spinal cord of a cat which are sensitive to acetylcholine (cholinoceptive).

The effects of acetylcholine itself in the central nervous system are best studied by iontophoretic injection[25,26]. A micro-electrode is filled with a strong solution of the drug and a brief electric current is passed, which carries the drug as ions into the tissue. In one form of the apparatus, five glass tubes are fused together and pulled out simultaneously. The centre tube is filled with saline and used for recording. The other tubes are filled with drugs, which can thus be delivered within a few microns of the recording electrode. The first clear results came from experiments on the spinal cord by workers in Canberra[27], who found that Renshaw cells were excited by acetylcholine and that other cells were not. These Renshaw cells have an inhibitory effect on spinal reflexes; they are supplied with fibres from motoneurones and provide negative feed-back. The action of acetylcholine on these cells resembles its action on voluntary muscle in the fact that it has a short latency and a brief after discharge, and is specifically inhibited by dihydroerythroidine, but not by atropine.

Krnjević and Phillis[28] have applied drugs to the cat's cerebral cortex. The highest concentration of cholinoceptive neurones was between 0·8 and 1·3 mm below the surface. Betz cells, identified by antidromic stimulation of the pyramids, were generally cholinoceptive. This action of acetylcholine developed slowly and had a prolonged after discharge; it was specifically antagonized by atropine, but not by dihydroerythroidine. The response of these Betz cells resembled the peripheral muscarine actions of acetylcholine, while the response of Renshaw cells resembled peripheral nicotine actions.

There is also evidence of cholinoceptive cells in the inferior colliculus, the medulla, the thalamus and elsewhere. Active work in this field is being done in many places.

Amines

The monoamines adrenaline, noradrenaline, 5-hydroxytryptamine (5HT, serotonin) and dopamine have attracted attention because they are drugs with powerful peripheral effects, and are found in extracts of the central nervous system.

Their actions on the central nervous system are complicated. When adrenaline or noradrenaline is injected intravenously it causes emotion; some say that noradrenaline causes anger and that adrenaline causes anxiety. These effects may well be due to reflexes arising from peripheral tissues. When Feldberg[29] injected them into the cerebral ventricles they caused inhibition. The action of noradrenaline on an individual cell may be either excitor or inhibitor[12].

There is no direct evidence that any of these amines is released by nerves in the central nervous system; but there is much indirect evidence from experiments on tissue extracts suggesting that they play an important part in controlling the brain. The first convincing experiments were done by Vogt[30], who showed that noradrenaline was present in the phylogenetically old parts of the brain, that it did not disappear when the cervical sympathetic nerve was cut and that its concentration could be altered by drugs. At about the same time the presence of 5HT in the central nervous system was announced simultaneously by two groups of workers at the International Physiological Congress in Montreal[31,32] and its distribution was shown to be similar to that of noradrenaline[33].

Table 3. MONOAMINES

Noradrenaline, adrenaline, dopamine and 5HT (serotonin)

Direct evidence	Increase	Decrease
(1) Release	Stimulation of nerves	Bretylium[36], etc.
(2) Action	Administration	Ergot derivatives, Phenoxybenzamine, etc.
Evidence from tissue extracts		
(3) Store	Monoamine oxidase inhibitors	Sham rage[30]
	Precursor	Reserpine[36]
(4) Formation	Decarboxylase	α-methyldopa[37,38]
(5) Destruction	Monoamine oxidase	Monoamine oxidase inhibitors
	Methylases	Pyrogallol

Vogt[30] showed that the store of noradrenaline is depleted by drugs which stimulate the centres controlling the sympathetic system, causing sham rage. This may have been due to release by adrenergic nerves, but the evidence is indirect; exhaustion of the store might also be due to decreased synthesis, decreased uptake from the blood, or increased destruction. These drugs do not affect the concentration of 5HT.

The concentration of noradrenaline in peripheral adrenergic nerves is increased by local synthesis and also by uptake from the tissue fluids. It is probable that, after it has been liberated, some of it is taken up by the nerve and used again[34]. Amines do not penetrate the blood brain barrier, but the amino-acids from which they are formed are taken up and the amines are formed inside the cells.

The concentration of monoamines in the brain is lowered by reserpine, which appears to have a direct action in blocking the storage sites. Their concentration is raised by drugs which inhibit the monoamine oxidase, which normally destroys them. Reserpine causes sedation, and monoamineoxidase-inhibitors, such as iproniazid, cause excitement and are used in the treatment of depression. It has been claimed that the accumulation of amines causes excitement, and their loss has the opposite effect. This simple story is inconsistent with Vogt's findings already described. There was controversy about whether the state of the brain depended on noradrenaline or 5HT, and it was decided that excitement was due to the accumulation of noradrenaline and that depression was due to the release of 5HT. It was then found that dopamine was present in large amounts. This amine is formed by the decarboxylation of dopa (dihydroxyphenylalanine) and is the precursor of noradrenaline in the body, but it has functions of its own and some think it is more important than the other amines in the central nervous system.

The fundamental facts about these monoamines were discovered by bioassay, but fluorimetric methods are quicker and more versatile, since they may be used to estimate not only the amines but also their metabolic products[35]. Amino-acids, such as dopa, are taken up by the brain and decarboxylated inside the cells to form amines. The administration of dopa increases the amount of both dopamine and its metabolites found in brain extracts. Drugs, such as α-methyldopa, which block the decarboxylase, have the opposite effect and lower both amines and metabolites. Reserpine blocks storage and so lowers the amine concentration and increases the metabolites. Drugs which block amine–oxidase increase the amines and presumably lower some of the metabolites.

It is thus possible to produce all combinations of change in the amounts of four different amines and their metabolites, inside and outside the cells. It is still true that the accumulation of amines is often associated with excitement and depletion of amines with depression, but the mechanism is not as simple as was once thought. The turnover may be more important than the store.

Histamine is an amine present in the same parts of the brain as the monoamines and in similar concentrations[39]. Little is known about its possible function.

Other Substances

More than half the neurones in the central nervous system do not liberate acetylcholine and it seems likely that they liberate other substances, and that these substances will be found in extracts. γ-Aminobutyric acid (gaba) is formed in the brain from glutamate (as dopamine is formed from dopa) and like dopamine it is liberated by reserpine[40]. Glutamate is a general stimulant of nerve cells and gaba a general depressant. When glutamate is applied by iontophoretic injection it causes depolarization and the initiation of impulses. Gaba prevents this depolarization but does not cause hyperpolarization. The Renshaw cells cause hyperpolarization in motoneurones, so that gaba cannot be the chemical transmitter liberated by Renshaw cells. Glutamic acid and gaba are both present in high concentrations in the central nervous system, and it seems unlikely that they are specifically released from active nerve endings.

Substance *P* is an active polypeptide found only in vertebrates and with a specific distribution in the mammalian brain. There is no good evidence of its function, but it has been suggested that it controls the circulation. Other active substances in extracts include fatty acids, adenosine triphosphate and many more[41]. There is no good evidence that any substance, except acetylcholine, is liberated as a chemical transmitter in the central nervous system. There must be other transmitters, but it is probably unreal to speak of 'the inhibitory transmitter'. Acetylcholine itself is inhibitory at some sites. If the Renshaw cells liberate a substance which inhibits motoneurones, this substance may well be found to excite other neurones in the central nervous system.

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CRANIAL CAPACITY OF *ZINJANTHROPUS* AND OTHER AUSTRALOPITHECINES

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THE cranial capacity of the fossil hominoids, the Australopithecinae, has always been a problem of great interest, especially since several workers such as Keith[1] and Vallois[2] have recognized a 'Rubicon' of brain size between apehood and manhood. Keith's 'Rubicon' was 750 c.c., while Vallois's was 800 c.c. Other workers, including Straus[3] and Dart[4], have emphatically rejected this notion of a cerebral 'Rubicon'. Recent studies (for example, that of Schultz[5]) have shown that there is no clear dividing-line, the largest gorilla cranial capacity (752 c.c.) differing scarcely at all from the smallest capacity of a Javanese *Pithecanthropus* (775 c.c.). Even if we do not recognize a dividing-line, it is of interest to see where the cranial capacities of the australopithecines lie in relation to those of other hominoids. It is the purpose of this article to review the recorded cranial capacities of australopithecines and to place on record the cranial capacity of the Tanganyikan australopithecine, *Zinjanthropus*.

At the outset, it should be stressed that cranial capacity is not the same as actual brain-size, since the cranial cavity accommodates as well as the brain the dura mater and the other coverings of the brain, cerebrospinal fluid and blood. The relationship between endocranial volume and brain volume has been thoroughly reviewed by Zuckerman[6]; there is no consistent ratio between the two values, and it has long been customary to accept the cranial capacity as an approximation to brain-size. For example, instead of comparing brain-weights with body-weights of living primates, as Connolly[7] has done, Schultz[8,9] expresses cranial capacity in cubic centimetres as a percentage of body weight in grammes. In the work recorded here, cranial capacity alone is considered, with no attempt to convert it to brain volume or brain weight.

The Australopithecinae are now known from at least seven, if not eight, sites in Africa: five of them within the Republic of South Africa, one or possibly two in Tanganyika and one in Chad. In addition, Robinson has claimed that the Javanese fossil, *Meganthropus*, belongs to the same sub-family[10,11]. All these fossil ape-men fall into two fairly distinct categories: a more gracile form, sometimes considered a separate genus, *Australopithecus*, and a more robust form, to which has been given the generic name *Paranthropus*[12]. There is a yet more robust form from Olduvai Gorge in Tanganyika, to which Leakey[13] has given the name *Zinjanthropus*, but which some workers regard as closely related to *Paranthropus* (for example, Robinson[14]).

A detailed examination of the cranium of the Olduvai australopithecine has been undertaken by me, at Dr.

Leakey's generous invitation. The resulting report will form the major part of the third volume of a new work which is being prepared by the Leakeys and their collaborators. Meanwhile, however, owing to the considerable interest in the cranial capacity, it was deemed important to place this on record in advance of the larger publication.

Cranial Capacity of the Gracile Australopithecines

Six of the gracile australopithecine crania are sufficiently well preserved to have made good estimates of the cranial capacity possible. Four of these are adult specimens from Sterkfontein, and their capacities are 435, 480, 480–520 and 530 c.c. (refs. 15, 16 and 17). The fifth is the child skull from Taung which, at about 5 years of age, has a capacity of 500–520 c.c. (refs. 15 and 18). The capacity which the Taung individual would have attained to, had it lived to adulthood, has been variously estimated at 570, 600 and 624 c.c. (refs. 1, 4, 19 and 20). The central value of 600 c.c. is accepted here. The sixth specimen is the recently discovered, tolerably complete cranium (*MLD* 37/38) from Makapansgat[21]; according to unpublished information kindly supplied by Prof. R. Dart, this cranium agrees in its dimensions so closely with the very complete cranium (*Sts* 5) from Sterkfontein that the same cranial capacity should be attributed to it, that is, 480 c.c. Thus, six specimens of *Australopithecus* range from 435 to 600 c.c. in cranial capacity, giving an average value of 504 c.c. (Table 1).

Cranial Capacity of the Robust Australopithecines

Until recently, no single specimen of the large-toothed, heavily muscled form of australopithecine had been discovered which was sufficiently complete and undistorted to make possible a reliable estimate of the cranial capacity. True, some guesses had been made: the robust dimensions of the teeth, jaws and crania of *Paranthropus* had led to estimates such as 650 c.c. for the Kromdraai specimen[14], 700 and 750–800 c.c. for two child crania from Swartkrans and "probably over 800" and "probably over 1,000" for two adult specimens[12]. All these estimates agreed in giving the robust ape-man an appreciably bigger cranial capacity than the gracile form; insecurely based as these guesses were, it had virtually become accepted by some workers that the robuster australopithecine was not only bigger-toothed, bigger-jawed and bigger-muscled, but also bigger-brained than the gracile hominid from Taung, Sterkfontein and Makapansgat.

The Leakeys' new fossil cranium from Bed I in the Olduvai Gorge, called by Dr. Leakey *Zinjanthropus boisei*[13], is the first of the robuster forms of australopithecine which is sufficiently complete and well preserved to have enabled an accurate assessment of the cranial capacity to be made.

Endocranial Cast of *Zinjanthropus*

No natural endocast was found with the cranial remains of *Zinjanthropus*. However, so much of the endocranial surface of the calvaria was preserved as to make possible the preparation of a plaster endocast. This was effected by me, with the invaluable expert assistance of Messrs. A. R. Hughes and T. W. Kaufman of the staff of the Department of Anatomy. Approximately the posterior two-thirds of the brain-case was virtually complete; likewise, the frontal poles and rostral regions. The areas between had to be reconstructed. There is little doubt as to the intervening distance, because of a satisfactory approximation between the anterior and posterior calvarial parts. Furthermore, there were available for comparison a large collection of endocranial plaster casts of gorilla, chimpanzee and orang-utan, of cercopithecoids, of *Pithecanthropus* and other fossil hominines, of modern man, and of the australopithecine natural endocasts hitherto discovered, as well as plaster casts in those instances where no natural cast had formed or been found. In the circumstances, it is felt that the plaster endocast of *Zinjanthropus* cannot be far from the mark and cannot deviate volumetrically from the true cranial capacity by more than perhaps 20 c.c. in either direction.

Table 1. CRANIAL CAPACITY OF *Zinjanthropus* AND OTHER AUSTRALOPITHECINES

Zinjanthropus		530	Tobias 1962
Australopithecus (*A*) Sterkfontein	*Sts* 1	435	Schepers (1946)
	Sts 5	480	Broom, Robinson and Schepers (1950)
	Sts 7	480–520	Broom, Robinson and Schepers (1950)
	Sts 8	530	Broom and Robinson (1948)
(*B*) Makapansgat	*MLD* 37/38	480	Dart, (personal communication)
(*C*) Taung	Taung juv.	500–520	Dart, Schepers (1946).
	Taung adult (estimate)	570–600–624	Le Gros Clark (1947) Le Gros Clark (1955) Dart (1956)
Australopithecus	adult mean ($n = 6$)	504	Present work
Australopithecinae	adult mean ($n = 7$)	508	Present work

Cranial Capacity of *Zinjanthropus*

The volume of the endocast was determined by displacement of water, after the cast had been varnished. In seven determinations, the displaced water was measured by volume and in three further determinations by weight. The mean of the first seven estimations was 529·7 c.c., the mean of the next three estimations 528·1 c.c.—and the overall mean for ten estimations is 529·2 c.c. For practical purposes, we may call the cranial capacity of *Zinjanthropus* 530 c.c.

The Tanganyikan creature falls somewhat short of full adulthood, the third molars being not yet in the fully erupted position of occlusion. The age of the youth may be 15–16 years. Very little, if any, further growth of the brain occurs after this age: in man, for example, brain growth is said to end at about 20–21 years of age[4,23,24], although a few workers would extend the period of growth somewhat longer[4]. In the chimpanzee, Zuckerman found that "in the resting stage after the eruption of the first molar", the capacity is already 90 per cent of the adult capacity in males and 95 per cent in females (*op. cit.*, p. 34). Since *Zinjanthropus* is practically dentally mature, the value of 530 c.c. may be accepted as very close to the adult cranial capacity.

In other words, its capacity is exactly the same as that of the largest adult *Australopithecus* from Sterkfontein, but falls short of the value estimated for the adult capacity of the Taung ape-man, that is, 600 c.c. Although the Olduvai creature is by far the biggest-toothed and biggest-muscled of all the robust ape-men thus far discovered, its brain-size proves to have been no larger than that of its smaller-toothed gracile cousins.

If this is true of the most robust of all the australopithecines, it is probably equally true of the somewhat less robust South African forms of *Paranthropus*. In this regard, it is interesting to note that, since the discovery of *Zinjanthropus*, Robinson has revised his earlier estimates of the capacity of *Paranthropus* from the high values quoted above to a modest 450–550 c.c., that is, the same order of size as obtains in the gracile *Australopithecus*[12,14].

Of course, the single valid estimate of 530 c.c. in one robust australopithecine cannot provide us with any estimate of the range of capacities. It may yet prove that the range and the average capacity of the robust forms differ from those for the gracile forms, although the ranges probably overlap to a considerable extent. Only the discovery of more and better specimens can answer this question. Meanwhile, there is no reason why at this stage the six capacities of the gracile *Australopithecus* and the single capacity of the robust *Zinjanthro-*

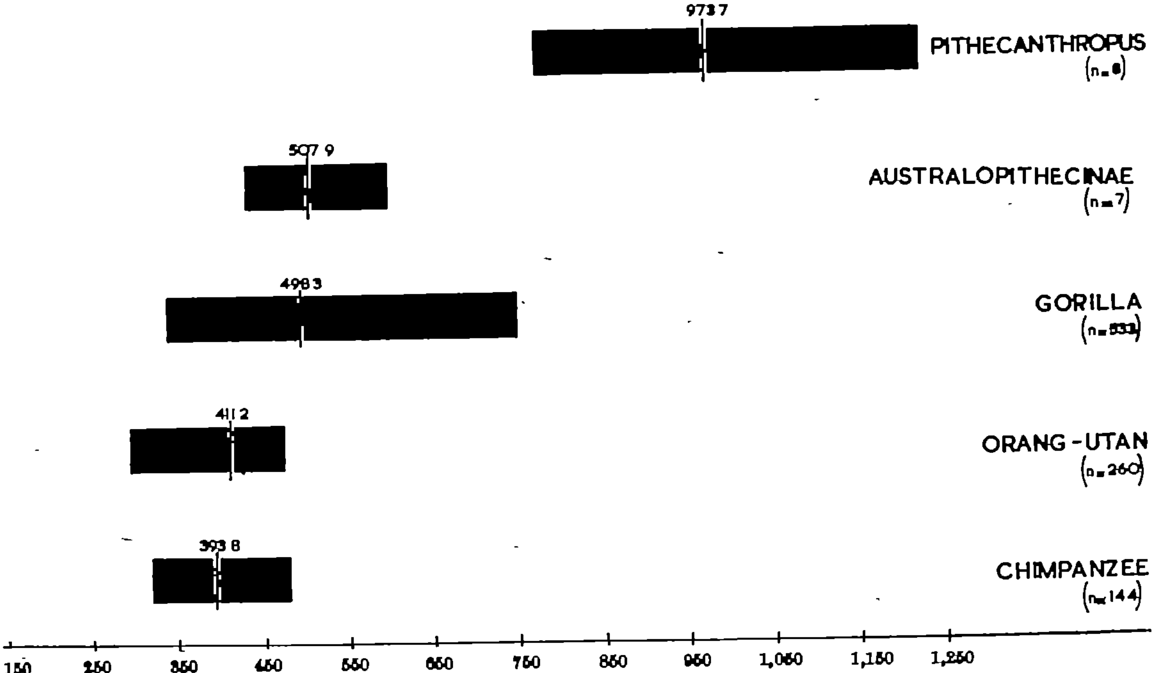

Fig. 1. Cranial capacities of hominoids

pus should not be combined as a single sample of seven australopithecine capacities. The mean of this combined sample is 507·9 c.c. (Fig. 1).

Australopithecine Capacities compared with those of Other Hominoids

The australopithecine mean of 507·9 c.c. is slightly larger than the mean for the biggest-brained of the anthropoid apes, the gorilla, which has a mean capacity of 497·8 c.c. (ref. 2), or 498·3 c.c. if an additional gorilla cranium of very large capacity is included (see following). The mean value for the orang (411·2 c.c.) is 100 c.c. smaller than the australopithecine mean, while the mean for chimpanzee is somewhat smaller again (398·8 c.c.). Nevertheless, the biggest orang and chimpanzee crania (about 480 c.c.) overlap the lower part of the australopithecine range (435 c.c. +).

Ashton's[26] analysis of the endocranial capacities of the Australopithecinae was based on seven crania. He included the Taung child, unfortunately not adjusted for age, and two crania estimated to have capacities of 650 c.c. which are not accepted in the present study. He compared these values with means for male and female chimpanzee and gorilla, as well as for *Pithecanthropus*. (Unfortunately, Ashton has apparently erroneously included the six Ngandong crania under *Pithecanthropus pekinensis*, of which he states Weidenreich[27] gave the cranial capacities of 11 specimens. In fact, Weidenreich tabulated the capacities of only five specimens of *Pithecanthropus pekinensis* (although mentioning another two estimates, 850 and 1,300, in the text). When to these 5 Peking cranial capacities are added Weidenreich's figures for 6 Ngandong crania and 3 Javanese *Pithecanthropus* crania, Ashton's total of 14 crania and his mean of 1,026 c.c. are obtained. Since there is no agreement with Ashton that the Ngandong crania belong to *Pithecanthropus pekinensis* (Campbell[28] places them in *H. sapiens soloensis*), I have excluded them from my *Pithecanthropus* group, including in the latter group only 5 Peking skulls and 3 *Pithecanthropus* skulls from Java.)

Ashton found that all the australopithecine capacities but one were significantly greater than those of the chimpanzee. None differed significantly from those of male gorilla, while only the two estimated capacities of 650 c.c. were significantly greater than those of female gorilla. *Zinjanthropus* thus provides one more australopithecine datum well within the gorilloid range of cranial capacities.

On the other hand, the australopithecine mean falls far short of the smallest hominine mean, which, according to my calculations, is 973·7 c.c. for eight crania of *Pithecanthropus* (=*Homo erectus*). Ashton found that all the australopithecine capacities, even including the estimates of 650 c.c., were significantly smaller than those of *Pithecanthropus*, as computed by him (see footnote). There is a gap of 175 c.c. between the largest australopithecine capacity accepted in the present study (600) and the smallest known pithecanthropine capacity (775). At the same time, the gap between the largest gorilla cranium and the smallest pithecanthropine cranium has been progressively reduced from 152 c.c. (ref. 27) to only 90 c.c. (ref. 2), and now to a mere 23 c.c., by the finding of a single large male zoo specimen with a capacity of 752 c.c. (ref. 5). No australopithecine skull yet discovered is as large as the biggest-brained gorilla cranium.

This raises the point whether the range of presently known australopithecine capacities, in which 165 c.c. separates the largest and the smallest, can be regarded as a reasonable approximation to the population range. In his definitions of the proposed genera, *Paranthropus* and *Australopithecus*, Robinson[13] ascribes to both an endocranial volume "of the order of 450–550 cm^3". This range of only 100 c.c. is clearly too small, as a glance at Table 2 will confirm.

The range between the maxima and minima for a large gorilla sample is 340–685 c.c. (or 340–752 if Schultz's outsize specimen is included); for a large orang-utan sample

Table 2. RANGES AND MEANS OF CRANIAL CAPACITIES OF HOMINOIDS

Species	Size of sample	Range	Difference between minimum and maximum capacities	Mean	Range/mean (%)
Gibbon	86	87–130	43	89 3	48·1
Siamang	40	100–152	52	124·6	41·7
Chimpanzee	144	320–480	160	393 8	40 6
Orang-utan	260	295–475	180	411·2	43·8
Gorilla	533	340–752	412	498·3	82·3
Australopithecinae	7	435–600	165	507·9	32·5
Pithecanthropus (*Homo erectus*)	8	775–1,225	450	973·7	46·2
Modern Man	—	±1,000–2,000	±1,000	±1,300	±77

295–475 c.c.; for a large chimpanzee sample 320–480 c.c.; and for a small sample of *Pithecanthropus* crania 775–1,225 c.c. (refs. 2 and 27). The volumetric differences between these pairs of maxima and minima are: chimpanzee 160, orang 180, gorilla 345 (or 412) and *Pithecanthropus* 450 c.c. (all figures relate to males and females combined). The range of variation seemingly increases as the mean cranial capacity increases: this being so, we might expect the australopithecines, with a mean capacity slightly greater than that of the gorilla, to have a range between those of gorilla and *Pithecanthropus*, though much closer to that of gorilla. Instead, the range for the presently known Australopithecinae is 435–530, a volumetric difference of only 95 c.c. If we accept the central estimate for the Taung adult capacity, the australopithecine range becomes 435–600, a difference of 165 c.c. However, even the range of 165 c.c. is on the small side as compared with those of other higher Primates. This range is about as extensive as the ranges in the smaller-brained chimpanzee and orang, but it is less than half the range in gorilla and *Pithecanthropus*.

Accordingly, estimates have been made of the probable population range for the Australopithecinae. Although the method and detailed results will be presented elsewhere[29], it may be stated here that even the most generous estimate yields a maximum value of 848 c.c. at the australopithecine grade of hominid organization. We may conclude that the estimates of 1,000 c.c. for the upper limits of the australopithecine cranial capacity[4,12] are excessive and highly unlikely.

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ROLE OF OPTICAL ISOMERS IN DETERMINING THE ANTIGENICITY OF SYNTHETIC POLYPEPTIDES

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IT has been recently demonstrated that a variety of synthetic polypeptides are antigenic in rabbits and other mammals[1–5]. Since the synthesis of the polypeptides is under careful control, certain molecular parameters can be systematically varied in order to explore the minimal requirements for eliciting an immunological response and for determining antigenic specificity. It has been shown, for example, that no more than two types of amino-acids are needed in the polypeptide chain[6] and that the antigenic sites probably consist of short amino-acid sequences in the disordered, non-helical regions of the chain[7]. In this investigation we have examined two polypeptides differing only in the residue configuration of the amino-acids in order to determine how this parameter affects the antibody production and the antigenic specificity.

The two polypeptides were synthesized from reaction mixtures of 60 mole per cent glutamic acid and 40 mole per cent lysine. The physical and chemical properties of the polymers are summarized in Table 1. The molecular weights were measured by sedimentation using the approach-to-equilibrium method. The compositions were determined by acid-base titration. The α-helical contents were obtained from measurements of optical rotatory dispersion in the conventional manner[8].

Rabbits were immunized subcutaneously with 10 mg of the antigen in Freund's complete adjuvant, followed three weeks later by another 10 mg in saline–phosphate buffer. Two weeks after the second injection the animals were bled (bleeding I). Some of the animals in both groups then received three further injections of 10 mg of the antigen in saline–phosphate buffer on alternate days after bleeding I, and were bled again one week after the last injection (bleeding II). The antibody concentrations of the individual sera were measured by the precipitin method, which is sensitive to a minimum of 1 μg antibody nitrogen per ml. of serum.

The polypeptide composed of D-amino-acids failed to elicit an immune response detectable by the precipitin method in any of the 24 rabbits tested. Moreover, none of these sera, tested individually, reacted with either the D- or the L-polypeptide. Contrasting with this, the polypeptide composed of L-amino-acids elicited an average of 13 μg antibody nitrogen per ml. of antiserum. (This average value included the 6 rabbits which gave no detectable response out of 12 immunized.) The average antibody concentrations are presented in Table 2.

It was further found that the D-polypeptide did not cross-react with any of the individual sera to the L-poly-

Table 1. PROPERTIES OF THE OPTICALLY ISOMERIC SYNTHETIC POLYPEPTIDES

Formula	Acid-base titration					Molecular weight	Optical rotatory dispersion				
	Mole per cent glutamic acid	Mole per cent lysine	pK_A	pK_B	Iso-electric point		$[\alpha]_D$	a_0	b_0	Per cent helix	Helix sense
$Glu_{58}Lys_{42}$	57·8	42·2	4·45	10·97	5·57	37,000	−101	−606	−122	20	Right
$D\text{-}Glu_{57}D\text{-}Lys_{43}$	57·2	42·8	4·35	11·00	5·65	46,000	+90	600	95	15	Left

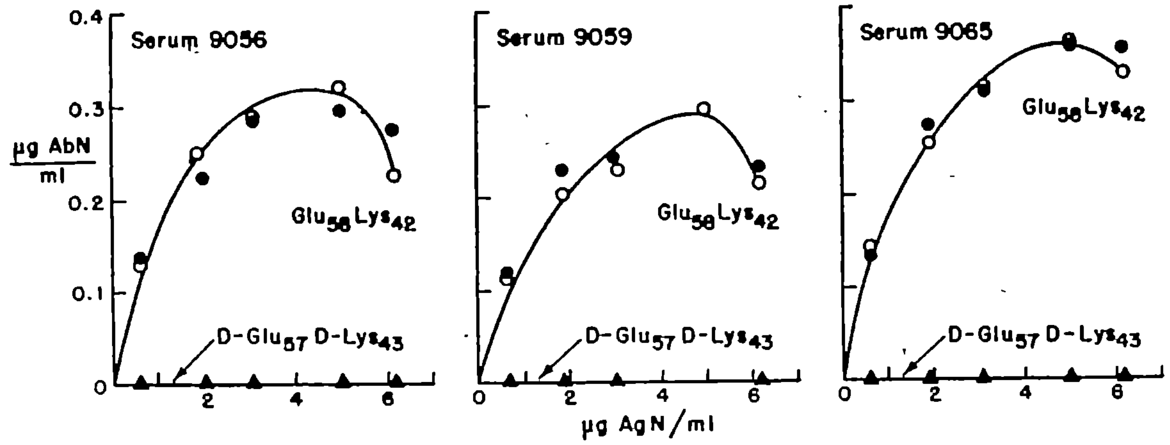

Fig. 1. The reaction of three individual antisera to the L-polypeptide with the L-polypeptide (open circles), the D-polypeptide (solid triangles), and the L-polypeptide in the presence of an equal concentration of the D-polypeptide (solid circles)

Table 2. IMMUNOLOGICAL RESPONSES TO THE OPTICALLY ISOMERIC SYNTHETIC POLYPEPTIDES

Polypeptide	Bleeding I			Bleeding II		
	Animals responding	μg AbN/ml. average	± *S.D.*	Animals responding	μg AbN/ml. average	± *S.D.*
Glu$_{58}$Lys$_{42}$	6/12	13	15	2/8	13	14
D-Glu$_{57}$D-Lys$_{43}$	0/24	0		0/9	0	

peptide, nor did the D-polypeptide inhibit the homologous reaction with the L-polypeptide. Three experiments showing this result are summarized in Fig. 1. In these experiments equal volumes (0·4 ml.) of a solution containing the D-polypeptide at five concentrations and antisera to the L-polypeptide were mixed and allowed to stand at 2° C for five days. At the end of this period the samples were centrifuged and the supernatants decanted. There was no precipitation (solid triangles in Fig. 1). The supernatant (0·6 ml.) and a solution of the L-polypeptide (0·3 ml.), to give a final concentration equal to that of the D-polypeptide, were then mixed and allowed to stand at 2° C for 5 days. The amount of precipitate formed was the same as that obtained when only the L-polypeptide was present (cf. solid and open circles, respectively, in Fig. 1). This result eliminates the possibility that the D-polypeptide reacts with the antisera to the L-polypeptide to form soluble complexes.

The physical and chemical properties of the two polypeptides are very similar. The small compositional differences should not affect the cross-reaction in view of the cross-reactivity between polypeptides with more disparate compositions[4,5]. The molecular weight difference is negligible in view of the cross-reactions between the L-polypeptide (molecular weight = 37,000) and a similar polypeptide of 111,000 molecular weight[10]. The α-helical contents of the two polypeptides are seen to be essentially the same, lying within the accuracy of the determination, although the helical senses are opposite. Finally, the finding that the acid and base dissociation constants and the overall shape of the titration curves are the same indicates that the average distribution of the two amino-acids, that is, the primary sequences, are not grossly different. These considerations demonstrate that the molecules of the two polypeptides are essentially mirror images. Yet one is antigenic and the other is not, and the latter does not cross-react with antisera to the former.

The failure of the cross-reaction leads to the conclusion that the amino-acid side-groups at the spacing characteristic of the peptide chain do not completely determine the antigenic specificity of the polypeptide. Classical studies on the antigenic specificity of proteins have involved modifying the side-groups, and showing that the specificity is changed[11,12]. Indeed, the modification may be the attachment of polypeptides[11,13] or of D- and L-amino-acids[14] to the protein. The present work contrasts with these studies in that the amino-acids in the two polypeptides are the same, but their spacial orientation on the peptide chain is different. Thus, the discrimination displayed by the antibody extends to a section of the peptide chain in combination with its attached groups.

The observation that the D-polypeptide did not elicit antibody production does not at present have a unique explanation. The failure may be either in the sequence of steps leading to antibody production, or in the inability of the γ-globulin chain to fold around the determinant portion of the D-polypeptide. The first explanation includes the possibility that the D-polypeptide cannot be transported to the site of antibody production, or that some prerequisite hydrolysis cannot be performed by the usual enzymes. Some of these possibilities are open to experimental investigation. The second possibility cannot be tested directly, but an exploration with models may offer some test of its validity. Of course, the result is also compatible with the 'selective' hypothesis of antibody formation since the genetic code for antibody directed against the 'unnatural' D-polypeptide would be lacking.

In summary, we have found that the L-amino-acid configuration is essential for a polypeptide to display antigenicity. This suggests that the immune response would not operate against 'proteins' constructed of D-amino-acids, that is, mirror images of those which are normally antigenic. In addition we have demonstrated that the D-polypeptide does not precipitate with antisera to the corresponding L-polypeptide. This failure reveals that the antibody senses the geometrical construction of a region of the peptide chain and not only the amino-acid residues in particular arrangements.

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DESTRUCTION OF PASTURES BY PARAQUAT AS A SUBSTITUTE FOR PLOUGHING

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PARAQUAT (1,1′-dimethyl-4,4′-bipyridylium dichloride) is very active against graminaceous species[1,2]. Because it is rapidly adsorbed by all but the sandiest of soils, and thereby inactivated, it can be used for killing grasses in pastures which may be re-sown immediately without ploughing[3].

To test the use of paraquat for killing swards prior to growing arable crops, two field experiments were carried out at Jealott's Hill. One was permanent pasture followed by winter wheat sown in autumn 1961; the other was three-year ley followed by spring wheat sown in 1962.

Experiment 1. On perennial ryegrass (*Lolium perenne*) dominant permanent pasture, the three main treatments were paraquat (at 3 lb. ion per acre in 24 gallons water containing 0·1 per cent 'Agral' 90) applied on September 19, a similar treatment followed by ploughing on October 7 and a no chemical treatment ploughed on September 12.

On October 12, Hybrid 46 winter wheat was sod-seeded on the first treatment and drilled on the other two treatments.

The three main treatments were duplicated in randomized blocks and each plot was split into four sub-plots, each of 0·016 acre, for nitrogen manuring of the wheat in the spring.

Paraquat completely killed all foliage within seven days. Wild white clover (*Trifolium repens*) and some broad-leaved weeds recovered but were controlled by 'Methoxone' *C* spraying in the spring. Only a few grass plants, mainly meadow foxtail (*Alopecurus pratensis*), rough-stalked meadow grass (*Poa trivialis*), bent (*Agrostis* spp.) and perennial ryegrass, re-appeared on the sprayed/sod-seeded and the no spray/ploughed plots. The plots sprayed before ploughing remained virtually free of weed grasses throughout.

The season was particularly favourable to winter wheat and the rich soil produced outstanding yields with no response to nitrogen fertilizer (Table 1). The sod-seeding treatment was outyielded significantly by both other treatments but still gave a highly satisfactory yield. The seeder used had blunt hoe-type coulters which tore up the turf, made rough furrows and caused patchy establishment.

Table 1. GRAIN YIELDS OF WINTER WHEAT IN CWT./ACRE (ADJUSTED TO 85 PER CENT DRY MATTER)

(1) Sprayed/sod-seeded	55·4
(2) Sprayed/ploughed and drilled	60·7
(3) Not sprayed/ploughed and drilled	58·3
Significant difference (*P* = 0·05)	2·9

Experiment 2. On timothy (*Phleum pratense*)/meadow fescue (*Festuca pratensis*)/white clover ley, the three main treatments were paraquat (as in Exp. 1) on February 19, sod-seeded with 'Jufy I' spring wheat on February 20, paraquat (as in Exp. 1) on February 19, sod-seeded with the same variety on March 13 and no chemical treatment ploughed on February 6 and drilled with the same variety on March 13.

The three main treatments, duplicated in randomized blocks, were each split into three sub-plots of 0·017 acre for nitrogen manuring of wheat in the seed-bed.

All foliage was killed by paraquat within about ten days. White clover and some broad-leaved weeds re-grew, but were controlled by 'Methoxone' Extra spraying.

Germination and brairding of wheat following the February sod-seeding were delayed by cold weather throughout February and March and so any advantage from early sowing in this season was probably less than would be expected in more normal seasons. Final numbers of seedlings on the February or March sod-seeded plots were only about 60 per cent of those on the cultivated plots, possibly due to inadequate tilth in the slit-like furrows made by the disk coulters on the sod-seeder used (which was different from that used in Exp. 1) and to depredations by slugs and other soil pests.

Paraquat killed sown grass species, but had only a temporary effect on couch (*Agropyron repens*), which re-grew over large areas and competed severely with the crop. Ploughing, on the other hand, reduced couch infestation considerably.

Ploughing and drilling in March outyielded sod-seeding at all levels of nitrogen fertilizer application and was also superior to February sod-seeding at 0 and 60 but not at 90 units nitrogen/acre, at which level an inexplicable depression in yield occurred on the ploughed plots (Table 2).

Table 2. GRAIN YIELDS OF 'JUFY I' SPRING WHEAT IN CWT./ACRE (ADJUSTED TO 85 PER CENT DRY MATTER)

	Units fertilizer N/acre		
	0	60	90
(1) Sprayed/sod-seeded February	18·7	28·9	31·4
(2) Sprayed/sod-seeded March	17·2	26·8	26·2
(3) Ploughed/drilled March	27·8	32·0	29·8
Significant difference (*P* = 0·05)		2·0	

Sod-seeding was markedly inferior to conventional practice at the no nitrogen fertilizer-level: this was thought to be due to the effects of competing couch grass particularly in the absence of fertilizer nitrogen rather than to inadequate release of mineralized nitrogen from the uncultivated, dead turf. The advantage from ploughing and drilling over sod-seeding declined with increasing nitrogen rate.

These experiments confirm the suggestion of Arnott and Clement[4] that satisfactory crops may be grown in pastures which, instead of being ploughed and cultivated, have been destroyed by herbicidal sprays; they also point to such practical problems as the design of an efficient coulter for sod-seeding and the control of couch grass and of various soil pests, including slugs, which seem to thrive in unploughed land. Nevertheless, the results of these first experiments are promising for this new technique. As nothing is yet known about the effect of repeated sod-seeding on soil and crops, the present work is being continued. Further experimentation is required to assess the general applicability of this technique in agricultural practice.

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[2] Jeater, R. S. L., and McIlvenny, H. C., *Proc. Fifth Brit. Weed Control Conf.*, 1, 311 (1960).

[3] Jones, L., *J. Brit. Grassl. Soc.*, 17, 1, 85 (1962).

[4] Arnott, R. A., and Clement, C. R., *Nature*, 195, 1277 (1962).

NEWS and VIEWS

Association of the British Pharmaceutical Industry :
G. E. Yates

MR. G. E. YATES has been appointed director of the Association of the British Pharmaceutical Industry. Mr. Yates, who is fifty-five, was educated at George Watson's College, Edinburgh, and the University of Edinburgh, taking his M.A. with honours in history. He entered the Home Civil Service in 1931 and served in the Ministry of Health, the Ministry of Home Security, and the Office of the Minister of State in the Middle East. Mr. Yates was a member of the United Kingdom delegations to the United Nations and other international conferences at the end of the War, and, as a delegate to the International Health Conference in New York in 1946, signed the treaty establishing the World Health Organization. He transferred to the Secretariat of the United Nations in 1946; served as secretary of the United Nations Economic and Social Council, and later as senior director in the Department of Economics and Social Affairs. He was executive secretary of many United Nations conferences, including the special commission of the United Nations Economic and Social Council which set up the United Nations expanded programme of technical assistance in 1949 in Geneva, and of the plenipotentiary conference for the Single Convention on Narcotic Drugs, New York, 1961.

Mr. A. Duckworth continues as secretary of the Association of the British Pharmaceutical Industry.

The Public Health Laboratory Service Board :
Prof. J. W. Howie

PROF. JAMES WILLIAM HOWIE has been appointed director of the Public Health Laboratory Service Board in succession to Sir Graham Wilson, who retires from the post in September 1963. Prof. Howie, who is fifty-five years of age, has held the chair of bacteriology in the University of Glasgow since 1951, and is also senior consultant bacteriologist to the Western Infirmary, Glasgow. He is vice-president of the College of Pathologists, and is a member of the Agricultural Research Council. He graduated in medicine at the University of Aberdeen, gaining honours both in his first degree and in his doctorate. He became a lecturer in the Universities of Aberdeen and Glasgow, and then served in the Royal Army Medical Corps during the Second World War as a specialist pathologist in Nigeria and at the War Office. Thereafter he was for five years head of the Department of Pathology and Bacteriology in the Rowett Research Institute, Aberdeen.

The Medical Research Council

IN written answers to questions about the Medical Research Council in the House of Commons on January 29, the Parliamentary Secretary to the Minister for Science, Mr. D. Freeth, stated that in the past five years the Medical Research Council had set up twenty-seven research units and nine research groups, and had also decided to establish a Clinical Research Centre to be linked with the new district hospital to be built at Northwick Park, Harrow. The Council's staff had increased from 2,213 in 1957–58 to 2,772 in 1961–62, its total expenditure on current grants had risen from £201,483 to £481,038, and the number of awards from 138 to 259. This does not take account of support provided for medical research through its research units and groups, training awards and grants for special departmental apparatus. Expenditure on research grants to universities had risen from £201,483 to £561,700.

Psychiatric Research in Britain

IN a written answer in the House of Lords on February 7, the Minister for Science, Viscount Hailsham, stated that the expenditure of the Medical Research Council on psychiatric research had risen successively from £63,000 in 1957–58 to £130,000 (1958–59), £170,000 (1959–60), £228,000 (1960–61) and £247,000 in 1961–62. The money was used during 1961–62 to support the Council's eight psychiatric research units as well as investigations relevant to mental illness being undertaken in other units in social medicine and genetics. It included a special grant to the Institute of Psychiatry, Maudsley Hospital, London; support for five members of the Council's external scientific staff studying problems of mental illness; and nineteen research grants and eleven training awards. More recently the Council had established a research group at King's College, Newcastle upon Tyne, to study the relation between organic and functional mental disorders, and research in psychiatry was also undertaken in university departments and in teaching hospitals with support from the University Grants Committee as well as in the National Health Service.

The Commonwealth Scholarships (Amendment) Bill

AT the Committee stage of the Commonwealth Scholarships (Amendment) Bill in the House of Commons on January 28, the Government was strongly pressed regarding the eligibility for scholarships of those who had not received formal education of university standard, and the Commission was criticized as too strongly representative of the academic outlook. In reply, the Under-Secretary of State for Commonwealth Relations, Mr. J. Tilney, quoted a directive of March 4, 1960, from the Secretary of State for Commonwealth Relations: "Awards tenable at residential colleges of adult education which provide one-year or two-year residential courses may also be considered. These would be especially suitable for students of mature age who have not attended a university". It was therefore open to the overseas countries to nominate non-graduates, but they could not be forced to do so. Other scholarships were available, including those from the British Council. [See also pp. 731 and 752 of this issue of *Nature*.]

The Commonwealth Scholarship Commission

THE third annual report of the Commonwealth Scholarship Commission, covering the year ended September 30, 1962, records a total of 381 scholars in attendance at United Kingdom universities and colleges in the session 1961–62 (Pp. 44. London: H.M.S.O., 1963. 2*s*. 6*d*. net). As expected, more than half the scholars for 1960 applied for extension of tenure of their awards into 1962–63, and accordingly the Commission decided it could not offer more than 209 new awards, for which 396 nominations were submitted. Owing to late withdrawals, only 188 candidates accepted scholarships and the statutory limit of 500 held in 1962–63 was not reached. This limit will be removed under the Bill now before Parliament (see pp. 731, 752 and preceding note). Some 232 applications were received from United Kingdom candidates for awards tenable in eleven different Commonwealth countries, and during the period covered United Kingdom students held Commonwealth Scholarships in nine Commonwealth countries. Four invitations to Commonwealth visiting fellowships for tenure in the session 1961–62 were accepted, but one was afterwards agreed for tenure at a later date. Total expenditure on the Plan in the United Kingdom in the year was £290,000, and the Commission is satisfied that the Plan is making a real and valuable contribution to educational co-operation in the Commonwealth at a high level.

Improving Britain's Overseas Technical Co-operation

In reply to a question in the House of Commons on January 29, the Secretary of Technical Co-operation, Mr. D. Vosper, said that he had reviewed how far Britain's overseas information met the changing needs of the day and had decided to extend the effort in three main ways. First, an increase was proposed in the British Council's English language teaching and training work, including greater use of modern methods. The Council's representation in new and developing Commonwealth and foreign countries would also be strengthened, together with some expansion in Eastern Europe. Secondly, British Information Services in some Commonwealth countries in Asia and Africa would be improved and extended. Thirdly, supplies of television material to the ever-growing number of stations overseas would be increased. This and other changes would involve extra expenditure of about £1 million a year. He also undertook to consider any request from an overseas Government or Governments to send teams of British journalists overseas to conduct short training courses in developing countries if this appeared to be the most suitable way of meeting the need.

Research and Development in Automation

The *B.C.A.C. Record*, 1961–62, published for the British Conference on Automation and Computation through the Institution of Production Engineers, describes the work of the Conference during the past two years. Besides Lord Hailsham's address to the Harrogate Conference on "Automation—Men and Money" in June 1961, it includes the text of the first two Annual Lectures: by Dr. D. C. Christopherson, "Mathematics—Friend or Foe", and by Dr. B. V. Bowden, "Automation—the Next Phase: Can Managers be Automated?". Prof. G. D. S. MacLellan describes the work of a Panel in the field of education and training, and J. E. Coales of a Panel set up to deal with research and development in automation. Copies of the *Record* are available from H. K. Lewis, Ltd., 136 Gower Street, London, W.C.1 (3*s*. 6*d*., postage paid 3*s*. 10*d*.).

The Federal Institute of Industrial Research, Nigeria

The annual report for 1961–62 of the Federal Institute of Industrial Research, Nigeria, covering the initial period of establishment of the Institute, includes a review of the period 1956–62 (Pp. 21 + 6 plates. Lagos: Federal Ministry of Commerce and Industry, 1962. 9*d*. net). The Institute was established to undertake research on all Nigerian raw materials and to develop effective industrial processes by pilot- or large-scale trials if required. About two-thirds of the senior staff posts have now been filled, but less than 60 per cent of the complement of twenty-eight scientists and engineers. The major research project has been the mechanized processing of gavi, a fermented dried meal from cassava, and this has led to the successful development of a pilot-plant producing one ton a day. Other main projects in 1961–62 were on fish drying, leaf proteins, investigations on pulp and paper, solar water heating, plant gums and the utilization of cashew apple. A pilot plant producing one ton of coir fibre a day was set up in a coconut producing area and is now running as a successful commercial venture.

The World Health Organization International Centre for Authentic Chemical Substances

The function of the Centre is to assure the collection, storage and international distribution of chemical reference substances under the authority of the World Health Organization. Recently, the collection of chemical substances available from the Centre has been extended by a set of thirteen substances intended for checking the accuracy of different instruments and methods for determining melting temperatures. The set of World Health Organization melting-point reference substances includes:

	M.p.		M.p.
Azobenzene	69° C	Salophen	192° C
Vanillin	83° C	Sulphapyridine	193° C
Benzil	96° C	Dicyandiamide	210° C
Acetanilide	116° C	Saccharin	229° C
Phenacetin	136° C	Caffeine	237° C
Benzanilide	165° C	Phenolphthalein	263° C
Sulphanilamide	166° C		

The substances are available in a polystyrene plastic package together with a leaflet on "Directions for Use". The following authentic chemical substances are also available:

	Size (g)		Size (g)
Chloramphenicol	0·8	Oestrone	0·03
Digitoxoside	0·05	Progesterone	0·065
Ergometrine maleate	0·02	Tubocurarine chloride	0·03
Melarsen	1	Vitamin A acetate	Five capsules, each containing 0·25 g of a solution in cottonseed oil containing 0·0344 g of vitamin A acetate in each gram of solution. In terms of formerly used International Units, the solution represents 100,000 I.U. per gram.

All the substances are sent free of charge to national administrations or laboratories and institutes working on a non-profit basis. Commercial laboratories are charged 10 dollars (U.S.) or the equivalent amount in other currency per set of melting-point reference substances and 4 dollars (U.S.) per package of the other substances. Orders for the substances should be sent to: WHO International Centre for Authentic Chemical Substances, Apotekens kontrollaboratorium, Box 30019, Stockholm 30. During 1963 the following substances are expected to be issued as authentic chemical substances: cortisone acetate, deoxycorticosterone acetate, dienoestrol, diethylstilboestrol, oestradiol benzoate, ethinyl oestradiol, ethisterone, hydrocortisone, hydrocortisone acetate, liothyronine, methyltestosterone, prednisolone, prednisone and testosterone propionate.

The Zoological Society of London: Awards

The following awards of the Zoological Society of London will be presented by H.R.H. The Duke of Edinburgh, president of the Society, at the annual general meeting on May 16: *Scientific Medal*, to Dr. J. W. L. Beament, of the Department of Zoology, University of Cambridge, for his outstanding work on the detailed physical and chemical study of the insect cuticle and egg shell and the discovery of important general principles concerning water conservation in small terrestrial mammals; and to Dr. N. A. Mitchison, of the National Institute for Medical Research, for his outstanding work on immunology and tissue transplantation; *Stamford Raffles Award*, to Mr. R. E. Moreau, in recognition of his contributions to zoology—as an amateur zoologist, Mr. Moreau has acquired an international reputation as an authority on the birds of Africa; *Thomas Henry Huxley Award*, to Dr. J. Machin, of Queen Mary College, now working at the University of Toronto, for original work submitted as a Ph.D. thesis to the University of London on "The Water Relations of Snail Integument"; *Prince Philip Prize* (open for competition to pupils of zoology, taking the General Certificate of Education Advanced-Level or equivalent examination in any school in Great Britain, Northern Ireland and the Channel Isles and Isle of Man), to Wendy A. Freaker, of St. Swithun's School, Winchester, for her entry, "An Investigation into the Casing Habits of the Caddis-fly Larva, *Limnophilus vittatus*".

The Australian Polarographic Society

The Australian Polarographic Society was founded at the inaugural meeting held at the University of New South Wales on December 3, when the relative merits of an electrochemical and of a polarographic society were discussed. It was agreed that the initial viability of the Society would be enhanced by concentrating the endeavour in such a way that the activities would tend to be of immediate interest to the great majority of members. On this basis, the foundation of a Polarographic Society was preferable at this stage. At a later time, when the Society had proved its viability and value, the prospects for an Electrochemical Society could be reassessed. The objective of the Society is: "To promote the development of polarography in Australia by affording opportunities for contact between those interested in polarography and related fields and by any other means that the Society shall from time to time determine". A Provisional Council has been elected: *President*, Prof. B. Breyer; *Vice-President*, G. S. Buchanan; *Secretary-Treasurer*, J. W. Hayes; *Councillors*, G. H. Aylward, Dr. H. H. Bauer and T. M. Florence. State Representatives are to be co-opted from each State/Territory. Membership is open "to all persons interested in furthering the aims of the Society"; details are available from the secretary-treasurer, Department of Analytical Chemistry, University of New South Wales, Box 1, P.O. Kensington.

The Institute of Mathematical Sciences, Madras

On the occasion of the first anniversary of the Institute of Mathematical Sciences, Madras, announcements were made of two annual visiting professorships entitled: (1) the Niels Bohr visiting professorship; (2) the Ramanujam visiting professorship. The first is a tribute to the memory of one of the creators of modern physics and that of the founder of quantum theory. His interest in the advancement of science in India and in particular the work of the young group of theoretical physicists at Madras was the immediate stimulus for the creation of this Institute. The second professorship is in memory of the Indian mathematician Dr. Srinivasa Ramanujam. Prof. R. E. Marshak, chairman of the Department of Physics and Astronomy, University of Rochester, New York, has accepted the first Niels Bohr professorship in February 1963. The award of the Ramanujam visiting professorship for this year will be announced later.

Sixth International Symposium on Free Radicals

The sixth International Symposium on Free Radicals will be held at the University Chemical Laboratories, Lensfield Road, Cambridge, England, during July 2–5. Papers will be presented by authors from ten countries. The plenary lectures will be given by Dr. R. Livingston (United States), Dr. G. Wilse Robinson (United States) and Prof. J. Weiss (Great Britain). Following the tradition of these meetings, the emphasis will again be on the preparation and properties of trapped radicals, including fluorescence, ultra-violet and electron spin resonance spectra, structural studies and many aspects of reaction kinetics. In addition to the formal sessions, a full programme of social events has been organized, including special entertainment for wives and friends of participants. The symposium will commence with an introductory lecture on the afternoon of July 2, followed by a reception at the Guildhall. Accommodation for men and women will be available in the colleges of the University. Further information can be obtained from Dr. A. B. Callear, Department of Physical Chemistry, Lensfield, Cambridge.

Eleventh International Congress of Applied Mechanics

The eleventh International Congress of Applied Mechanics will be held in the Deutsches Museum at Munich (Germany) during August 30–September 5, 1964. Apart from a number of invited general lectures, the technical sessions of the Congress will be held in two sections: Section 1, mechanics of solids; Section 2, mechanics of fluids and gases. The Congress will, however, not include thermodynamics or numerical methods and computing machines, with the exception of specific applications to pertinent problems of one of the two sections mentioned above. Those persons wishing to present papers to the Congress should submit them to the Programm-Sekretariat des Mechanik-Kongresses, Stuttgart (Germany), Technische Hochschule, Institut für Mechanik, before February 1, 1964 (except that by arrangement with the U.S. National Committee on Theoretical and Applied Mechanics, contributions from the United States will be by invitation, and likewise with the exception of papers submitted by the authors from the U.S.S.R., for which a special arrangement has been made with the U.S.S.R. National Committee on Theoretical and Applied Mechanics). Further information can be obtained from the Organisations-Secretariat des Mechanik-Kongresses, München 2 (Germany), Institut für Mechanik, Arcisstrasse 21.

Naturally Occurring Peptides, Peptide Antibiotics, Proteins and Nucleoproteins

Prof. K. T. Yasunobu and L. K. Ramachandran have written to the Editor stating that they are compiling data on the physical, chemical, functional and structural properties of: (*a*) naturally occurring peptides; (*b*) proteins and nucleoproteins; (*c*) enzymes. They anticipate eventual publication of the data in the form of a book, and ask for any pertinent data to be forwarded for them at the Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii.

Announcements

Dr. T. H. Benzinger, director of the Bio-Energetics Laboratories of the Naval Medical Research Institute, National Naval Medical Center, Bethesda, has been awarded the Golden Scheele Medal of the Chemical Society of Stockholm, for his work on "Calorimetric Analysis of Chemical Change".

The sixth International Mineral Processing Congress will be held in Cannes during May 26–June 2. Further information can be obtained from the Sixth International Mineral Processing Congress, 28 rue Arthur-Rozier, Paris XIX^e.

A conference on "Inert Gas Compounds" will be held at Argonne National Laboratory during April 22–23. Further information can be obtained from Herbert H. Hyman, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois.

The fifth International Conference on Coal Science will be held in Cheltenham, Gloucestershire, during May 28–30. Further information can be obtained from Mr. R. G. J. Kingsmill, National Coal Board Research Establishment, Stoke Orchard, near Cheltenham, Gloucestershire.

The South African National Conference on Nuclear Energy will be held in Pretoria during April 8–11. The main object of the meeting is to make a survey of isotopes and radiation research in South Africa and its future. Further information can be obtained from the Atomic Energy Board, Post Bag 256, Pretoria.

An international symposium on "Humidity and Moisture—Measurement and Control in Science and Industry", sponsored by the National Bureau of Standards, the United States Weather Bureau, the Instrument Society of America, the American Meteorological Society and the American Society of Heating, Refrigerating and Air Conditioning Engineers, is to be held in Washington during May 20–23. Further information can be obtained from Mr. A. Wexler, National Bureau of Standards, Washington 25, D.C.

THE SECOND COMMONWEALTH EDUCATION CONFERENCE

THE brief report of the Second Commonwealth Education Conference*, held at New Delhi during January 11–25, 1962, is accompanied by various annexes. To three of these—the reports of the committees set up by the Conference to study and made recommendations on the Commonwealth Scholarship and Fellowship Plan, the training and supply of teachers, and technical education and the provision of text-books and other books—reference is made elsewhere (see p. 731 of this issue of *Nature*). Others comprise the reports of committees dealing with social education, with education in rural communities, and with financial problems of educational expansion. A further annex details the composition and functions of a Commonwealth Education Liaison Committee and a Commonwealth Liaison Unit.

The Conference was encouraged by the progress already made since the first Conference at Oxford in 1959, particularly under the Commonwealth Scholarship and Fellowship Plan and in the further training of teachers from the newer Commonwealth countries. Notable was the progress under the Bursary Scheme introduced by the British Government. It was agreed that in the years ahead the main emphasis should be placed on the further development of the schemes for the supply and training of teachers and for the further training of those who are to occupy key posts in developing educational systems. However, some of the developing countries were quite clear that scholarships at undergraduate level and awards in professional, technical and other fields would meet their present needs more effectively than awards at post-graduate level. The Conference decided that arrangements established tentatively at Oxford to facilitate Commonwealth co-operation in education should be confirmed as appropriate for constructive use by Governments.

It was recommended that a Commonwealth information centre on the teaching of English as a second language should be established in London, and that the establishment of regional English language centres closely linked with universities and training colleges be given urgent attention. Development of the practice and techniques of social education is at different stages throughout the Commonwealth, and any programme of social education requires the active support of Government, voluntary organizations and voluntary community leaders. Voluntary organizations, above all, must have adequate facilities to play their part effectively. Social education should receive high priority in educational development programmes.

The problems of rural education in different parts of the Commonwealth have much more in common than differences of latitude, climate or history might suggest. Although it is necessary first to establish the generally desirable objects of rural educational policy, even at this early stage recommendations are warranted concerning the systematic exchange of information, arrangements of visits by experts, conferences of experts on matters of common interest, etc. Agricultural education should be considered as a part of technical or vocational education as a whole and not treated in isolation.

Financial problems of educational expansion were not discussed quantitatively, but the Committee considering this aspect recommended that countries requiring assistance in respect of capital or recurrent expenditure for educational expansion should seek it through bilateral arrangements. Close attention should be given to educational planning to secure the most effective use of the available funds and every effort made to assure the interest of the community.

There is thus wide scope for co-operation among Commonwealth countries. This is especially so in meeting the major needs of the various countries in respect of educational and training opportunities, the supply of teachers, particularly in science and mathematics, and of specialists in technical and higher education, and the supply of specialized equipment and text-books.

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* Ministry of Education. Report of the Second Commonwealth Education Conference. Pp. iii+86. (Cmnd. 1655.) (London: H.M.S.O., 1962.) 5s. 6d. net.

SCIENTIFIC RESEARCH IN BRITISH COLONIAL TERRITORIES

ALTHOUGH reduced in scale and scope, *The Colonial Territories, 1961–62**, now compiled with the help of the Department of Technical Co-operation, continues on similar lines, but there is a marked absence of information of particular interest to the scientist or technologist. Here, as elsewhere, a close veil is drawn over all research work proceeding overseas, and only in the short notes on technical and higher education and on medical and health services are matters of primary interest mentioned.

Campaigns against malaria and yaws have in some territories reached the stage at which maintenance services can be established and more attention devoted to problems of environmental sanitation in rural areas. Rural water supply and sanitation schemes continued in Kenya with aid from the World Health Organization and United Nations Children's Fund so satisfactorily that it is planned to extend these activities to other areas. Although little progress was made in providing facilities for the mentally ill, problems of mental health were more prominent. Dental health services steadily developed. Malaria and tuberculosis continued to be major preoccupations in many territories, but in some very substantial progress was made towards control and eradication of malaria, and although leprosy continued to be a serious problem in certain territories there was some evidence of reduced incidence. Following active eradication campaigns the importance of yaws has greatly declined in most territories, but trypanosomiasis is a continuing threat necessitating consistent and careful measures, and the report indicates the extent to which preventive medicine in industry and health education offer opportunities for expansion and assistance.

Enrolment at universities continued to increase and reached 1,600 at Hong Kong and 1,257 at the University College of the West Indies. A provisional council has been set up for the proposed University of East Africa, and to meet the urgent need for veterinary practitioners in East Africa, improved facilities for veterinary education are being developed near Nairobi, and responsibility for the new veterinary faculty is being transferred from the Makerere University College to the Royal College, Nairobi.

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* Colonial Office. *The Colonial Territories, 1961–1962*. Pp. vi+136. (Cmnd. 1751.) (London: H.M.S.O. 1962.) 8s. 6d. net.

THE BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE

THE seventh annual conference of the British Society for the Philosophy of Science was held during September 21–23, in Tetley Hall, University of Leeds. It comprised a colloquium of four symposia and was attended by some sixty members and guests. Mr. F. T. C. Harris of Middlesex Hospital Medical School was conference secretary.

The four symposia were entitled: "The Idea of a Physical Quantity", "Fundamental Particles and Identity", "Kant's Regulative Principles and their application to Science", and finally, "The Nature of Law in Social Science". In conformity with the Society's usual practice each symposium heard two papers and then entered into a discussion of them.

With the Society's president, Prof. R. B. Braithwaite, in the chair, Dr. J. R. Ravetz (University of Leeds) introduced the first symposium with a historical paper "Galileo and Physical Quantities", which discussed the belief in the existence of physical quantities and the problem of assigning measures to these quantities. Dr. Ravetz claimed that the first of these was an essential assumption in Galileo's investigations, while the second is necessary for the construction of a fruitful natural philosophy. There followed an inquiry into the relationship between the concepts of 'quality' and 'quantity' in which the speaker, Dr. H. V. Stopes-Roe (University of Birmingham), argued that the former concept included the latter. For example, the quality brightness can be given a measure and so construed as a quantity. Gallileo's idea of fundamental individual quantities was claimed by Dr. Ravetz to be inadequate, leading Galileo away from the formulation of a coherent approach to units and thus away from numerical results. On the other hand, Dr. Stopes-Roe suggested that Fourier's approach to this problem similarly failed to treat the unit satisfactorily. He went on to suggest that the concept of quantity should be taken as basic and combined with a liberal approach to the analysis of the concept 'quantity'.

Dr. C. W. Kilmister (King's College, London) took the chair at the second symposium, the first paper of which was a very thorough survey by Dr. H. R. Post, of the Chelsea College of Advanced Technology. Dr. Post explained that elementary particles of one type had no individuality. It was a fallacy to derive this non-individuality from Heisenberg's Principle. In fact it derived from the nature of the theoretical structure of quantum mechanics. Fundamental particles are without 'secondary qualities', he insisted, and their lack of individuality derives less from their ontological status than from their high level of abstraction.

In the second paper Dr. E. W. Bastin (King's College, Cambridge) took the point of view of ontological realism. He argued that in electron spin one had a property which was uniquely identifiable, but must, of course, be attributed to individual events, particles, which existed as such. In discussion Prof. Braithwaite and Dr. Kilmister took the side of Nature, the latter with currently new empirical data.

Prof. S. Korner was chairman at the third symposium, which was opened by Mr. Jonathan Bennett with an argument directed to disproving Kant's view that a science in which some generalizations have exceptions is intellectually disreputable. Contrary to this, Mr. Bennett held that the scientist must qualify his generalizations if he is to avoid confusion. Mr. Bennett explained a 'regulative principle' as one that had no other justification than its utility. Thus, standing unanalysed, it bears comparison to a logical axiom. The Kantian view that everything has a cause is just such a principle. Mr. Bennett went on to show that Kant's claim that the principle of causation had such a status is in fact at variance with that writer's attempted proof elsewhere, of that very same principle: but under the name 'the second analogy from experience'. Entirely to the contrary, Dr. G. Buchdahl (The Whipple Museum, Cambridge) accepted Kant's distinction between a general principle of causation and a 'regulative' principle of determinism. He held that, of the two versions of the deterministic principle discussed by Mr. Bennett, only the first is truly regulative and it is this version that permits the law-like nature of empirical generalization. The chairman introduced the discussion and directed attention to the philosopher's distinction between logical implication and nomical implication, or implication reflecting things as they are. He pointed out that Kant did not regard Newtonian physics as a regularity which happened to be observed, but did in fact believe it to be true. As Prof. Korner remarked, "The opus of great philosophers is a kind of philosophical Rorschach test".

The discussion of the final symposium was carried out under the guidance of Mr. J. W. N. Watkins (London School of Economics). The symposium opened with a paper by Mr. J. G. H. Newfield (London School of Economics), which criticized the views expressed in Mr. P. G. Winch's *The Idea of a Social Science and its Relation to Philosophy* (London, Routledge and Kegan Paul, 1958). Mr. Newfield argued that to criticize sociology by means of *a priori* philosophical criteria was to make a category mistake. For example, it was not a prerequisite for sociology that sociologists study the language of their science, a test procedure for laws was simply to distinguish between their valid and invalid applications and this did not necessarily involve formulating the law. Mr. P. G. Winch (University of Wales) argued that the Humeian position taken by the previous speaker was fully answered by Kant, and it was this philosopher that Mr. Winch followed. It was not the case that regularities were first observed and then logic entered the field. It was more cogent to argue that the observation of empirical regularities was only possible within a preconceived frame-work of order, which was an argument in accord with the hypothetic-deductive point of view of Prof. K. R. Popper. Mr. Watkins agreed with the second speaker that phenomena arise from language. He also argued that Mr. Winch's approach did not admit of social criticism in that it had not been equipped with a metalanguage. Prof. W. C. Kneale, the president-elect, took up a point concerning the reducibility of sociological concepts to dispositional statements about individuals. He pointed out a fundamental difference between the reduction of a behavioural science, sociology, to psychology, and of a physical science, chemistry, to physics, in that the former reduction would have to embrace two different conceptual schemes. For example, it was not possible to describe the spread of economic consumption in psychological terms.

In his closing address the president of the Society, Prof. R. B. Braithwaite, thanked the conference secretary and Mr. B. C. Brookes, University College, London, for their efforts on behalf of the conference, the warden of Tetley Hall, Miss E. M. McAlpine, for the care taken by her staff and herself for the comfort of the participants and the Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, for secretarial assistance.

D. G. HARRIS
F. T. C. HARRIS

EDUCATION OF SCIENTISTS AND TECHNOLOGISTS FOR THE NEW AGE

IN his presidential address to the Science Masters' Association in Manchester on January 2, Dr. B. V. Bowden, pointing out that Britain is already spending more than £800 million a year on education, or 4 per cent of the gross national product, questioned whether all this money was being spent properly, efficiently and on the right things, and whether enough or too much was being spent. In this context, he challenged strongly the meagre expenditure of about £20,000 in 1962 by the Ministry of Education to investigate the efficiency of educational progress. Even making the most generous allowance for expenditure by university departments of education, he doubted whether Britain's expenditure on educational research reached £250,000 a year, and while he valued the impetus from the United States and the initiative and generosity of the Nuffield Foundation, most of the research into new syllabuses and text-books had still to be done. Education was, he thought, as profitable an investment as was open to any nation to-day, and this was especially true in respect of education in the under-developed countries.

Challenging next the belief of many schoolmasters that the universities provide courses in technology for boys who have no aptitude for science, Dr. Bowden, noting that science began in an attempt to solve practical problems and paying tribute to Sanderson's work at Oundle, insisted that science was much more than a body of knowledge; it was a way of life, a way of thought, and at its best only understood by active practitioners. He was concerned that so many of Britain's ablest schoolboys were interested in science but not in technology and engineering. While a successful engineer or technologist must know his science and be able to work with people, even to lead them, it was vital that schoolmasters should strive to persuade more of their pupils to take up the life of the creative technologist rather than that of the scientist. Few boys, in fact, even understood that technologists could be trained at a university. Finally, he thought there was room for a huge programme of research into methods of teaching and the possible effect of new techniques based on modern technology, including the study of the processes of learning and the use of television and films.

The full text of Dr. Bowden's address will be published in the March issue of *The School Science Review*, published by the Science Masters' Association.

RHEOLOGY OF MOTOR OILS

AT the meeting of the Cambridge Rheology Club held on October 29, Mr. J. F. Hutton ('Shell' Research, Ltd.) spoke on "The Rheology of Motor Oils". He started by describing briefly the manufacture of lubricating oils from crude petroleum, continued with a discussion of lubricating oils as Newtonian liquids, and ended with a description of experimental work which has shown that lubricating oils, which are generally regarded as Newtonian liquids, can exhibit non-Newtonian effects and behave as visco-elastic liquids at very high rates of shear.

The most important property of a lubricating oil is its viscosity and the temperature of the oil is the external factor which has the greatest influence on viscosity. A rise in temperature of 1 deg. C causes a decrease in viscosity of the order of 5 per cent. It is desirable that the dependence of the viscosity of an engine lubricating oil on temperature should be reduced and this is achieved primarily by suitable refining. An additional reduction is obtained by dissolving in the oil about 1–3 per cent of a polymer having a weight-average molecular weight of $1–5 \times 10^5$. The most effective type of polymer is a copolymer made from oil soluble and oil insoluble monomers and so designed that its intrinsic viscosity, or thickening effect, increases as the temperature increases.

Addition of a polymer to an oil introduces non-Newtonian properties which, being of importance in lubrication, have been studied by many workers. However, in the short time available, Mr. Hutton chose to talk about the more interesting problem, from the point of view of liquid structure, of non-Newtonian effects in lubricating oils not containing polymers. The detection and measurement of such effects in capillary or Couette viscometers is a most difficult undertaking. Rates of shear greater than 10^5 sec^{-1} are necessary, and in this range it is difficult to separate true shear rate thinning from a fall in viscosity due to viscous heating. A different approach to the problem has been made, notably by J. Lamb and A. J. Barlow, who, from measurements of the propagation of ultra-sonic shear waves through the oils have calculated their relaxation spectra. The results clearly demonstrate the visco-elastic nature of lubricating oils and indicate that the peaks in the spectra are characteristic of the types of molecule present.

In the discussion the comments and questions ranged widely from technological ones concerning the best oil to use in a motor-car to more fundamental ones concerning heating effects in viscometry and the relation between experiments carried out in continuous shear and experiments carried out in oscillatory shear.

The next meeting of the club will be held on March 4 at the Technological Research Station, Spillers, Ltd., Station Road, Cambridge, when Mr. G. G. Zahler and Mr. G. R. Murfitt (Instron, Ltd.) will speak on "The *Instron* High Shear Capillary Rheometer". H. G. MULLER

THE FISHERIES BIOCHEMICAL RESEARCH UNIT

FOR some years the U.K. Development Commission has sponsored a Unit for Biochemical Research bearing on the problems of fisheries, and at present located within the National Institute for Research in Dairying, Reading. In view of the retirement of the honorary director, Dr. S. K. Kon, within the next few years, the Development Commission has decided that it should be transferred to Aberdeen. The advantages of this new location are that the reconstituted Unit will be in close contact with the fishing industry, the University and the existing marine research institutes in Aberdeen. These are the Scottish Department of Agriculture and Fisheries Marine Laboratory and the Torry Research Station of the Department of Scientific and Industrial Research.

After consultation between the Development Commission and the University of Aberdeen, it was agreed that the Unit should be established within the University under the ægis of the professor of biological chemistry, Prof. W. O. Kermack. It is envisaged that initially the staff would consist of five scientific workers, including a director, who would be a reader in the University, and that members of the staff might take some part in the teaching activities of the University, although their primary obligation would be towards research. By this arrangement, the integration of research and advanced teaching would be assisted, and a mutually stimulating relationship established between the staff of the Unit and their colleagues in the University.

The two existing marine laboratories in Aberdeen deal respectively with the scientific and technological problems concerned with living fish in the sea, and with those involved in the storage and processing of fish in the period between catching and marketing. The object of the Fisheries Biochemical Research Unit will be the investigation of the fundamental biochemistry of both marine and freshwater fish. Although the emphasis will be on the basic scientific aspects, it is envisaged that this work will assist in the solution of problems of practical importance to the fishing industry.

It is proposed to plan and build a new laboratory to house this Unit in close proximity to the existing marine laboratories at Torry in Aberdeen. In order to facilitate this development, the Court of the University of Aberdeen has appointed Dr. P. T. Grant to the post of director of this Unit. Dr. Grant is at present a member of the staff of the Department of Biological Chemistry. It has been arranged that this appointment will become operative at a later date, but meanwhile he will be directly concerned with the planning and equipping of the new laboratory.

Dr. Grant was a member of the staff at the National Institute for Medical Research during 1951–56 and worked with Sir Charles Harington and Dr. H. R. V. Arnstein on the biosynthesis of penicillin, and afterwards with Dr. J. D. Fulton on the comparative biochemistry of pathogenic protozoa. Since he has been in Aberdeen he has worked on the enzymology of the protozoa and in particular has been concerned with the components and mechanism of the unusual respiratory enzyme systems present in the *brucei* group of pathogenic African trypanosomes. He has also been interested in the isolation and biosynthesis of mucopolysaccharides present in connective tissue.

POSITIONS OF THE OXYGEN ATOMS IN $UO_{2 \cdot 13}$

By B. T. M. WILLIS

Metallurgy Division, Atomic Energy Research Establishment, Harwell

URANIUM dioxide, UO_2, can dissolve oxygen at high temperatures to form the single phase UO_{2+x}, where x assumes any value in the range $0 \leqslant x \leqslant 0 \cdot 25$. For $UO_{2 \cdot 10}$ the temperature must exceed 600° C and for $UO_{2 \cdot 25}$ the minimum temperature is about 1,050° C (ref. 1).

Neutron diffraction investigations have been made on a single-crystal of composition $UO_{2 \cdot 13}$ with the aim of determining the positions of the oxygen atoms. The ratio of the slow neutron coherent scattering amplitudes of uranium and oxygen is 1·47, so that, unlike for X-rays, an appreciable proportion of the scattered intensity comes from oxygen. However, we must distinguish between oxygen atoms present in the UO_2 crystal before oxidation and 'extra' oxygen atoms; as the former predominate, the location of the extra atoms is analogous to a light-atom determination with X-rays. In these circumstances very accurate neutron structure-factors are required, and it is necessary to correct the observed intensities for extinction (affecting the strong reflexions) and double-Bragg scattering (mainly affecting the weak reflexions).

All non-equivalent *hkl* planes with an interplanar spacing greater than 0·53 Å were measured at 800° C, inside the single-phase region of the phase diagram. No reflexions were observed other than those with *h*, *k* both odd or both even, and so it was assumed in the analysis by least-squares and Fourier methods that the space-group is *Fm3m*, as for UO_2.

Table 1 summarizes the results of the least-squares analysis, which was carried out using a *Mercury* computer programme specially written for this work by Dr. J. S. Rollett. The oxygen atoms in $UO_{2 \cdot 13}$ occupy three different kinds of crystallographic site, and, as there are more equivalent positions for each site than oxygens to fill them, the oxygens are distributed at random among these positions. The programme allowed as adjustable parameters the probability of occupation of each atomic site, as well as the atomic co-ordinates and temperature factors. Altogether 11 parameters were obtained from 33 independent data, and the final 'discrepancy factor' $(\Sigma||F_{obs}| - |F_{calc}|| \div \Sigma|F_{obs}|)$ was 2·2 per cent, which is close to the estimated accuracy of the individual data.

Table 1. CRYSTAL STRUCTURE OF $UO_{2 \cdot 13}$

Atom	Unit cell co-ordinates	Contribution to formula unit U_mO_n		Temperature factor B (Å²)
Uranium	0 0 0...	$m =$	1·00 (0·01)	1·18 (0·06)
Oxygen O	u u u...		1·82 (0·02)	1·45 (0·08)
Oxygen O′	0·5 v v...	$n =$	0·08 (0·05)	1·8 (1·6)
Oxygen O″	w w w...		0·23 (0·11)	7·5 (2·7)

($u = 0 \cdot 267$ (0·001), $v = 0 \cdot 39$ (0·01), $w = 0 \cdot 38$ (0·01). Space group *Fm3m*. Standard deviations of parameters in brackets.)

The principal conclusions from Table 1 are as follows:

(1) The composition determined from the scattering results is $UO_{2 \cdot 13}$ in agreement with that found chemically. A more complete expression for the formula unit is:

$$U_{1 \cdot 00}O_{1 \cdot 82}O'_{0 \cdot 08}O''_{0 \cdot 23}$$

where O, O′, O″ refer to oxygen atoms in the three kinds of site.

(2) The uranium atoms occupy the face-centred positions 000, $\frac{1}{2}\frac{1}{2}0$, $\frac{1}{2}0\frac{1}{2}$, $0\frac{1}{2}\frac{1}{2}$, and all these sites are filled, as in UO_2.

(3) The O atoms at uuu . . . are close to the fluorite positions $\frac{1}{4}\frac{1}{4}\frac{1}{4}$ . . . , but are shifted slightly along <111> towards the interstitial holes at $\frac{1}{2}\frac{1}{2}\frac{1}{2}$ . . . (the $\frac{1}{2}\frac{1}{2}\frac{1}{2}$, $\frac{1}{2}00$, $0\frac{1}{2}0$, $00\frac{1}{2}$ positions are the largest holes in the UO_2 lattice). The O atoms represent approximately nine out of ten of the original oxygens in UO_2 before oxidation to $UO_{2 \cdot 13}$; the remaining one-tenth are transferred during oxidation to O′ or O″ positions.

(4) The interstitial atoms O′ and O″ do not occupy the large interstitial holes at $\frac{1}{2}\frac{1}{2}\frac{1}{2}$ . . . , but positions about 1 Å away.

Fourier analysis confirms the least-squares results. Fig. 1 is a Fourier projection, based on a Fourier series with the F_{obs} data as coefficients, showing the uranium and oxygen O atoms. Fig. 2 is a difference Fourier projection, using $F_{obs} - F_{UO}$ as coefficients, where F_{UO} is calculated for the uranium and O atoms only. The contours in Fig. 2, which are on a much finer scale than in Fig. 1, show peaks representing the O′ atoms at 0·5 vv . . . and O″ atoms at www . . . .

The least-squares and Fourier results, based on an analysis of the coherent Bragg reflexions only, give the contents and atomic co-ordinates of the average unit cell of the disordered compound: they do not define the local atomic arrangement. However, a plausible interpretation in terms of the local relaxation around an isolated interstitial atom is as follows. This interstitial atom enters the lattice at the O′ position 0·5 0·4 0·4; to maintain charge balance the two nearest U^{4+} ions at $\frac{1}{2}\frac{1}{2}0$ and $\frac{1}{2}0\frac{1}{2}$ are converted to U^{5+}, and the polarizing influence of these U^{5+} ions prevents the interstitial atom occupying the hole at $\frac{1}{2}\frac{1}{2}\frac{1}{2}$. The two nearest oxygens at $\frac{1}{4}\frac{1}{4}\frac{1}{4}$ and $\frac{3}{4}\frac{1}{4}\frac{1}{4}$ are then displaced along <111> to O″ positions and oxygens further out relax a much shorter distance along <111> to O sites at *uuu* . . . . The large 'temperature factor' of 7·5 Å² for the O″ atoms is to be ascribed to the disorder accompanying the relaxation of the nearest-neighbour oxygens.

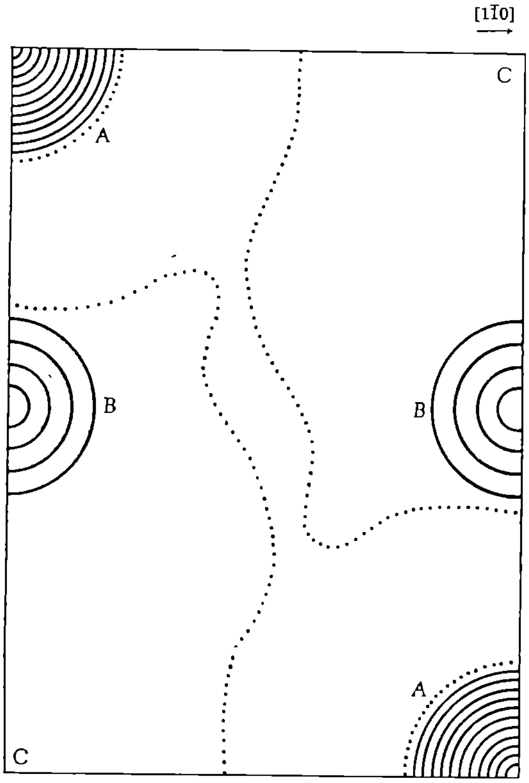

Fig. 1. Fourier projection along [110] of nuclear density of $UO_{2\cdot 13}$ at 800° C. Peaks at *A* represent the uranium atoms at 000 . . . and peaks at *B* the oxygen atoms O at 0 27 0 27 0 27 . . . The interstitial holes are at *C*. Zero contours dotted

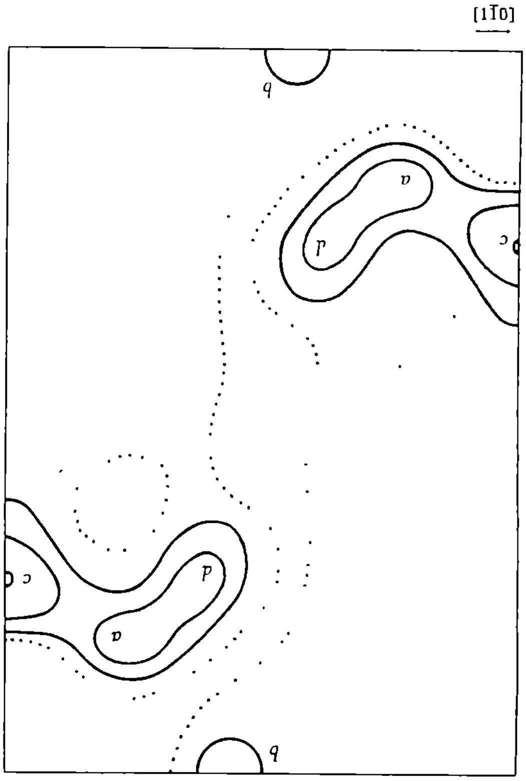

Fig. 2 Fourier projection along [110] with contributions from uranium and oxygen atoms O removed. Interval between contours is one-sixth of that in Fig. 1. Peaks at *a*, *b* represent oxygen atoms O′ at 0 5 0 4 0·4 . . . and peaks at *c*, *d* oxygen atoms O″ at 0,4 0 4 0·4 . . .

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ULTRA-VIOLET-ABSORBING COMPOUND(S) REPORTED PRESENT IN THE MURRAY METEORITE

By PROF. J. ORÓ

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PRELIMINARY investigations[1–9] have shown that when a water extract of the Murray meteorite is applied to a column of 'Dowex 1' resin and eluted with water or with 0·05 N hydrochloric acid, the eluates contain ultra-violet-absorbing compound(s).

Since some ion-exchange resins are known to yield water-soluble ultra-violet-absorbing substances[10] the investigations reported here were undertaken to ascertain whether the compounds detected in meteorite extracts are indigenous to the meteorite or are the result of contamination by the analytical technique used. These show that the ultra-violet spectra obtained from meteorite extracts passed through 'Dowex 1' and the spectra given by blank eluates from 'Dowex 1' are essentially identical. Observations concerning the presence of similar water-soluble ultra-violet-absorbing substances in 'Dowex 2' resin are also described.

The observations made by Vaughn[1–4] on the water extract from the Murray meteorite can be summarized as follows:

(1) The spectrum obtained directly with the water extract of the meteorite showed no maxima in the ultra-violet region. A uniform absorption at the short wave-length end of the spectrum and a very weak shoulder in the 260–280 mμ region were only observed[1,2].

(2) No peak was found in the ultra-violet spectrum of the distillate obtained at 300° C in vacuum from the dry residue of the water extract[1].

(3) When the water extract was applied to a 'Dowex 1' column and the column eluted first with water and then with 0·05 N hydrochloric acid, a small amount of ultra-violet-absorbing material was observed to emerge with the water eluate and a larger amount with the acid eluate.

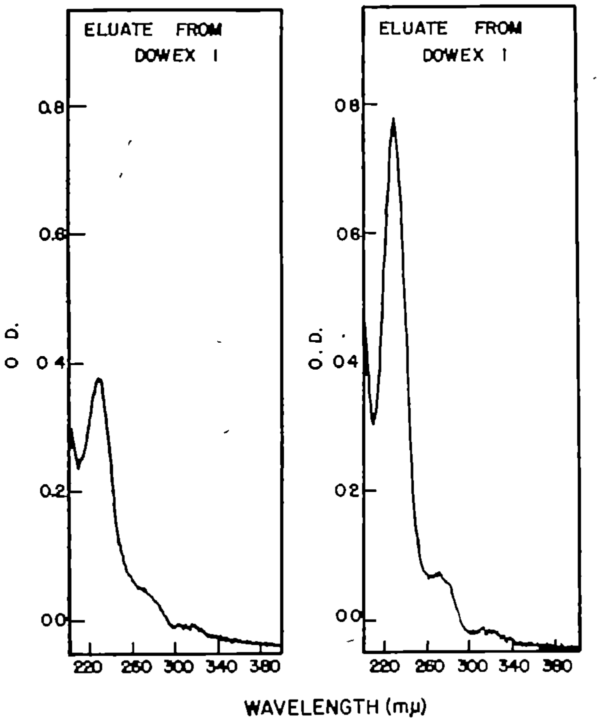

Fig. 1. Ultra-violet absorption spectra of blank eluates from columns of 'Dowex 1' resin (Cl^- form). The eluates were obtained with 0·1 N hydrochloric acid at room temperature (left) and at 90° C. (right)

(4). The ultra-violet-absorbing spectra of the eluates, in particular of the acid eluate rechromatographed a second time through 'Dowex 1', showed at *p*H 2 two absorption peaks, a strong one with a maximum at about 230 mμ and a smaller one with a maximum at about 273 mμ (refs. 2 and 3) (the maxima from the water eluate and from the first acid eluate were less well defined, yet in agreement with the above maxima).

(5) At neutrality and under basic conditions (*p*H 9–10) the 230 mμ maximum shifted towards shorter wave-lengths (about 222 mμ) while the 273 mμ maximum was very much decreased and in some cases disappeared.

(6) The ultra-violet spectrum in the region 290–250 mμ, observed in CCl_4 and benzene extracts of the meteorite, was found to be caused by the presence of elemental sulphur[4].

The 'Dowex 1' ion-exchange resin which I used was obtained from a source identical to that of the resin that had been used previously in the meteorite investigations.

A small column (5 c.c.) of 'Dowex 1' (200–400 mesh, × 8, Cl^- form) was eluted with 10 ml. 0·1 N hydrochloric acid at room temperature. The ultra-violet spectrum of the eluate was recorded in a Cary spectrophotometer (Fig. 1, left). Alternatively, an acid eluate of the same resin was obtained by passing 10 ml. of hot (90° C) 0·1 N hydrochloric acid through a small column (1 c.c.) of 'Dowex 1'. The ultra-violet spectrum of the eluate is shown in Fig. 1 (right). A strong absorption peak at about 230 mμ and a smaller one at about 273 mμ can be observed in the two spectra.

The relatively larger ultra-violet absorption of the eluate obtained at 90° C compared with that obtained at room temperature indicates that the extraction of the ultra-violet-absorbing material from the resin is enhanced by the hot acid.

Elution of the same ultra-violet-absorbing material was also observed from samples of resin which were extracted with ether, regenerated with saturated sodium bicarbonate, washed with distilled water and finally eluted with 0·1 N hydrochloric acid. Fig. 2 shows the spectrum of one such eluate. The fact that this ultra-violet-absorbing material is present after regeneration of the resin indicates that the ultra-violet-absorbing compounds are released from the inside of the resin beads. This observation is also in line with the results obtained in the extraction with hot acid.

Since 'Dowex 2' ion-exchange resin might be used in similar analytical applications, the presence of ultra-violet-absorbing compounds in the acid eluates from this resin was also examined.

A small column (5 c.c.) of 'Dowex 2' (200–400 mesh, × 8, OH^- form) was eluted with 10 ml. of 4 N acetic acid.

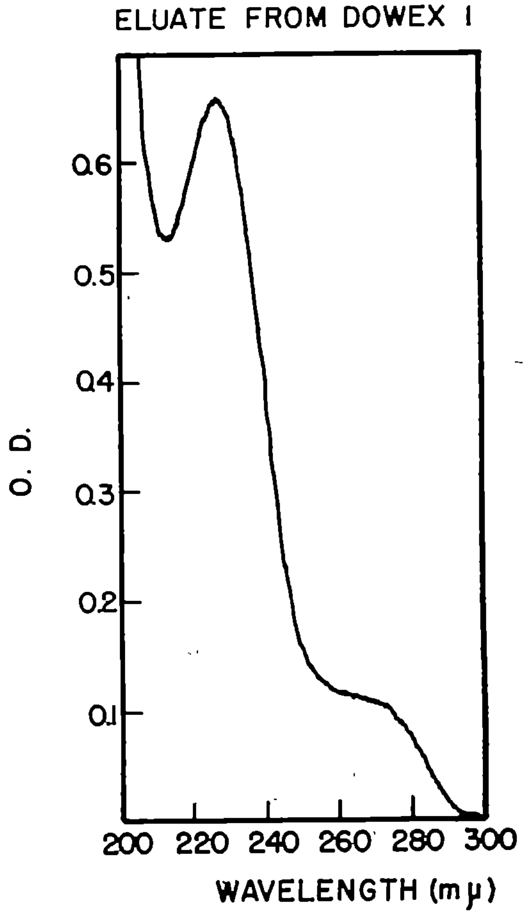

Fig. 2. Ultra-violet spectrum of a 0·1 N hydrochloric acid eluate from a column of 'Dowex 1' resin. The resin has been extracted previously with ether, treated with saturated sodium bicarbonate and washed with distilled water. (Data provided by, Dr. J. Ullrich)

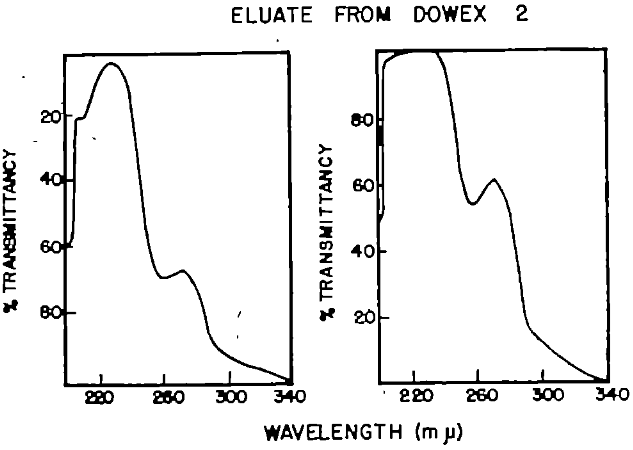

Fig. 3. Ultra-violet absorption spectra of a 4 N acetic acid eluate from a column of 'Dowex 2' resin (OH^- form). The acetic acid was evaporated and the residue dissolved in 0·1 N hydrochloric acid. (Spectra obtained by L. Engberg)

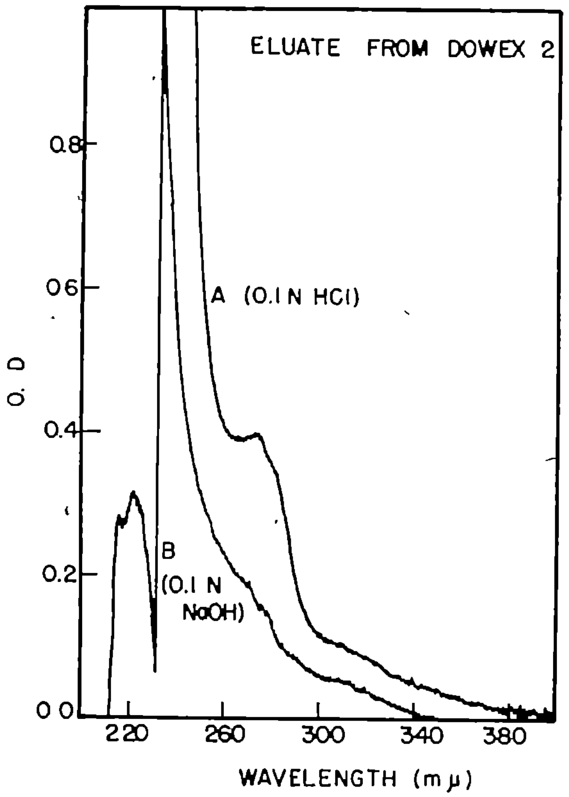

Fig. 4. Spectrum of a 0·1 N hydrochloric acid eluate from a column of 'Dowex 2' resin (curve *A*) and spectrum of the same eluate after it was made 0·1 N basic with sodium hydroxide (curve *B*)

After evaporation of the acetic acid the residue was dissolved in 0·1 N hydrochloric acid. Fig. 3 shows two of the typical spectra obtained from a number of 'Dowex 2' acetic acid eluates by this procedure. The same two absorption peaks at about 230 and 273 mμ can be observed.

The identity of the absorption peaks obtained from resins of type 1 and type 2 is not surprising. In fact, it should be expected since these two types of resins have the same structural polystyrene backbone and are manufactured following essentially the same procedure[11].

The effect of *p*H change on the ultra-violet absorption curve of a hydrochloric acid eluate from 'Dowex 2' is shown in Fig. 4. Curve *A* shows the ultra-violet absorption of the 0·1 N hydrochloric acid eluate, whereas curve *B* shows the ultra-violet absorption of the same eluate after it was made 0·1 N basic with sodium hydroxide. It can be seen that in 0·1 N sodium hydroxide the 273 mμ absorption peak disappeared and the 230 mμ maximum was shifted to about 222 mμ. These changes are identical to those observed by Vaughn with the 'Dowex 1' eluates of a water extract of the Murray meteorite[4].

When 'Dowex 2' (OH⁻ form) columns were eluted with water and then with 4 N acetic acid the ultra-violet-absorbing material was found in the water eluates as well as in the acid eluates, thus paralleling also the elution behaviour described for the meteorite extracts.

On the basis of the identity of the maxima, identity of shifts caused by changes in *p*H and similarity of elution pattern (observations 4, 5 and 3 of Vaughn, respectively) it is concluded that the ultra-violet-absorbing compound(s) detected in water extracts of the Murray meteorite after treatment with 'Dowex 1' came from the resin and not from the meteorite. This conclusion is also in line with the other observations made by Vaughn[1,2].

These results do not exclude the possibility that some ultra-violet-absorbing substances, other than elemental sulphur, are present in the Murray meteorite in amounts below the limit of detectability by the procedure used by Vaughn. The very weak shoulder observed in the 260–280 mμ region in the water extracts may be indigenous to the meteorite or may be due to contamination from organic solvents (benzene, etc.) with which the meteorite samples were extracted prior to the extraction with water. Analysis of the Murray meteorite using more sensitive spectrophotometric methods is in progress.

I thank Prof. M. Calvin for providing the laboratory facilities and the stimulus to carry out this work. The data provided by J. Ullrich and L. Engberg are also gratefully acknowledged.

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ELECTRON DONATION AND ACCEPTANCE BY CARCINOGENIC COMPOUNDS

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AND

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In view of the low chemical reactivity of carcinogenic compounds (apart from alkylating agents) under physiological conditions, attention has been focused on their physical properties. Recently, the capacity of carcinogens to donate electrons, and so form charge-transfer complexes, has been discussed. Szent-Györgyi *et al.*[1] have shown that a number of carcinogens can contribute electrons to iodine. Formation of charge-transfer complexes between carcinogenic hydrocarbons and quinones has been described by Laskowski[2] and by Liao and Williams-Ashman[3], and complexes with trinitrofluorenone have been investigated by Takemura *et al.*[4]. Calculations from charge-transfer spectra of ionization potentials of hydrocarbons, including some that are carcinogenic, have been presented[5–7]. The theoretical results of Pullman *et al.*[8] can also be interpreted in terms of the charge density distribution in the molecule of a carcinogen and its capacity to donate electrons. The first part of the investigation reported here was undertaken in order to ascertain how general the property of electron donation is among carcinogens of widely different chemical structure. Particular attention has been given to closely related compounds where some are carcinogenic and others are not.

Electron donation by carcinogens. This property has been measured by formation of charge-transfer complexes with chloranil in acetonitrile and also, in some cases, with 1,3,5-trinitrobenzene in benzene. In the electronic absorption spectrum of a charge-transfer complex, one electron is excited, as a rule, from the highest filled orbital of the donor molecule to the lowest empty orbital of the acceptor. The energy transition of the wave-length of longest absorption is given by:

$$E_{CT} = I_D - E_A + \Delta$$

where I_D is the gaseous ionization potential of the donor, E_A is the electron affinity of the acceptor and additional energy changes Δ are due mainly to solvation and coulombic effects, which are reasonably constant when similar molecules in the same solvent are considered[9,10]. From the equation it will be seen that there should be a simple relationship for a series of complexes when donors of the same general type and the same acceptor and solvent are used (E_A and Δ nearly constant). Since the energy E_{CT} is proportional to the wave number, plots of the wave number of maximum absorption of the charge-transfer band, $\bar{V}$, against I_D should give a straight line. The validity of this interpretation has been demonstrated by many workers (see review of Briegleb[10]), and plots of this type have been obtained with some substances of biological importance by Beukers and Szent-Györgyi[7].

Under conditions similar to those described by Foster[8], we have measured by difference spectrophotometry in a Unicam *SP* 700 or Beckman recording spectrophotometer the maxima of charge-transfer absorption bands of various complexes. The results are presented in Table 1, together with some indication of the relative carcinogenicities of different compounds. For the hydrocarbons this has been taken from Iball[11] and von Haam[12], and for most other compounds from the *Survey of Compounds which have been Tested for Carcinogenic Activity*[13]. Other references are given when necessary. Since compounds have been tested for carcinogenicity under very different conditions, the comparisons between different groups must be at best a rough approximation; but they are most useful when related compounds tested under comparable conditions show marked differences in carcinogenicity. Although the overall picture is important, these provide the most critical tests for any hypothesis of carcinogenesis.

The results summarized in Table 1 confirm and extend the view that there is a broad correlation between electron-donating capacity and carcinogenicity. However, as will be discussed here, at certain points the correlation breaks down in detail, and factors other than electron donor strength clearly have to be taken into consideration.

Electron donation by carcinogens studied by electron spin resonance technique. So far the ability of carcinogens to donate electrons has been deduced from the formation of charge-transfer complexes, and it is important that these results be checked by other techniques which provide quantitative measurements of electron donation and acceptance. Polarographic measurements are presented in the following article, and similar results have been obtained by electron spin resonance measurements of complexes made in collaboration with Drs. W. Slough and D. H. Whiffen of the National Physical Laboratory, Teddington. Following the work of Kainer *et al.*[14], electron spin resonance signals have been reported in a number of charge-transfer complexes, including complexes of indole and iodine[15] and have been measured in a series of complexes of non-carcinogenic aromatic hydrocarbons with iodine[16] and tetracyanoethylene[17].

Table 1. WAVE NUMBERS OF MAXIMUM ABSORPTION OF CHARGE-TRANSFER BANDS, $\bar{\nu}$, OF VARIOUS COMPOUNDS IN COMPLEXES WITH CHLORANIL IN ACETONITRILE AND WITH TRINITROBENZENE (TNB) IN BENZENE

Electron donor strength increases as $\bar{\nu}$ and E_{CT} decrease. In the last column reactions with acridine are listed

Compound	Carcino-genicity	$\bar{\nu} \times 10^{-3}$ chloranil	E_{CT} eV	$\bar{\nu} \times 10^{-3}$ TNB	E_{CT} eV	Acri-dine
Hydrocarbons						
Naphthalene	–	21·3	2·64			–
Anthracene	–	16·0	1·98	21·9	2·72	–
1 : 2-Benzanthracene	–	16·5	2·05	22·2	2·75	–
1-Methyl-1 : 2-benzanthracene	–	16·7	2·07			+
2-Methyl-1 : 2-benzanthracene	–	16·7	2·07			+
3-Methyl-1 : 2-benzanthracene	–	16·7	2·07	22·2	2·75	+
4-Methyl-1 : 2-benzanthracene	++	16·8	2·08	21·9	2·72	+
5-Methyl-1 : 2-benzanthracene	++	16·7	2·07	21·8	2·70	+
6-Methyl-1 : 2-benzanthracene	–	16·6	2·06			+
7-Methyl-1 : 2-benzanthracene	–	16·8	2·08	21·7	2·69	+
8-Methyl-1 : 2-benzanthracene	–	16·7	2·07			+
9-Methyl-1 : 2-benzanthracene	+	16·7	2·07			+
10-Methyl-1 : 2-benzanthracene	++	16·6	2·06			+
1′-Methyl-1 : 2-benzanthracene	–	16·8	2·08			+
2′-Methyl-1 : 2-benzanthracene	–	16·7	2·07			+
3′-Methyl-1 : 2-benzanthracene	–	16·8	2·08			+
4′-Methyl-1 : 2-benzanthracene	–	16·7	2·07			+
9,10-Dimethyl-1 : 2-benzanthracene	+++	14·8	1·83	20·7	2·57	+
5,7-Dimethyl-1 : 2-benzanthracene	+++	15·1	1·87			++
1 : 2,5 : 6-Dibenzanthracene	++	17·4(*c*)	2·16			+
1 : 2,7 : 8-Dibenzanthracene	++	17·6(*c*)	2·18			+
20-Methylcholanthrene	+++	14·2	1·76	20·3	2·52	++
Pyrene	–	17·2	2·13			+
1-Methylpyrene	?	16·7	2·07			+
3-Methylpyrene	?	16·7	2·07			+
4-Methylpyrene	–	16·8	2·08			+
1 : 2-Benzpyrene	–	17·6	2·18	24·2	3·00	–
3 : 4-Benzpyrene	+++	14·4	1·79	20·6	2·55	++
1 : 2,3 : 4-Dibenzpyrene	++(69)	15·4(*c*)	1·91			+
3 : 4,8 : 9-Dibenzpyrene	++	14·7(*c*)	1·82			+
3 : 4,9 : 10-Dibenzpyrene	+++	15·4(*c*)	1·91			+
Coronene	–	16·8(*c*)	2·08			–
Chrysene	–	20·3(*c*)	2·52			–
Naphthacene	–	13·5(*c*)	1·67			+
Fluorene	–	20·6	2·55			–
3 : 4-Benzfluorene	–	17·8	2·21			–
Fluoranthene	–	21·3	2·64	26·3	3·26	–
3 : 4-Benzfluoranthene	–	21·4	2·65			–
Phenanthrene	–	22·0	2·72	27·1	3·36	–
Perylene	–	14·8(*c*)	1·83			+
1 : 2-Benzperylene	?	14·9(*c*)	1·85			+
Triphenylethylene	+	19·7	2·44			+
Diphenyl	–	23·1	2·86			–
cis-Stilbene	–	19·7	2·44			–
trans-Stilbene	–	19·8	2·45			–
Stilboestrol	++	15·6	1·93			+
Steroids						
Equilenin	+	17·6	2·18			+
Oestrone	++	18·0	2·23			+
Oestradiol	++	18·5	2·29			–
Cortisone	–	–	–			–
Testosterone	–	–	–			–
Aromatic amines						
1-Naphthylamine	–	15·4	1·91	21·2	2·63	++
2-Naphthylamine	+	16·2	2·01	21·5	2·67	++
2-Aminoanthracene	++	13·9	1·73	19·6	2·43	?
2-Aminofluorene	++	15·5	1·92	19·0	2·36	++
2-Acetylaminofluorene	+++	18·2	2·26			++
N-Hydroxy-2-acetyl-aminofluorene	+++	18·0	2·23			+
3-Acetylaminodibenzofuran	+	19·6	2·43			+
2-Acetylaminodibenzothiophen	+	19·2	2·38			+
2-Acetylaminodibenzothiophen sulphone	–	21·3	2·64			–
4-Dimethylaminoazobenzene	+++	16·5	2·05	20·7	2·57	?
4-Diethylaminoazobenzene	–	16·3	2·02	20·3	2·52	?
3′-Fluoro-4-dimethylamino-azobenzene	–	16·9	2·10			?
2-Methyl-4-dimethylamino-azobenzene	–	16·0	1·98			?
4-Aminostilbene	++	14·4	1·79	20·3	2·52	+
4-Aminodiphenyl	++	16·5	2·05	21·7	2·69	+
o-Tolidine	+	13·8	1·71	18·8	2·33	+
Benzidine	+	14·4	1·79	20·0	2·48	+
Diphenylamine	+	16·9	2·10	21·3	2·64	+
3-Hydroxyanthranilic acid	+	21·9	2·71			+
Heterocyclic N compounds						
Acridine	–	22·9	2·83			
1 : 2-Benzacridine	–	21·8	2·58			–
5,7-Dimethyl-1 : 2-benzacridine	++	20·0	2·48			+
Indole	*	20·6	2·54	25·3	3·14	–
Carbazole	–	18·8	2·33			–
5,6-Benzpyrido-(2′,3′ : 1,2)-carbazole	+++	17·5	2·17			+

* Reported carcinogenic (ref. 70), but this may be due to metabolites.

c, These compounds were poorly soluble in acetonitrile and charge-transfer spectra were measured in chloroform; with other compounds $\bar{\nu}$ is similar in acetonitrile and chloroform.

Table 2. ELECTRON SPIN RESONANCE MEASUREMENTS OF COMPLEXES OF CARCINOGENIC AND RELATED NON-CARCINOGENIC COMPOUNDS WITH TETRACYANOETHYLENE

Donor molecule	Spin concentration of charge transfer complex	Unpaired spins per mole complex	Line-width (gauss)	Ratio of spin concentration
Azobenzene	7×10^{15} spins/g	$3·7\times10^{-6}$	7·5	1
1,2-Benzpyrene	8×10^{16} spins/g	$5·2\times10^{-5}$	7·1	11
3,4-Benzpyrene	$1·8\times10^{17}$ spins/g	$1·1\times10^{-4}$	7·1	25
1,2-Benzanthracene	$2·0\times10^{17}$ spins/g	$1·2\times10^{-4}$	2·4	27
9,10-Dimethyl-1,2-benzanthracene	$2·4\times10^{18}$ spins/g	$1·3\times10^{-3}$	4·1	340
4-Dimethylamino-azobenzene	$2·5\times10^{18}$ spins/g	$1·4\times10^{-3}$	3·2	350

In our experiments 1 : 1 molecular complexes of highly purified donor molecules and tetracyanoethylene were formed in glass tubes and sealed under nitrogen. The electron spin resonance measurements were made at room temperature at 9,000 Mc/sec using a magnetic-field modulated spectrometer of conventional type. Spin concentrations were measured by comparison with standard samples which in turn were standardized against weighed amounts of diphenyl picryl hydrazyl. Closely similar instrumental conditions were maintained for these measurements.

Preliminary results are given in Table 2. There is clear evidence of increased donor strength of *p*-dimethylaminoazobenzene as compared with azobenzene, 9,10-dimethyl-1 : 2-benzanthracene as compared with 1 : 2-benzanthracene, and 3 : 4- as compared with 1 : 2-benzpyrene. In Table 2 the compounds are placed in order of increasing donor strength.

Electron acceptance by carcinogens. One factor that may help to explain the discrepancies mentioned here is the possibility that electron acceptance by carcinogens is important for activity. This was suggested by Nash[18] and by Mason[19] on different theoretical grounds, and recently some carcinogenic compounds have been shown to have an affinity for electrons while in the gaseous phase. This is true of the hydrocarbons[20] and also aromatic amines (N. L. Gregory and A. C. Allison, unpublished results). The polarographic measurements in solution presented in the following article also show that many carcinogens are both donors and acceptors of electrons.

Szent-Györgyi and McLaughlin[21] observed that a number of carcinogenic compounds give a yellow colour with acridine. They pointed out that the colour could not have been due to charge-transfer as usually understood since it was always the same (yellow) instead of being characteristic of the test compound. However, besides π–π charge-transfer between two molecules, both of which are aromatic, there is another type of charge-transfer illustrated by that between the simple aliphatic amines and trinitrobenzene, with the reversible production of a brown colour. In this case the electron is transferred from the lone-pair on the amino nitrogen, and the interaction is known as n–π charge-transfer. Slifkin[22] has pointed out that with aromatic amines both π–π and n–π charge transfer can occur simultaneously. With regard to the latter, acridine is in a particularly favourable position because the nitrogen atom is negatively charged and has a fractional electron due to pyridine-type mesomerism, besides the usual lone-pair. We can presume that this fractional electron is more readily transferred to another molecule than the corresponding fraction of a 'straight pair' such as is found in the aliphatic amines.

We have previously described the changes produced in the acridine–carcinogen interaction by the addition of a weak organic acid[23]; the 'weak chemical link' formed in the absence of acid can now be more positively identified as n–π charge-transfer localized at the nitrogen atom of the acridine.

We have examined the interaction of carcinogens with acridine by drying down solutions on filter paper[21] and also by analysis of absorption spectra. Concentrated solutions of acridine in chloroform show a yellow colour and shift of absorption to a longer wave-length on addition of carcinogens. Difference spectra between the absorption of the complex and that of the carcinogen and acridine alone show a sharp peak with a maximum at $\bar{\nu} = 24{,}600$ in every case. This peak is in exactly the same position as that seen in the difference spectrum of acridine/acridine-acetic acid, when the acridine in the latter must have been the positively charged partner of the H-bonded complex. This is strong presumptive evidence that the band with carcinogens is due to n–π electron transfer from the acridine, the carcinogen behaving as electron acceptor.

The results summarized in the last column of Table 1 show that the correlation between a positive acridine reaction and carcinogenicity is as good as that between the latter and electron donation. We therefore suggest that carcinogenicity arises as a result of a suitable combination of both electron-donating and electron-accepting properties in a compound. Huggins and Yang, on the other hand, have recently suggested that either property is sufficient[24]. Their suggestion is based on Szent-Györgyi's work and on the observed carcinogenicity of the strong electron acceptor 2,4,7-trinitrofluorenene, but it is possible that this compound is rapidly metabolized to others with very different properties. In any case, evidence is summarized elsewhere[25] that many drugs which are apparently not carcinogenic are either donors or acceptors of electrons, so that either of these properties *per se* is obviously insufficient for carcinogenicity. The combination of both electron-donating and electron-accepting properties in the same molecule is, however, relatively rare, and it is remarkable that it should recur in so many carcinogens with widely differing structures.

Quinonoid character due to lone-pairs. In an earlier communication[18] it was shown that some of the properties of quinones could arise in compounds containing, not opposed carbonyl groups, but opposed lone-pairs, that is, in peroxides, hydrazines, hydroxylamines and their vinylogues, in which the electrical strain set up by neighbouring atomic dipoles could not be relieved by mesomeric charge transfer such as is found in amides or esters. Pseudo-quinonoid character would also be expected, in other compounds containing directly linked oxygen and nitrogen, that is, in nitro compounds and nitrosamines. The quinones themselves have a very high affinity for electrons, but probably little tendency to donate them. In the pseudo-quinones, on the other hand, some degree of electron affinity should be possible without greatly affecting their ability to donate electrons. For example, 2-naphthylamine is a strong electron donor, and *N*-hydroxylation such as occurs *in vivo*[26] will increase its electron affinity by virtue of the hydroxylamine system while leaving the electron-donating capacity of the aromatic amine portion largely unchanged. The compounds should therefore have a much better balance of donor and acceptor properties than the original amine. The following other known carcinogens are pseudo-quinonoid by the aforementioned definition, or have electrical strain of an analogous kind: cholesterol hydroperoxide[27], safrole[28], urethane[18], actinomycin *D* (ref. 29) and dinaphthazine[30].

Hydrocarbons. The ability of condensed ring hydrocarbons to donate electrons from their delocalized π-orbitals has been known for many years. Recent observations on electron affinity in the gaseous phase[20], polarographic measurements (following article) and the interaction with acridine reported here show that the hydrocarbons are also electron acceptors. From the results shown in Tables 1 and 2 and polarographic measurements (following article) it is clear that as a rule the carcinogenic hydrocarbons are stronger electron donors than other hydrocarbons. Thus, there is a progressive increase in donor strength in the series anthracene, 1 : 2-benzanthracene, monomethylbenzanthracene and dimethylbenzanthracene, and in 3 : 4- as compared with 1 : 2-benzpyrene. Methylcholanthrene is a powerful donor.

Nevertheless there are important exceptions. In keeping with previous observations of Takemura *et al.*[4] on complexes with trinitrofluorenone and of Birks and Slifkin[5] on complexes with chloranil, we have found only trivial differences between the electron-donating capacity of various monomethylbenzanthracene derivatives, although some of these are carcinogenic and others are not[12]. Moreover, the carcinogenic dibenzanthracenes are weaker electron donors than some non-carcinogenic hydrocarbons, notably naphthacene and perylene.

At first sight these exceptions seem so impressive as to cast doubt on the importance of electron donation in the carcinogenic process. However, apart from electron acceptance, at least two additional factors must be considered. The first is the steric factor. For charge transfer to occur there must be a very close fit of donor and acceptor molecules, with actual overlap of electron orbitals; bulky substituents are known to decrease charge transfer in carefully studied systems[31]. This might account for such findings as that 20-methyl, -ethyl, -isopropyl and *t*-butyl-derivatives of cholanthrene show progressively decreasing carcinogenicity[32]; that higher alkyl derivatives at position 5 of 1 : 2-benzanthracene are less carcinogenic than the methyl derivative[33]; and that the larger polycyclic hydrocarbons are not carcinogenic[12,33].

That steric factors might be important in carcinogenesis should occasion no surprise, since this is known to be the case with hormones and drugs. It need only be recalled that D-thyroxine fails to alleviate the symptoms of hypothyroidism and that the position of the carbonyl and α, β-unsaturated ketone systems in ketosteroids profoundly affects their biological activities.

In this connexion, the possibility that polycyclic hydrocarbons are steric analogues of steroids has been raised again recently[23]. This suggestion should be treated with reservation, since X-ray analysis shows that steroids are non-planar molecules[34] whereas the carcinogenic hydrocarbons so far studied are essentially planar[35]; 9,10-dimethyl-1 : 2-benzanthracene has one ring at an angle to the others, which are planar (J. Iball, private communication). The structures of carcinogenic hydrocarbons and steroids must therefore be quite different. Nevertheless, many groups of carcinogens (polycyclic hydrocarbons, some aromatic amines and heterocyclic nitrogen-containing compounds, œstrogenic steroids and stilbœstrol) have similar overall dimensions, and it seems possible that these are particularly favourable for carcinogenesis. However, other carcinogens, including naphthylamine and urethan, are much smaller, so there is no universal rule.

The second important additional factor is the stability of hydrocarbons under physiological conditions. It is reasonable to suppose that in order to be carcinogenic the hydrocarbons must persist in active form in the tissues for relatively long periods. Available evidence indicates that metabolites of hydrocarbons are not carcinogenic, and that the amount of hydrocarbon persisting in the tissues is related to carcinogenity[36]. Furthermore, polarographic observations summarized in the following article suggest that carcinogenic and non-carcinogenic hydrocarbon ions are likely to differ in stability, and differences in their metabolism have also been described[37]. Further observations on the stability of various hydrocarbons in tissues under comparable conditions would be of considerable interest.

With regard to electron acceptance in hydrocarbon carcinogenesis, the recent observations of Lacassagne *et al.*[38] on carcinogenicity of aldehyde derivatives of anthracene and of dibenzanthracenes are of interest, since the aldehyde substituents, particularly when hydrogen bonded, would increase electron acceptance. Despite the qualifications that have been necessary, it seems clear that polycyclic hydrocarbons could exert their carcinogenic effects by virtue of their capacity to function both as donors and acceptors of electrons. The calculations of the Pullmans[8] on the necessity of a particular charge-density distribution in the molecule of a carcinogenic hydrocarbon may reflect a capacity for both electron and acceptance, as well as stability. However, it must be recognized that such calculations depend on assumptions that are not at present verifiable; the difficulties in this field are emphasized by the recent controversy with Mason[39]. We also feel that reservations are necessary about the relevance of Birks's[40] suggestion that in carcinogenic hydrocarbons the first singlet π-electron absorption band strongly overlaps the emission spectrum of tryptophan. This is not true of some carcinogenic hydrocarbons[41], and the energies necessary to raise electrons to excited states are rarely available in most organs, so that Birks's suggestion is difficult to translate into biochemical terms.

Steroids. The only steroids that are highly carcinogenic in appropriate test systems are œstrogens[18], and it is of interest that these are the only members of the group to possess aromatic systems and to be relatively strong electron donors (Table 1 and following article). The same is true of the synthetic œstrogen, stilbœstrol, and this compound and most œstrogenic steroids show also easily measurable electron acceptor properties (following article). Cholesterol itself is only weakly carcinogenic (if the unmetabolized compound is carcinogenic at all), but there is good evidence that the products of dehydrogenation of cholesterol (1 : 2-*cyclo*pentadieno-phenanthrenes) are carcinogenic[42], as is also cholesterol hydroperoxide[27]. Though we have had no opportunity to test these compounds, it is virtually certain that both would be stronger donors and acceptors of electrons than cholesterol itself.

Aromatic amines. These are among the classical electron donors known to form charge-transfer complexes[37]. It is therefore of interest that many aromatic amines are carcinogenic, including 2-naphthylamine, 2-aminoanthracene, 2-aminophenanthrene[25], 2-aminofluorene and derivatives, *p*-dimethylaminoazobenzene, aminostilbenes and aminobiphenyls. All these are strong electron donors, as shown in charge-transfer experiments (Table 1) and polarographic measurements (following article).

There is evidence that *N*-hydroxylation (and possibly *o*-hydroxylation in some cases) of aromatic amines increases their carcinogenicity, or may even be essential for it. Thus, *N*-hydroxy-2-acetylaminofluorene is locally carcinogenic when administered to various sites, whereas it seems that 2-acetylaminofluorene itself is metabolized by the liver before it is carcinogenic elsewhere[43]. Possibly deacylation occurs, and the immediate carcinogen is 2-hydroxylaminofluorene; *N*-hydroxylation of 2-naphthylamine also occurs in man and other animals[26], and *N*-hydroxy-4-acetylaminobiphenyl is more carcinogenic than 4-acetylaminobiphenyl[44]. The evidence that aromatic hydroxylamines are more carcinogenic than the parent compounds is of interest in relation to the argument presented here that in hydroxylamines there will be a better balance of electron donor and acceptor properties than in the corresponding amines. That aromatic hydroxylamines retain potent electron-donating properties is readily shown in appropriate experiments, for example, on electron transfer to aromatic diazonium compounds[45].

There is no doubt that carcinogenic aromatic amines and derivatives have both electron donor and acceptor properties, but here again certain qualifications are necessary. No important difference in donor or acceptor strength exists between the carcinogenic *p*-dimethylaminoazobenzene and its inactive diethyl-analogue. It is possible that the bulky ethyl substituent reduces charge transfer with a biologically important compound, or affects the rate of metabolism of the azo carcinogen; and this may also be true of certain substituted dimethylaminoazobenzenes that are not carcinogenic. Again, there are only small differences in donor strength between the carcinogenic 2-naphthylamine and the non-carcinogenic 1-naphthylamine; but in this case it is likely that the hydroxylamine of the latter would be less stable than that of the former. The rates of oxidation or specific hydroxylation of aromatic amines, without loss through coupling to glucuronic or sulphuric acid and solubilization in the aqueous phase, could well be of as much importance in determining whether or not a given amine is carcinogenic as its electron-donating capacity. Here again there is a close analogy with what is known of the duration of action of aromatic amine hormones and drugs.

In all aromatic amines and derivatives, electron-donating capacity is much greater than electron-accepting capacity. On our hypothesis, introduction of appropriate substituents which increase electron acceptance without markedly reducing donor strength would be expected to increase carcinogenicity. This has been achieved by Brown *et al.*[46], who have replaced the unsubstituted ring of *p*-dimethylaminoazobenzene with quinoline and pyridine derivatives which would be acceptors as a result of immobilization of nitrogen lone-pair electrons through hydrogen bonding.

Heterocyclic nitrogen compounds. Nitrogen can enter the aromatic ring in two different ways, first, as in pyridine, where the nitrogen lone-pair of electrons does not take part in the primary aromatic conjugation and, secondly, as in pyrrole, where the nitrogen lone-pair is an essential part of the aromatic system and is therefore too de-localized to be available for hydrogen bonding. In the first case, the nitrogen carries a negative charge and the molecule as a whole is a weak donor or acceptor of an electron. In the second case, the nitrogen carries a positive charge and the molecule as a whole becomes a relatively strong electron donor, for example, indole (Table 1). In larger molecules any π-electron donor or acceptor capacity already present will be modified in the appropriate direction by the entry of nitrogen into the molecule, so that acridine, for example, is a weaker donor than anthracene, whereas carbazole is a stronger.

On the hypothesis advanced here, the introduction of nitrogen into aromatic ring systems would therefore be expected sometimes to increase and sometimes to decrease their carcinogenicity. This is, in fact, the case. Acridine and its derivatives are not carcinogenic[13], and this is also true of pyrenoline, a heterocyclic *N*-derivative of 3,4-benzpyrene[47]. On the other hand, many heterocyclic compounds of nitrogen are carcinogenic, including dinaphthazine, compared with the inactive parent compound pentacene[48], and a series of dibenzocarbazoles[49], angular benzacridines[50], angular benzpyridocarbazoles[51] and tri*cyclo*quinazolines[52]. As already discussed, hydrogen bonding of the nitrogen atom would increase electron acceptor character in all such compounds of the pyridine type. It is suggestive that in the former group only relatively large molecules, which will be π-electron donors as well as hydrogen acceptors, are carcinogenic. Where donor and acceptor properties are very well marked, and the donating and accepting groups are readily identifiable, as in the benzpyridocarbazoles (see polarographic measurements in following article) the compounds are among the most potent carcinogens known[51].

Solubilization of carcinogens by purines. Boyland and Green[53] have recently re-examined the solubilization of polycyclic aromatic hydrocarbons by caffeine and other purines. They concluded that the interaction is due mainly to polarization forces. However, in view of the results with acridine discussed here, the case for *n*–π charge transfer from one or more lone-pairs on the caffeine molecule to the carcinogen is now stronger. The spectral shifts observed by Boyland and Green would agree with Tsubomura and Lang's evidence[54] that *n*–π interaction results in a shift to the red of the main band of the acceptor. If this is so, solubilization of carcinogens by caffeine would be related to electron affinity of the former, although other factors would also affect the solubilities of the complexes in water. Possibly there is a continuous gradation between *n*–π charge transfer and polarization forces.

The complex formation between carcinogenic hydrocarbons and nucleic acid[55] could have a similar basis. The presence of sodium chloride markedly depresses complex formation, which would be expected since the sodium ions would compete for potential purine electron-donating sites. However, this fact implies that the amount of hydrocarbon so solubilized *in vivo* will be very small.

This reaction may be of interest in demonstrating the presence of minor components of potentially carcinogenic hydrocarbons in mixtures, as the results of Eby *et al.*[56] suggest. Only hydrocarbons that are electron acceptors would be solubilized by caffeine in the aqueous phase.

Relevance to biological systems: the 'sandwich' model. The finding that most, if not all, carcinogens (other than alkylating agents) are both donors and acceptors of electrons leads to a model that may help to explain their mode of action *in vivo*. According to this 'sandwich' model the carcinogen lies between two neighbouring active sites in a metabolic system, one an electron donor and the other an acceptor, forming relatively stable charge-transfer complexes with both; in some cases at least, complex formation will be reinforced by hydrogen bonds. For example, electron transfer may normally occur between nitrogen and oxygen on different substrate molecules. The carcinogen would form an *n*–π complex with, say, the nitrogen, and the transferred electron would now be so diffused as no longer to be able to pass over to the oxygen. This may be called the 'rectifier' model, in that π–*n* transfer is not normally observed (so far as we are aware, polycyclic hydrocarbons do not interact with electron-deficient substances such as the Lewis acids). Alternatively, the carcinogen could form a π–π complex on one side of the substrate, and this might prevent electron transfer by reducing the availability of an electron at a lone-pair site elsewhere in the substrate.

The search for biological systems markedly affected by carcinogens has not yet progressed far, but two systems can serve as examples. One is a purified soluble enzyme system from rat kidney, having flavine mononucleotide (FMN) as coenzyme, which oxidizes reduced ribosyl nicotinamide in the presence of 2-methyl-1,4-naphthoquinone (menadione). This is powerfully inhibited by carcinogenic hydrocarbons ($K_i < 10^{-7}$ M)[3]. Both FMN and menadione are electron acceptors, and it seems likely that charge transfer of one or other with carcinogens occurs, the latter perhaps accepting also an electron from some donor (such as a tryptophan residue in the enzyme). This is a highly specific effect, since a similar enzyme from rat mammary tissue is not inhibited by carcinogens[57]. The second example is inhibition of reduced diphosphopyridine nucleotide oxidation in rat liver enzyme systems by carcinogenic hydrocarbons and aromatic amines[58], where similar mechanisms could be involved. These mechanisms are not entirely speculative since complex formation of hydrocarbons with FMN has been reported[59], and charge-transfer of a variety of carcinogens with menadione, coenzyme *Q* and tryptophan can readily be demonstrated spectrophotometrically (unpublished).

From this and other evidence which cannot be reviewed here *in extenso*, there is good reason to believe that carcinogens might affect oxidative phosphorylation, and a plausible case can be made for this being the primary change in carcinogenesis. Prolonged inhibition or uncoupling of oxidative phosphorylation might initiate the neoplastic change through transformation of normal cells into tumour cells, or by providing a metabolic environment favourable for proliferation of tumour cells, or both. The first suggestion, due originally to Warburg[60], has been the subject of much debate, and the fact that the debate continues with undiminished vigour[61] means that the arguments against the hypothesis are not as compelling as its antagonists claim. The hypothesis should in any case be restated to take into account important recent observations on differences between normal and tumour cells, including the utilization of energy for protein synthesis[62] and lipid synthesis[63], and metabolism of reduced diphosphopyridine nucleotide[64]. According to the metabolic selection hypothesis[65], inhibition of oxidative phosphorylation places tumour cells (which efficiently use glycolytic energy for protein synthesis[62] and probably for other important synthetic reactions) at a competitive advantage relative to normal cells; the tumour cells can thus form self-perpetuating clones, in which later modifications leading to malignancy can occur. A variant of this hypothesis is that the normal cells, which are at a competitive disadvantage owing to the presence of carcinogens, are those concerned with the elimination of any tumour cells arising and differing antigenically from cells originally present.

However, the observed effects of carcinogens on oxidative phosphorylation might be epiphenomena, while the fundamental changes occur in other systems. Other possibilities should constantly be borne in mind. The carcinogens might, for example, produce specific changes in permeability of nuclear or other membranes, or interfere with some aspect of nucleic acid or protein metabolism occurring at a lipid–aqueous interface, thereby interfering with normal mechanisms of growth control or differentiation. But our information about these mechanisms in mammalian cells is too scanty to allow analysis of the effects of carcinogens at present.

Some observations which are otherwise puzzling are explained by recognizing that carcinogens act as electron donors and acceptors. One is the marked reduction in carcinogenicity of polycyclic hydrocarbons by pretreatment with halogenated aromatic compounds, benzoquinone and cantharidin[66]; the latter are strong electron acceptors and could prevent the hydrocarbons from reaching their normal sites of action. Charge transfer may also precede the specific binding of carcinogens by proteins of liver[67] and skin[68], irrespective of whether this is directly involved in carcinogenesis.

Considering all the factors that intervene, the correspondence over a wide range of compounds between carcinogenicity and electron donor and acceptor capacity is remarkably good, so that this seems to be the most attractive theory of their mode of action at present available. The theory explains many facts that are otherwise obscure, but not all, and further work is required to ascertain whether the explanations offered for the apparent exceptions are valid.

With regard to screening of potentially carcinogenic compounds, it is clear that simple tests demonstrating charge transfer with trinitrobenzene and acridine would have picked up all the major carcinogens listed in this paper. Although some non-carcinogenic compounds would also have been included, it seems fair to say that any new compound showing these effects should be treated with suspicion until there is good evidence that it is not carcinogenic. We feel that these tests, and others for alkylation of some test compound such as cysteine, should be of value in screening for potential carcinogens.

We thank our colleagues who supplied compounds for testing, in particular Prof. J. A. Miller, Prof. A. Haddow, Prof. M. S. Newman and Prof. N. P. Buu-Hoï. We also thank Drs. D. H. Whiffen and W. Slough for allowing us to quote electron spin resonance measurements made in their laboratory, and Dr. J. W. Cornforth for helpful discussion.

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POLAROGRAPHIC MEASUREMENTS OF ELECTRON DONATION AND ACCEPTANCE BY CARCINOGENIC COMPOUNDS

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EVIDENCE has been presented in the preceding article that a wide range of carcinogenic compounds are able both to donate and to accept electrons. They are thus potentially capable of forming charge-transfer complexes with electron donors as well as with electron acceptors and this dual property may help to explain how they exert their carcinogenic effects. Electron-donating and -accepting power is determined respectively by the ionization potential and electron affinity of the molecule; direct measurement of ionization potentials can be made in favourable cases but not, at present, of electron affinities. Donating power has also been investigated in relation to charge-transfer spectra using theoretical calculations of energy-levels[1,2]. In this article, the results of experiments with a polarographic technique will be described. Such results have already proved of value in measuring electron-accepting capacity[3-5], as well as electron-donating capacity[6], in connexion with investigations of charge-transfer interaction.

A number of workers have shown that electron-transfer between an organic substance and an electrode surface results initially in the formation of the mono-negative or -positive ion (or the dipositive ion in some cases), which have appreciable lifetimes in a relatively inert solvent[7]. These properties have recently been utilized in the study of organic free-radical anions and cations by electron spin resonance[8]. Thus, at a moving electrode, as used in polarography, the lifetime of the electrode products may be large (some seconds) in comparison with their rate of diffusion from the electrode surface. As a consequence, the current-potential curves will be close to the redox curves for the reactions $A + e \rightleftharpoons A^-$, $D - e \rightleftharpoons D^+$, where A and D are the acceptor and donor molecules. Even when the electrode products A^- and D^+ are highly unstable, the half-wave potentials are not generally shifted from the E_1 values by more than a few hundredths of a volt.

The half-wave potentials can be identified with corresponding redox potentials E_1^{red} and E_1^{ox}. The E_1 potentials are related in a simple manner to the gaseous ionization potential I_D and electron affinity E_A by the equations[9]:

$$E_1^{\text{ox}} = I_D - \Delta E_{\text{sol}}^{\text{ox}} - \text{constant}$$

$$E_1^{\text{red}} = E_A + \Delta E_{\text{sol}}^{\text{red}} - \text{constant}$$

where ΔE_{sol} is the solvation energy contributed by the solvent (the constant is appropriate to the reference electrode). The main contribution to ΔE_{sol} lies in the solvation energy of the ion A^- or D^+, and decreases as the delocalization of the charge increases[4,9]. Recent work shows that with the exception of large condensed aromatic systems the solvation energy term is remarkably constant[4-6]; it varies approximately linearly for large aromatic systems[4,5,9]. Measurement of E_1 values thus provides a convenient method obtaining a parameter linearly related to I_D and E_A, the electron-donating and -accepting energies of the molecule.

We have used as solvent acetonitrile, which has been shown by a number of workers to be convenient for polarography of this type[7,9]. Briefly, measurements were made with 0·1 N tetraethyl ammonium perchlorate as supporting electrolyte, with a Cambridge Instrument pen-recording polarograph, using either a dropping mercury or rotating platinum electrode (600 r.p.m.). Initial polarization of the platinum electrode at $-2·4$ V for 5 min gave a reproducible electrode surface. Potentials are referred to an aqueous saturated calomel reference electrode using a three-electrode cell with compensator[4,10]. The limits of measurement are from about $-2·8$ V to 2·4 V. Roughly, this corresponds to the reduction of fluorene at one extreme and oxidation of benzene at the other. Poorer electron acceptors or electron donors cannot therefore be determined.

The results for a variety of carcinogenic and chemically related non-carcinogenic compounds are given in Table 1. The compounds are grouped in such a way that comparable solvation energy terms are involved. Our results for aromatic hydrocarbons are in good agreement with those of Lund[11], who used a vibrating platinum electrode. Plots of donor strength measured polarographically against those calculated from charge-transfer bands (see preceding article) are shown in Fig. 1. These fall reasonably well on a straight line with a slope of 45°, but the curve for hydrocarbons is clearly different from that for amines.

Most carcinogens show both acceptor and donor properties well within the limits of the technique, being

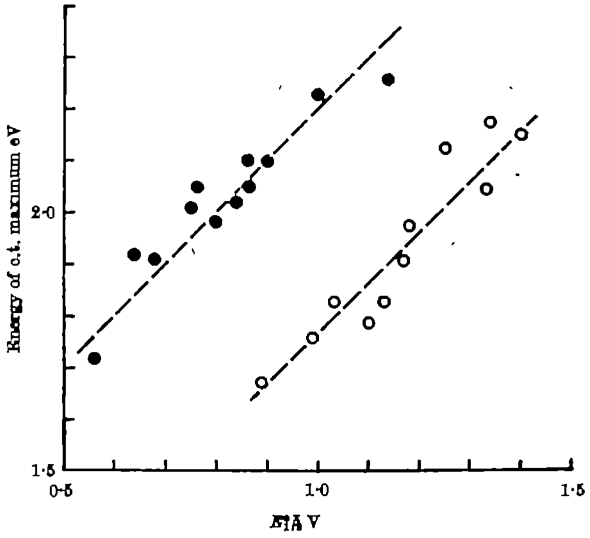

Fig. 1. Plots of energies of charge-transfer (c.t.) maxima of complexes of hydrocarbons (○) and aromatic amines (●) with chloranil against first half-wave potentials for oxidation measured polarographically

Table 1. E_1 VALUES FOR SOME CARCINOGENIC AND RELATED NON-CARCINOGENIC COMPOUNDS

Comparable solvation energy terms are involved in each of the groups. Donor and acceptor strengths increase as E_1^{ox} decreases and E_1^{red} becomes less negative

Compound	V versus S.C.E.		Carcinogenic activity
	E_1^{ox}	E_1^{red}	
3,4-Benzpyrene	1·35	−2·22	−
1,2,5,6-Dibenzanthracene	1·34*	−2·04†	+
1,2-Benzanthracene	1·33*	−2·11†	−
Pyrene	1·22	−2·18	−
Anthracene	1·19	−2·07	−
1,2,5,6-Dibenzpyrene	1·17	−1·90	+
9,10-Dimethyl-1,2-benzanthracene	1·12	−2·16	+
1,2-Benzpyrene	1·10	−1·96	+
5,6-Benzpyrido-(2′3′ : 1,2)-carbazole	1·06	−1·98	+
5,6-Benzpyrido-(3′2′ : 1,2)-carbazole	1·04	−1·95	+
Perylene	1·04	−1·73	−
20-Methylcholanthrene	0·99*	?	+
2-Aminoanthracene	0·56	−2·19	+
Cortisone	>2·2	−2·12	−
Oestrone	1·19	−1·98	+
Stilboestrol	0·90	−2·30	+
Benzene	2·38	N‡	−
Fluorene	1·60	−2·79	−
3-Acetylaminodibenzothiophene sulphone	1·59	−1·79	−
3-Acetylaminodibenzofuran	1·40	−2·21	+
3-Acetylaminodibenzothiophene	1·39	−1·99	+
2-Acetylaminofluorene	1·14	−2·01	+
N-Hydroxy-2-acetylaminofluorene	1·00	−2·12	+
4-Aminobiphenyl	0·93	N‡	+
2-Naphthylamine	0·75	−2·8	+
1-Naphthylamine	0·68	−2·8	−
2-Aminofluorene	0·64	−2·8	+
Azobenzene	1·80	−1·45	−
3′-Fluoro-*p*-dimethylaminoazobenzene	0·90	−1·50	−
p-Dimethylaminoazobenzene	0·86	−1·58	+
p-Diethylaminoazobenzene	0·84	−1·62	−
2-Methyl-*p*-dimethylaminoazobenzene	0·80	−1·60	−

* From Lund, Ref. 11.

† In 96 per cent dioxane, Hoijtink, G. J., *Rec. Trav. Chim.*, **74**, 1525 (1955).

‡ N—not reduced.

relatively good donors[8] and showing measurable acceptor properties, which are nevertheless weaker than those of common acceptors such as quinones[3,4]. It is striking that so diverse a series of functional groupings in the carcinogens produces such a similar pattern of electron donor and acceptor strengths. The only carcinogen listed not showing measurable acceptor property is 4-aminodiphenyl. However, in this case, and that of 2-naphthylamine, it is likely that the active compound is a hydroxyl derivative, which would be an acceptor by virtue of the hydroxylamine system.

In the non-carcinogenic compounds exhibiting both donor and acceptor properties these functions are in general weaker than in carcinogens. However, non-carcinogenic azo-compounds (*p*-diethylaminoazobenzene, 3′-fluoro-*p*-dimethylaminoazobenzene and 2-methyl-*p*-dimethylaminoazobenzene) were exceptional in that their donor and acceptor strengths were of the same order as those of the carcinogenic *p*-dimethylaminoazobenzene. Moreover, the carcinogenic 1,2,5,6-dibenzanthracene is a weaker donor than certain non-carcinogenic hydrocarbons. It should be recalled that the stability of charge-transfer complexes is not always simply related to donor and acceptor strengths[6,12]. Moreover, steric and metabolic effects have to be taken into consideration, as discussed in the preceding article. The polarographic characteristics of the various compounds will be discussed in detail elsewhere. However, it is worth noting here that some differences between carcinogenic and non-carcinogenic compounds were observed. Thus, in the series of pyrene derivatives only those that were carcinogenic were found to undergo one electron oxidation. Pyrene itself was oxidized by a two-electron step, and 3 : 4-benzpyrene by a three-electron step, with the formation of insoluble products. Thus the products of oxidation of the carcinogens are likely to be more stable than those of the non-carcinogens.

Finally, we may point out a further application of the E_1 potentials. From the relevant Nernst equations it is easily shown that:

$$E_1^{red} - E_1^{ox} = \frac{RT}{F} \ln \frac{[D^+][A^-]}{[D][A]} = \frac{RT}{F} \ln K$$

where K is the equilibrium constant of the reaction:

$$D + A \rightleftharpoons D^+ + A^-$$

Thus for any pair of D and A, it is possible to calculate the concentration of free D^+ and A^- ions, in the solvent in which the potentials are measured. This should be of value in the elucidation of electron spin resonance and spectrophotometric studies of such systems[13,14].

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EVIDENCE FOR TWO TYPES OF MEDIATION OF NEUTRAL AMINO-ACID TRANSPORT IN EHRLICH CELLS

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BECAUSE many of the neutral amino-acids compete with one another for transport[1–3], at least in mammalian systems, a common mediation has generally been taken to serve for them all. A lack of strict quantitative agreement between the affinities indicated by initial rates, and those indicated by competitive action or extent of accumulation has, however, been apparent to us for some time[4,5]. If only one mediating site or carrier serves for transport of all the neutral amino-acids, then they will yield a consistent set of relative affinities whatever amino-acid is used to compare their inhibitory actions. The present results show that at least two sites with overlapping specificities must be operative to account for the relative inhibitory actions observed in the Ehrlich ascites tumour cell.

The techniques of propagating, collecting and separating the Ehrlich ascites tumour cells have been described elsewhere[6]. Uptake of amino-acids was studied at 37° C by incubating 4 per cent suspensions of tumour cells for 1 min in Krebs–Ringer bicarbonate buffer media containing amino-acids labelled with carbon-14. At the end of the incubation time the suspension was diluted with 1 volume of ice-cold buffer and centrifuged for 2 min at 2,500*g*. Radioactivity in a hot acetic acid extract of the cells was counted in a liquid scintillation spectrometer. The results for the packed cells were corrected for 20 per cent extracellular space and expressed as a distribution ratio, that is, c.p.m. per ml. intracellular fluid divided by c.p.m. per ml. extracellular fluid.

Fig. 1 shows the results of an experiment in which the uptake from 1 mM solutions of the amino-acids listed at the top of the figure was measured in the presence of 5-mM levels of the amino-acids listed in the body of the figure. The effectiveness of leucine as an inhibitor may be seen to decrease from left to right, while that of alanine increases. If the competition were for a single site, the trends in the inhibitory action would be parallel and not opposite. Valine appears to follow the same trend as leucine, and glycine the same as alanine. Although a quantitative test has not been completed, two transport-mediating sites promise to be enough to explain the results. One of these we may call the leucine-preferring or *L* site, the other the alanine-preferring or *A* site. Alanine, glycine and α-aminoisobutyric acid have affinities predominantly for the latter site, and leucine and valine for the former. A third type of amino-acid is illustrated in Fig. 1 by methionine, which is strongly inhibitory to all five of the amino-acids selected here for comparison. Accordingly, it must have high affinity for both the postulated sites.

Although these transport mediators are clearly distinguishable, their specificities are nevertheless so broad that all the naturally occurring, neutral amino-acids tested have some affinity for both. Of the poorly metabolized model amino-acids proposed from this laboratory α-aminoisobutyric acid may be recommended for investigating the *A* mediation, and tertiary leucine, for the *L* mediation. Curiously, 1-amino*cyclo*pentanecarboxylic acid is a model for the third type of amino-acid with high affinity for both mediations.

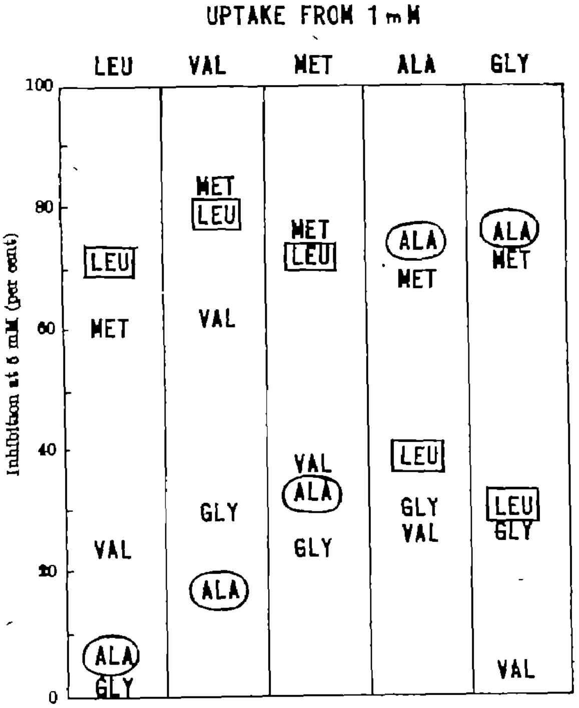

Fig. 1. Relative inhibitory actions for transport among five amino-acids. The position of each amino-acid in the body of the illustration represents quantitatively its percentage inhibition of the uptake of the amino-acid listed at the top of each column

For the same levels, the effluxes of amino-acids using site *A* for entry, such as glycine and alanine, are considerably smaller than their influxes; therefore, they are accumulated to high chemical gradients. The amino-acids preferring the leucine site show effluxes much closer to their influxes; accordingly, they give much lower steady-state distribution ratios, between 1 and 5. Because efflux among all the amino-acids varies independently of influx, their initial rates of uptake bear no fixed relationship to their steady-state distribution ratio. The change of the distribution ratio of representatives of the three groups of amino-acids with time is presented in Fig. 2. Glycine has the slowest initial entry rate but is eventually accumulated to a high degree. In contrast, leucine has one of the fastest initial rates, but it reaches a final distribution ratio of only 3. Methionine and 1-amino*cyclo*pentanecarboxylic acid have high initial rates of entry, and are accumulated to relatively high distribution ratios. Obviously if glycine is finally concentrated so strongly, despite its comparatively slow uptake it must be lost from the cell even more slowly.

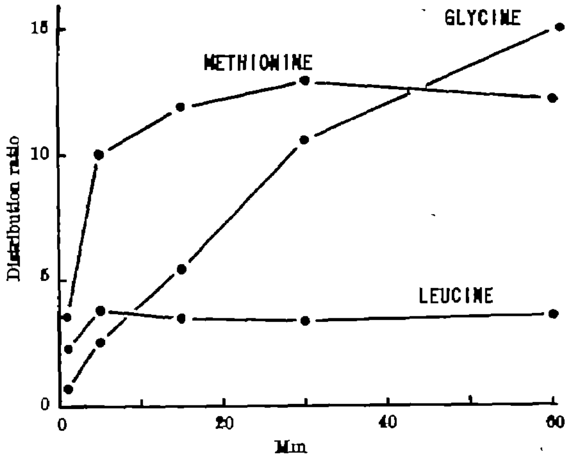

Fig. 2. Time-course of the uptake of three amino-acids by Ehrlich tumour cells at 37°. Initial level of amino-acid labelled with carbon-14 in Krebs–Ringer bicarbonate medium, 1 mM; 25 volumes of suspending medium used

These differences in efflux among the amino-acids indicate that they escape from the cells largely by mediated processes, a conclusion supported by the following findings (to be published): the escape process is subject to saturation and to competition by analogous amino-acids; it is largely stereospecific; in some cases it can be accelerated by the external presence of another amino-acid.

Fig. 3 compares the ability of amino-acids of the three groups discussed here to stimulate amino-acid entry by exchange. In this experiment the amino-acid listed at the top had been permitted to accumulate within the Ehrlich cell from a 10 mM solution. The length of the bars, in relationship to the horizontal line, show whether the subsequent uptake of the amino-acids listed at the bottom during 1 min was faster or slower than it was in the absence of the intracellular accumulation. Valine, 1-amino*cyclo*pentanecarboxylic acid and methionine can be seen to serve for exchange with each other, thereby accelerating uptake. In contrast, α-aminoisobutyric acid and glycine (and alanine, to be published) in our hands give little or no stimulation of exchange unless their internal level is very high (70–100 mM). The results indicate that the *L* site serves far more effectively for exchange; its reversible action may well account for all the exchange-diffusion of neutral amino-acids.

We may tentatively summarize the characteristics of the two sites. First, the steady-state distribution ratios of the group of amino-acids using site *A* (10–20) are much higher than those using site *L* (1–5). Secondly, site *L*

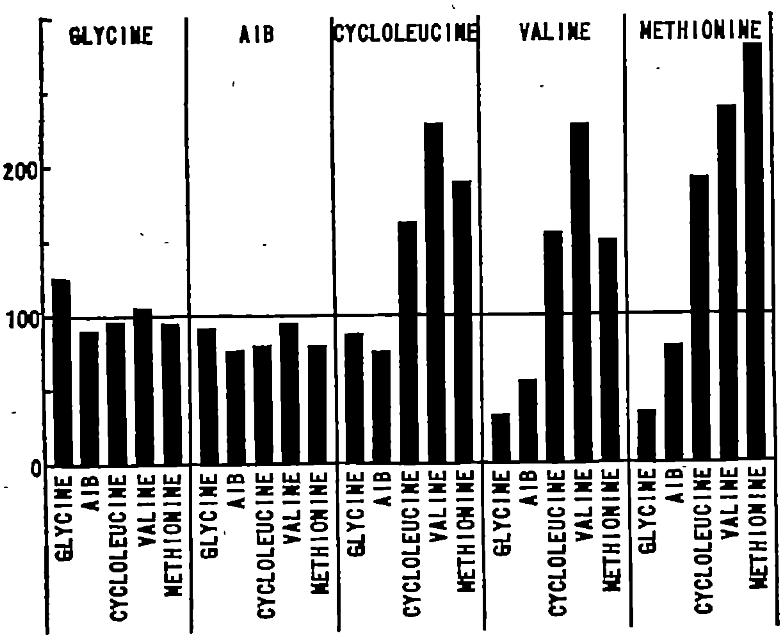

Fig. 3. Effect of five previously accumulated amino-acids on uptake of each other. The amino-acid listed at the top had been allowed to accumulate for 40 min from a 10 mM solution. The cells were then washed and resuspended for 1 min at 37° in fresh medium containing 1 mM levels of the labelled amino-acids listed at the bottom of the figure. The amount of radioactivity taken up is compared with the amount taken up by control cells, setting the latter at 100 per cent

serves for exchange and perhaps for all the mediated exit of the amino-acids from the cells, whereas site *A* appears to operate largely for entry. Thirdly, raising the temperature from 27° C to 37° C accelerates the entry of glycine and α-aminoisobutyric acid by about 3·5 times, whereas under the same conditions the Q_{10} of valine and leucine entry is only about 2·5.

The site serving best for the amino-acids with large hydrocarbon side-chains such as valine and leucine shows increased affinity with both the length and bulk of that side-chain. The drop-off in affinity is very sharp as the number of carbon atoms in the amino-acids falls below 5.

The other site, serving best for glycine and alanine entry, appears to discriminate against amino-acids with 5–6 carbon atoms, especially when their chains are branched. As the number of carbon atoms in a straight chain increases beyond 4, the affinity is again increased. This effect appears to account for the affinity of methionine for this site; the sulphur atom of methionine seems to be approximately equivalent to an additional methylene group in establishing affinity. One possibility being considered is that the *E* site, by the expenditure of energy, can be converted into the *A* site, thus increasing its affinity for certain amino-acids, including methionine, alanine and glycine. The reconversion of *A* to site *L* on passing the osmotic barrier of the membrane would cause the unloading of these amino-acids.

Preliminary results suggest that much the same pair of sites with overlapping affinities participate in amino-acid transport in other cells and tissues[7]. In the mature human red blood cell uptake appears to be largely limited to the *L* mediation[8].

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SUPPRESSION OF INFECTIONS RESULTING FROM A DEOXYRIBONUCLEIC ACID VIRUS (VACCINIA) BY SYSTEMIC ADMINISTRATION OF 5-IODO-2′-DEOXYURIDINE

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THE ability of 5-iodo-2′-deoxyuridine (IUDR or IDU) to inhibit plaque formation of vaccinia and herpes simplex viruses *in vitro*[1] led to investigations with animal and clinical trials which have demonstrated that IUDR is capable of arresting and curing herpes simplex infections of the cornea in both rabbits and man[2]. These observations have been confirmed and extended to related compounds[3] and other DNA viruses[4]. Since high local concentrations of drug can be achieved by frequent topical application, without systemic toxicity, the eye presents a unique site for therapy with potent anti-viral agents[2]. While this approach has demonstrated impressive clinical value[2,4], it is necessarily limited to ophthalmic and possibly other superficial infections. We have explored the feasibility of inhibiting DNA-viral disorders by the systemic administration of iodinated pyrimidine deoxyribonucleosides. This preliminary communication reports favourable results obtained with parenterally administered IUDR in the treatment of intradermal vaccinia infection.

The rationale for the design and synthesis of IUDR and 5-iodo-2′-deoxycytidine (ICDR) has been described[7–10], as have work on their mechanisms of action[10–12] and the results of initial clinical trials[13,14]. A brief summary of our present knowledge of these agents is important, nevertheless, for understanding of the anti-viral findings to be reported. Each of these compounds, originally synthesized and studied as potential anti-neoplastic agents, is converted enzymatically within cells into the 5′-monophosphate of IUDR (IUDR 5′P) and, presumably, to the di- and tri-phosphate esters. ICDR, a more soluble and thermostable compound than IUDR, must be deaminated, either before or after 5′-phosphorylation, to form either IUDR or its phosphorylated derivatives. These substances not only interfere with the utilization by cells of thymidine and its corresponding derivatives

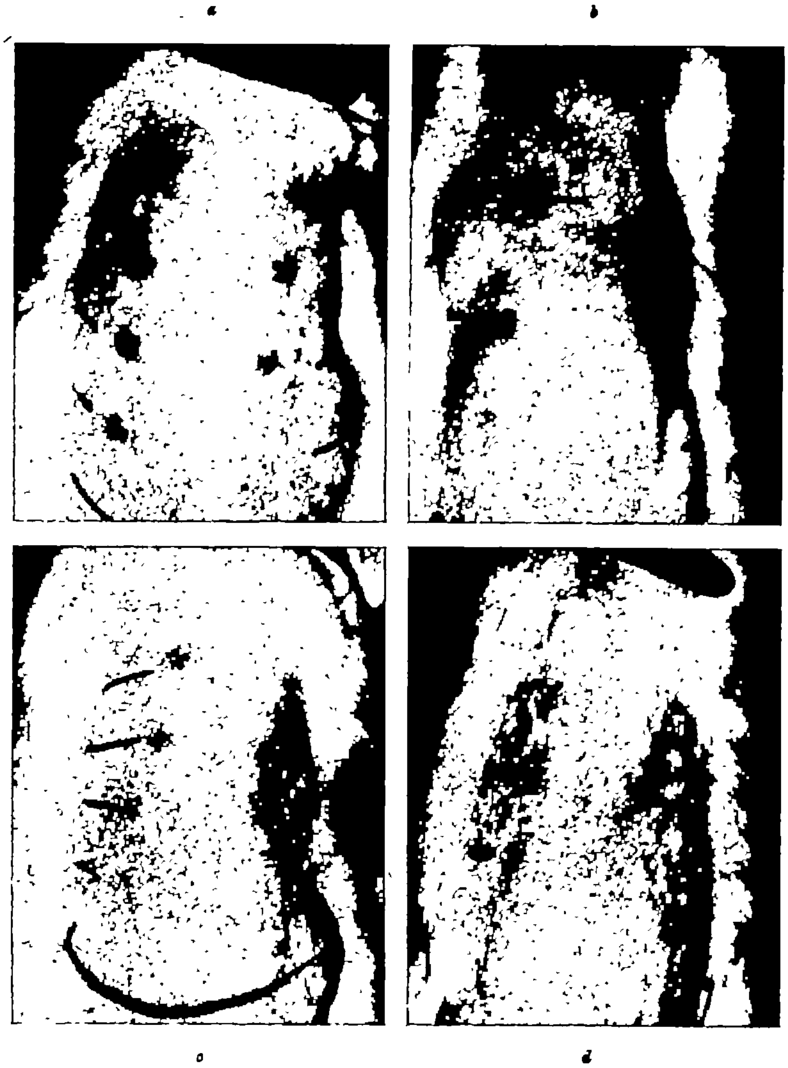

Fig. 1. Dorsal aspect of four rabbits, each representing one of the groups in Exp. 1, on the sixth day after para-spinal intradermal injections of vaccinia virus. Sequential inoculations of the virus preparation in ten-fold dilutions were started at the upper left, progressing downward (caudally), these were continued on the right, again progressing caudally. Although the inoculations were started with dilutions of 10^{-1} in the control animals (*a*), the sequence was begun with undiluted stock virus in the rabbits given IUDR (*b*, *c* and *d*). In each series the last position (lower right in each illustration) was used for vehicle control. The rabbits shown above received IUDR daily for 5 days according to the following dosage schedules (see also Table 1): *a*, control group – no IUDR; *b*, group I – 120 mg IUDR/kg once daily, *c*, group II – 40 mg IUDR/kg every 8 h; *d*, group III – 80 mg IUDR/kg every 8 h

but also lead to the formation of 'fraudulent DNA', in which IUDR replaces thymidine; either mechanism, or both, can inhibit normal replication of DNA and reproduction of cells. In addition, and perhaps with greater selectivity, the replication of DNA in certain viruses, or their cytopathogenicity, may be affected by the drug. The susceptibility of certain actively reproducing human tissues to these agents can lead to alopecia, stomatitis and hæmatopoietic suppression; these effects can be prevented by concomitant regional administration of small amounts of thymidine. Thus, in patients receiving IUDR parenterally in doses of 120 mg/kg daily for 5 days, regional protection has been achieved by daily administration of 4–8 mg of thymidine per kg[15,16]. Because of such toxic manifestations and the possible danger that incorporation of IUDR into DNA could lead to increased mutation rates, and conceivably, neoplastic change, we have administered these drugs systemically only to patients with advanced neoplastic disease; nevertheless, in potentially lethal diseases of man, caused by DNA viruses, such as vaccinia gangrenosum, smallpox, simian *B* virus and herpes simplex encephalitis, chemotherapeutic trial of IUDR deserves consideration.

The present work was initiated to explore quantitatively the significance of preliminary observations in two patients with advanced neoplastic disease, who were undergoing treatment with IUDR or ICDR and who had not been vaccinated against smallpox for at least 20 years. In one patient who was receiving a 2-h intravenous infusion of 80 mg of ICDR/kg daily for 5 days, the standard dose of a commercial calf-lymph smallpox vaccine was applied directly to the skin of both arms 1 h after the first intravenous infusion was begun, and the stratum corneum was penetrated 20–40 times with the sterile scarifying needle. The other patient was vaccinated in a similar manner 1 h after initiation of a total dose of 150 mg of IUDR/kg; the compound was administered as a continuous intravenous infusion for 36 h. In both patients, 'takes' were not obtained; however, revaccination with the same lot of vaccine, after an interval of 3 weeks, caused typical primary reactions in both individuals. In two other patients ICDR failed to prevent 'takes'; but in these subjects studies of the pattern of metabolic alteration of ICDR–^{131}I have indicated that an adequate conversion of ICDR to IUDR did not occur and significant antineoplastic effects were not seen[17]. On the other hand, in the patient given ICDR for 5 days in whom a primary 'take' was prevented by the drug, sustained blood-levels of IUDR were found, the typical toxic effects were seen and a marked regression in a carcinoma of the vulva occurred[17]. In two other patients, who were given IUDR by continuous intravenous infusion (300 mg/kg during a period of 72 h) and vaccinated as above, but with a different commercial vaccine prepared from infected chick embryo tissue, 'takes' were not prevented. Thus, it is clear that because of the complexities posed by possible differences in the potency of commercial preparations of vaccinia virus, the regimens of dosage of these drugs, as well as significant variations in rates of metabolic degradation and utilization of these compounds, more clinical studies are needed to determine the significance of these preliminary findings in man.

In rabbits infected intradermally with vaccinia and treated systemically with IUDR, the results have been much more definitive. The virus used was derived from a pool representing the fourth chick embryo tissue culture passage of a vaccinia strain previously carried through an unknown number of passages on the chorioallantoic membranes of embryonated eggs. Vaccinia hæmagglutinin used in the serological tests was prepared from the egg-passage line; serial two-fold dilutions of serum were challenged with four agglutinating units. In each of two experiments, twelve rabbits were injected intradermally in parallel para-spinal rows of inoculations of the virus preparations in sequential ten-fold dilutions ranging from undiluted stock virus suspension to 10^{-8}. IUDR was administered subcutaneously at sites well removed from the intradermal inoculations of vaccinia virus. The regimens of dosages of IUDR and the results obtained are shown in Table 1.

In both series the treatment increased the amount of virus required to produce dermal lesions; the degree of

Table 1. EFFECTS OF SUBCUTANEOUSLY ADMINISTERED IUDR ON INTRADERMAL TITRATION OF VACCINIA VIRUS IN RABBITS

Experiment*	Group†	Treatment‡ (daily for 5 days)	IUDR (mg/kg daily	(total))	Endpoints of intradermal titrations observed on day :§ 3	5	7	Serum HA-I ¶ titre 18–21 days after inoculation
	Control	5 per cent dextrose in water, 30 ml. every 8 h	none	(none)	$10^{-7\cdot8}$	$10^{-7\cdot6}$	$10^{-6\cdot6}$	1:160
	I	IUDR, 120 mg/kg once daily	120	(600)	$10^{-4\cdot8}$	$10^{-5\cdot6}$	$10^{-5\cdot3}$	1:160
1	II	IUDR, 40 mg/kg every 8 h	120	(600)	$10^{-5\cdot5}$	$10^{-4\cdot8}$	$10^{-4\cdot1}$	1:80
	III	IUDR, 80 mg/kg every 8 h	240	(1,200)	$10^{-5\cdot1}$	$10^{-3\cdot8}$	$10^{-3\cdot4}$	1:160
	IV	IUDR, 120 mg/kg every 8 h	360	(1,800)	$10^{-6\cdot6}$	$10^{-3\cdot5}$	$10^{-2\cdot6}$	1:80
2	V	IUDR, 60 mg/kg every 4 h	360	(1,800)	$10^{-6\cdot4}$	$10^{-3\cdot6}$	$<10^{-1\cdot0}$	1:160
	VI	‖IUDR, 120 mg/kg every 8 h, starting 48 h after virus inoculation	360	(1,080)	$10^{-6\cdot6}$	$10^{-5\cdot8}$	$10^{-3\cdot3}$	1:320
	Control	5 per cent dextrose in water, 30 ml. every 8 h	none	(none)	$10^{-6\cdot8}$	$10^{-6\cdot3}$	$10^{-6\cdot6}$	1:320

* In Exp. 1 treatment with IUDR was started 2 h before, and, in Exp. 2, 6 h after virus inoculation (except in Group VI).
† Each group consisted of three rabbits without demonstrable vaccinia antibodies prior to inoculation.
‡ IUDR was administered in a concentration of 5 mg/ml. in solutions of 5 per cent dextrose in water adjusted to a pH of 8·6.
§ Calculated on the basis of an area of induration and erythema not less than 4 mm.
¶ HA-I, hæmagglutination inhibition. Figures represent average maximal serum dilution inhibiting 4 agglutinating units of antigen.
‖ Treated for only 3 days.

suppression of the response to the virus was greater with increasing dosages of IUDR. In addition, central necrosis in dermal lesions was greatly reduced (Fig. 1) and, even when IUDR therapy was not initiated until 48 h after inoculation with the virus (Group VI), a marked increase in amount of virus required to produce infection was observed. There was no evidence of drug toxicity, as indicated by weight loss, hæmatopoietic depression or change in physical appearance.

These preliminary studies suggest that adequate levels of IUDR in the skin can interfere with development of the dermal lesions ordinarily caused by vaccinia virus. Further investigations are in progress to determine the chemotherapeutic value of IUDR in generalized vaccinia infections produced in rabbits previously treated with adrenocorticosteroids. Studies of this nature, as well as comparative trials with isatin β-thiosemicarbazone and related compounds[18,19], will be necessary to determine the advisability of clinical trials of IUDR in generalized and potentially lethal infections with such DNA viruses as smallpox, vaccinia and simian B virus. In addition, studies of the effect of IUDR on the oncogenicity of a variety of DNA viruses (murine, simian and human) in animal species will be carried out.

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PENICILLINASES FROM GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA: A THERMODYNAMIC DIFFERENCE

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WORK in this laboratory indicates that considerable differences occur in the physical and chemical properties of penicillinases from different bacterial species, for example, in substrate specificity and affinity, inducibility, susceptibility to inhibition, cellular location and pH-activity characteristics. In addition to these, we have found a general difference between penicillinases from Gram-negative and Gram-positive sources, as regards the values of the apparent activation energies for the hydrolysis by them of the β-lactam ring in benzylpenicillin.

Cell-free penicillinase preparations were made from clinically isolated Gram-negative bacteria by disrupting the organisms in a liquid extrusion press[1,2] and sedimenting debris at 20,000g for 50 min. Staphylococcal penicillinase was prepared by growing organisms overnight at 37° C in infusion broth containing 0·5 μg/ml. of methicillin as inducing agent; the organisms were collected and washed by centrifugation and sodium p-chlormercuribenzoate added to 0·001 M to prevent further induction[3]. *B. cereus* was grown overnight at 37° C in infusion broth containing 5 μg/ml. of methicillin as inducing agent, organisms removed by centrifugation and the supernatant fluid used as the penicillinase preparation. *B. licheniformis* penicillinase was obtained from Wellcome Research Laboratories, Beckenham.

Rates of the hydrolytic cleavage of the β-lactam ring of penicillin G (at an initial concentration of 2 mg/ml., a sufficient concentration to saturate all enzymes tested) were determined at various temperatures by the hydroxylamine method[4]. An integrated form of the Arrhenius equation is $\ln\frac{k_2}{k_1} = \frac{A}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$, where k_1 and k_2 are rate constants at temperatures T_1 and T_2, A = apparent activation energy and R = the gas constant. Therefore, when the logarithms of the rate constants are plotted against the reciprocals of the absolute temperature a straight line is obtained, from which the apparent energy of activation can be calculated. The hydrolyses of benzylpenicillin, studied here, were found to be zero order reactions and hence the rate constants are equal to the reaction velocities, so these values were plotted as the rate constants. Activity-temperature data given by Royce *et al.*[5] and Novick[6] for *B. subtilis* and *Staph. aureus* 524 *SC*, respectively, were similarly processed. The value of 7,500 cal/mole for *B. cereus* is that quoted by Henry and Housewright[7], and was determined by the manometric assay. Preliminary experiments showed that induction of *Staph. aureus* and *B. cereus* penicillinases did not alter the value of their apparent activation energies.

The proportion of molecules in a system possessing a given energy can be calculated by evaluating the exponential term in the equation: $N = N_0 e^{-E/RT}$ (derived from the Maxwell–Boltzmann distribution law[8]), where N, number of molecules possessing sufficient energy to react; N_0, total number of molecules present; E, required energy-level; R, gas constant; T, absolute temperature (taken as 310° K). The rate constant for a reaction is proportional to the number of activated molecules. Thus, in this case the rates of reaction are also directly proportional to both the number of activated molecules and the exponential term $e^{-E/RT}$. Now if the energy E is the apparent activation energy, the corresponding exponential term can be used to measure the effect produced in a system by an enzyme which, by lowering the activation energy, increases the proportion of molecules possessing sufficient energy to react.

The exponential terms were calculated for each individual penicillinase, and the results are shown, together with the values for the energies of activation, in Tables 1 and 2. Energies of activation were also found for the non-enzymatic hydrolysis of benzylpenicillin in the presence of different buffers at different pH values[9]: these results, together with the values of the exponential terms, are shown in Table 3; the mean value for the activation energy (13,467 cal/mole) agrees well with that reported by Abraham and Newton[10] (14,500 cal/mole). The mean values of all the apparent activation energies are shown in Fig. 1.

Table 1. SHOWING THE EXPERIMENTALLY DETERMINED APPARENT ACTIVATION ENERGIES AND THE DERIVED EXPONENTIAL FUNCTIONS OF PENICILLINASES FROM GRAM-POSITIVE ORGANISMS

	Activation energy (cal/mole)	Exponential function ($\times 10^4$)
Staph. aureus E3	7,200	9·05
Staph. aureus 524 *SC* (ref. 6)	6,600	23·62
Staph. aureus 13137	8,500	1 11
B. licheniformis	8,900	0·68
B. subtilis (ref. 5)	7,700	4 04
B. cereus N.R.R.L. 569	7,700	4 04
B. cereus (ref. 7)	7,500	5 58
Mean	7,714	6·90
S.D.	706	10 86
S.E.	267	4·10

Table 2. SHOWING THE EXPERIMENTALLY DETERMINED APPARENT ACTIVATION ENERGIES AND THE DERIVED EXPONENTIAL FUNCTIONS OF PENICILLINASES FROM GRAM-NEGATIVE ORGANISMS

	Activation energy (cal/mole)	Exponential function ($\times 10^4$)
K. aerogenes 418	3,400	41·54
K. aerogenes 366	4,000	15·78
K. aerogenes 402	3,400	41·54
K. aerogenes 415	3,400	41·54
E. coli 214	4,600	6·00
E. coli 419	5,300	1·94
A. cloacae 53	4,700	5·10
Mean	4,114	21·92
S.D.	767	18·84
S.E.	290	7·12

Table 3. SHOWING THE EXPERIMENTALLY DETERMINED APPARENT ACTIVATION ENERGIES AND THE DERIVED EXPONENTIAL FUNCTIONS FOR THE NON-ENZYMATIC HYDROLYSIS OF BENZYLPENICILLIN

Buffer	Molarity	Ionic strength	Activation energy (cal/mole)	Exponential function ($\times 10^{10}$)
Veronal *p*H 9·2	0·04	0·042	12,600	14 94
*Tris p*H 7·4	0·05	0·065	14,100	1 83
Phosphate *p*H 7 4	0 025	0·065	13,700	2 53
		Mean	13,467	6 27
		S.D.	778	7 53
		S.E.	449	4 34

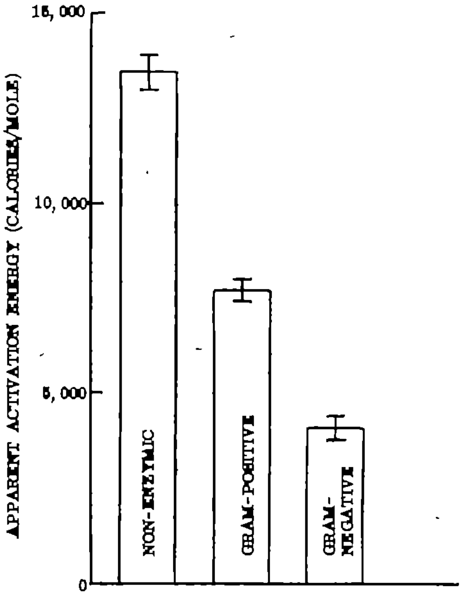

Fig. 1. Showing the relationships between the mean energies of activation ± *S.E.* for the non-enzymatic and penicillinase catalysed hydrolysis of benzylpenicillin

The results obtained were analysed statistically; the difference between the apparent activation energies of penicillinases from Gram-negative and Gram-positive organisms was significant at a probability of < 0·001 (t = 8·89 with 12 *d.f.*), and the difference in magnitude of the corresponding exponential terms was found to be significant at a probability of < 0·01 (t = 3·07 with 12 *d.f.*). As the mean exponential function derived from the apparent activation energies of penicillinases of Gram-negative organisms was 318 times the corresponding exponential function for Gram-positive organisms it follows that penicillinases from the former sources were more efficient catalytic agents than those from the latter sources. However, as only a relatively small number of penicillinases have been studied so far, it is too early to say if this is a general rule.

From the mean values of the exponential functions it was possible to calculate the increase, brought about by the presence of the enzymes, in the proportion of activated penicillin molecules as compared with the fraction present in non-enzymatic hydrolysis mixtures. This increase for each source of penicillinase was found to be: (*a*) using Gram-positive organisms $1·1 \times 10^4$ times; (*b*) using Gram-negative organisms $3·5 \times 10^6$ times; (*c*) using all penicillinases $1·8 \times 10^6$ times.

Further work is at present being carried out on the penicillinases produced by members of the Enterobacteriaceae, and reports have been[11,12] or will be made elsewhere.

We thank Prof. R. Knox and Dr. W. L. G. Gent for advice and criticism.

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EFFECT OF HYPOXIA ON BRAIN GANGLIOSIDES

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GANGLIOSIDES are a group of complex, acidic, water-soluble glycosphingolipids occurring predominantly in membrane constituents of a microsomal fraction from grey matter of vertebrate brain[1–3]. The presence of *N*-acetylneuraminic acid (NANA) distinguishes gangliosides from other brain glycolipids. Recent investigations indicate that gangliosides may be involved in the maintenance of the excitable state in cerebral tissues[4–9]. Our present work reports a decrease in the NANA content of isolated and purified gangliosides obtained from human and cat brains which were hypoxic before death.

Gangliosides were extracted from cerebral cortex by a modification of the tetrahydrofuran extraction procedure of Trams and Lauter[10]. Pieces of cerebral cortex were homogenized in 2 vol. of 0·01 M phosphate buffer, *p*H 7, and the mixture blended with 12 vol. of ice-cold tetrahydrofuran. This mixture was filtered on a Büchner funnel and the residue re-extracted with a further 3 vol. of tetrahydrofuran. The volume of the combined clear yellow filtrates was measured and 1/4 of this volume of ether added. The mixture was shaken vigorously and allowed to stand for 2 h at 0°–4° C. The lower phase was removed, the upper phase washed with 100 ml. of water, and the lower phase again removed. The combined lower phases were filtered and passed through an 'Amberlite *MB*-3' ion-exchange column, previously washed with 50 per cent ethanol. To the eluate 0·2 N barium hydroxide was added in the cold until the *p*H was between 10 and 11. 4 vol. of cold absolute ethanol (– 20° C) were added and the barium salts of the gangliosides allowed to precipitate overnight at 0°– 4° C. The precipitate was collected by centrifugation, washed with cold 90 per cent ethanol and suspended in 50 ml. of water. Sulphuric acid (1 N) was added, with continuous stirring in an ice bath, to bring the *p*H to 2·3. The mixture was brought to *p*H 7 with 2 M sodium carbonate and centrifuged at 10,000*g* to obtain a clear supernatant. The supernatant was passed through a fresh, water-washed 'Amberlite *MB*-3' column. To the cooled eluate, 1/10 vol. of 1 N hydrochloric acid was added and the solution placed at 0°– 4° C for 4 h. It was then dialysed in the cold for 48 h against four changes of 5 l. of deionized water. The sac contents were freeze-dried.

Gangliosides were isolated by this method from normal human cerebral cortex obtained at post-mortem within 6 h of death and from fresh specimens obtained at craniotomy. No significant difference was found in the composition of gangliosides from these two sources. Thus gangliosides are not altered in the first few hours after death. The extracted, unfractionated gangliosides are white, water-soluble powders containing 3·2–3·3 per cent nitrogen and less than 0·1 per cent phosphorus. No peptides, amino-acids, sodium or potassium were found. The NANA content of these gangliosides was 1·00 ± 0·05 μM/mg (6 preparations). The NANA content of gangliosides was not altered in brain specimens kept at – 20° C for several weeks before extraction, if they were cut for homogenizing while the brain was still frozen. Thin layer chromatography was carried out, using 100 μg samples of these gangliosides, on silica-gel plates, pre-washed with chloroform, and developed with chloroform/methanol/water (65 : 35 : 7 v/v/v). The plates were sprayed with 0·005 per cent 'Rhodamine-6*G*' in 80 per cent acetone and examined under ultra-violet light or with the aniline–diphenylamine reagent[11]. Five distinct spots were resolved in this system with R_F's of 0·06, 0·11, 0·19, 0·26 and 0·35 respectively.

Table 1. GANGLIOSIDES EXTRACTED FROM HUMAN BRAIN IN DIFFERENT PATHOLOGICAL STATES

Group	Diagnosis	NANA content μM/mg	Age/sex
1	Severe atherosclerotic vascular disease	0·88	73/M
		0·75	76/F
		0·72	83/F
		0·56	60/M
		0·89	67/F
2	Anæmic anoxia	0·71	57/M
		0·85	30/F
		0·88	60/M
3	Severe chronic respiratory disease	0·44	66/M
		0·78	67/M
4	Cardiac arrest and mechanical respiration	0·76	32/F
		0·53	3/F
5	Expanding intracranial lesion	0·75	45/F
		0 65	54/F
			Age-range (yr.)
Altered human gangliosides		0·73 ± 0·12(14)*	3–83
Normal human gangliosides		1·00 ± 0·05(6)*	14–88

* Mean value ± *S.D.* with number of cases in parentheses.

In the course of extraction of a large number of human brains, gangliosides from some specimens were found to contain considerably less NANA than our normal series (Table 1). Repeated extraction and re-purification of these abnormal gangliosides did not increase their NANA content. Furthermore, thin layer chromatography of these gangliosides showed the absence or marked decrease of the slowest moving spot (R_F 0·06). A common feature in the clinical history of the patients, from which the abnormal gangliosides were obtained, was the occurrence before death of some degree of local or generalized hypoxia. Age was not important as the normal series covered an equally broad range. The interval between death and post-mortem did not differ in the two groups and was never more than 6 h. Two samples of brain were obtained at craniotomy from one patient with a large intracerebral hæmatoma. Gangliosides isolated from an area of cortex, compressed locally by the hæmatoma, contained 0·75 μM NANA/mg. Gangliosides from a second sample from an area of the frontal lobe not compressed by the hæmatoma contained 1·02 μM of NANA/mg. In normal brain, gangliosides extracted from these two areas did not differ in NANA content. These findings suggest that hypoxic or anoxic situations in brain, regardless of cause, decrease the neuraminic acid content of gangliosides afterwards isolated and purified.

Further support for the theory that hypoxia or anoxia alters brain gangliosides was obtained from experiments with cats. Adult female cats (approximately 3 kg weight) were anæsthetized with 'Nembutal' administered in a single intraperitoneal injection. A Y-shaped tracheotomy tube was inserted and connected by one of the free arms to a mechanical respirator. The animal's head was fixed in a Horsley–Clark stereotaxic apparatus. A 6–8 cm midline incision was made in the scalp. Electroencephalographic leads were inserted into the skull over the sensory area of the brain, bilaterally. The reference electrode was attached to one ear. An electrocardiographic tracing was made between the left chest and the right fore-limb.

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* Holder of a fellowship from the Queen Elizabeth II Canadian Research Fund.

After control tracings of the EEG and EKG patterns were taken, the tracheotomy tube was clamped for specified periods and the electrical recordings followed. At the end of the period of asphyxia, the tracheotomy tube was unclamped, the animal examined, and the respirator started, if necessary. The cranium was removed with bone rongeurs and the cerebral hemispheres lifted out immediately on the death of the animal or within 1 h of re-establishment of the airway, if the animal survived the insult. Removal of the cerebrum required about 5 min. Control animals were anaesthetized with 'Nembutal' and the brains then removed. Brain gangliosides were either extracted immediately or after storage at − 20° C.

Normal cat brain gangliosides contained 1·03 ± 0·01 μM of NANA per mg (Table 2). This value is similar to that for other species[3]. In the experimental animals, between 90 and 120 sec after clamping the tracheotomy tube there was complete loss of electrical activity over the brain. 4 min of clamping could be withstood by most animals and was followed by complete recovery of EEG and neurological responses within a few minutes of restoration of a patent airway. After 5 min of clamping, recovery was much slower and abnormal slow wave activity persisted long after the tracheotomy tube was unclamped. Animals subjected to 6 min or more without air did not survive for longer than 20 min following restoration of the airway. The NANA content of the gangliosides extracted from the brains of animals subjected to various periods without air was significantly lowered, as shown in Table 2. Further experiments are planned using nitrogen and nitrogen–carbon dioxide mixtures in a closed ventilating system connected to the tracheotomy tube.

The foregoing findings suggest that gangliosides are irreversibly altered in hypoxic or anoxic conditions. Li and McIlwain[4] recorded depressed resting membrane potentials in cerebral cortex slices in the absence of substrate under hypoxic conditions. Using similar preparations Hillman[7] found that some cells are more sensitive to these conditions than others, and suggested that some cells are permanently depolarized by hypoxia. The addition of the basic protein, clupein, hypopolarized cerebral cells in tissue slices. These cells could be repolarized by the addition of gangliosides. Thomson and McIlwain[8] were also able to restore the metabolic responsiveness of cerebral cortex slices to electrical pulses by a similar treatment. N-acetylneuraminic acid is the only acidic group in the structure of gangliosides. If part of the neuraminic acid occurring in gangliosides in the neuronal membranes is removed an irreversible alteration in the net negative charge of these membranes would be expected to occur. This change could well lead to a loss in excitability. A parallel situation has been demonstrated in erythrocytes where the removal of neuraminic acid by the enzyme neuraminidase leads to a marked decrease in electrophoretic mobility and a loss of surface negative charge[12]. The sensitivity of cerebral tissue to hypoxia and the irreversible nature of hypoxic changes may in part result from the removal of NANA from membrane gangliosides. Of interest here are the *in vitro* studies of Korey and Stein[13], who reported the degradation of gangliosides, only under anaerobic conditions, by a postulated gangliosidase.

Table 2. *N*-Acetylneuraminic Acid Content of Gangliosides Extracted from Normal and Asphyxiated Cats (mean ± *S.D.* with the number of animals in parentheses)

Time tracheotomy tube was clamped (min)	NANA content μM/mg ganglioside
0	1·03 ± 0·01(3)
5–6	0·80 ± 0·03(3)
7–8	0·62 ± 0·04(3)

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EFFECTS OF HIGH PARTIAL PRESSURES OF OXYGEN ON FUNGI

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In 1931, I postulated that high partial pressures of oxygen had a toxic effect on the enzyme systems in apple tissues[1]. A short paper describing the experiments then carried out was published many years later[2]. I showed[1] that the effect was specifically due to high oxygen pressures and not to actual pressure as such. For example, the output of carbon dioxide in 20 atm. of 5 per cent oxygen was substantially the same as that in 1 atm. of 100 per cent oxygen and no toxic effects of the high actual pressure were observed, though 20 atm. of 100 per cent oxygen was toxic to the tissues.

This view has been confirmed by many subsequent workers and the enzyme systems actually affected have been studied by others[3,4].

I also noted[1] that, whereas plant tissues which have become damaged in respiration chambers are normally attacked by fungi and bacteria giving a greatly increased output of carbon dioxide, in the tissues exposed to high partial pressures of oxygen there was no evidence of invasion by bacteria or fungi, despite the obvious damage. I suggested that high partial pressures of oxygen might have a toxic effect on these organisms also.

It was to examine this problem that the experiments reported here were carried out. If, in fact, fungal tissues and bacteria were destroyed by exposure to reasonably high partial pressures of oxygen this method of sterilization might be of some practical importance.

For the experiments a container of very much the same type as that used in the earlier experiments was made[1].

In the first group of experiments two fungi were used, namely, *Aspergillus niger* and *Penicillium digitatum*. The fungi were grown on 2·5 per cent malt agar on Petri dishes 9·0 cm in diameter. After inoculation the control plates were kept in a box at room temperature and at atmospheric pressure—four plates being usually kept as controls. The others were put into the container and oxygen passed in to expel the air. Thereafter the outlet valve was closed and further oxygen was added until the desired pressure was obtained.

At the end of each experiment the oxygen was allowed to escape slowly (during a period of about 15 min) to reduce the possibility of mechanical damage to the fungal tissues.

Growth of a colony was estimated by measuring its diameter before and after the experiments and, where possible, during them. This, it is realized, is not a very precise measure of growth since the mycelium in some instances, after exposure to pressure, seemed to be rather

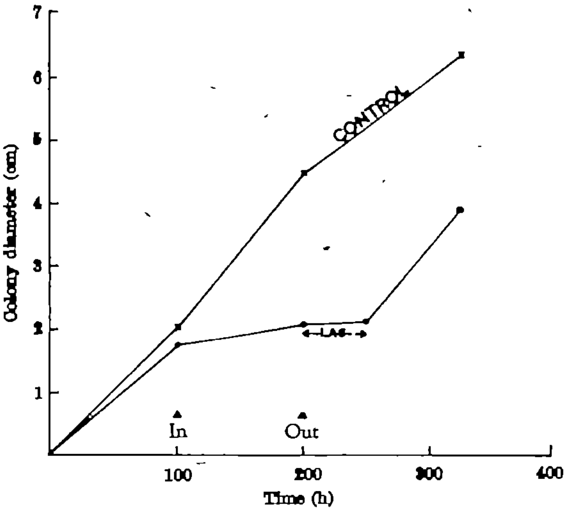

Fig. 1. Colony growth of *Penicillium digitatum* in air at 15 atm. pressure = 300 per cent oxygen

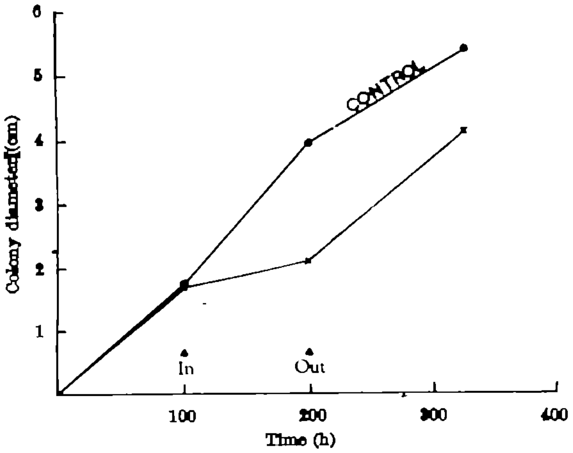

Fig. 2. Colony growth of *Aspergillus niger* in air at 15 atm. pressure = 300 per cent oxygen

below the surface of the agar, though later there was normal surface growth and in any event no allowance is made for the thickness of the mycelial growth. Within the limits of the experiment, however, the diameter of a colony seemed to give a reasonably accurate indication of the effect of the various treatments.

Various preliminary tests were carried out to ascertain whether rapid compression and decompression had any marked effect on the tissues or the agar medium, but in general no obvious effects were observed. Compressed air was used in the first group of experiments, and the growth-rate, both of the cultures exposed to pressure and of the controls, seemed to be normal. Small slits sometimes appeared in the agar at high pressures when decompression was very rapid, but they disappeared after an interval of less than 0·5 h. These were probably caused by gas bubbles.

Various gas mixtures were used at different pressures and with consequently different partial pressures of oxygen. In general, it was found that what controlled the effect on growth was again the partial pressures of oxygen and not the actual pressure of the gas to which the cultures were exposed. This confirms the early conclusions for apple tissue.

In the first set of experiments, using *Aspergillus* and *Penicillium*, the colonies were allowed to grow before the Petri dishes were put into the pressure-chamber, thus ensuring that the mycelium and not merely spores was exposed to the pressure.

After treatment at high pressures it was found that no growth had occurred in the treated colonies, while the controls had grown normally. After the lapse of some period of time, growth of the treated colonies began again. It was clear, therefore, that the effect of high partial pressures of oxygen was, in the case of these two fungi, to suspend the metabolic processes, but not to kill the tissues. In general, it was found that the higher the partial pressure or the longer the exposure to high pressure the greater the time-lag before growth recommenced. *Penicillium* seems more sensitive than *Aspergillus* in this respect.

Brown[3] reported that within wide limits oxygen pressure has little effect on the germination of spores or on fungal growth. This does not hold for partial pressures of oxygen above 100 per cent as growth was always considerably slower or was completely stopped in these circumstances. It is noteworthy that in *Penicillium* especially, some mycelial growth occurred at fairly low partial pressures of oxygen (for example, 3 atm. pure oxygen).

Figs. 1 and 2 show the effect of 15 atm of air (= 3 atm. oxygen) on the two fungi under discussion.

Aspergillus, as has been noted, seemed less sensitive to high partial pressures of oxygen than did *Penicillium*.

Clearly one or two questions still remained unanswered. It was not possible to establish whether growth under pressure stopped instantaneously (or almost so) or whether it continued for a shorter or longer period at a greatly reduced rate before it stopped completely. Nor was it possible in these experiments to determine the partial pressure of oxygen which actually halted the growth of the mycelium. In one experiment 8 atm. of air (1·6 atm. oxygen) seemed to kill the *Penicillium* mycelium after exposure for 500 h.

It was with the view of examining this latter effect that the experiments were continued using a different fungus, namely, a strain of *Phoma violacea* (Bertel) Eveleigh (syn. *P. pigmentivora* Massee), isolated in this Department.

In these experiments pure oxygen was used and the culture plants were put into the container as soon as the colonies had reached a diameter of about 1 cm. These experiments were carried out in a constant-temperature room at 25° C whereas the earlier series were conducted at laboratory temperature of about 18° C.

The results are given in Figs. 3 and 4. It can be seen that the effect of 2 atm. oxygen was very slight indeed. In 4 atm. oxygen, however, the lag is again roughly proportional to the period of exposure and five days exposure seemed to have no permanently deleterious effect. On

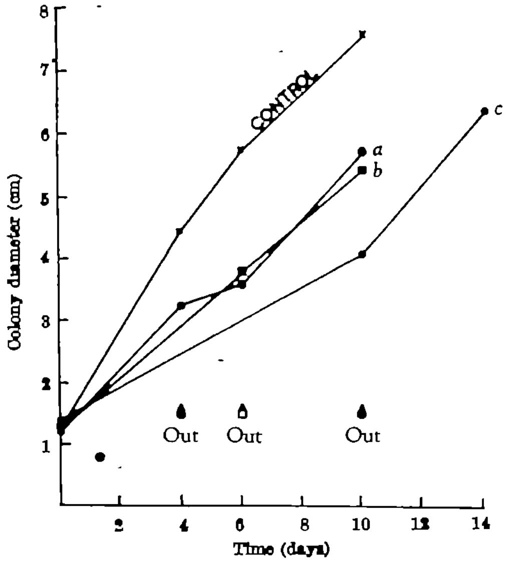

Fig. 3. Colony growth of *Phoma violacea* in 100 per cent oxygen at 2 atm. pressure

a, Four days under pressure; *b*, Six days under pressure; *c*, Ten days under pressure

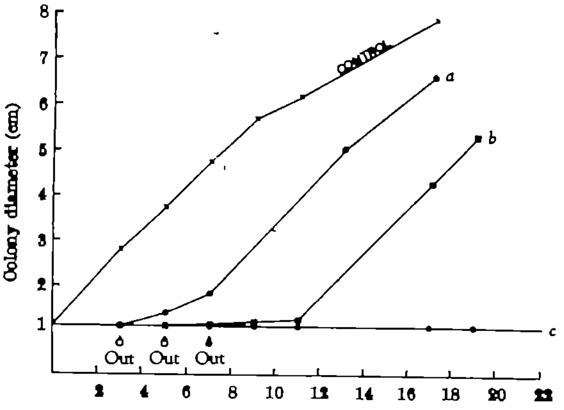

Fig. 4. Colony growth of *Phoma violacea* in 100 per cent oxygen at 4 atm. pressure

a, Three days under pressure; *b*, five days under pressure; *c*, seven days under pressure

the other hand, seven days exposure resulted in no growth of the colony even after a lapse of twenty-five days at normal atmospheric pressure.

In yet another series of experiments, five different fungi were used, namely, *Aspergillus niger*, *Penicillium cyclopium*, *Phoma violaceae*, *Aspergillus wentii* and *Merulius lacrymans*. These were inoculated on to 2·5 per cent malt agar plates on December 26 and kept in an incubator at 25° C until December 30, when the experimental plates were placed in the pressure chamber at 10 atm. 100 per cent oxygen, and the controls were put into a copper box at normal pressure. Both lots of material were kept in a constant temperature room at 25° C. All the colonies had grown during the period in the incubator. On January 9, after ten days exposure to pressure, the pressure was released slowly, and the plates returned to the incubator at 25° C. All the controls had grown normally, but the colonies under pressure had not increased in size at all. After the lapse of 8 days, however, apparently normal growth of the mycelium was observed in the colonies of *Aspergillus niger* but not in any of the others.

In a second experiment, the same five fungi were used in the same medium and with similar treatments. The period under pressure at 10 atm. pure oxygen was, however, reduced to seven days when the cultures were returned to air in the incubator at atmospheric pressure. *Aspergillus niger* and *Penicillium cyclopium* began to grow after the lapse of 6 and 10 days respectively, and did so apparently by the growth of the hyphæ and not of germinating spores. The other fungi did not grow.

In a third experiment with the same five fungi on the same medium and with similar treatment, after 10 days at 10 atm. pure oxygen the cultures were removed to the incubator. Only *Aspergillus niger* and some of *Penicillium cyclopium* cultures grew, the former after an interval of 8 days and the latter after an interval of 12 days. None of the others showed any sign of growth even after four weeks in the incubator.

It appeared, therefore, that 10 days' exposure at 10 atm. pure oxygen was just about critical for *Penicillium cyclopium* but that *Aspergillus niger* was substantially more resistant.

In yet another series of experiments, cultures of *Aspergillus niger* on the same medium were exposed to pressures of 10 atm. pure oxygen for 14 days and while no growth occurred during the period under pressure, growth of the mycelium began again after 10 days at atmospheric pressure in an incubator at 25° C.

Finally, a set of eleven culture plates with well-grown colonies of *Aspergillus niger* were left in 10 atm. of pure oxygen for 28 days before being returned to atmospheric pressure. None of these grew after this treatment, and not even the conidia grew on fresh agar plates, though conidia from control cultures germinated at once on a similar medium under the same conditions.

Preliminary tests by Dr. G. G. Pritchard show that there is a marked accumulation of pyruvic acid in the hyphæ of the fungi exposed to high partial pressures of oxygen, and that this pyruvic acid is removed after recovery and growth in air. It appears, therefore, that the effect of the high oxygen pressure is on similar enzyme systems in fungi to those in other plant tissues[6].

The effect of pressure on the metabolism of the fungi is being studied in detail and will be reported in another paper.

I thank Misses S. M. Robb and E. J. Bowers and Messrs. D. Tilney and M. E. J. Daft for their help in this work or in the preparation of this article.

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ANTIPHONAL SINGING IN BIRDS AS EVIDENCE FOR AVIAN AUDITORY REACTION TIME

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IN a small minority of bird species, the females 'sing' as well as the males, and where this happens it is not unusual for there to be vocal elements in the 'courtship' displays, with the result that a rather haphazard and ill-timed duet may occur. However, in many tropical (and some sub-tropical) areas, both of the Old and New Worlds, a much more elaborate form of mutual vocal display is known to occur. In this type the notes of the male and female are different, the sexes alternating so as to sing antiphonally, often with extraordinary precision. This antiphonal singing of tropical birds is most in evidence in species which live in dense scrub jungle or undergrowth and it is a natural assumption that under these conditions mutual recognition and maintenance of contact by visual display is ineffective, and consequently mutual auditory display has tended to accompany it and in some cases take its place.

During a recent period of investigation in Uganda, I was enabled to make a start with the recording and observation of such antiphonal singing in three widely separated groups of birds, one non-passerine and two passerine, namely, the barbets (*Trachyphonus*—family Capitonidae), the fantail warblers, genus *Cisticola* (family Muscicapidae, sub-family Sylviinae) and shrikes of the genus *Laniarius* (family Laniidae, including Laniariidae). It is the singing of this last genus which raises problems of special interest since, in some species, both male and female have a considerable vocabulary which it seems they utilize so as to work out, with practice, a series of duet patterns by which presumably the two can recognize each other individually when out of sight. From preliminary study and recording it seems that each pair of a species such as *Laniarius aethiopicus* tends to develop a particular repertoire of duet patterns by which they can be dis-

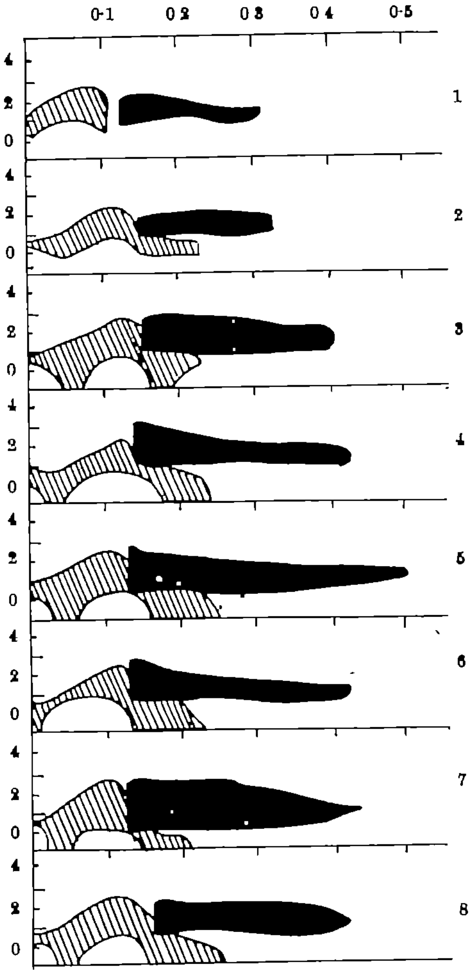

Fig. 1. Diagrammatic sound spectrograms of eight consecutive duet patterns of a pair of *Laniarius erythrogaster*. Old Entebbe, Uganda, March 6, 1962 The note of bird *A* is shown cross hatched; that of bird *B* in black. All higher harmonics and overtones are omitted. Vertical scales· frequency in kc/sec, horizontal scale, time (sec)

tinguished from other pairs in the neighbourhood. The problems which such a performance raises for the student of animal learning and particularly for those interested in vocal imitation and communication are extensive and intriguing. Research on these has only begun and it is hoped that, since I am fortunate to have as a collaborator Mr. Myles E. W. North of Nairobi, a highly expert ornithologist and recorder, it will be possible in due time to carry out a useful field and experimental study of these matters. However, during the first steps of this work some data were obtained which seem to throw new light on an interesting problem in avian physiology and this is accordingly being presented in outline now.

In one of the shrikes studied, *Laniarius erythrogaster*, the black-headed gonolek, the duet is extremely simple. The first bird, presumably the male, makes a 'yoick'-like sound which is followed immediately by a tearing hiss-like sound from the second bird (Fig. 1). In all the birds so far heard (three species of *Laniarius*, one of *Cisticola* and the *Trachyphonus*), the timing of the singing is normally so perfect that in no case would one suppose that two birds were involved unless one sees them or until one happens actually to come between them and hear the sound coming from two different directions. In the particular case of *L. erythrogaster*, a number of observations led me to suppose that the individual peculiarity of the duet was expressed not in peculiarity of pattern, for the bird apparently lacks the ability to vary this, but in a very precise and exactly maintained time-interval between the contributions of the two sexes. The precision of this time-keeping at once raises questions of the auditory reaction time as an expression of the powers of temporal discrimination of the avian ear. There is already circumstantial evidence for supposing[1,2] that the speed of response of the avian ear must be of the order of ten times that of the human ear. The reaction time of human beings, the 'personal equation', has been a concern of psychologists since the famous case, in 1795, when Maskelyn, head of the Greenwich Observatory, discharged an otherwise capable assistant because of a habit of recording all transits about half a second 'too late'. The study of human reaction to an auditory stimulus commenced in the 1860's with the work of a Swiss astronomer Hirsch and was quickly followed by a Dutch physiologist Donders in 1868. Since that time there has been a great deal of work by applied psychologists and physiologists on human reaction times, but I am aware of no information as to the bird's ability in this respect.

In the case of human beings it has been found that in studies in which the subject has to respond by pressing a key, the reaction time to a visual stimulus is of the order of 200 msec, and that to an auditory stimulus of 150 msec. Donders[3] found that if the stimuli used were simple speech sounds and the subject had also to respond by making the same or a similar speech sound, the reaction time was longer, varying according to conditions as follows. In experiment type *A*, the stimulus was always the sound 'ki' and the response also was always to be 'ki'. In experiment type *B*, the stimulus was any one of five syllables and the subject had to respond with the same syllable. In experiment type *C*, the stimulus was again any one of five given syllables but the subject had to respond only to the sound 'ki', using this syllable as his response. Under these conditions the results were found to be as follows: experiment type *A*, 197 msec; experiment *B*, 285 msec; experiment *C*, 243 msec. Modern work on the subject has been summarized by Teichner[4]. Among a number of conclusions he shows that simultaneous stimulation of more than one sense modality produces faster reaction times than stimulation of just one. On the other hand, successive stimulation of different senses produces slower reaction times than stimulation of a single sensory channel. Broadbent and Gregory[5] found that if the verbal response was compatible with the stimulus, in the sense that the subject had to respond by making the same sound as the stimulus, the mean response time was 284 msec, with a *S.D.* of 55·4 msec, whereas if the response was incompatible, that is to say, the subject had to reply with a sound different from that of the stimulus (as does *Laniarius*) the reaction time rose to a mean of 444 msec, with a *S.D.* of 31·1 msec.

Laniarius erythrogaster is of interest in that it is a species which habitually duets in extremely dense foliage and it seems that for a great proportion of the time the birds must be out of sight of each other. The complicating element of a visual stimulus is thus often ruled out. Fig. 1 shows sound spectrographs of a consecutive series of eight duets of a pair of *L. erythrogaster* recorded at Old Entebbe, Uganda, on March 6, 1962. The fundamentals only are shown, the harmonics having been omitted from Fig. 1. The method is accurate to 1·5 msec. Table 1 shows the timing of these eight consecutive duets. Column 1 indicates the duet series, column 2 shows the time in seconds at which the note of bird *A* (the initiator) commences and the figure following, in brackets, shows the time in seconds which has elapsed since the previous note of bird *A*. This figure provides us with what psychologists call the 'fore-period', or amount of warning which the second bird may have in the task of giving its reply. It will be seen at a glance that the calling of bird *A* is not markedly regular, yet in spite of this the reaction time of bird *B* remains extraordinarily constant. It is clear from the study of the sound spectrograms of this and other similar series of duets, of this and other species, that the second bird is taking its time from the start of

Table 1. Timing of Eight Consecutive Duets of a Pair of *Laniarius erythrogaster*

Duet No.	Time (sec) at which note of bird *A* commences	Reaction time of bird *B* (= time in msec between start of the note of *A* and that of *B*
1	0	125
2	2·5 s. (2·5)	145
3	5·7 s. (3·2)	150
4	10·5 s. (4·8)	160
5	15·0 s. (4·5)	150
6	19·3 s. (4·3)	150
7	23·7 s. (4·4)	125
8	29·3 s. (5·6)	150
	S.D. of reaction time	12·6

The figures in brackets show the time in sec since the previous note of bird *A*. This could be regarded as the 'fore-period' for bird *B*. (Old Entebbe, Uganda. March 6, 1962).

the note of the first bird. This is what happens in human beings; for it has been shown[6] that the human subject begins his response as soon as he hears the first phoneme, without waiting for the completion of the letter or number, or whatever the signal may be. As Table 1 shows, the mean reaction time of the bird *B* in this series was a little more than 144 msec, with a *S.D.* of 12·6 msec. This mean reaction time is the fastest yet found for any of these duetting birds, but this speed is not a species-characteristic. As has been indicated here, different pairs of *erythrogaster* may show different reaction times; but once a pair gets under way in a series of duets, the second bird maintains a constant response time, even though this may be very much longer than the minimum possible. Thus in another series recorded by Mr. Myles E. W. North at Entebbe a few months previously, the mean reaction time over a series of seven consecutive duets was 425 msec, but the standard deviation (4·9 msec) was less than that of the birds we have just been discussing. It seems from this that this species must have an extremely precise time sense, the accuracy of which does not decrease even if the response time is extended by a factor of 4. I am not aware of any auditory reaction-time experiments with humans in which the standard deviation is less than about 20 msec.

Although the figures given in Table 1 show a minimum reaction time of 125 msec, it may well be that even shorter times are possible, although they have not been recorded. In fact it is clear that the reaction time of this individual is, if anything, an overestimate since no allowance has been made for the time taken for the sound to travel from one bird to the other. Any estimate of this figure is bound to be somewhat speculative. I think in general it is safe to assume that the birds were about equidistant from the microphone, so no differential need be allowed for in this respect. I have certainly heard these birds duetting and apparently keeping excellent time when they must have been about 20 yards (18 m) apart. Assuming the speed of sound to be 1,130 ft./sec (344 m/sec), we should have to allow 53 msec for the sound to reach bird *B*. In that event, the true mean reaction time in this instance would have been 90 msec and the shortest 72 msec. To be on the safe side in this particular case and to assume that the birds were only 10 ft. (3 m) apart would involve a correction to 135 msec mean and 116 msec minimum.

L. erythrogaster is from this point of view the most interesting of the species so far studied since one can often be virtually certain that the birds are unable to see each other. On the human analogy, this might have been expected to have lengthened the reaction time since Todd (1912; ref. in Teichner[4]) found the reaction time to combined sound and light was not only faster than to light alone but also faster than to sound alone. I have, however, recorded a long series of responses of Chubb's Cisticola (*Cisticola chubbi*) and D'Arnaud's barbet (*Trachyphonus d'arnaudii*), and have not found a response time as fast as that of *L. erythrogaster*. In the case of *Cisticola chubbi* a series of six consecutive duets, recorded when the birds were in sight of one another, gave a mean reaction time of 396 msec but with the remarkably small standard deviation of 2·9 msec—about ⅛ that of which a human would be capable in similar circumstances.

It is to be hoped that it may one day prove possible to keep these birds in captivity under conditions so good that they develop their duets normally. If this can be done it should be feasible to establish a number of points much more accurately than is possible at present. Although it should be possible with a Skinner box type of apparatus to secure in, say, a pigeon, the action of pecking in response to a sound stimulus at the lowest possible reaction time by steadily reinforcing for a quick response, this never seems to have been done and virtually nothing in the way of experimental investigation of the whole animal has been carried out with birds. With any species other than these duetting shrikes it would probably be very difficult indeed to secure a regular vocal reaction in minimum time as a response to sound stimulus. Although we cannot do more than guess at the physiological basis for the rapid and accurate response of this bird, a response about three times faster and much more accurate than that of which a human being is capable in similar circumstances—and with a striking independence of fore-period—it is perhaps plausible to assume that the performance is primarily the expression of superior powers of the avian ear in the matter of time discrimination.

I thank Prof. L. C. Beadle, professor of zoology at Makerere, for the hospitality of his Department and of Makerere University College during the first three months of 1962.

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SEASONAL VARIATIONS OF AVIAN AND HUMAN METABOLIC ACTIVITY IN RELATION TO THE DAYLIGHT/DARKNESS RATIO

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FOR the past three decades it has been known from the work of N. Rollin[1] and others that the dawn song of many different species of passerine birds is subject to wide fluctuation of the time of onset for individual birds within each group.

Nevertheless, by examining the results for each species collectively, a systematic difference in the times of onset of their dawn song is revealed. Thus it is possible to allocate to each species a characteristic 'dawn penetration' (conveniently expressed in terms of the angular position of the Sun below the horizon at the time of onset of dawn song) that is repeated with great regularity from year to year at similar phases of the breeding cycle.

As examples of the wide range of dawn penetration actually observed we may cite two species, *Alauda arvensis* L., and *Fringilla coelebs* L. While the former is consistently an early songster, with a mean dawn penetration of −9°, the latter is far more lethargic with a dawn penetration of only −2°, many birds of this species failing to commence song before sunrise. There is thus a dawn penetration specific for each species lying within the broad interval, −18°–0° solar depression, namely, from the beginning of astronomical twilight to the end of dawn.

On posing the question: How do such differences occur among the different species?, the probability of finding a satisfactory answer would appear highest if the problem is considered from the point of view of metabolic activity. As Rollin[3] has already pointed out, the amount of singing performed by an individual bird is related to season, phase of sexual cycle, development of gonad and endocrine activity, as well as to several meteorological factors. But, overriding these sources of variable behaviour, there is evident in the timing of dawn song an instinctive act linked in some complex manner with the bird's basal metabolism. Such response may be used, under controlled conditions, as a reliable, though indirect, measurement of metabolic activity in the particular avian species under observation.

In the investigation now to be described the most suitable species for the test was *Erithacus rubecula* L., and this for the obvious reason that, of all our resident song-birds, Redbreast alone sings at dawn with great regularity throughout each of the four seasons, save for a brief respite in late summer.

The large collection of observational data used in the analysis which follows constitutes one section of a general study of dawn-song made at Sidmouth, over a long period, for six representative species indigenous to the district. This had for its aim the accumulation of new facts concerning the effects of meteorological and other external factors on the degree of sky brightness at the time of onset of singing.

Analysis of the raw data for *E. rubecula* has revealed a marked influence of the daylight/darkness ratio (in computing this ratio, the times of sunrise and sunset at an altitude of 65 km above the Earth's surface, namely, 8° solar depression, were used; this depression angle corresponds to the observed mean dawn/dusk penetration for *E. rubecula*) on the observed dawn penetration, an effect that persists after applying corrections to the 481 observations for the combined influence of cloud-cover, ambient air temperature, wind force, barometric pressure and atmospheric humidity.

On plotting the observed sky brightness, *B* (on a logarithmic scale) as ordinate, and the log ratio (hours of daylight/hours of darkness) as abscissa, a hysteresis loop is obtained as in Fig. 2*b*. Plotted points refer to group mean values of log *B* for each of the twelve months enumerated, I . . . XII. The mean standard error of a single ordinate value is indicated in the figure by the vertical bar alongside the scale.

The unexpected nature of the relationship between the aforementioned parameters suggested an attempt to ascertain whether or not basal activity in man is also affected in like manner. For this investigation it appeared likely that the simplest and most reliable index of basal metabolism readily available for daily measurement is the pulse. Accordingly, daily records of the radial pulse, with myself as subject, were commenced in March 1959; and these have been continued without intermission.

Since it was realized that significant changes are likely to occur in the pulse-rate records arising from the operation of a variety of conditioning factors—some psychological, and others physical, or physiological, in origin—particular care was taken to ensure so far as was possible a standard pattern of behaviour immediately prior to each daily count. It was at first thought that standardization could best be achieved by making the daily measurement of pulse rate in the first few minutes after waking from the night's rest. However, preliminary tests soon showed that, for this particular subject, a longer post-waking period was more conducive to reproducible counts, especially so if the period was one of moderate indoor exercise.

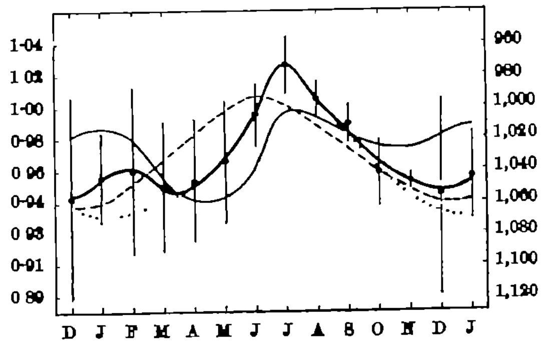

Fig. 1. Observed annual variation of basal metabolism in man (derived from measurements of basal pulse), bold curve. Dotted portions of the curve indicate the expected profile if the secondary maximum is eliminated. Continuous, ——, and broken, - - - lines represent semi-annual and annual components, respectively

Accordingly, the following procedure was adopted. At each daily session, commencing about 1 h after rising at 0640 ± 5 min G.M.T., the subject sits in a chair facing east while a total of one hundred pulse beats is timed with an accurately regulated chronometer to within ± 0·5 sec. From this record, the value of the mean interval, τ, between successive pulse beats is derived, and measured to the nearest millisecond. The *S.E.* of a single measurement of τ is 10 msec, or ~ 1 per cent of the actual value. Ambient room temperature, and prevailing weather conditions, are noted alongside each entry of τ in the log-book. If, for any reason, it is known beforehand that the pulse-rate is likely to be affected on a particular occasion, the record for this date is entered in the normal way together with an explanatory note; but it is rejected in any subsequent analysis of the comprehensive records.

In the discussion which follows, basal metabolic activity is measured by the reciprocal ($1/\tau$) of the mean pulse interval.

Since the data thus obtained now cover more than three complete 12-monthly periods, it has been possible to compute a series of provisional monthly mean values of τ with their standard errors to include the entire period of March 1959–February 1962. These results are presented graphically in Fig. 1, the vertical bar through each monthly mean value denoting the attached standard error. A marked annual variation of basal metabolic activity is shown by the bold curve passing through the experimental points. Characteristic features of this variation are the sharp primary maximum centred on mid-July, and flanked on either side by shallow minima centred on March–April, and mid-December respectively, and a secondary maximum of activity in mid-February that may, or may not, prove to be real.

The reality of the primary maximum and minimum present in the curve of annual variation has been confirmed statistically by a Student's *t*-test. The relevant data for this test are summarized in Table 1.

Table 1

Year	1959	1960	1961	Mean value (msec)
Maximum monthly value	1,052	1,046	1,098	1,065
Variance	−13	−19	+33	
Minimum monthly value	950	986	984	973
Variance	−23	+13	+11	

The mean range is +92, and with four degrees of freedom the estimated standard deviation is equal to 17·46. Thus 't' = + 92/17·46 × $\sqrt{2/3}$ = + 6·46, a value which is significant at the 1 per cent probability-level. The reality of the primary annual variation of τ is thus confirmed.

The secondary maximum of basal activity, prominent in both the 1959 and 1960 data, cannot be traced with certainty in the later 1960–61 records. Should future observations fail to confirm its presence, the annual variation would then be expected to follow those portions of the smoothed curve that are shown dotted in Fig. 1. The two dominant features of the annual variation remaining are the sharp maximum in July and the shallow minimum in December.

If, however, subsequent results confirm the correctness of the provisional curve, a combination of an annual with a semi-annual component is suggested.

The annual component, the curve of fluctuation of which is shown by the broken curve in Fig. 1, may be expected to follow the seasonal change of solar distance and altitude, taking the form represented by the equation $\tau_\gamma = \tau_0(1 - \alpha\gamma)$, where $\gamma = \cos\psi \times (1/\rho^2)$, ψ being the solar zenith distance, and ρ the solar distance modulus. Both τ_0 and α are empirical constants. For the curve of Fig. 1, $\tau_0 = 1047{\cdot}5$, and $\alpha = 0{\cdot}173$.

On subtracting the annual variation from the observed variation, a semi-annual fluctuation of τ is disclosed. This follows a curve denoted by the thin, continuous line in Fig. 1.

Both winter and summer maxima have approximately equal values (τ = 1,008 ± 5 msec); but the intervening minima are quite dissimilar, the corresponding values of τ being 1,063 and 1,027 msec, respectively.

Of particular interest in the present investigation is the possibility of a relationship between human basal metabolism (as measured here by the index, $1/\tau$), and the seasonal changes that occur in the daylight/darkness ratio, R, already defined.

To ascertain whether such a relationship exists, reciprocals of the smoothed monthly values of τ obtained from the bold curve of Fig. 1 were plotted against corresponding values of log R. The result was a looped curve (Fig. 2*a*) which, although inverted, bears some resemblance to the curve obtained from the unpublished dawn-song data for *E. rubecula* previously mentioned. This latter curve is reproduced in Fig. 2*b* to the same log ratio scale so as to facilitate a direct comparison. The average *S.E.* of a single ordinate value is indicated in both diagrams by a vertical barred line alongside the appropriate scale. Monthly sequences are indicated by the Roman numerals I–XII.

It is readily seen that the 'hysteresis loop' for *Homo sapiens* is a much more open one than that for *E. rubecula*. Owing to the far greater asymmetry in the ascending and descending branches of the first-mentioned curve, the observed difference between the respective values of τ, at log R = 0·25, for example, is as much as 43 msec, or about 4 per cent of the mean value.

An inspection of this curve shows a higher level of activity to persist in the period during which the value of log R is falling, namely, from July to October. An asymmetry of this type could result from the incidence of enhanced solar ultra-violet radiation during the months immediately preceding the afore-mentioned interval.

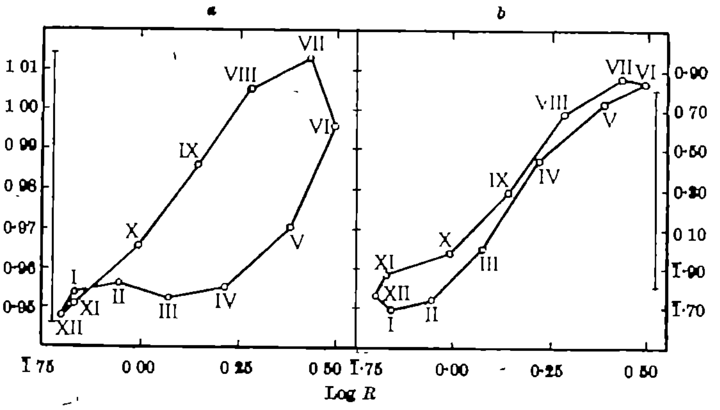

Fig. 2. Hysteresis loops obtained by plotting (*a*), $1/\tau$ for man, and (*b*) log B for *E. rubecula*, versus log ratio (daylight hours/hours of darkness). The relevant *S.E.* is shown by the vertical bar alongside ordinate scale

By contrast, the 'hysteresis loop' for *E. rubecula* suggests a greater song activity in the spring and early summer when the value of log R is rising. This is followed by a spell of lowered activity in late summer and autumn. Partially at least, this sequence may be explained by reference to the avian breeding cycle, since sexual maturity is reached just at that time of year when the rate of increase of the daylight/darkness ratio is most rapid.

When taken together, the results just described—referring as they do to a natural environment in both cases—suggest that the rhythmic seasonal fluctuation of natural illumination exerts a control of basal metabolic activity in both man and birds that is certainly less simple than the type of light control already demonstrated for several different species of birds[3] and mammals under controlled artificial conditions in the laboratory. In experiments with *F. coelebs* L., Thorpe[4] has noted, however, that the time of onset of artificially stimulated song is more nearly related to the rate of change of artificial illumination than to the total period of illumination to which the bird is exposed.

In conclusion, the strictly provisional nature of the results for the human subject must be emphasized. It cannot be too strongly stressed that, before any far-reaching conclusions can be drawn from the results of the investigation described here, much more data are needed to construct a definitive curve of annual variation of the human pulse. Not until then will it be possible to correct the raw experimental data for this particular source of observational scatter; and afterwards to examine the corrected daily values of τ for any residual effects that may then be evident.

Meanwhile, it is thought that the novelty of the experimental approach to this problem alone justifies the publication of a certain amount of information not previously available.

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EFFECT OF GIBBERELLIC ACID ON THE PRODUCTION OF PRODIGIOSIN BY *SERRATIA MARCESCENS*

By PRENDIS O. BENNETT and HORACE D. GRAHAM

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INVESTIGATIONS of the effect of gibberellic acid on micro-organisms are few. Stowe and Yamaki[1] state that "no convincing action of the gibberellins has yet been shown on any organism except higher plants". Some authors have claimed that bacteria and fungi are not affected by gibberellic acid, while others have suggested that these substances are antagonistic to several fungi[2]. Inhibitory effects on the alkaloid content of and spore

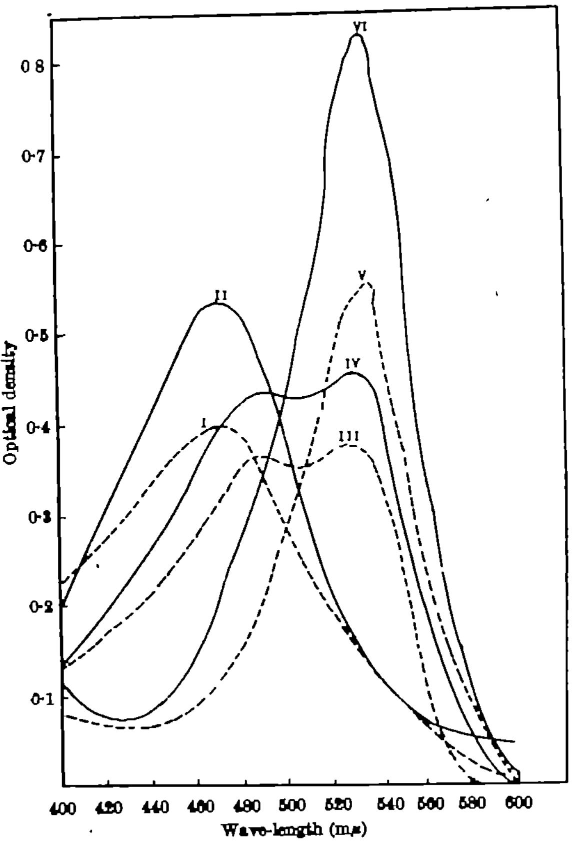

Fig. 1. Absorption spectra of authentic and extracted prodigiosin. ——, Authentic ; – – –, extracted. I and II, in absolute ethanol + sodium hydroxide ; III and IV, in absolute alcohol + water ; V and VI, in absolute alcohol + hydrochloric acid

formation by ergot, a parasitic fungus, have recently been reported by N. I. Ostrovskii *et al.*[3]. Lu *et al.*[4] have reported varying effects of gibberellic acid on soil micro-organisms, particularly the *Azotobacter* types. In their work, terminal respiration, nitrogen fixation and cell numbers were found to be increased under the influence of added gibberellic acid. Decomposition of gibberellic acid in the soil was also reported. The most recent experimentation on micro-organisms dealt with *Azotobacter indicus*, which Greenberg and Tirpak[5] found to be stimulated by gibberellic acid. It was implied that the effect might be a complex physiological one.

This article is concerned with the effect of gibberellic acid on the production of the blood-red pigment, prodigiosin, produced by *Serratia marcescens*. This pigment has attracted much interest recently because it is thought to be the only naturally occurring tripyrrylmethene, and, as such, its biosynthesis may possibly be related to the biosynthesis of the porphyrins[6].

Serratia marcescens, strain Nima, obtained from Dr. Robert P. Williams, Department of Medicine, Baylor University, Houston, Texas, was used. The organism was grown on a modification of the medium of Bunting[7], containing: glycerol, 0·5 per cent; dipotassium phosphate, 1·0 per cent; ferric ammonium citrate, 0·005 per cent; magnesium sulphate, 0·05 per cent; and sodium chloride, 0·05 per cent. The medium was sterilized by autoclaving for 10 min at a pressure of 15 lb. When a solid medium was needed, 1·5 per cent agar was added.

For comparison, a sample of prodigiosin, supplied by Parke, Davis and Co., was used. For convenience, this has been named 'authentic prodigiosin'. In order to compare the spectral characteristics of this so-called authentic prodigiosin and that of the laboratory acetone-extracted pigment, spectral curves of each were prepared. The supposedly pure, authentic prodigiosin was dried for 12 h in a vacuum oven at 40°. A stock solution was then prepared by weighing out 0·25 mg of the substance on an analytical balance, dissolving it in absolute ethanol and making the volume up to 100 ml. in a volumetric flask. Spectra of the acidic, basic and neutral solutions were therefore needed. 10 ml. of stock solution was added to each of three 'Pyrex' glass-stoppered tubes. 1 ml. of 1 N hydrochloric acid was added to the first tube, 1 ml. of 1 N sodium hydroxide to the second tube and 1 ml. of water to the third tube in order to obtain acidic, basic and neutral solutions, respectively. Distinct colours were developed for each of the solutions; acid red, base yellow and neutral, brown. The optical density of each solution was measured over the wave-length range of 400–600 mμ by the use of the Coleman universal spectrophotometer. Readings were begun approximately 1 min after colour development for each solution. A similar procedure was followed for the extracted substance except no actual weighing was done since not enough pigment was extracted. The extractions were taken up in the appropriate solution after evaporation and pooled. Fig. 1 summarizes the results, which compare well with those obtained by Williams *et al.*[8].

Since the acidic solutions of both the authentic and the extracted prodigiosin exhibited sharp spectral peaks (absorption maxima at 535 mμ), it was decided to use an acidic solution in the preparation of the standard curve. 9 ml. of hydrochloric acid–ethanol solution (0·10 N with respect to the acid) was placed in consecutive 'Pyrex' glass-stoppered test-tubes containing 1 ml. of prodigiosin dissolved in absolute ethanol. The final concentrations of prodigiosin ranged from 0 to 2·5 μg/ml. of the hydrochloric acid–ethanol solution. The optical densities of the colours developed were measured with a Coleman universal spectrophotometer, model 14, at 535 mμ. The results are shown in Fig. 2.

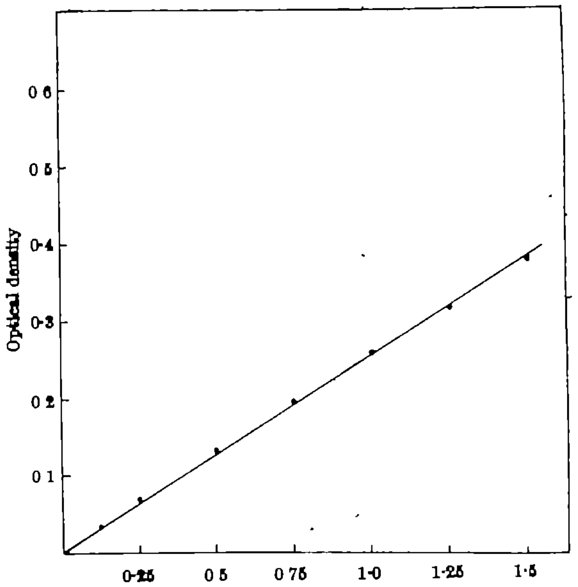

Fig. 2. Standard curve of prodigiosin in 0·09 N ethanol–hydrochloric acid

1 ml. of an 8-h culture of *Serratia marcescens* grown at 30° C, containing 1 × 10^8 cells, was inoculated into 5 ml. of double-strength sterile culture medium. Aqueous solutions of potassium gibberellate (75 per cent potassium salt, Nutritional Biochemicals Corp., Cleveland, Ohio), sterilized by filtration, were added to give final concentrations of 5–1,000 p.p.m. The final volume of all culture tubes was 10 ml., attained by adding distilled

water, where necessary. The cotton-plugged inoculated tubes were incubated at 30° C for varying periods of time.

After incubation, the pigment produced was extracted by a modification of the method of Williams *et al.*[9] and then determined spectrophotometrically. The samples were taken from the incubator and transferred to 300-ml. Erlenmeyer flasks with 35 ml. of acetone. The pigment adhering to the sides of the tubes was dislodged by the use of a bent spatula and rinsed out with a portion of the acetone. After remaining in the acetone solution for 6 h, which was established to be the most advantageous time for optimum pigment extraction, the samples were filtered under vacuum using medium sintered glass filters. To ensure quantitative transfers, the flasks and cell debris were washed with two 5-ml. portions of acetone. After this, 80 ml. of ligroin was added to each sample in a different separation funnel which was stoppered and shaken vigorously to permit extraction of the pigment by the ligroin from the acetone. 350 ml. distilled water was immediately added, which created a separation of the ligroin-pigment layer and the acetone–water layer. After 5 min the acetone–water layers were drawn off and discarded, while the ligroin extracts were drawn off into 300-ml. Erlenmeyer flasks and taken to dryness (one by one) in an oven at 120° C. The samples were taken up in 0·09 N hydrochloric acid–ethanol and, after cooling to room temperature, the optical densities of the solutions measured at 535 $m\mu$. The amount of prodigiosin was calculated from the standard curve.

The results obtained and summarized in Fig. 3 indicate that gibberellic acid inhibited the production of prodigiosin by *Serratia marcescens*. This effect was observed after 72 h of incubation. No effects were observed at 36 h, or at 144 h. At levels of 5–20 p.p.m., the decrease in prodigiosin content was almost linear, but the effect tapered off between levels of 20–50 p.p.m. From 50–200 p.p.m. levels, there was considerable suppression of the prodigiosin content caused by added gibberellic acid. These results are somewhat comparable with the findings of Greenberg and Tirpak[5], who were unable to obtain linearity while examining the effect of gibberellic acid on *Azotobacter indicus*. The period of incubation after which the inhibitory effect was noted is interesting. Possibly, at the shortest period of 36 h, no effect was noted because the pigment was not yet being biosynthesized by the cells at a very great rate. According to Williams *et al.*[9], the time of maximal pigment production coincides with the stationary phase of the growth-cycle, and, in their opinion, the pigment-forming system is not exclusively a property of the viable cells. Therefore, at 72 h, in the senescent phase of the growth-cycle and, concomitantly the period of maximal pigment production, the effect of gibberellic acid was pronounced. Quite likely, the greatest inhibition of prodigiosin production by gibberellic acid occurred at the period of maximum activity of the enzymatic systems concerned with the biosynthesis of prodigiosin by *Serratia marcescens*.

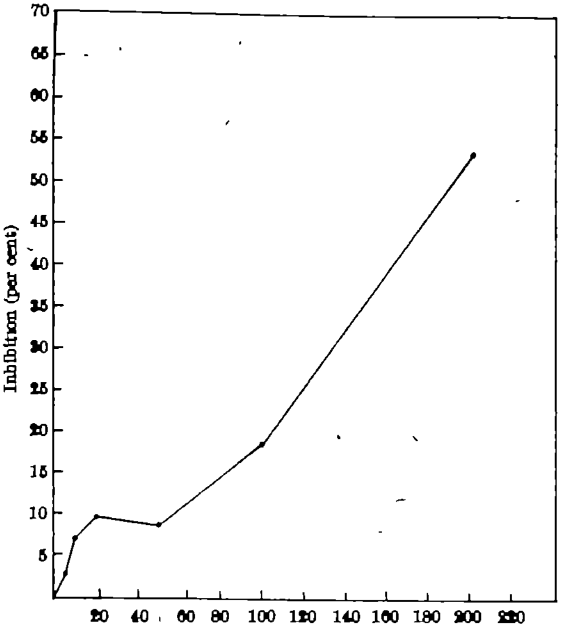

Fig. 3. Inhibition of prodigiosin production in the presence of gibberellic acid

Since gibberellic acid is antagonistic to several fungi[10], this constitutes evidence for a similar effect on the metabolic activity of a bacterium. Stowe and Yamaki[1] referred to reports on the growth-promoting effects of gibberellic acid on liverworts, mosses, algae and ferns, and to brief reports on a mushroom and on yeast. Reference was also made to results indicating that hormonal levels of gibberellin promote the growth and respiration of the *BCG* strain of *Mycobacterium tuberculosis*. Although the major effects of gibberellins on plants have been reported as producing increased rate of growth and increased size and weight, turbidity measurements and plate counts indicated no increase in protoplasmic mass or cell numbers when *Serratia marcescens* was grown in the presence of varying concentrations of potassium gibberellate. However, reports of inhibitory effects of gibberellic acid are not novel. Paleg and Aspinall[11] reported inhibition of the development of the barley spike by gibberellic acid and a decrease in the alkaloid content of plants has been reported[12,13]. Of particular interest is the report on the inhibition of the biosynthesis of rutin and rhamnose[14]. Although total chlorophyll content was increased, the number of leaves per plant also increased. It was not stated whether or not the chlorophyll content per unit weight or area of leaf was affected. The inhibition of rutin biosynthesis by gibberellic acid was thought to be effected through its action on the enzyme or enzymes functioning in the biosynthetic pathway.

In this work it seems likely that the added potassium gibberellate exerted its influence on some enzyme or enzymes concerned with the biosynthesis of prodigiosin without exerting any detectable effects on cell reproduction. Uptake of oxygen examined by using both washed cell suspension and cell-free extracts also indicated an inhibitory action of potassium gibberellate. This further substantiates the view that the added plant growth regulator was probably acting on some enzymatic system(s). The exact mechanism(s) is still unknown, and more fundamental research is contemplated to gain further information.

We thank Miss Gwendolyn Howze, formerly of the Biology Department, Tuskegee Institute, Alabama, for advice. This work was sponsored by a grant from the Brown–Hazen Fund, Research Corporation, New York City.

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LETTERS TO THE EDITOR

ASTRONOMY

Impacts on the Earth and Moon

ALTHOUGH nine out of ten of the average meteorites are stoney, the larger objects which collide with the Earth may be predominantly iron. This reversal in characteristics is suggested by the different size law for stone and iron meteorites. The number of impacts on the Earth's surface, N (km^{-2} year^{-1}), with initial mass greater than m_∞ (kg), is at the present time:

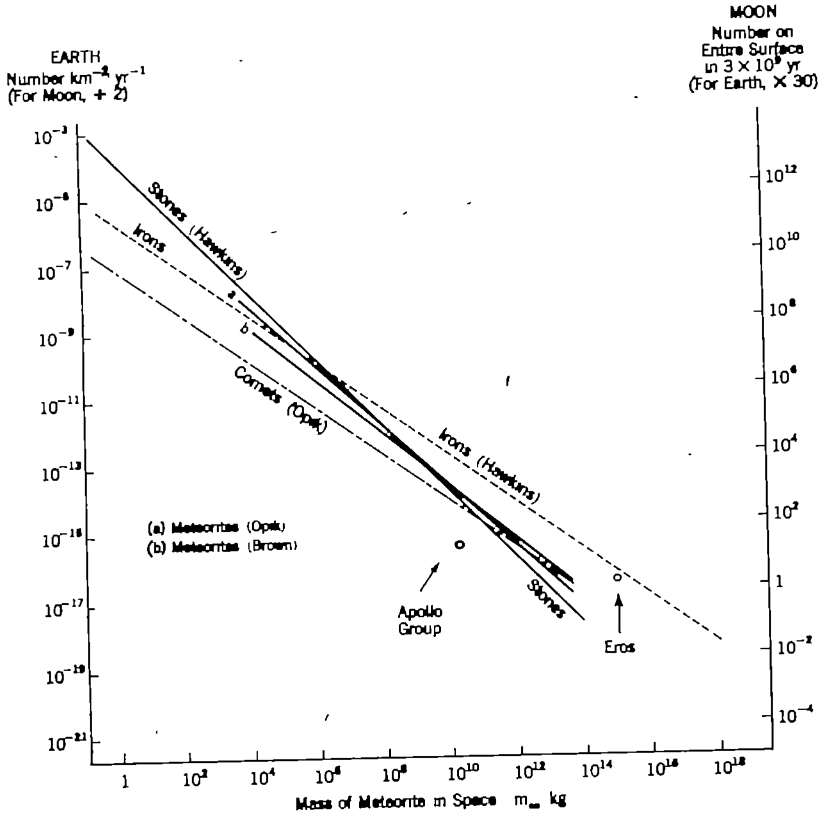

Fig. 1

$$\text{stones: } \log_{10} N = -3{\cdot}73 - \log_{10} m_\infty \quad (1)$$

$$\text{irons: } \log_{10} N = -5{\cdot}61 - 0{\cdot}7 \log_{10} m_\infty \quad (2)$$

These equations can be derived from existing meteorite data[1,2] by assuming that 90 per cent of a stone and 80 per cent of an iron are ablated.

In Fig. 1 the values found from equations (1) and (2) are compared with the data of H. Brown[3] and Opik[4]. The influx rate of stones (1) agrees with other determinations over the range 10^4–10^{14} kg. However, the new value for iron meteorites represents an upward revision of the estimated rate. At $m_\infty = 10^6$ kg the number of irons is equal to the number of stones and at $m_\infty = 10^{16}$ kg the irons outnumber the stones by 10 : 1. It is interesting to note that the rate of collision with cometary nuclei is comparable with that of stony meteorites at the upper end of the size range. We may assume the rates for the surface of the Moon to be 1/2 of the rates for the Earth, thus making allowance for the effect of concentration caused by the Earth's gravitational field.

The extrapolation beyond the range of observation is considerable and it is of interest to attempt to confirm this result by other methods. The largest object passing close to the Earth is the asteroid Eros with a mass of approximately 3×10^{15} kg. The distance of closest approach is $2{\cdot}2 \times 10^7$ km, and, since the search for close approaching asteroids has been confined mainly to the northern hemisphere, the collecting area is $2{\cdot}1 \times 10^{16}$ km^2. The periodicity is 2 years and the rate for $m_\infty \geqslant 3 \times 10^{15}$ kg is $2{\cdot}4 \times 10^{-17}$ (km^{-2} year^{-1}). This is in fair agreement with equation (2). It is probable that very few objects with mass greater than or equal to Eros remain undetected in the vicinity of the Earth's orbit. However, there remains the statistical uncertainty in the small number of events. A similar calculation for the Apollo group gives a value that is some 10^3 times lower than the prediction. Although the number of events is greater this value is probably less reliable than the value derived from Eros. The search for the smaller and faster moving objects is by present estimates far from complete.

We may tentatively conclude that the near-approaching asteroids are iron objects, though, of course, the bulk of the material which remains in the asteroid zone may still be of non-metallic composition. For impacts that occur on the Earth's surface at a rate of 1 every 5×10^6 years, such as the terrestrial events suggested by some authors as the cause of tektite fields, the irons may outnumber the stones by as much as 100 : 1. If we assume that equations (1) and (2) are approximately true for the past then we may estimate the maximum size of object that has collided with the Earth during its geological history. The largest object to collide with the Earth is of mass 10^{17} kg. The Moon has probably suffered one collision with an object comparable in size to Eros, 10^{16} kg.

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RADIOPHYSICS

Long Baseline Interferometry of Jovian Decametric Radio Bursts

DURING the 1962 opposition of Jupiter we made some exploratory observations with a long baseline interferometer in an attempt to obtain information on the sizes and distribution of the decametric burst sources. The observations were made at 19·7 Mc/s with a north–south baseline of 32·3 km, equivalent to 1940 λ at the declination of the planet. They have shown that successful interferometry is possible at these large distances in spite of complications due to ionospheric irregularities. The angular diameter of a burst source was found to be

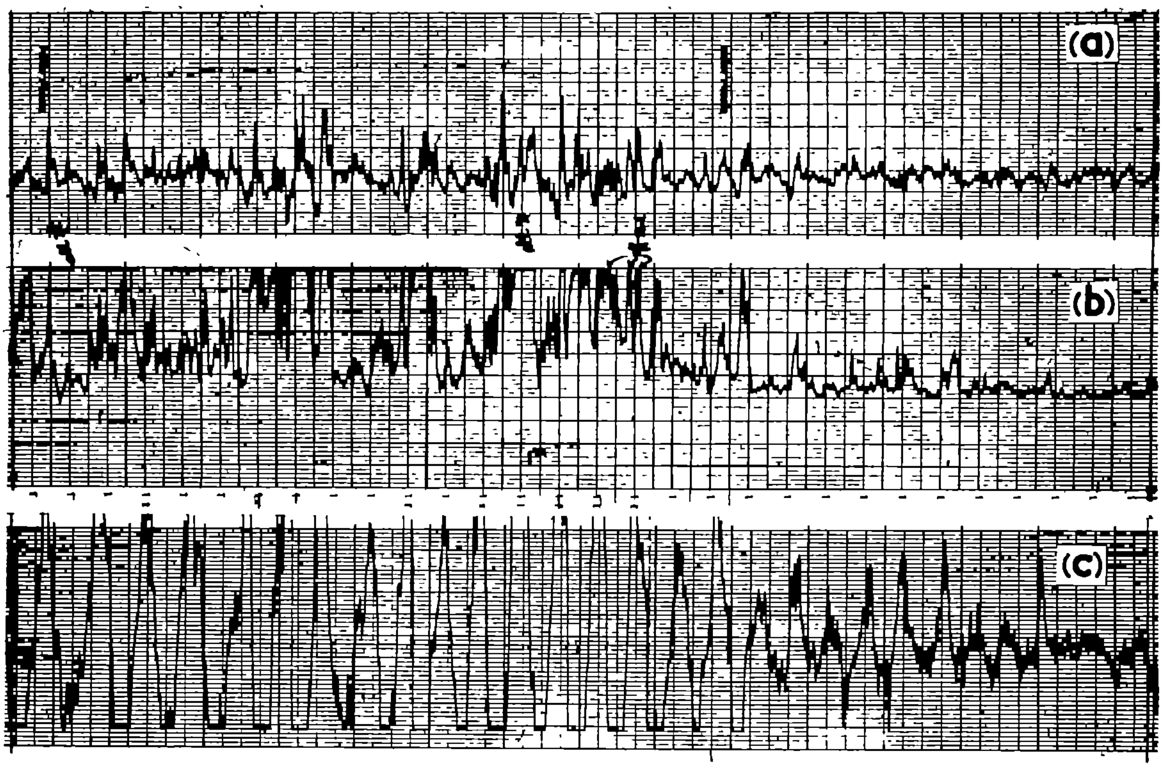

Fig. 1. A 15-min section of the Jovian noise storm recorded on September 24, 1962, at 19·7 Mc/s. Records (*a*) and (*b*) show the total power signals received at the remote and home sites respectively; the local record is a factor of two higher in sensitivity. Record (*c*) is the corresponding phase sensitive interferometer pattern

less than a third of the planet's diameter, and all bursts contributing to a noise storm originated in a single area less than a half of the size of the planet's disk.

Prior observation established that the radiation was predominantly circularly polarized so that differential Faraday rotation was not expected to prejudice interferometry. Consequently, a square array of $\lambda/2$ dipoles was used at each end of the baseline which accepted all components of the radiation. A radio-link, operating at a higher frequency than the signal frequency, was used to transmit the Jovian noise signals from the remote site to the home site at Fleurs, near Sydney. At Fleurs, the noise signal was reconverted to 19·7 Mc/s, using the local oscillator signal, which was also transmitted from the remote site. The remote noise signal and the suitably delayed signal from the home aerial were multiplied by the conventional phase switching method. The total powers received at each end of the baseline and the sine and cosine Fourier components were continuously recorded at the home site. Variations in propagation over the radio-link and in the receiver gains were controlled by means of A.G.C. systems with time constants of 1 min. The time constants of the standard recording systems, determined by the response times of the recorder pens, were 0·5 sec for full-scale deflexion. A special four-channel recorder with a response time of 0·02 sec was used occasionally to examine the few bursts with durations less than 0·5 sec. Two values of receiver band-width, namely, 4 kc/s and 200 kc/s to half-power points, were used at different stages of the observations, which extended from August 16 until October 6, 1962.

The effective interferometer spacing of 1940 λ produced an interference pattern with a fringe separation of 1·7 arc. The low primary directivity of the system permitted observations to be made for a period of 5 h each night near transit. During this period, the rotation axis of the planet changes by 94° with respect to the interferometer baseline, so that over extended periods of time, the high resolving power of the interferometer could be applied to both polar and equatorial directions on the planet.

The degree of correlation between the total power signals from the two ends of the baseline varied considerably from night to night. An example of good correlation is shown in Fig. 1*a* and *b*. These records illustrate one of the difficulties in this type of measurement, namely, the very large dynamic range of the Jovian radiation. With a pre-selected intensity range, only a small percentage of the recorded events are suitable for direct comparison. Where detailed comparison is possible, the high degree of correlation shows beyond any reasonable doubt that the decametric radiation is already in the form of bursts with durations ranging from 0·1 sec to about 60 sec when the waves impinge on the Earth's atmosphere. On these nights, as can be seen from Fig. 1*c*, regular interferometer patterns were produced as the apparent motion of Jupiter carried the burst source through the fringes.

On those occasions when the total power signals were poorly correlated, presumably due to the effects of uncorrelated ionospheric scintillations, the fringe periods were very irregular, reflecting the apparent position changes associated with the scintillations. However, interferometer deflexions were always obtained of amplitude consistent with the instantaneous values of the total power signals even when the latter had no other directly comparable features. The interesting conclusion to be drawn from these results is that the Jovian radiation remains coherent at the ends of a long baseline in the presence of severe uncorrelated scintillations.

Size of burst sources. If p_1 and p_2 are the instantaneous powers delivered to the two receivers during a Jovian noise burst, then the deflexions of the total power recorded are proportional to $\alpha_1 p_1$ and $\alpha_2 p_2$, while their combination results in a deflexion $\beta V(p_1 p_2)^{\frac{1}{2}}$, where the constants α_1, α_2, β may be determined from the receiver calibrations. The factor $V \leqslant 1$ is a measure of the coherence of the radiation over the baseline and the amount by which it departs from unity depends on the type of brightness distribution and its dimensions relative to the fringe spacing.

For the purpose of estimating the angular sizes of the burst sources, six Jovian noise storms were examined. Two of these were recorded with a receiver band-width of 4 kc/s and four with a band-width of 200 kc/s. From the narrow-band records, analysis of 35 bursts led to a value of $\overline{V}_n = 1{\cdot}00$ for the mean fringe visibility, with an *S.E.* of 0·03. From the wide-band interferometer, 35 bursts resulted in a mean fringe visibility of $V_w = 1{\cdot}04$, with an *S.E.* of 0·06. Much of the larger error in the wide-band result may be due to the necessity of applying corrections to the observed fringe visibilities for band-width dispersion. Consideration of the standard errors involved in these mean values leads to estimates of an upper size limit for the burst-emitting sources. From the narrow-band results, for a Gaussian brightness distribution, the angular size of the sources was $< 13'$ arc at the 5 per cent confidence-level. The wide-band result indicates that the source size was $< 19'$ arc. There were no detectable systematic differences in source size between the polar and equatorial directions.

Size of the burst-emitting regions. Restricting consideration to those observations for which the absence of marked ionospheric effects results in a high degree of correlation between the total power records at both sites, it is possible to set an upper limit on the size of the region responsible for the bursts in a storm. In these circumstances and where the bursts are sufficiently frequent, they give rise to a continuous series of fringes. For example, during a storm on September 25, 1962, 27 such fringes were counted over a period of 9 min. The mean fringe period was 19·8 sec with an *S.E.* of 12 per cent for individual measurements. Hence it was most unlikely (at the 5 per cent confidence-level) that independently radiating sources, if indeed there was more than a single source, occupied an area with dimensions greater than ¼ of the fringe separation or 25′ arc. A number of such occurrences showed that there were no differences between equatorial and polar directions.

There seems to be no reason, from the results recorded here, why interferometer observations should not be continued with longer baselines until the Jovian burst sources are resolved. It is our present intention to make similar measurements over baselines of 80 km or more during the 1963 opposition.

We thank Mr. C. F. Fryar for assistance in operating the interferometer during the present experiment.

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Sudden Changes in Amplitude and Phase of the Very-Low-Frequency Signal from Station *GBR* at Salisbury, South Australia

A peculiar phenomenon, to our knowledge not reported so far, has been observed on the signals of Station *GBR* on 16 kc/s.

The signals of this Station, monitored for frequency standardization purposes, are mixed with a suitable frequency derived from a 100-kc/s standard oscillator and a record, as the one shown in Fig. 1, is produced. The number of beat cycles over 24-h intervals is counted and the drift of the local source determined.

At about 12.30 h local time (9½ h ahead of U.T.) on May 31, 1962, the number of beat cycles suddenly increased and this faster beat persisted for about half an hour, as shown in the figure.

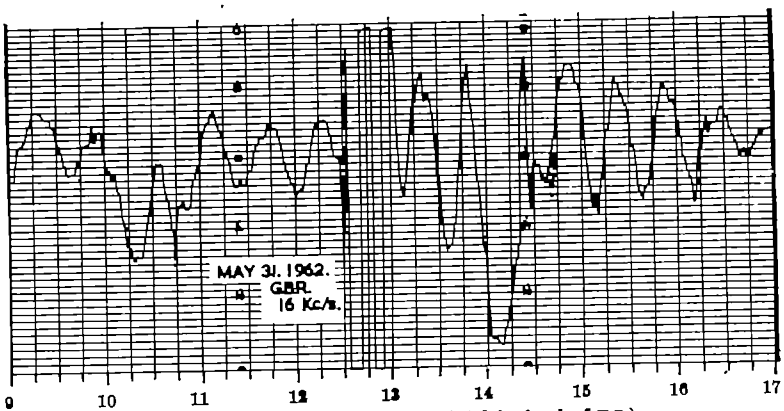

Fig. 1

Similar occurrences were recorded on June 11 at approximately 12.30 h local time, on June 12 at 09·45 h and on June 25 at 09·40 h. These lasted approximately 15, 30 and 8 min respectively.

All possibility of instability of the local source must be ruled out as the same derived signal is mixed also with the signals of the United States transmitter *NBA* on 18 kc/s and no trace of a similar effect has been noticed on the record of this station.

The beat frequency, while the described phenomenon lasted, was of a high stability not likely to be possible to derive from a local source switched on for this period.

We thank the Chief Scientist, Department of Supply, for his permission to publish this communication.

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GEOPHYSICS

Lithium Emission in Twilight at Uppsala during November 1962

A patrol spectrophotometer (*SP*3) has been operating at this Observatory for a long period to record lithium emission in twilight at 6708 Å. In spite of the high sensitivity of the instrument no lithium was recorded until a strong emission suddenly occurred on November 7, 1962, in the evening twilight. The last record before this date was made on October 19, 1962, when no emission at all was detected. In the days before and after November 7 the weather conditions were unfavourable, but in spite of this a number of good records have been received.

The spectrophotometer was adjusted on November 7 to sweep over a range of ~ 50 Å each fourth minute, and the spectral line is recorded via photomultiplier and rate-meter on an x–y recorder. The resolution was about 2·5 Å and the instrument was directed 25° elevation north-north-west. Fig. 1 shows the first record of the lithium flash.

After November 7, records were made in zenith both in the morning and evening twilight. Simultaneous records were made with another spectrophotometer of somewhat less light power including the sodium lines for comparison. From these records one can get some idea of the ratio and the time difference of the lithium flash with respect to the sodium flash. Regarding the intensity of the lithium emission a comparison with sodium shows that the lithium emission at the beginning of the period seems to have been at least of the same order as the sodium emission. Regarding the time difference with respect to sodium, the lithium flash follows very near that of sodium, but both are displaced in time with respect to the *OI* 6300 Å flash, which

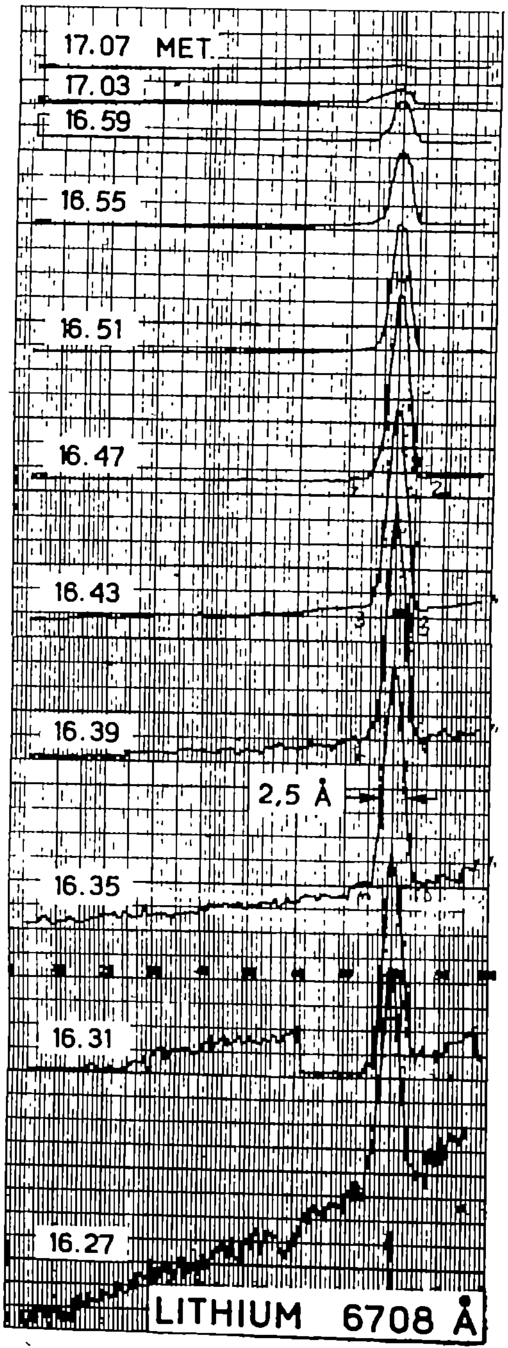

Fig. 1. Sample of lithium twilight record on November 7, 1962, at Uppsala

was recorded separately. The records for November 15 and November 23 are shown in Fig. 2. These records were made with 1-min intervals and are good enough for an analysis. After having normalized the curves for these two days one sees a striking similarity between the lithium and sodium flashes. When discussing the height of the sodium and lithium layers respectively one has to assume a screening height, which for sodium is about 27 km according to Bullock and Hunten[1]. For lithium a screening height was chosen 5 km lower than for sodium, as used by Gadsden and Salmon[2]. Then using the method for determining the height of the maximum density of the sodium-layer proposed by Hunten[3], and Hunten and Shepherd[4], the height (km) for lithium and sodium was found to be approximately:

	Li	Na
November 15	91 km	94 km
November 23	87 km	95 km

Thus it seems that the height of lithium was lower on November 23 than on November 15, corresponding to a decrease ~ 0·5 km a day. A similar decrease of the height of lithium was reported by Gadsden and Salmon[2].

The observations during November 1962 at Uppsala give, in spite of very variable weather conditions, a clear picture of the lithium : sodium ratio with time. At the end of November the lithium had nearly disappeared and the decrease during the actual 4 weeks was very near the same as that reported by Gadsden and Salmon[5], 1961. In Fig. 3 the lithium values are given showing the decrease of the intensity over the whole period. The mean level of sodium-emission relative to lithium is indicated by a

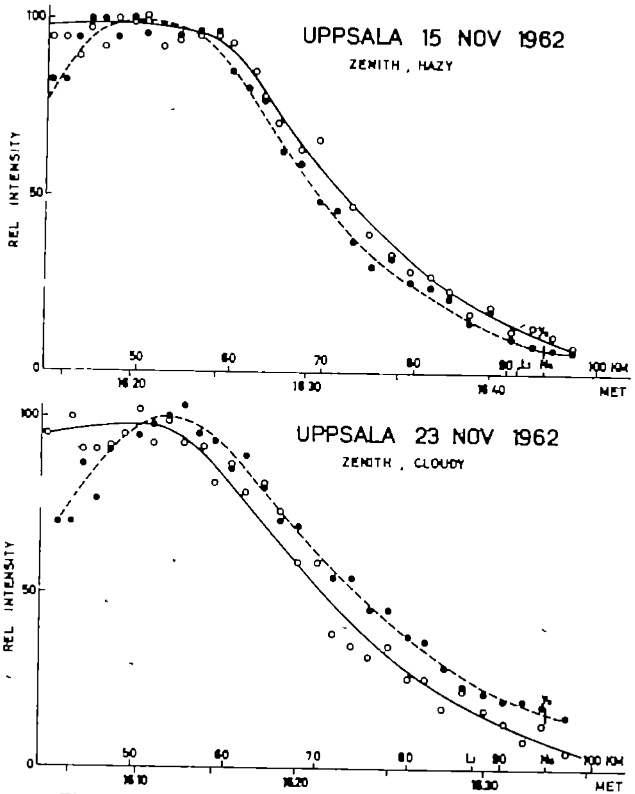

Fig. 2. Comparison of lithium and sodium twilight record. O, Lithium; ●, sodium

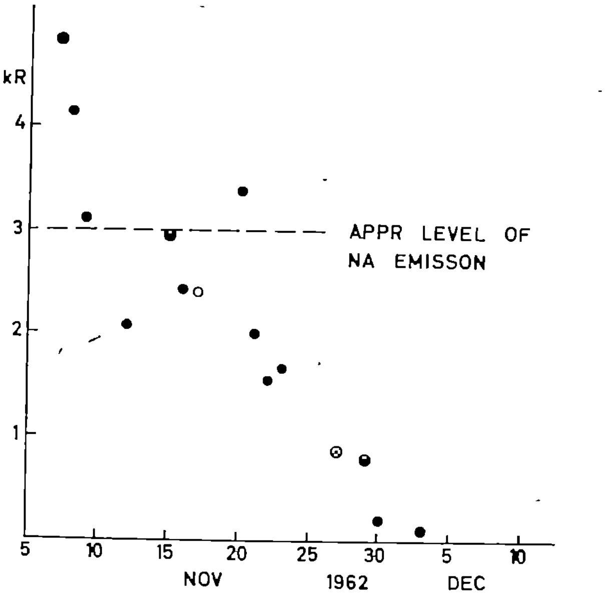

Fig. 3. Diagram showing the decrease of lithium emission during the observing period of about 25 days. O, Clear; O, hazy; ●, cloudy

dashed line. The intensity scale has an uncertainty of about ± 40 per cent.

Earlier enhancement of lithium emission was reported by Barber[6], and related to the nuclear explosion at high altitude over the central Pacific Ocean. From the high intensity and the short life-time of the emission of lithium reported here, it seems with little doubt that the emission is related to some of the U.S.S.R. nuclear explosions.

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Earth's Gravitational Potential: Evaluation of Even Zonal Harmonics from the 2nd to the 12th

The Earth's gravitational potential U at an exterior point distant r from the Earth's centre, and having geocentric latitude φ, may be written in a series of spherical harmonics as:

$$U = \frac{GM}{r}\left\{1 - \sum_{n=2}^{\infty} J_n \left(\frac{R}{r}\right)^n P_n(\sin\varphi)\right\} \tag{1}$$

where G is the gravitational constant, M the mass of the Earth, and R the Earth's equatorial radius. $P_n(\sin\varphi)$ is the Legendre polynomial of degree n and argument $\sin\varphi$, and the J_n are constant coefficients. Equation (1) does not take into account the small variation of U with longitude, and represents an average over all longitudes.

In the past five years the first few J_n have been evaluated by various authors from the perturbations of the orbits of artificial satellites. The arithmetic means of the more complete determinations[1–7], with root mean square scatter, are:

$$\begin{aligned} 10^6 J_2 &= 1082{\cdot}7 \pm 0{\cdot}2 & 10^6 J_3 &= -2{\cdot}44 \pm 0{\cdot}08 \\ 10^6 J_4 &= -1{\cdot}6 \pm 0{\cdot}2 & 10^6 J_5 &= 0 \pm 0{\cdot}2 \\ 10^6 J_6 &= 0{\cdot}7 \pm 0{\cdot}2 & 10^6 J_7 &= -0{\cdot}45 \pm 0{\cdot}1 \end{aligned} \tag{2}$$

Some tentative values of J_8 and J_9 have also been obtained. In all these determinations, harmonics of degree higher than those evaluated were necessarily ignored, though they cannot be proved to be negligible, and their neglect contributes towards the scatter among the values derived by the different authors.

We have recently made a new determination of the even harmonics in the Earth's gravitational potential, from the motion of seven satellites, chosen with the view of sampling the gravitational field as thoroughly and evenly as possible: their orbits were inclined to the equator at angles between 28° and 97°. The potential experienced by these satellites is found to be best represented by the following set of coefficients:

$$\begin{aligned} 10^6 J_2 &= 1082{\cdot}78 \pm 0{\cdot}05 \\ 10^6 J_4 &= -0{\cdot}78 \pm 0{\cdot}2 \\ 10^6 J_6 &= 0{\cdot}70 \pm 0{\cdot}1 \\ 10^6 J_8 &= 0{\cdot}24 \pm 0{\cdot}2 \\ 10^6 J_{10} &= -0{\cdot}50 \pm 0{\cdot}2 \\ 10^6 J_{12} &= 0{\cdot}28 \pm 0{\cdot}2 \end{aligned} \tag{3}$$

The quoted errors are tentative estimates of the departures of the quantities from the true physical values, making some attempt to allow for the neglect of J_{14}, J_{16} . . . If the potential is to be represented by fewer even harmonics, the best number is found to be three and the values obtained are:

$$\begin{aligned} 10^6 J_2 &= 1082{\cdot}79 \pm 0{\cdot}05 \\ 10^6 J_4 &= -1{\cdot}09 \pm 0{\cdot}20 \\ 10^6 J_6 &= 0{\cdot}73 \pm 0{\cdot}20 \end{aligned} \tag{4}$$

To complete the potential, values of J_3, J_5, and J_7 must be added to (3) and to (4), from equation (2).

The values of J_2 and J_6 in (3) and (4) are consistent with each other and with those in equation (2). J_2 was found to be almost unaffected by the evaluation of higher harmonics, virtually identical values being obtained when either 3, 4, 5 or 6 harmonics were included. The same cannot be said for J_4, which suffered a sharp change when J_{12} was included: J_4 has in equation (3) a smaller numerical value than any hitherto obtained. Even in (4) the value of J_4 is significantly smaller than in (2), probably because near-polar satellites, which tend to give small values of J_4, were not included in previous determinations. Although the values of J_8–J_{12} in (3) must be regarded as tentative, it appears likely that J_{10} is strongly negative, and may be numerically almost as large as J_4 or J_6.

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OCEANOGRAPHY

Definition of the 'Salinity' of Sea-water

For sixty years oceanographers have accepted and used Knudsen's[1] relationship defining salinity in terms of the chlorinity of sea-water. The validity of this relationship has several times been questioned, for example by Carritt and Carpenter[2]. These doubts have been strengthened by the work of Cox, Culkin, Greenhalgh and Riley[3].

To consider the problems involved, a panel has been appointed jointly by the International Council for the Exploration of the Sea, the Special Commission for Oceanographic Research, the International Association of Physical Oceanography and Unesco. The members are: Prof. G. Dietrich (Germany, *chairman*); Prof. D. E. Carritt (United States); Dr. R. A. Cox (United Kingdom); Dr. N. P. Fofonoff (Canada); Mr. F. Hermann (Denmark); Dr. G. N. Ivanoff-Frantzkevich (U.S.S.R.); Dr. Y. Miyake (Japan).

This panel met in Paris in May 1962, when the problems were discussed and a report was drafted which includes certain recommendations. These may be summarized:

(*a*) That salinity should be re-defined in terms of density at 0° (sigma 0); the relationship being chosen to preserve, on average, the present relationship between salinity and chlorinity as closely as possible.

(*b*) That new tables should be computed relating salinity so defined to conductivity and refractive index.

(*c*) That as soon as practicable Copenhagen Standard Sea-Water should be certified in conductivity as well as chlorinity.

The full report has been distributed by Unesco to most oceanographic institutions. Copies of the report can be obtained from the Office of Oceanography, Unesco, Place de Fontenoy, Paris, 7.

It is hoped to debate these recommendations at the International Association of Physical Oceanography meeting in San Francisco in August 1963. In the meantime, the panel would like to receive and consider comments, criticisms and suggestions relating to the report. These should be sent to me.

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CHEMISTRY

Influence of Polyvalent Cations on the Rate of Electro-reduction and Aquation of $Co(NH_3)_5X^{n+}$ Complexes

IN previous communications[1,2] the influence of the nature and concentration of anions on the rate of electro-reduction of $Co(NH_3)_5X^{n+}$ complexes has been reported. The influence exerted depends on the nature of the ligand X. This phenomenon has been explained by the assumption that the complex particle does not behave as a conducting sphere with uniform charge distribution (as assumed usually) but that the non-homogeneous charge distribution contributes considerably to the electrostatic free energy of activation and has to be taken into account. The ligand X represents the region of maximum non-homogeneity which induces an opposite non-homogeneity in the ionic atmosphere, the cations of the supporting electrolyte being preferentially attracted into the parts of the ionic atmosphere near the negative pole of the central particle. This increased concentration of cations shields the negative pole of the complex particle or might lead to the formation of an ion-pair between the positively charged complex particle and the cation. Both these effects make the attainment of the favourable orientation (that is, with ligand X removed from the positively charged electrode surface[2]) of the particle in the activated complex depolarizer-electrode[2] much easier and increase the rate of electro-reduction.

Such an acceleration is caused by H^+-ions for complexes with $X = F^-$, formate, acetate, propionate, butyrate. In this case the association of the complex with protons might be governed by the basic properties of the ligand and not by the non-homogeneity of the charge distribution. The later effect can be expected for complexes with ligands X carrying a higher fraction of the negative charge (for example, F^-, SO_4^{2-})[1] and polyvalent cations like Th^{4+}, La^{3+} or Ba^{2+}.

The influence of Th^{4+} on the rate of reduction of various complexes at the dropping-mercury electrode is represented in Fig. 1. A similar but much smaller influence has been observed with La^{3+} ions. Ba^{2+} influences the rate of reduction to a negligible extent. The rate constant of reduction (k_e) in presence of Th^{4+} and La^{3+} ions obeys the equation:

$$k_e = {}_0k_e + {}_Mk'_e\,[M^{n+}] \qquad (1)$$

${}_0k_e$ being identical with the rate constant of the reduction in a solution without polyvalent cations. The experimental value of the constants calculated with the use of the value 7×10^{-6} cm^{2}s^{-1} (ref. 3) for the diffusion coefficient are given in Table 1.

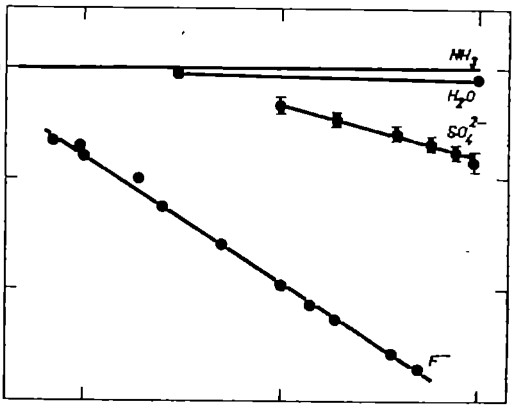

Fig. 1. The change of the half-wave potential of $Co(NH_3)_5X^{n+}$ complexes with Th^{4+} concentration. $\mu = 2$; 1 M H^+; nitrate. Concentration of the complex. 7×10^{-4} M

From the form of the rate law it follows that:

(1) The reduction follows two paths, the first being the reduction of the 'free' complex, the second the reduction of the 'ion-pair' complex-cation.

(2) The ion-pair is reduced much more rapidly than the free complex.

(3) The concentration of the ion-pair is very small and its formation very rapid.

(4) The rate constant ${}_Mk'_e$ equals ${}_Mk_e \cdot K_M$, K_M being the association constant of the ion-pair.

The difference in the influence of La^{3+} and Th^{4+} is greater than would be expected on the basis of shielding effect of the ionic atmosphere only and points to the conclusion that specific affinity ligand-cation contributes considerably to the stability of the ion-pair, especially in the case of $[Co(NH_3)_5F^{2+} \cdot Th^{4+}]$.

The much smaller ratio ${}_{Th}k_e : {}_0k_e$ for $X = SO_4^{2-}$ than for F^- is due to a relatively small affinity of sulphate ions to Th^{4+}. The association of Th^{4+} and $Co(NH_3)_5SO_4^+$ is governed from a great part by the electrostatic effect and the concentration of the ion-pair $[Co(NH_3)_5SO_4^+ \cdot Th^{4+}]$ is much smaller than that of $[Co(NH_3)_5F^{2+} \cdot Th^{4+}]$.

To prove the foregoing conclusions and to show that the effects observed are not due to the change of the electrode double layer caused by polyvalent cations, the rate of aquation of $Co(NH_3)_5F^{2+}$ under the influence of Th^{4+} ions was followed. The aquation follows the rate law:

$$k = k_H + k_{Th} \cdot [Th^{4+}] \qquad (2)$$

k_H is identical with the rate constant observed in a solution of the same acidity without Th^{4+} ions and depends on the concentration of hydrogen ions, increasing with increasing acidity of the solution. The values of k_H and k_{Th} are given in Table 1. The accelerating influence of Th^{4+} ions on the aquation of $Co(NH_3)_5F^{2+}$ is analogous to the influence exerted by Hg^{2+} ions on the aquation of $Co(NH_3)_5Y^{2+}$, Y being Cl^-, Br^- or I^- (ref. 4). In the latter case the accelerating effect is due to the high affinity of halogen and mercuric ions, as is shown by the increase of the rate in the order $Cl^- > Br^- > I^-$ (ref. 4).

Table 1. SUMMARY OF RATE CONSTANTS FROM EQUATIONS (1) AND (2)

Solution: 1 M HNO_3 + $NaNO_3$ up to $\mu = 2$ M. ${}_0k_e$, ${}_Mk_e$ at 0° C, k_H, k_{Th} at 20·5° C. ${}_0k_e$, ${}_Mk'_e$ related to the potential of 0·1 M calomel electrode at 0° C

Complex	${}_0k_e$	${}_{Th}k'_e$	${}_{La}k'_e$	${}_{Th}k_e : {}_0k_e$	k_H (s^{-1})	k_{Th} (s^{-1} mol^{-1} l.)
$Co(NH_3)_5F^{2+}$	$1·4 \times 10^{-6}$	$9·7 \times 10^{-3}$	$5·7 \times 10^{-5}$	6,800	$7·0 \pm 0·2 \times 10^{-5}$	$3·1 \times 10^{-4}$
$Co(NH_3)_5SO_4^+$	$9·55 \times 10^{-6}$	$5·3 \times 10^{-4}$	$1·06 \times 10^{-5}$	56	—	—
$Co(NH_3)_5H_2O^{3+}$	4×10^{-6}	$6·6 \times 10^{-6}$	—	1·5	—	—

Dimensions: ${}_0k_e$ = cm × s^{-1}; ${}_Mk_e$ = cm × s^{-1} × l.mole^{-1}.

These experiments show that an association of the complex cation with the metallic cation occurs, the ligand *X* acting as a bridge between the two metals. In the ion-pair formed a certain fraction of electron density is transferred to the bond *M*–*X*, so that the bond *X*–Co is loosened which facilitates the aquation. The electrochemical reduction of the ion-pair does not proceed via the aquated ion or via a particle with the co-ordination number 5, for the rate of reduction is higher than it would correspond to a process preceded by a homogeneous splitting off the ligand *X*.

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Evaporation of Langmuir – Blodgett Monolayers in Vacuum

While there is an extensive literature on the methods of depositing monomolecular films of fatty substances on solid surfaces by the Langmuir–Blodgett method[1], there seems to be little information available on the stability of the films once prepared. Gregg and Widdowson[2] studied the evaporation of built-up multilayers of fatty acids, but their results were restricted to layers many molecules thick. More recently, Karle[3] and Menter and Tabor[4] have obtained some evidence of the evaporation of fatty acid and soap molecules from monomolecular films in electron diffraction investigations.

The availability of radioactively labelled fatty acids in recent years has permitted a more direct approach to assessing the stability of such deposited monolayers. We have recently reported observations on the stability *in vacuo* of stearic acid and barium–copper stearate monolayers deposited on quartz, soft glass and gold[5]. The monolayers were labelled with carbon-14, and the rate at which they were desorbed at room temperature and a pressure of ~10^{-8} torr was measured radiometrically. The barium–copper stearate films on soft glass lost about 10 per cent of their initial activity in 90 h pumping; stearic acid films on gold, on the other hand, were about half removed after 5 h evacuation. These investigations have now been extended.

The most stable monolayers which we have prepared are those of *n*-octadecylamine hydrochloride, deposited on either mica or evaporated gold films from the surface of 10^{-3} N hydrochloric acid. Such monolayers retain more than 90 per cent of their initial activity after 40 h of evacuation. Autoradiographs of these monolayers, both before and after evacuation, show a uniform density of activity over the entire sample. We have found the deposition of *n*-octadecylamine hydrochloride monolayers, however, to be more erratic than that of the stearates, perhaps partly due to the greater tendency to expansion of the amine hydrochloride monolayers on the aqueous surface[6]; we have also found evidence of slow solution of these monolayers while spread on the acid subphase. In several cases, we have obtained transferred monolayers, the initial activity of which was appreciably below that to be expected from a homogeneous monolayer; autoradiographs of the samples show an inhomogeneous distribution of activity over the surface. The free amine, spread on 10^{-3} N sodium hydroxide solutions, should form a more compact monolayer[7]. We have prepared a few samples of free *n*-octadecylamine monolayers on gold, quartz, and mica, and their stability in vacuum appears to be appreciably lower than that of the corresponding amine hydrochloride films. This result is not surprising, since the free amines have much lower melting points and higher vapour pressures than do their salts.

For many types of experiments, only short-term stability to vacuum exposure may be required. The removal of the first small fraction of a monomolecular film may be governed by factors quite different from those controlling the removal of the major portion of the monolayer. In the case of soap monolayers, for example, it is possible that any free fatty acid in the film would evaporate more readily than the soap itself[7].

Table 1. SHORT-TERM STABILITY OF MONOLAYERS IN VACUUM. FRACTION OF MONOLAYER REMAINING AFTER 30 MIN PUMPING IS TABULATED. MONOLAYERS WERE DEPOSITED UNDER 17 DYNES/CM SURFACE PRESSURE

Substrate Monolayer	Quartz	Mica	Soft glass	Gold on quartz	Gold on mica
Barium–copper stearate	0·93 ± 0·02 (4)	0·97 ± 0·03 (5)	0·96 ± 0·02 (3)	0·94 ± 0·01 (3)	0·91 ± 0·04 (3)
Stearic acid	0·31 ± 0·04 (2)	—	—	0·85 ± 0·01 (2)	—
n-Octadecylamine hydrochloride	—	1·00 ± 0·02 (4)	—	—	0·96 ± 0·01 (4)

Figures in brackets indicate number of samples.

In order to investigate this behaviour, we examined a number of samples after they had been exposed to vacuum for 30 min. The results are presented in Table 1. Both barium–copper stearate and *n*-octadecylamine hydrochloride monolayers are fairly stable over this short period; stearic acid films have already lost an appreciable amount of their activity in 30 min. Mica substrates seem to show the highest resistance to desorption of the solids which we have used, although the differences observed with the barium–copper stearate and *n*-octyldecylamine hydrochloride monolayers are small.

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Schmidt Reaction

Since the advent of commercial polyphosphoric acid, a number of novel procedures have been developed for the Schmidt reaction using polyphosphoric acid as the solvent and catalyst[1–3].

It was interesting to note that in nearly all the reactions run, temperatures of 100° or higher were used. One of these[3] reported that benzoic acid gave aniline in 48 per cent yield, along with a considerable amount of diphenylurea, while *p*-nitrobenzoic acid failed to react. The conditions of these reactions were not clearly defined.

We found that aromatic carboxylic acids can be converted to the corresponding amines at room temperature (25°) in good yields using polyphosphoric acid and excess sodium azide. Table 1 shows our results. The yields are in agreement with the fact that acids with electron donator groups give better yields and have faster rates[4].

Table 1

Acid	Product	Yield (%)	Derivative	Melting point	Infra-red
Benzoic	Aniline	71·2	Benzamide	161–162°	2·82, 2·91, 3·08, 6·19, 7·68µ (film)
p-Nitro-benzoic	*p*-Nitro-aniline	48·2	—	146°	2·82, 2·94, 3·09, 6·19, 7·72µ (Nujol mull)
p-Methoxy-benzoic	*p*-Methoxy-aniline	79·7	—	55–56°	2·82, 2·91, 3·07, 6·17, 7·70µ (Nujol mull)
Mesitoic	Mesidine	90·6	Benzamide	203°	2·83, 2·90, 3·07, 6·18, 7·70µ (film)

Apparently no side-reaction occurred, such as urea formation, because the amine products, which were solid, melted sharply within two degrees of the reported melting points[7] without recrystallization. Those amines which were liquids were also uncontaminated because their infra-red curves[8] were identical to original samples.

It appears that urea formation is decreased to a considerable degree when the Schmidt reaction is run at room temperatures. Furthermore, the increase of yields in polyphosphoric acid over conventional methods seems to imply the formation of the more reactive oxocarbonium ion in this medium.

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Effect of Naphthalene on the Quenching of Liquid Scintillation Solutions

IN liquid scintillation counting the passage of β-particles through the phosphor solution results in a complex series of events, the useful ones culminating in the emission of photoelectrons at the photocathode of the photomultiplier. A number of parasitic events occur concomitantly that lead to a lowering of the yield, or total loss of the photoelectrons, that is, quenching. Possible sequences for these events, which occur during a time-interval of approximately 10^{-9} sec, have been outlined by several authors[1-3]. The addition of naphthalene to some liquid phosphors improves their counting characteristics. The enhancement in counting efficiency has been ascribed by Fust and Kallmann[4] to the ability of the naphthalene to act as an intermediate solvent that is capable of transmitting the energy from the primary solvent to the scintillators, thereby by-passing the quenching agent. If this is true the series of events leading to photoelectron production could be outlined as follows: (*A*) emission of β-particle; (*B*) excitation of primary solvent molecules (toluene); (*C*) excitation of secondary solvent molecules (naphthalene); (*D*) excitation of primary solute molecules (PPO); (*E*) excitation of secondary solute molecules (POPOP); (*F*) production of photons.

I have found that the addition of naphthalene to some liquid scintillator systems containing tritium can have a marked effect on the quenching curves. The effect depends entirely on the chemical nature of the quenching agent. With some substances the quenching effect is almost entirely removed; with others, it is enhanced.

The continuous curves shown in Figs. 1 and 2 were prepared by adding inactive benzoic acid and methyl benzoate to a liquid scintillator (4 g/l. PPO and 0·04 g/l. POPOP in toluene) containing approximately 10^{-8} g of tritiated benzoic acid. The broken curves were prepared by repeating the experiments with the same liquid phosphor solutions containing in addition 100 g/l. of purified naphthalene.

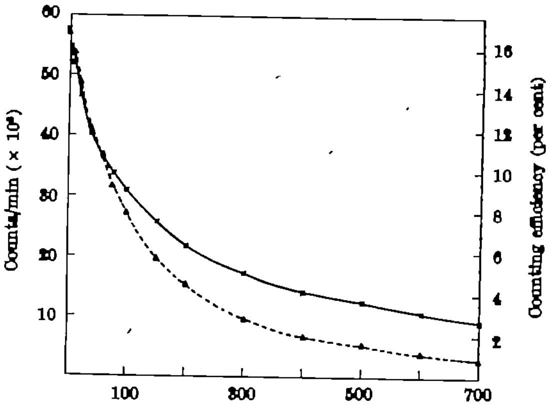

Fig. 1. Graph showing the effect on the count-rate of adding inactive benzoic acid to liquid scintillators containing a very small quantity of tritiated benzoic acid. ×, Normal scintillator solution; ▲, scintillator plus naphthalene

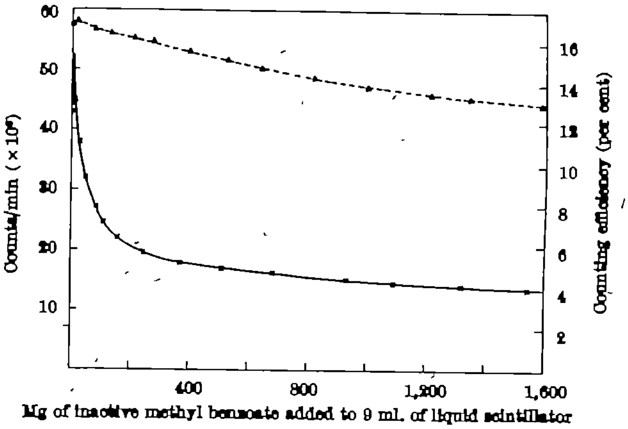

Fig. 2. Graph showing the effect of adding naphthalene to the scintillator on the tritium–methyl benzoate quenching curve. The small increase in count-rate recorded on the broken curve for very small amounts of methyl benzoate is due to desorption of active tritiated benzoic acid from the walls of counting vessel (ref. 5). ×, Normal scintillator; ▲, scintillator plus naphthalene

It can be seen from the results that addition of naphthalene to the liquid scintillator containing benzoic acid results in a small increase in counting efficiency at low concentrations of benzoic acid, and a decrease in efficiency at high concentrations. The addition of naphthalene to a benzoate-quenched solution, on the other hand, results in a considerable increase in counting efficiency at all quencher concentrations.

These results would seem to indicate that the quenching effects of benzoic acid and methyl benzoate are basically different. Two possible explanations are: (*a*) that the quenching phenomena for the two quenching agents occur at different points in the chain of events outlined here; or (*b*) that the quenching phenomena operate at the same stage in the scintillation process (between events *B* and *D*) and are the result of different rates of energy transfer. Thus, if quenching occurs between events *B* and *C* the rate of transfer of energy from the primary solvent to benzoic acid is greater than its rate of transfer to naphthalene, which in turn is greater than the transfer rate to methyl benzoate. If, however, quenching occurs between events *C* and *D*, the rates of energy transfer from naphthalene would be in the order benzoic acid > PPO > methyl benzoate.

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Use of Modified 'Bentone-34' for the Gas Chromatographic Separation of Aromatic Hydrocarbons

The use of 'Bentone-34' (dimethyldioctadecylammonium bentonite) for the separation of aromatic hydrocarbons has been described previously[1-3], but the chromatograms published have either exhibited considerable tailing[3] or, where symmetrical peaks have been shown, these have been obtained using small sample volumes (about 0·02 µl.) and ionization detectors[3,4].

In an effort to obtain a stable column packing suitable for use with µl. samples and thermal conductivity detection the performance of 'Bentone-34' when modified with silicone oil and dispersed on 'Celite' has been investigated.

Equal parts by weight of 'Bentone-34' (F. W. Berk and Co., Ltd.) and 'Silicone Oil *MS 555*' (Midland Silicones, Ltd.) were dispersed in benzene and added to 10 parts by weight of 'Celite' (60–100 mesh). The resulting mixture was evaporated to dryness, sieved to break up any lumps present, and was packed into a 12-ft. copper column (internal diameter $\frac{1}{8}$ in.) at a packing density of 1·85 g/ft. A chromatogram, obtained using this column at 70° C, illustrating the separation of the C_8 aromatics and other hydrocarbons is shown in Fig. 1. The separation of the xylenes was completed in less than 30 min and symmetrical peaks were obtained. It will be noted, however, that the elution order of the *m*- and *o*-xylenes was the reverse of that normally obtained using 'Bentone-34'/'Celite' columns[3]. The relative retention volumes for seventeen hydrocarbons are given in Table 1.

The same column has been used at 90° C to separate all but two of the C_8–C_9 aromatics and the relative retention volumes obtained are given in Table 1. The inlet pressure was as before, that is, 22 lb./in.^{2}, but the flow-rate 106 ml./min.

X-ray examination of the 'Bentone-34' before and after modification indicates that the basal spacing of the organo-clay has been expanded from 12·3 Å to 38·5 Å. Thus, in the modified column packing the 'Bentone-34' is likely to be present as a thixotropic gel. With a 1 : 1 mixture of 'Bentone-34' and silicone oil the separation factor for the *meta*- and *para*-xylenes is 1·13 at 70° C and, as the proportion of silicone oil is decreased, the separation factor increases but the basal spacing remains unchanged. It would appear, therefore, that the inter-lamellar distances within the clay complex play only a minor part in the separation of the xylene isomers but the need for the xylenes to expand the lattice prior to adsorption may explain the serious tailing experienced when using the unmodified 'Bentone-34'.

Other modifying solvents have been used successfully including squalane and polyethyleneglycol and it appears that the separation possible can be controlled by careful selection of the type and proportion of the modifying solvent. Also, there seems no reason why the technique should be limited to modified organo-bentonites and other

Table 1. RELATIVE RETENTION VOLUMES OF HYDROCARBONS ON A 'BENTONE-34'/'SILICONE OIL' (EQUAL PARTS BY WEIGHT) COLUMN

Hydrocarbon	Relative retention volume at 70° C	Relative retention volume at 90° C
Isopentane	0·022	
n-Pentane	0·029	
n-Hexane	0·071	
*Cyclo*hexane	0·133	
n-Heptane	0·160	
Methyl*cyclo*hexane	0·217	
Benzene	0·244	0·273
n-Octane	0·366	
Toluene	0·506	0·528
n-Nonane	0·843	
Ethylbenzene	1·00	1·00
p-Xylene	1·08	1·07
m-Xylene	1·22	1·12
o-Xylene	1·41	1·38
Isopropylbenzene	1·52	1·46
Styrene	1·93	1·82
n-Propylbenzene	2·08	1·93
1-Methyl-4-ethylbenzene		1·96
1-Methyl-3-ethylbenzene		2·26
1-Methyl-2-ethylbenzene		2·43
1,3,5-Trimethylbenzene		2·67
1,2,4-Trimethylbenzene		2·95
1,2,3-Trimethylbenzene		3·42

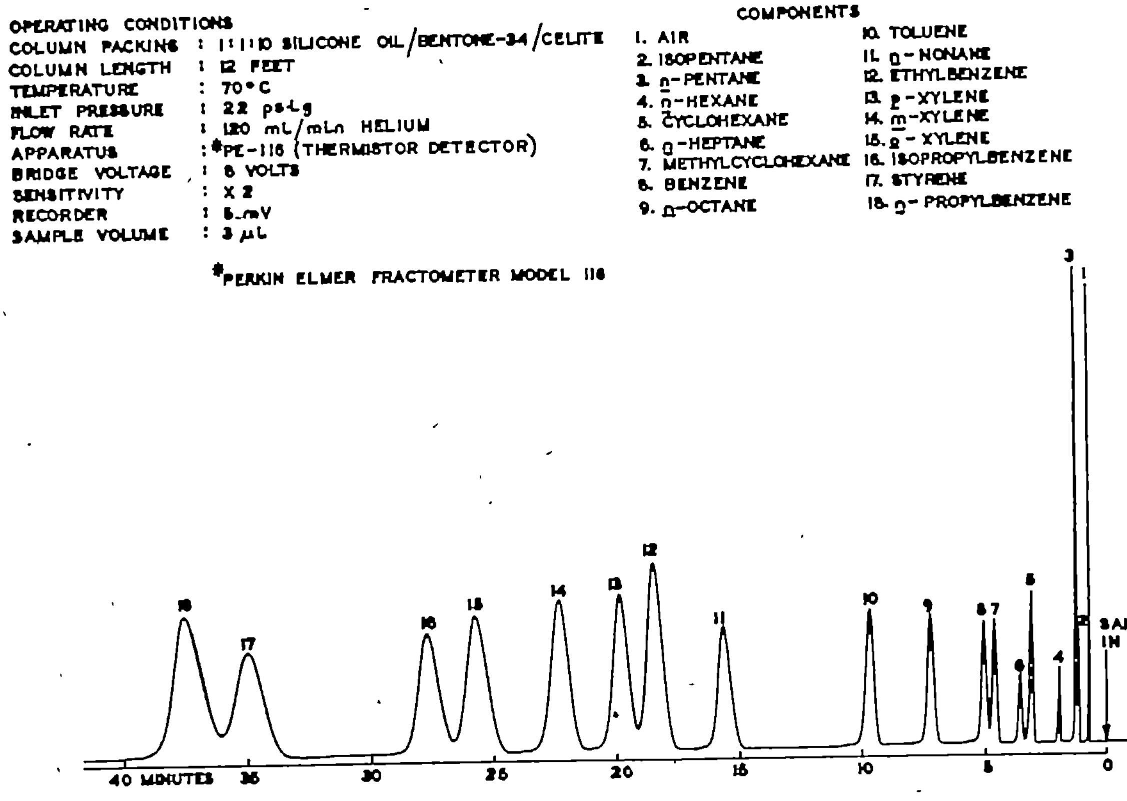

Fig. 1. Separation of hydrocarbons including xylenes

easily expandable clays possessing exchangeable interlamellar cations should be equally applicable.

It is clear that the use of modified organo-clays as gas chromatographic stationary phases may have wide possibilities. Their application is certainly not limited to the separation of the C_6–C_8 aromatics described here, and many naphthenic and substituted hydrocarbon mixtures for which separation by conventional gas liquid partition chromatography has involved extended retention times may be more rapidly resolved on columns containing modified organo-clay packings.

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Thin Layer Chromatography of the Gibberellins

PAPER chromatography of the gibberellins has been investigated by many investigators but no comprehensive study of all the known gibberellins has been reported. MacMillan, Seaton and Suter[1] described four solvent systems which separate gibberellins A_5, A_6, A_8 and A_9 but not the pairs gibberellin A_1 and gibberellic acid (A_3) or gibberellins A_4 and A_7. The solvent systems used by Takahashi *et al.*[2] and Phinney *et al.*[3] do not separate gibberellins A_1, A_2 and A_3. Gibberellins A_1 and A_3 can be separated on paper which is developed by overflow elution with benzene/acetic acid/water (4 : 1 : 2); this method is lengthy when conducted under gravity as described by Bird and Pugh[4] and resolution is poor when development is hastened by centrifugal force[5].

Thin layer chromatography of gibberellins A_1 and A_3 has been described by Kutacek, Rosmus and Deyl[5]; good resolution was obtained on a layer of aluminium oxide, developed by overflow elution with benzene/acetic acid (100 : 23).

We have found that the nine known gibberellins can be separated by thin layer chromatography (Table 1).

Table 1. R_F VALUES OF THE GIBBERELLINS

Gibberellin	Silica gel Solvent system 1	Silica gel Solvent system 2	Kieselguhr Solvent system 3	Kieselguhr Solvent system 2
A_1	0·11	0·0	0·54	0·26
A_2	0·04	0·0	0·64	0·30
A_3	0·11	0·0	0·42	0·18
A_4	0·37	0·82	1·0	1·0
A_5	0·31	0·85	1·0	0·88
A_6	0·25	0·21	0·95	0·76
A_7	0·37	0·70	1·0	1·0
A_8	0·04	0·0	0·28	0·06
A_9	0·75	1·0	1·0	1·0

For solvent systems, see legend to Table 2.

Thin layers of silica-gel *G* or kieselguhr *G* were prepared[6] on glass plates; they were spotted with not more than 10 μg gibberellic acid and the solvent was run for 15 cm at room temperature (17°–21° C). Running times for solvent system 1 were 25–50 min and for solvent systems 2 and 3 were 45–70 min. With solvent systems 2 and 3 plates were equilibrated overnight with lower phase then developed with upper phase.

Two sprays were used: (*a*) ethanol/concentrated sulphuric acid (95 : 5); (*b*) water/concentrated sulphuric acid (30 : 70).

After spraying with (*a*) the plates are heated at 120° for 10 min. The gibberellins are then visible in ultra-violet light. Gibberellins A_1, A_3, A_5, A_6 and A_8, which possess a 7-hydroxyl group, give blue fluorescent spots of different intensities; gibberellins A_2, A_4, A_7 and A_9, which have no 7-hydroxyl group, give purple spots.

With spray (*b*) gibberellins A_3 and A_7 are visible in ultra-violet light as yellow-green fluorescent spots before heating. After heating at 120° for 10 min, all are visible in ultra-violet light. Gibberellins A_1, A_5, A_6 and A_8 give green-blue fluorescent spots; gibberellins A_2, A_4 and A_9 give blue-purple fluorescent spots; gibberellins A_3 and A_7 are exceptions and give light yellow and brown fluorescent spots respectively.

Both sprays show similar sensitivity ranging from 0·00025 μg gibberellin A_3 to 0·01 μg gibberellin A_8. In mixtures 0·5 per cent gibberellin A_1 can be detected in 1 μg gibberellin A_3 and 0·05 per cent gibberellin A_1 in 10 μg gibberellin A_3. Similar quantities of gibberellin A_4 can be detected in gibberellin A_7.

Methyl esters (Table 2) give the same coloured spots as the corresponding acids but with weaker fluorescence.

Table 2. R_F VALUES OF METHYL ESTERS OF GIBBERELLINS

Methyl ester	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
Solvent system 2	0·33	0·34	0·26	1·0	1·0	1·0	1·0	0·1	1·0
Solvent system 1 (2% for 5% acetic acid)	0·18	0·08	0·16	0·50	0·38	0·31	0·48	0·08	0·60

Solvent systems: (1) Di-isopropyl ether/acetic acid (95 : 5); (2) Benzene/acetic acid/water (8 : 3 : 5); (3) Benzene/propionic acid/water (8 : 3 : 5).

No one solvent system separates all the gibberellins or their methyl esters. Gibberellin A_5, A_6 and A_9 are separated from each other and from the unresolved pairs gibberellins A_2 and A_8, A_1 and A_3, and A_4 and A_7.

Gibberellins A_4 and A_7 are separated on silica-gel *G* by solvent system 2. The pairs, gibberellins A_2 and A_8 and gibberellins A_1 and A_3, are separated on kieselguhr *G* by solvent system 2; all four (A_1, A_2, A_3 and A_8) are separated on kieselguhr *G* by solvent system 3; but this system is unreliable since streaking of the plates frequently occurs for reasons unknown.

Gibberellins A_3 and A_7 can be further distinguished from the other gibberellins by means of spray (*b*); they give characteristic yellow-green fluorescent spots without heating while the other gibberellins fluoresce only after heating for 10 min at 120°.

Indolyl-3-acetic acid has an R_F value of 0·60 on silica gel in solvent system 1 and so can be separated from the gibberellins.

All methyl esters except those of gibberellins A_4 and A_7 can be resolved on silica-gel *G* using solvent systems 1 and 2.

Advantages of these methods are convenience, speed and sensitivity. The sulphuric acid sprays, which cannot be applied to paper chromatograms, are to be preferred to the perchloric acid spray[5] and are more sensitive and specific than the 0·5 per cent aqueous potassium permanganate spray[1]. The latter reagent does not detect gibberellin A_9 or less than 5 μg of the other gibberellins.

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CERAMICS

Deformation of Quartz Crystals at the Transformation Temperature

The recent demonstration by Gorum, Parker and Pask[1] that magnesium oxide crystals could be bent plastically at room temperature has stimulated considerable interest in ductile ceramics. The plastic behaviour of alumina crystals at elevated temperatures as shown by Wachtman and Maxwell[2] and Kronberg[3] also directed attention to the fundamental behaviour of refractory oxides.

No data concerning deformation of quartz appear to have been published. This communication describes attempts to deform this material at the α ⇌ β transformation temperatures (571° and 573° C).

The specimen was cycled between two temperatures, 565° and 585° C, as the exact transformation temperatures could not be maintained for a period of time.

Rectangular bars (approximately 2·5 cm × 0·180 cm × 0·17 cm) of single-crystal quartz of random orientation were loaded in a three-point bending device, the details of which will be published elsewhere[4]. Each specimen, subjected to a maximum fibre stress of 5,000 lb./in.^{2} (occasionally 7,000 lb./in.^{2}), was cycled several times between 565° and 585° C with a very slow rate of heating and cooling. A typical curve is shown in Fig. 1 for one cycle of heating and cooling. The temperature at the maximum point of deflexion was found to be 573° C on the heating cycle and 571° C on the cooling cycle for all specimens. The specimen had apparently deformed. Assuming the complete reversibility of the transformation process, this may be considered as a true plastic deformation. The appearance of the samples under a polarizing microscope was the same before and after testing. The total permanent deformation was calculated from the difference of the original and final reading at 565° C. Similar calculation considering 585° C as the line of reference was not so successful, due to the drift on the line at 585° C (Fig. 2). This drift probably was produced by the temperature gradient existing across the furnace wall to the ceramic thrust column and was more pronounced in the heating cycle. However, calculation, if made on the basis of the dotted line (Fig. 2, drawn from the points at which drifting started) as the true base line at 585° C, produced results which are in good agreement with those obtained from 565° C as the line of reference.

In addition to the direct measurement of the deformation, the data were analysed as follows. The maximum deflexion (as in Figs. 1 and 2) is a cumulative effect of the change of elastic modulus with temperature (which exhibits a minimum value at the transformation temperature) and the change of volume accompanying the transformation and permanent deformation. After approximately the fourth cycle of heating and cooling, no further permanent deformation was observed and the total deflexion was found to be constant for the heating as well as for the cooling cycles. The deflexion produced by the change of elastic modulus was reduced during heating due to volume expansion. It was increased by the same magnitude during cooling due to volume shrinkage. Knowing the values of deflexion at the heating and cooling cycles, the deflexions due to the change of modulus of elasticity and change of volume were calculated. Then, subtracting from the total deflexion those constant contributions produced by the elastic modulus and volume change, values for the permanent deformation in first, second and third cycle were obtained.

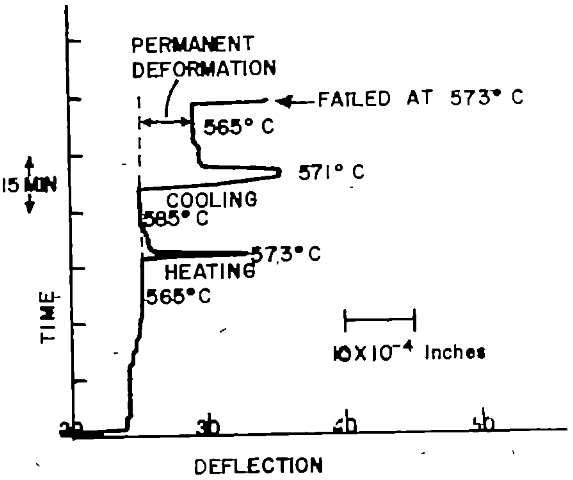

Fig. 1. Temperature cycling-deflexion curve of quartz under a maximum fibre stress of 5,000 lb./in.^{2}

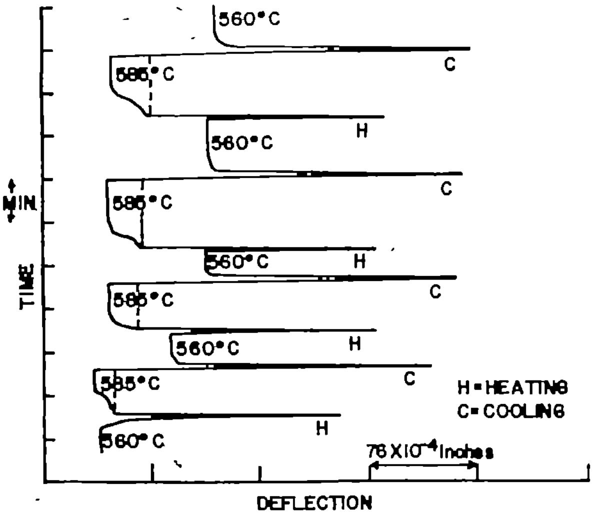

Fig. 2. Effect of temperature cycling on the permanent strain of quartz under a maximum fibre stress of 5,000 lb./in.^{2}

The modulus of elasticity and the per cent linear expansion or contraction were also calculated for different specimens. Typical values are shown in Table 1.

The values for the modulus of elasticity are in quite good agreement with those obtained by other workers[5].

The residual strain was found to vary from 0·055 to 0·030 per cent for different specimens on the first cycle. This variation is possibly due to the different orientation of crystals in respect to the direction of the applied stress. A somewhat higher permanent strain was obtained (as much as 0·07 per cent) with a maximum fibre stress of 7,000 lb./in.^{2}. However, frequent failure of the specimen under this load at the transformation temperature restricted further investigation.

Fig. 2 shows the effect of successive cycling of heating and cooling on the permanent deformation. The residual strain was very similar for all the specimens tested for the second and third cycles, being 0·013 and 0·004 per cent respectively. Permanent deformation of 0·002 per cent could be calculated in the fourth cycle in some cases. However, in most cases no noticeable deformation could be observed in the fourth or succeeding cycles.

Table 1

Specimen No.	Total deflexion (in.) Heating (average of three cycles)	Total deflexion (in.) Cooling (average of three cycles)	Deflexion due to change of elastic modulus (in.)	Deflexion due to volume change (in.)	Linear change (%)	Modulus of elasticity (lb./in.^{2}) 565° C.	Modulus of elasticity (lb./in.^{2}) 573° C
1	$13·6 \times 10^{-4}$	$26·2 \times 10^{-4}$	$20·0 \times 10^{-4}$	$6·2 \times 10^{-4}$	1·0	$8·0 \times 10^{6}$	$4·2 \times 10^{6}$
2	$14·8 \times 10^{-4}$	$25·8 \times 10^{-4}$	$20·3 \times 10^{-4}$	$5·5 \times 10^{-4}$	0·83	$10·2 \times 10^{6}$	$3·7 \times 10^{6}$

This effect was further studied by successively cycling a specimen (6 times) with an applied maximum fibre stress of 5,000 lb./in.² to obtain complete hardening. Afterwards, another series of temperature cycling was carried out with the same specimen at 7,000 lb./in.². A permanent strain of 0·035 per cent was obtained on the first cycle at 7,000 lb./in.² for the hardened specimen. However, with continued cycling at 7,000 lb./in.² the specimen again lost its ductility.

Annealing of the strained specimen for 15 h at 800° C completely restored the original ductility. However, no recovery of its original ductility was observed with a similar annealing treatment of the strained specimen below the transformation temperature.

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BIOCHEMISTRY

Succinic Dehydrogenase Activity and Oxidative Phosphorylation of the Mitochondria in the Crystalline Lens of Bovines

It is well established that only a small amount of the glucose consumed by the eye lens supplies carbon dioxide. The part played by the Krebs cycle in this oxidization and even the presence of the enzyme of this cycle have given rise to much controversy. According to Kinsey[1] oxidative degradation occurs almost exclusively in the epithelium. However, the report of succinic dehydrogenase activity in eye lens homogenates[2] and the inhibition of energy production in the fibres by potassium cyanide[3] suggests the possible presence of an oxidative process outside the epithelium.

In the course of an earlier investigation[4] we established the presence of mitochondria in the various zones of the lens and attempted the study of their metabolic activity. This communication attempts to define the succinic dehydrogenase activity of the mitochondria in the various zones of the lens and particularly the aptitude to oxidative phosphorylation of the mitochondria in the anterior (*E*) and cortical equatorial (*CE*) zones.

Our experiments have been carried out on lenses of calves, taken from the animals immediately after death and placed into a container at 0°. Various zones of the lens were separated by dissection at 0°: an anterior zone comprising the capsule, the epithelium and some fibres (*E*), an equatorial cortical zone (*CE*), an anterior cortical zone (*CA*) and a posterior cortical zone (*CP*); the central part which remained frozen during the manipulation was then rejected. The various zones were homogenized for 5 min, after the addition of nine volumes of 0·3 M sucrose containing 10^{-4} M of ethylenediamine tetraacetic acid. Subcellular fractionation was carried out by differential centrifugation according to the technique of Hogeboom, Schneider and Pallade[5], as modified by Aldridge and Johnson[6]. The combined mitochondrial fractions were washed in 0·3 M sucrose and 10^{-4} M ethylenediamine tetraacetic acid.

In a previous investigation we established by electron microscopy the presence of mitochondria in the pellets thus isolated; we found that these mitochondria were very few in number, more or less swollen, but recognizable by their peripheral cristae.

An incubation mixture almost similar to Aldridge's[7] (see legend, Table 3) was adopted, but we had to add cytochrome *c*. The choice of the incubation mixture allowed us to eliminate the inhibition of succinic dehydrogenase by endogenous oxaloacetate or by that produced by the degradation of sodium succinate[8]. The quantity of oxygen consumed by the mitochondrial fractions was determined according to the classic manometric method of Warburg. Incubations were performed for 1 h at 37° and in the presence of air[9]. Finally we evaluated the quantity of adenosine triphosphate (ATP) produced, by measuring the amount of mineral phosphorus which had disappeared from the incubation mixture[10]. The technique for evaluating the quantity of ATP by enzymatic dosage of glucose-6-phosphate[11] could not be applied because of the glucose-6-phosphate dehydrogenase activity of the lens[12,13]. For phosphorus assay we used the technique of Martin and Doty[14].

Our results are presented in Tables 1, 2 and 3.

Table 1. ACTION OF CYTOCHROME *c* AND INHIBITORS ON SUCCINIC DEHYDROGENASE ACTIVITY OF CALF LENS MITOCHONDRIA

Incubation mixture	Q_{O_2} μl /g wet weight
Sodium succinate 5×10^{-2} M + cytochrome *c* 1×10^{-4} M	3·46
Sodium succinate 5×10^{-2} M without cytochrome *c*	0
Sodium succinate 5×10^{-2} M + cytochrome *c* 1×10^{-4} M + Na malonate 5×10^{-2} M	0
Sodium succinate 5×10^{-2} M + cytochrome *c* 1×10^{-4} M + potassium cyanide 1×10^{-3} M	0

Incubation mixture, see Table 3.

Table 2. SUCCINIC DEHYDROGENASE ACTIVITY OF VARIOUS ZONES OF CALF LENS

Zones	*E*	*CE*	*CA*	*CP*
No. Exp.	3	5	2	4
Q_{O_2} μl./g wet weight	14·51	3·46	2·41	2·04

E, epithelial zone; *CE*, equatorial fibres; *CA*, anterior fibres; *CP*, posterior fibres. Incubation mixture, See Table 3.

Table 3. OXYGEN CONSUMPTION, ATP PRODUCTION AND P/O RATIO OF ISOLATED MITOCHONDRIA FROM EPITHELIAL AND CORTICAL ZONES OF BOVINE LENSES

Exp.	μm ATP produced (μatom g P)	μatom g O	P/O
1	3·00	2·67	1·12
2	3·55	2·75	1·29
3	2·18	1·70	1·28
4	3·55	2·85	1·25

ADP, 0·0025 M; ATP, 0·0006 M; KCl, 0·044 M; $MgCl_2$, 0·006 M; PO_4H_2Na and PO_4HNa_2, 0·0008 M; cytochrome *c*, 0·0004 M; KF, 0·025 M; glucose, 0·06 M; sodium succinate, 0·03 M, in main vessel, volume 2·3 ml, Hexokinase 200 u., 0·3 ml. in side-arm and 0·2 ml. NaOH 10 per cent in the centre well.

A survey of Tables 1 and 2 shows that the addition of cytochrome *c* is necessary to demonstrate the succinic dehydrogenase activity of the mitochondria *in vitro*. This, undoubtedly, is a consequence of the very small quantity of cytochrome *c* in the lens[15], a fraction of which has probably been lost during preparatory work; cytochrome thus appears as a limiting factor in the evaluation of respiration of the lens.

It is noticeable, moreover, that classic inhibitors such as that of the succinic dehydrogenase, sodium malonate, and that of electron carriers, potassium cyanide, suppress this respiration. But, and this is an essential fact, the succinic dehydrogenase activity actually exists in all the zones studied: in the epithelium where it is clearly highest as well as in equatorial, anterior or posterior fibres.

The parallel analysis of oxygen consumption and ATP production gives an average value of 1·2 for P/O. Now, the theoretical P/O ratio admitted for the succinic fumarate stage is two[16]. This figure, however, represents the maximum yield possible in optima conditions of coupling of the phosphorylations and oxidization. Experimental figures show the average yield of a preparation for a given tissue; they are generally inferior to 2 and oscillate between values approximating 1, obtained for sarcosomes, and 1·7, given by the same authors for the mitochondria in the liver[17].

We think that our comparatively small values can be explained by damage done to the mitochondria the separation of which from the lens is relatively long and difficult because in each experiment the zones of 120 crystalline lenses were used and separated. Thus taking into account these preparatory conditions which favour the uncoupling of phosphorylations and oxidations, we may admit that the P/O values *in vivo* are superior to those found *in vitro*.

In any event, our experiments indicate the actual existence of a reaction chain of the Krebs cycle in the lens fibres; it is more active in the epithelium, but is not negligible in the other zones of the lens; our P/O value can be considered as satisfactory considering the damages done to mitochondria.

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Axial Period of Fibrinogen and Fibrin

X-RAY diffraction was first applied to the analysis of blood clotting by Bailey, Astbury and Rudall[1]. The apparent persistence of the α-type wide-angle X-ray diagram when fibrinogen is converted to fibrin led these investigators to conclude that "fibrinogen is no other than an insoluble modification of fibrin without any fundamental change in molecular plan".

More recent investigations have given new insight into the structural basis of the α-diagram. This pattern may be interpreted as due to a coiled-coil conformation of α-helices[2,3]. In general, however, the wide-angle X-ray diffraction pattern of a polymer gives information about the chain configuration of ordered regions only. An estimate of α-helix content may be made by optical rotatory dispersion measurements: on this basis, fibrinogen has a helix content of about 30 per cent[4]. Thus, the coiled-coil region characterizes only a small part of the total molecular structure, so that conclusions on the clotting mechanism based solely on the wide-angle diagram are not rigorous.

Support for the picture of fibrinogen as a more complex molecule comes from the electron microscope observations by Hall and Slayter[5,6]. These investigators have proposed a model for the molecule consisting of three globular units about 60 Å in diameter, separated by a thin strand. This picture is consistent with a molecular weight of about 300,000[7]. The average length of dried fibrinogen monomer varies with pH from about 270 to 400 Å when measured in electron micrographs[8]. Light scattering measurements[8] at pH 6·2 and 8·2 give a radius of gyration for fibrinogen monomer of 150 Å. Assuming the general model proposed by Hall and Slayter this would correspond to an overall length of about 400 Å upwards, depending on the exact relative sizes of the three globular units, and the rigidity of the interconnecting strand. It has been postulated[9] that the thin rod seen in the electron microscope may have the coiled-coil conformation.

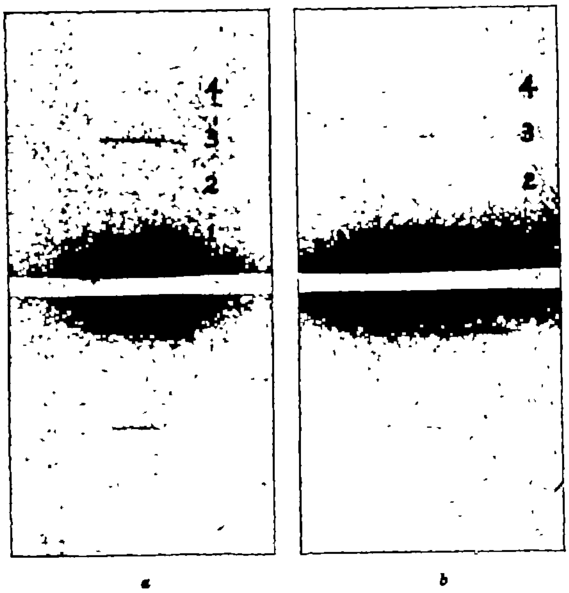

Fig. 1. *a*, Fibrinogen gel; *b*, fibrin clot. The specimen to film distances and magnifications are identical in these two photographs. The defining slit was longer in Fig. 1*b* than in Fig. 1*a*

In order to obtain further information on the structural basis of the clotting mechanism, low-angle X-ray diffraction investigations were begun. This technique reveals significant large-scale structural features; it may be carried out on hydrated 'native' specimens; and it affords the possibility of a quantitative determination of electron density. Here we report our preliminary observations.

Bovine fibrinogen ('Pentex Fraction I') was formed into gels by high-speed centrifugation of a 1–2 per cent purified solution in 0·3 M potassium chloride at pH 8–9. A portion of the birefringent pellet was aspirated into a thin-walled 1 mm diameter quartz capillary; second-order interference colours and good extinction were often observed. Fibrin was formed by the addition of bovine thrombin (Parke-Davis) to a fibrinogen solution in 0·3 M potassium chloride at pH 5–7. The clot was packed by centrifugation and a portion of the birefringent pellet inserted into a capillary. Most of the X-ray diagrams were obtained with nickel-filtered copper $K\alpha$-radiation and a low-angle camera with slit collimation, giving very high resolution along the meridian. Pictures were also taken using a bent quartz crystal focusing monochromator which gave more uniform overall resolution.

The low-angle diagrams of fibrinogen and fibrin gels both show four orders of a 226 ± 3 Å axial repeat (Fig. 1). There is some variation of the intensity distribution among different specimens, but the trend is similar in the two materials (Table 1). Pinhole collimation pictures show that the four layer lines have greatest intensity on the meridian. The patterns are characterized by very sharp layer lines indicating a considerable degree of crystallinity along the fibre axis; and very little arcing of the layer lines, indicating good orientation of the crystallites with respect to the fibre axis. The equator has no discrete low-angle reflexions.

Fibrin stained with phosphotungstic acid also shows a 230 Å period, and a characteristic striation in the electron microscope[10,11]. This period has been interpreted by some

Table 1. QUALITATIVE ASSIGNMENT OF INTENSITIES IN FIBRINOGEN AND FIBRIN GELS

Order (period 226 Å)	Fibrinogen	Fibrin
1	Strong	Strong to very strong
2	Very weak	Very weak or absent
3	Medium	Medium
4	Weak	Weak

authors[4] as directly representing the length of the fibrinogen monomer. By this hypothesis, an intramolecular contraction or folding must occur on clotting, since the monomer seen under the electron microscope is often longer than 230 Å. Our X-ray diffraction results make it appear more likely that the fibrin period represents some staggered arrangement of the fibrinogen monomer. For example, if the length of the fibrinogen monomer were 460 Å, a half-staggered arrangement would give the observed 230 Å period for fibrin.

The identity of the axial period of the fibrinogen gels and fibrin clots, and the similar trend of thoi diffracted intensities, strongly suggest that there is no major internal rearrangement of the monomer in the clotting process. This finding thus provides a more rigorous foundation for the original interpretation given by Bailey *et al.*[1].

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Replacement of Thymidylic Acid by Deoxyuridylic Acid in the Deoxyribonucleic Acid of a Transducing Phage for *Bacillus subtilis*

THE findings that the thermal denaturation temperature (T_m) of DNA from a wide variety of bacterial, plant and animal sources as well as the buoyant density in a caesium chloride gradient are linearly related to the guanine plus cytosine (G plus C) base composition[1,2] have made it possible to search for the existence of DNA samples which would show an anomalous behaviour with respect to these two parameters. Native DNA preparations which show no correlation between their buoyant density and T_m would be expected to have components other than those usually encountered in DNA[3]; this situation might arise with a high degree of probability in the DNA isolated from bacteriophages or viruses, which because of their specialized behaviour would be expected to possess distinctive structural features.

Bacteriophages *PBS*1 and *PBS*2 have been shown to transduce several genetic markers among strains of *Bacillus subtilis*[4,5]. The DNA's from these phages were very similar in their chemical composition and physical properties. Consequently only the data obtained with *PBS*2 will be presented in this communication. When native DNA from this phage was examined, it was found that its T_m in 0·15 M sodium chloride plus 0·015 M sodium citrate was 76·5° (corresponding to 17·5 per cent G plus C) and its buoyant density was 1·722 g/c.c. (corresponding to 62 per cent G plus C). This discrepancy prompted a chemical analysis of the nucleic acid constituents.

Purified *PBS*2 DNA was hydrolysed in 12 N perchloric acid for 60 min at 100° and liberated bases were separated by paper chromatography. Four spots were obtained by two-dimensional chromatography with solvents *A* and *B* and also with solvents *B* and *C* (Table 1). Three spots were identified as adenine, guanine and cytosine. Thymine was absent from the chromatograms. The designation of the fourth base as uracil was suggested by chromatographic and also spectrophotometric comparison with other pyrimidines (Tables 1 and 2). Further, this identification was supported by the fact that a uracil requiring *B. subtilis* strain (*A* 26) could grow on a minimal medium containing the compound eluted from the fourth spot but not on thymine.

Table 1. PAPER CHROMATOGRAPHY OF PYRIMIDINES*

Pyrimidine	R_F value Solvent *A*	Solvent *B*	Solvent *C*
Uracil †	0·69	0·30	0·81
Uracil ‡	0·69	0·29	0·80
Thymine ‡	0·81	0·45	0·80
Cytosine ‡	0·49	0·17	0·80
HMU ‡	0·63	0·18	0·60

* Descending, Whatman No. 1 paper was used.
† Obtained from purified *PBS*2 DNA by perchloric acid hydrolysis.
‡ Reference compounds.
HMU, 5-Hydroxymethyluracil.
Solvent *A*, Isopropanol–hydrochloric acid–water (170 : 41 : 39).
Solvent *B*, *n*-Butanol saturated with water–ammonium hydroxide (100 : 1).
Solvent *C*, Water adjusted to *p*H 10 with 1 N ammonium hydroxide.

Table 2. ULTRA-VIOLET ABSORPTION DATA OF PYRIMIDINES AND THEIR DEOXYRIBONUCLEOTIDES*

Free base

	Acid				Alkali			
Pyrimidine	Max. (mμ)	Min. (mμ)	280 mμ/260 mμ	250 mμ/260 mμ	Max. (mμ)	Min. (mμ)	280 mμ/260 mμ	250 mμ/260 mμ
Uracil †	258	227	0·17	0 85	283	240	1·46	0·64
Uracil ‡	259	227	0·17	0·84	284	241	1·40	0·71
Thymine ‡	264	233	0·53	0·67	291	244	1·31	0·55
Cytosine ‡	274	238	1·53	0·48	267	247	0·80	0 76
HMU ‡	261	231	0·37	0·75	284	248	—	—

Deoxyribonucleotide (5′)

	Acid				Alkali			
Pyrimidine	Max. (mμ)	Min. (mμ)	280 mμ/260 mμ	250 mμ/260 mμ	Max. (mμ)	Min. (mμ)	280 mμ/260 mμ	250 mμ/260 mμ
Uracil †	262	231	0·38	0·77	262	244	0·34	0·84
Uracil ‡	262	232	0·39	0·74	263	243	0·37	0·65
Thymine ‡	267	—	0 72	0 64	—	—	0·67	0·74
Cytosine ‡	280	239	2·12	0·46	—	—	0·99	0·82
HMU ‡	264	234	0·57	0·70	264	246	—	—

* The data for the reference compounds were taken from the literature; the data for deoxyuridylate (Sigma) were obtained in our laboratory.
† Obtained from purified *PBS*2 DNA either by perchloric acid hydrolysis or by snake venom phosphodiesterase hydrolysis.
‡ Reference compounds.

Enzymatic hydrolysis of thermally denatured *PBS*2 DNA with snake venom phosphodiesterase (Worthington) was carried out and the mononucleotides were separated by chromatography on 'Dowex-1' (formate form) and elution carried out with formate and bicarbonate[6]. The uracil-containing nucleotide was eluted from the column with 0·2 M ammonium bicarbonate (which normally elutes thymidylate). The spectrophotometric data of this nucleotide together with those of other pyrimidine deoxynucleotides are summarized in Table 2. The ultra-violet absorption spectrum of deoxyuridylate is indistinguishable from that of uridylate and additional tests were carried out to identify the sugar component. The nucleotide was treated with prostatic phosphomonoesterase. The uracil-containing nucleoside[7] thus obtained migrated with authentic deoxyuridine but not with the faster moving uridine in paper electrophoresis with 0·1 M borate buffer at *p*H 9·2. Furthermore, the sugar moiety of the nucleoside was identified as deoxyribose by the method described by Waravdekar and Saslaw[8] after an incubation with deoxynucleoside transglycosidase and adenine[7]. Thus the possibility that the uracil spot found in the paper chromatograms of perchloric acid hydrolysed *PBS*2 DNA might be a degradation product could be eliminated.

In addition to the replacement of thymine by uracil, the DNA from *PBS*2 has another unusual feature. The mole per cent base composition of *PBS*2 DNA determined by paper chromatography (average of 4 independent experiments) was adenine, 35·9; uracil, 35·9; guanine, 13·4; cytosine, 14·7 (G plus C : 28 per cent). This base composition differs significantly from that of the host DNA (G plus C : 43 per cent). The DNA's of known transducing phages such as *P*22 and λ have been shown to possess a base composition similar to that of bacterial host DNA[8,9]. This suggests that the mechanism by which *PBS*1 and *PBS*2 transfer the bacterial genome might be somewhat different from that in other transducing systems. Phages *PBS*1 and *PBS*2, therefore, may offer a unique situation not only for studying the biosynthesis of phage DNA but also the genetics and the DNA base sequence homology of host–virus relationships.

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Changes in the Ribonucleoproteins of the Nucleolus following Inhibition of Synthesis of Deoxyribonucleic Acid

BY means of an improved toluidine blue–molybdate staining procedure[1] two types of ribonucleoprotein (RNP) can be very clearly demonstrated in the nucleolus. One type is the pars amorpha and the other consists of minute granules or hollow spheres and appears to correspond to the nucleolini of classical cytologists[2]. Enlargement of the nucleolini has been observed in cells infected with viruses and in cells in which the onset of mitosis is blocked by large doses of colchicine[3]. The relationship between the changes in the nucleolini and nucleic acid metabolism is not clear in these systems. An examination of the nucleolar RNP was therefore undertaken in cells treated with 5-fluorodeoxyuridine (FUDR) since the metabolic changes produced by this antimetabolite have been extensively studied. FUDR inhibits synthesis of deoxyribonucleic acid (DNA) by blocking the formation of thymidylic acid[4,5]. When cells in tissue culture are treated with FUDR, DNA synthesis is prevented, cell division is arrested and the metabolism of ribonucleic acid (RNA) is disturbed[6,7]. During one generation time of HeLa cells, the DNA per cell remains constant, the cytoplasmic RNP doubles and the nuclear RNP is unchanged. Salzman and Sebring provide evidence that the effect of FUDR on RNA metabolism of HeLa cells is mediated through its effect on DNA synthesis since the process is reversed by thymidine, but not by uridine[7].

In the present work, a clone of *S*3 HeLa cells (kindly provided by Dr. N. P. Salzman) was grown in ring cultures[8] in Eagle's minimal essential medium containing 1 per cent glutamine and 8 per cent horse serum. Cell growth was inhibited by the addition of 10^{-6} M FUDR, which was changed daily (Fig. 1). The inhibitory effect was reversed by removal of the analogue and the addition of equimolar thymidine (Fig. 1). Examination of preparations stained to demonstrate the nucleolar RNP[1] indicated that the changes in the cells were not synchronous. FUDR inhibited the onset of prophase and exerted a *C*-mitotic effect on dividing cells. The earliest change in the nucleolus was noted in a few cells 4 h after addition of the inhibitor and consisted of fusion of the nucleolini to form large RNP bodies. Afterwards, the number of cells showing this change increased. During the succeeding 21 h, two processes occurred contemporaneously. The intensity of staining of the nucleolini decreased and many were extruded from the pars amorpha into the nucleoplasm until most of the nucleoli contained few or no nucleolini. Fragments of RNP staining like the nucleolini were seen in the nucleoplasm and, occasionally, even in the cytoplasm of some cells. In many cells the nucleoplasm appeared empty. 3–6 h after addition of thymidine numerous prominent nucleolini were reformed in the pars amorpha. Shortly afterwards, mitotic division was restored to normal (Fig. 1). In the cultures which remained exposed to the inhibitor mitosis was completely blocked and the nucleolini progressively disappeared, leaving only the pars amorpha surrounded by an empty nucleoplasm in which occasional RNP bodies remained.

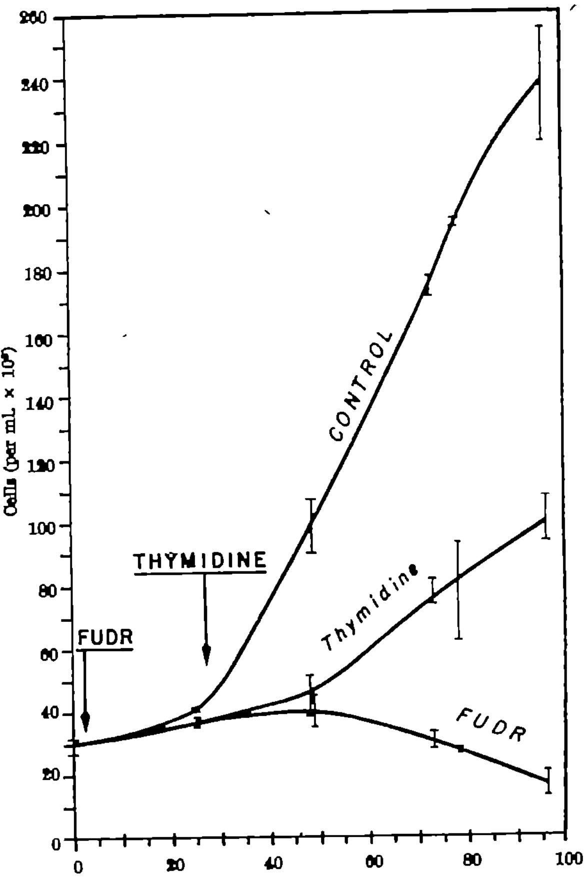

Fig. 1. Effect of 10^{-6} M 5-fluorodeoxyuridine on growth of HeLa cells and reversal by thymidine. The medium was changed daily on the control cultures. 10^{-6} M 5-fluorodeoxyuridine was applied to the experimental groups for the first 25 h. At this time, and daily thereafter, the medium was replaced by medium containing 10^{-6} M fluorodeoxyuridine or thymidine. The range of two replicate counts is indicated by the vertical bars

The mechanism of action of FUDR on RNP metabolism is indicated by reversal studies. The inhibitory effect of FUDR on cell growth and on the formation of RNP of the nucleolini was prevented by equimolar thymidine, but not by a 100-fold greater concentration of uridine in the FUDR solution. Since exogenous thymidine is incorporated solely into DNA thymine in HeLa cells[8], the reversal of the effect of FUDR on the RNP by thymidine appears to be secondary to reversal of the inhibition of DNA synthesis. Some FUDR is converted to fluorouracil; this in turn forms fluorouridylic acid which is incorporated into RNA[4,5,7]. If the inhibition of RNA synthesis were due to a block in the synthesis of uridylic acid by fluorouracil, it should have been reversed by exogenous uridine, which is a precursor of RNA uracil[9] and which reverses the effect of fluorouracil on *Candida albicans*[10]. Thus, the formation of RNP in the nucleolini appears to be dependent on DNA synthesis.

In normal cells, the nucleolini were small and numerous and occasional small granules of similarly staining RNP were present in the nucleoplasm. When DNA synthesis and mitosis were blocked by FUDR, the nucleolini and the granular RNP of the nucleoplasm were enlarged and occasional granules of similar RNP were seen in the cytoplasm. Afterwards, this RNP disappeared, first from the nucleolus and then from the nucleoplasm and cytoplasm. This would suggest the RNP of the nucleolini was passing from the nucleolus to the cytoplasm, probably in the same way as it does in the normal cell. When mitosis is blocked in cells infected with viruses or by treatment with colchicine or FUDR, the RNP may not be utilized normally or may move more slowly and accumulate in larger masses than in the normal cell. In the absence of further synthesis of RNP of the nucleolini type, this RNP disappears first from its site of formation, the nucleolus, and then from the nucleoplasm. The dependence of the synthesis of the RNP of the nucleolini upon DNA synthesis would suggest that its RNA may be the 'nucleolar' RNA II fraction which has been shown to have a high rate of synthesis and a base composition resembling DNA[11]. Further experiments are in progress to determine whether the RNA of the nucleolini may be the 'messenger' RNA which has been described by Sirlin *et al.* in the nucleolus[12].

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A Possible Role for Minor Bases in Ribonucleic Acid

MOST discussions on the nature of the code between RNA and protein have given consideration only to the four major bases and have ignored the existence of the minor bases known to occur in RNA. Bergquist and Matthews[1] have suggested on the basis of analytical data on RNA fractions from various tissues that all the methylated purines occur in soluble RNA and that the small proportion of these bases found in other fractions, for example ribosomal RNA, may be due to transfer RNA functionally associated with these particles at the time of extraction. We also calculated that there may be roughly 1–2 methylated purine residues in each molecule of *p*H 5·2 precipitate RNA, but at that time we could see no special function for such bases. However, two recent papers[2,3] on RNA make it possible to suggest such a function.

First, Spencer *et al.*[2] have described a structure for transfer RNA. The bulk of the molecule is made up of a regular double helix with hydrogen-bonded base pairing. Methylated bases would be unlikely to occur in this region of the molecule. They suggested that a coding triplet of nucleotides with bases exposed for hydrogen bonding might occur in the short non-helical region of the chain they describe. Secondly, Woese[3] suggested that only the 2′ and 6′ positions in the four major bases may be involved in coding; and that of the triplet of bases concerned with one amino acid, one base codes according to both the 2′ and 6′ position, one according to the 2′ position only, and one according to the 6′ position only. This scheme accounts nicely for a number of experimental observations.

I now wish to suggest that in the triplet of bases in transfer RNA, which may be at the non-helical end of the molecule, and which might hydrogen bond specifically with the appropriate triplet in the template RNA on the ribosome, two of the bases are methylated purines or one of the minor pyrimidine components. The structural differences in these bases might serve to block the reading of the redundant 2′ or 6′ positions suggested by Woese. If there was a unique minor base in transfer RNA for each of the 2′ or 6′ positions on the template bases that are read singly in Woese's scheme, then 5 different minor bases would be required as follows: three which are incapable of bonding with the 6′ position in the template base but distinguished between $=O$, $-NH_2$ or $-H$ on the 2′ position; and two that are incapable of bonding with the 2′ in the template base but distinguished between $-NH_2$ and $=O$ on the 6′ position. For example, 6-hydroxy-2-dimethyl-aminopurine would be unable to donate hydrogen from the 2′ position, but the $=O$ at position 6′ would be available as a hydrogen acceptor. The following minor bases have been found in soluble RNA from various sources: 6-amino-2-methylpurine[1,4–6], 6-methylaminopurine[1,4–6], 6-dimethyl-aminopurine[1,4], 6-amino-1-methylpurine[6], 2-amino-6-hydroxy-1-methylpurine[1,5,6], 6-hydroxy-2-methylaminopurine[1,5], 6-hydroxy-2-dimethylaminopurine[1,5], pseudouridine[6,7], thymine[4,6] and 5-methylcytosine[4,6].

Available analytical data are inadequate in several ways. All these bases have not been reported from all sources tested, but some may have occurred below the level of detection in the analyses. On the other hand, most analyses have been carried out either on whole soluble RNA or on unfractionated *p*H 5·2 precipitate RNA. The RNA in the supernatant fluid after precipitation of transfer RNA at *p*H 5·2 is richer in many of these bases than is the precipitated RNA[1]. Thus many of the published values may be higher than the actual proportions in transfer RNA. Further, there is the possibility that certain of the bases may arise by rearrangement from another base during hydrolysis of the RNA (for example, 6-methylaminopurine from 6-amino-1-methylpurine during alkaline hydrolysis[10].

Testing of the hypothesis outlined here could be made along two lines. First, more accurate analyses could be made on purified transfer RNA. The hypothesis predicts 2 minor base residues per 80 nucleotides, and if a transfer RNA specific for one amino-acid were analysed then there should be either 2 residues of one base per 80 nucleotides or one residue of each of two different bases, unless some of the bases function interchangeably or have some other function. Base sequence analysis on purified single transfer RNAs, when possible, should show whether two minor bases occur in the expected location near the centre of the chain.

Secondly, model-building, based on the structure of Spencer *et al.*[2] with two minor bases in the supposed coding triplet, and opposed to appropriate triplet sequences of major bases in a model or models of template RNA might reveal whether anything approaching the expected specificity can be obtained. A major base in the centre of the transfer RNA triplet flanked by two minor bases might tend to arrange the proposed four specific hydrogen bonding sites in the most effective pattern to give maximum stability to a molecule 100 Å long held by one end to the template and holding an activated amino-acid in line at the other.

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Comparison of the Caseins of Buffalo's and Cow's Milk

By comparing the major non-casein proteins of the milk of the Indian buffalo (*Bubalus bubalis*) with those of cow's milk, it has been found[1] that the single β-lactoglobulin component produced by the buffalo is very similar in properties to one (*B*) of the two genetically controlled variants (*A* and *B*)[2,3] produced by the cow. Likewise, the single α-lactalbumin of buffalo milk resembles one (*A*) of the two variants (*A* and *B*) of this protein which occur in the milk of African[4] and Indian humped (zebu) cattle[5]. (Non-zebu cows have only one α-lactalbumin which is like the *B*-variant of zebu milk.) Thus there are striking similarities in the corresponding non-casein milk proteins of buffalo and cattle, two species incapable of cross-breeding.

We have now extended the comparison to the casein complex to see whether such similarities might also pertain to the components of the principal milk protein. Milk samples from individual buffaloes, zebus, Jersey cows and some zebu–Jersey F_1 crosses were prepared for, and subjected to, paper electrophoresis in the manner recently described for cow's milk[6]. Examination of the results, of which an example is given in Fig. 1, showed that two of the three major components, β- and κ-casein, were of comparable electrophoretic mobilities. (The apparent slower movement of the Jersey β-casein in Fig. 1 is fortuitous: the particular animal happened to be homozygous for the slower-moving *B*-variant occurring in Jersey milks.) No evidence of β-casein polymorphism was seen with any of the buffalo and zebu milks, but too few samples were examined to exclude the possibility that variants exist. In contrast to the β- and κ-components, the α-caseins showed quite marked differences in their rates of migration, not only between buffalo and cattle but also, more surprisingly, between zebu and non-zebu cows. The differences are larger than those due to the α-casein polymorphism recently observed in the milk of Holstein cows[7], differences which cannot be resolved by the paper-electrophoretic technique as applied here.

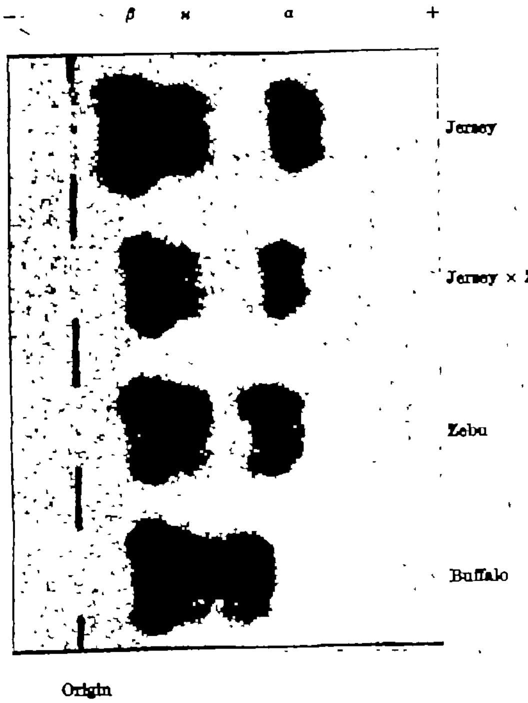

Fig. 1. Bovine caseins. Paper electrophoresis at pH 7·15

This preliminary study has shown that each of the main protein components of cow's milk has its counterpart in buffalo milk. More detailed physico-chemical studies are needed to establish the identity or near-identity of the isolated constituents, a finding which would lend support to the attractive hypothesis of a common origin of the bovine genera under discussion. Such a hypothesis might hold in spite of the non-identity of the α-caseins, since the difference in electrophoretic mobility between zebu and buffalo seems to be no greater than that between zebu and non-zebu cattle.

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Inherited α-Lactalbumin and β-Lactoglobulin Polymorphism in Indian Zebu Cattle. Comparison of Zebu and Buffalo α-Lactalbumins

It has been shown by paper electrophoresis that genetically different types of β-lactoglobulin[1,2] and α-lactalbumin[3,4] occur in cow's milk. While genetic polymorphism of β-lactoglobulin is general among the cattle of different countries, that of α-lactalbumin has only been observed in two breeds of African zebus, the Nigerian White Fulani[3] and the boran[4] cattle of Kenya, but not in Icelandic, British[3], Danish[5] and North American[6] (Holstein) cattle. Blumberg and Tombs[3] noted the Nigerian White Fulani cattle to be similar in appearance to the 'Brahmin' cattle of the East, which were probably brought into Africa by the nomadic Fulani people, and suggested that α-lactalbumin-*A* might serve as a marker by which the affinities of the zebu cattle, and indirectly of the Fulani people associated with them, could be traced. We have now examined the α-lactalbumin and β-lactoglobulin polymorphism in Indian zebu cattle with the view of getting further information on this aspect.

Moreover, from a comparison of electrophoretic patterns of non-casein proteins of African zebu[5] and Indian buffalo milk[7], the mobilities of zebu α-lactalbumin-*A* and of the single buffalo α-lactalbumin appeared to be approximately the same. This indicated that these two proteins might have closely similar physico-chemical properties as has been observed[7–9] in the case of cow β-lactoglobulin-*B* and the single buffalo β-lactoglobulin.

The cows are described according to the nomenclature of Aschaffenburg and Drewry[3,4] as of phenotypes β-lactoglobulin *A*/*A*, *B*/*B* and *A*/*B* and α-lactalbumin *A*/*A*, *B*/*B* and *A*/*B* and the proteins as β-*A*, β-*B* and β-*AB* and α-*A*, α-*B* and α-*AB*.

Samples of milk from 138 zebu cows of different breeds and from 15 crosses between Jersey sires and Hariana dams were examined. Interestingly, the crosses, all belonging to the F_1 generation, were humpless, resembling European cattle. The samples were prepared according to the method of Aschaffenburg and Drewry[3] and tested at *p*H 8·6 by vertical or horizontal strip paper electrophoresis in veronal buffer, $I = 0·05$, on Whatman *3MM* paper applying a voltage of 240 V for 17 h.

The distribution of α-lactalbumin and β-lactoglobulin phenotypes among different breeds is shown in Tables 1*a* and *b* respectively. The agreement between the observed and expected frequencies shows that the Hardy–Weinberg law applies quite well throughout. Except in the case of Hariana, however, the results may not be very significant owing to the small number of samples available for testing. Nevertheless, it is seen that the gene frequency for α-*A* (Table 1*a*) is the lowest in the crosses and that for β-*A* (Table 1*b*) the highest. Since the α-lactalbumin *A*-gene is not found in British breeds[5], a relatively lower gene frequency for α-*A* is expected in the Hariana–Jersey crosses than in the total Hariana or zebu populations. In the case of β-lactoglobulin-*A*, the gene frequency in British breeds has been shown[3] to be 28 per cent, which is a significantly higher figure than the 9·4 per cent found by us for the total zebu population. Therefore, the gene frequency of β-*A* in the crosses should be higher than that in the total zebu population. This seems to be the case from Table 1*b*.

There were 31 dam daughter pairs in the Hariana population. Separate estimates of gene frequencies from this relationship gave frequencies of α-*A* and α-*B* of 0·181 and 0·819 (*S.E.* 0·0422) respectively and of β-*A* and β-*B* of 0·090 and 0·910 (*S.E.* 0·0303), frequencies which agree reasonably well with those obtained by the application of the Hardy–Weinberg formula.

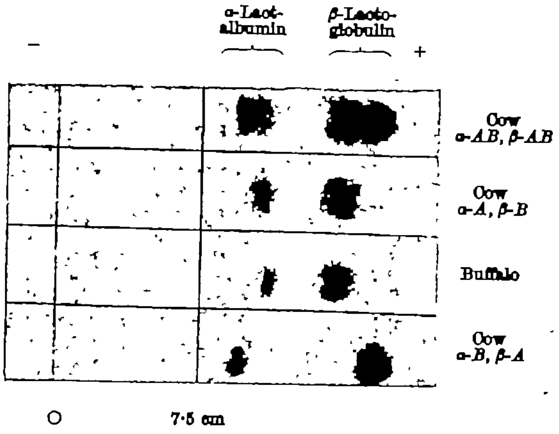

Fig. 1. Whey proteins of buffalo's and different types of cow's milk. O, Origin; bromo-phenol blue staining

Table 2 lists the gene frequencies of α-lactalbumin and β-lactoglobulin of cattle of different countries. The frequency of the *A*-gene of α-lactalbumin has the highest value in Indian zebu cattle with the African breeds having intermediate and the European breeds zero values. On the other hand, the frequency of the *A*-gene of β-lactoglobulin follows a reverse order with the Indian zebu cattle having the minimum and the European breeds the maximum value. The difference between the gene frequencies of α-*A* among Indian and African zebu cattle, though fairly large, is not significant according to the χ^2 test. The gene frequency of β-*A* for White Fulani, however, is significantly higher than that of the Indian zebu cattle. These observations appear to support the view[5] that the zebu cattle were probably brought into Africa by nomadic people.

Out of 7 α-*A* homozygotes, 4 had the combination of α-*A*, β-*B*. The electrophoretic patterns of buffalo whey proteins and cow whey proteins of types α-*AB*, β-*AB*; α-*A*, β-*B*; and α-*B*, β-*A* are shown in Fig. 1. The patterns

Table 1. OBSERVED AND EXPECTED FREQUENCIES OF α-LACTALBUMIN AND β-LACTOGLOBULIN IN INDIAN ZEBU CATTLE

(*a*) α-lactalbumin

Breed		Total	Phenotypes *A*/*A*	*A*/*B*	*B*/*B*	χ^2 (*d.f.*=1)	Frequency *A*-gene	*B*-gene
Hariana	Obs.	96	3	36	57			
	Exp.		4·59	32·81	58·59	0·91	0·219	0·781
Sahiwal	Obs.	15	1	9	5			
	Exp.		2·02	6·97	6·02	1·27	0·367	0·633
Deshi	Obs.	27	3	6	18			
	Exp.		1·33	9·33	16·33	3·47	0·222	0·778
Crosses	Obs.	15	0	6	9			
	Exp.		0·60	4·80	9·60	0·94	0·200	0·800
All breeds	Obs.	153	7	57	89			
	Exp.		8·24	54·53	90·24	0·31	0·232	0·768
All breeds excluding crosses	Obs.	138	7	51	80			
	Exp.		7·65	49·69	80·65	0·10	0·236	0·764
(*b*) β-lactoglobulin								
Hariana	Obs.	96	2	13	81			
	Exp.		0·75	15·49	79·75	2·51	0·089	0·911
Sahiwal	Obs.	15	0	2	13			
	Exp.		0·06	1·87	13·07	0·07	0·067	0·933
Deshi	Obs.	27	0	7	20			
	Exp.		0·45	6·09	20·45	0·61	0·130	0·870
Crosses	Obs.	15	0	4	11			
	Exp.		0·27	3·47	11·27	0 35	0·133	0·867
All breeds	Obs.	153	2	26	125			
	Exp.		1·47	27·04	124·47	0·24	0·098	0·902
All breeds excluding crosses	Obs.	138	2	22	114			
	Exp.		1·22	23·55	113·22	0·62	0·094	0·906

Table 2. COMPARISON OF GENE FREQUENCIES OF CATTLE OF DIFFERENT COUNTRIES

Breed	α-Lactalbumin		β-Lactoglobulin	
	A-gene	*B*-gene	*A*-gene	*B*-gene
British breeds (ref. 2)	0·00	1·00	0·28	0·72
Icelandic (ref. 3)	0·00	1·00	0·34	0·66
Holstein (ref. 6)	0·00	1·00	0·52	0·48
White Fulani (Nigeria) (ref. 3)	0·15	0·85	0·21	0·79
Boran (Kenya) (ref. 4)	0·13	0·87	0·19	0·81
Indian Zebu*	0·24	0·76	0·09	0·91

* From Table 1 *a* and *b*.

of whey proteins of buffalo's milk, and cow's milk with α-*A*, β-*B* seem to be almost identical, α-*A* migrating approximately the same distance as buffalo α-lactalbumin. Thus, α-lactalbumin polymorphism and existence of combinations such as α-*A*, β-*B* in zebu milk rule out the possibility of differentiation of cow's and buffalo's milk in a mixture, as previously suggested[7].

Cow α-lactalbumin-*A*, purified and crystallized by the method[10,11] used for α-*B*, and buffalo α-lactalbumin show, as expected, very similar mobilities (u) and shapes of patterns in free electrophoresis at *p*H 6·0, 7·58 and 8·6. The values of u (descending) for cow α-*A* and buffalo α-lactalbumin are $-2·40 \times 10^{-5}$ and $-2·43 \times 10^{-5}$ cm²/sec/V (*p*H 6), $-4·92 \times 10^{-5}$ and $-4·70 \times 10^{-5}$ cm²/sec/V (*p*H 7·58) and $-4·40 \times 10^{-5}$ and $-4·40 \times 10^{-5}$ cm²/sec/V (*p*H 8·6) respectively. In respect of other physical chemical properties, cow α-*A*, α-*B* and buffalo α-lactalbumin show close parallelism. They appear to have the same crystalline form, similar nitrogen contents, specific extinction coefficients at 280 mμ and tyrosine and tryptophan contents. Their sedimentation coefficients ($s_{20,w}$) fall within the range $1·87 \times 10^{-13}$ sec and $1·99 \times 10^{-13}$ sec. The molecular weight of buffalo α-lactalbumin, 16,200 g/mole, calculated from sedimentation and diffusion data is also close to that of the values[11,12] reported for cow α-lactalbumin. Some of the protein preparations, even after several recrystallizations, show the presence of a faster moving minor component (4–6 per cent) in electrophoresis and sedimentation, the mobilities and s values of which indicate it to be probably the serum albumin contaminant.

Thus, it is interesting to observe that cow α-*A* and buffalo α-lactalbumin show close similarity in their properties in almost the same manner as shown by cow β-*B* and buffalo β-lactoglobulin, which have approximately the same molecular weights[9] and are practically indistinguishable in crystal form[7], electrophoresis[9], sedimentation and ultra-violet absorption[9].

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Significance of the Michaelis Constant

THE Michaelis–Menten[1] equation relating initial velocity of a simple enzyme-catalysed reaction to initial substrate concentration is:

$$v = \frac{k_3 es}{K + s} \tag{1}$$

where v is the initial velocity of reaction, e is the initial enzyme concentration, s is the initial substrate concentration, k_3 is the velocity constant of the breakdown of the enzyme-substrate compound into enzyme and product of the catalysed reaction and K is the substrate concentration at which v is half-maximal.

Michaelis and Menten[1] supposed K to be the equilibrium constant of the reversible reaction between enzyme and substrate. Briggs and Haldane[2] derived an expression showing it to be the ratio of the sum of the two monomolecular velocity constants governing breakdown of the enzyme-substrate compound to the bimolecular constant governing association.

Both pairs of workers based their derivations on the equation:

$$(e - p)s = Kp \tag{2}$$

where p is the initial equilibrium or steady-state concentration of enzyme-substrate compound. The full equation is:

$$(e - p)(s - p) = Kp \tag{3}$$

and the assumption was made in both cases that p was negligible relative to s. However, there are now known numerous cases in which the half-saturation concentration of the enzyme is very low. For example, the half-saturation concentration of D-amino-acid oxidase with flavin adenine dinucleotide (FAD) was reported by Warburg and Christian[3] to be in the region of 10^{-7} M. Since this means that the practicable range of substrate concentrations that can be used for kinetic studies will extend over about one order either side of this, the validity of equation (2) in all cases as an approximation to equation (3) seems doubtful.

The purpose of this communication is to point out that when the quadratic (3) is solved for p, one of the roots gives a solution which approximates to the form of equation (1), but K is replaced by $(K + e)$.

It would seem wise, therefore, in experiments with enzymes of unknown molar concentration, which have low half-saturation concentrations of substrate, to perform at least two series of experiments at different enzyme concentrations before equating the half-saturation concentration with the Briggs–Haldane[2] K. Where the enzyme concentration is known, an approximate idea of the error involved in ignoring enzyme concentration can be obtained by inspection.

When the original data of Warburg and Christian[3] are re-examined using modern techniques of fitting the Michaelis–Menten[1] relation it is found that the fit is very close and that the original estimates of K and $k_3 e$ are fully confirmed. Using more modern data for the half-saturation concentration for alanine, the second substrate used by Warburg and Christian[3], and for the turnover number of the pure enzyme (corresponding to k_3) which are given by Long[4], it can be calculated that the enzyme concentration used was roughly $1·4 \times 10^{-7}$ M. Since the half-saturation concentration for FAD was found to be $2·5 \times 10^{-7}$ M, it follows that the Briggs–Haldane[2] constant is about half the published value for K_m.

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PHYSIOLOGY

Electrophoretic Behaviour of an Aminopeptidase of Human Tissues and Serum

A SERUM aminopeptidase[1] (LAP) that hydrolyses L-leucyl-β-naphthylamide migrates as a single component to the α_1-globulin region in the electrophoretic pattern of normal serum and the serum of the first and second trimesters of pregnancy. Additional components of LAP which migrate to other areas have been found in the serum of many patients with hepatobiliary and pancreatic diseases and in the third trimester of pregnancy[1-3]. The origin of these 'isoenzymes' of serum LAP was sought in an examination of the electrophoretic mobility of LAP from various tissues which exhibited high specific activity of this enzyme.

Crude extracts of apparently normal human liver, pancreas, kidney and placenta obtained at operation or immediately post-mortem were prepared by homogenizing each tissue in approximately 5 times its weight of distilled water (2° C) in a *TRI-R, STIR-R* homogenizer. The more fibrous tissues were prehomogenized in a 'Virtis 45' homogenizer. The homogenates were centrifuged at 5,000 r.p.m. (1,900*g*) for 30 min at 2° C. The residue was discarded and the supernatant centrifuged at 17,000 r.p.m. (20,000*g*) for 30 min at 2° C. The residue was again discarded and solid ammonium sulphate was then added to the supernatant (I) to yield a 65 per cent saturated solution. This solution was centrifuged at 10,000 r.p.m. (7,500*g*), the supernatant (II) separated and the residue suspended in cold distilled water (III) to a final volume equal to that of the original solution. Fractions I, II, and III were assayed for LAP activity[4] and the electrophoretic behaviour of the enzyme was determined on cellulose acetate strips.

For electrophoresis 10 μl. of solution were placed along the centre line of a 2·5 × 10 cm strip of cellulose acetate, which was divided into twenty equal sections by vertical pencil lines drawn at 0·5-cm intervals along the length of the strip. The strips were contained in a Shandon Universal electrophoresis apparatus. The samples were electrophoresed for 3 h in veronal buffer *p*H 8·6, ionic strength 0·075, at a constant current of 0·4 m.amp/cm width of paper. Samples of normal serum and serum treated as here with ammonium sulphate were electrophoresed simultaneously with the tissue extracts for comparison. Following electrophoresis LAP activity was localized on the cellulose acetate strips by a method described elsewhere for paper strips[5].

The electrophoretic mobilities of LAP in normal liver and serum were identical and coincided with that of the serum α_1-globulin (Fig. 1*A* and *B*). This observation supports the view that the liver is a primary source of serum LAP[1].

The pancreatic enzyme exhibited two overlapping peaks of activity, a major peak in the vicinity of the serum γ-globulin, and a minor peak in the region of the β-globulin (Fig. 1*C*). The latter peak has been observed in the serum of most patients with cancer of the head of the pancreas[3].

Placental LAP migrated as an α_2-globulin, as did the serum LAP in late pregnancy (Fig. 1*D*).

Crude kidney extract (I) displayed a broad peak of LAP activity in the α_2- and β-globulin regions (Fig. 1*E*). Kidney supernatant (II), unlike that of liver, pancreas, and placenta, showed considerable LAP activity. Electrophoresis of this supernatant (II) and of the resuspended residue (III) showed that the single broad peak consisted of two peaks, that of the soluble component in the

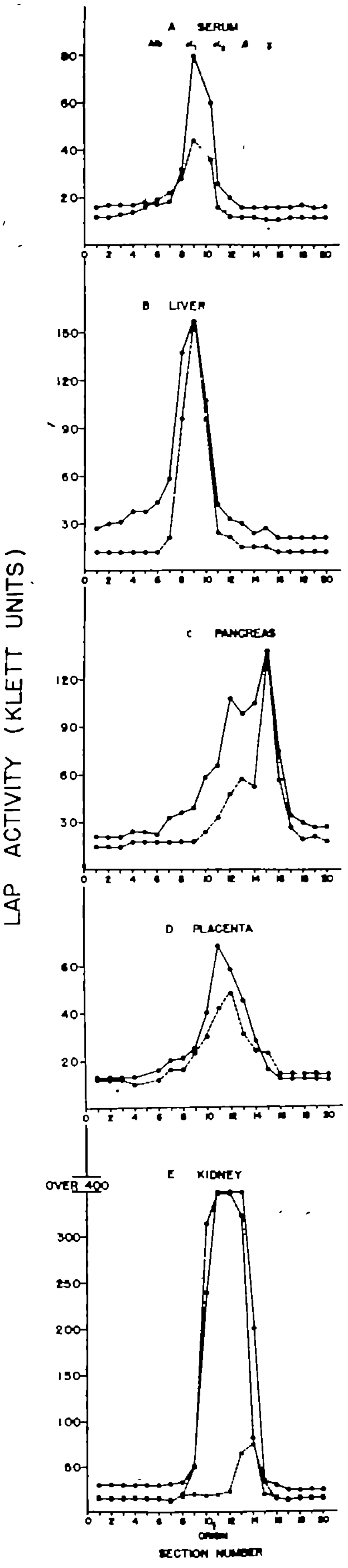

Fig. 1. Electrophoretic localization of leucine aminopeptidase in serum and tissue extracts before (solid line) and after (broken line) ammonium sulphate fractionation. The small peak in Fig. 1*E* represents kidney LAP soluble at 65 per cent ammonium sulphate saturation

β-globulin region and that of the insoluble enzyme in the α_2-globulin region. Preliminary studies showed that the LAP in normal urine migrates to the α_2-globulin region.

In a single observation, LAP from a breast cancer metastasis to the liver migrated as a β-globulin, whereas the enzyme from adjacent liver parenchyma migrated as an α_1-globulin. This finding suggests that the abnormal (β-globulin) LAP component in the serum of most patients with metastatic cancer of the liver[4,5] may be derived from the cancer rather than the liver parenchyma.

Since the results indicated different types of LAP in different tissues, the appearance in the serum of types other than the usual one may prove to be a useful indicator of disease.

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* This enzyme, commonly referred to as 'leucine aminopeptidase', differs from the 'leucine aminopeptidase' which hydrolyses L-leucineamide or L-leucylglycine.

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Sex Differences in the Pattern of Radio-iodine Excretion in the Urine of Intact Stilbœstrol-treated Guinea Pigs

The effects of œstrogen on intra-thyroidal iodine metabolism have been widely investigated[1-4], but very little is known about its influence on the excretion of iodine in urine. In thyroidectomized castrated male rats on a daily maintenance dose of *dl*-thyroxine there is some indication that 50 µg of œstradiol benzoate daily for one week causes a slight retention of radioiodine[1]. Feldman[2] has observed a decreased renal clearance of iodine-131 in both castrated and hypophysectomized rats receiving 500 I.U. of œstrone daily, but the decrease was not significant when compared with controls. It is most likely[1,4] that œstrogen influences thyroidal activity by depression of renal excretion of iodine-131. It has also been reported that œstrogen influences the distribution of radioiodine in castrated male rats[5].

In the experiments reported here, however, intact guinea pigs were used in order to observe physiological differences in reactivity to stilbœstrol dependent on sex and dose of œstrogen, which it is believed have not hitherto been described. Since the 24-h renal excretion test gives incomplete information about the course of iodine metabolism during this period the method of fractional urine examination was used[6].

Three groups, each containing 7 male and 7 female guinea pigs, were used in the experiment. All animals were fed *ad libitum* with bulky wet food. Stilbœstrol was injected in oil intramuscularly in doses of 0·5 mg/day for three days (group II) and 1·25 mg/day for five days (group III). The animals of the control group (group I) were injected intramuscularly with saline. These two doses of stilbœstrol were assumed to stimulate and depress thyroid activity respectively[7]. 24-h after the last injection of hormone the animals were given an intraperitoneal injection of 28 µc. of carrier-free $Na^{131}I$ in 0·5 ml. of saline. It was previously established that the most favourable fractionation of urine for the required observation should be 0–8 h, 8–14 h and 14–24 h (zero time = time of radioiodine injection). The daily volumes of excreted urine per animal were: 67–140 ml. in controls (group 1); 12–91 ml. (group II) and 17–104 ml. (group III). Following the addition of potassium iodide solution as carrier the radioactivity of the dried urine samples was estimated using the β-ray Geiger–Müller counter. A summary of the results is given in Table 1.

It will be noted that retention of radioiodine was greater in female guinea pigs (including controls) than males. This difference was statistically significant, and therefore comparison with the controls was possible only within each sex.

The 24-h test of urinary excretion used previously gives only slight information about the influence of stilbœstrol on the retention of radioiodine in the body. With the adoption of the method of fractionated urine examination it has become possible to observe differences between groups. Relative contents of radioiodine in separate fractions of urine show the largest differences between groups in the first fraction (0–8 h). The second fraction (8–14 h) does not show any significant inter-group differences. In the last fraction (14–24) there was a marked change in the urine excretion in the male but to a lesser degree in the female. In general, there was a decreased excretion of radioiodine in females, but only at the 1·25 mg dosage of stilbœstrol was the difference statistically significant ($P < 0·05$).

Table 1. Effect of Stilbœstrol on the Excretion of Radioiodine to Sex and Dose in Intact Guinea Pigs

No. of animals	Sex	Treatment	Body-weight (g)		Percentage of administered ^{131}I excreted in urine											
					0–8 h			8–14 h			14–24 h			Total 0–24 h		
			Mean	*S.E.*	Mean	*S.E.*	Range	Mean	*S.E.*	Range	Mean	*S.E.*	Range	Mean	*S.E.*	Range
7	♂	Controls	510·0	±28·89	41·5	±1·62	37·1 48·4	22·7	±1·59	19·8 31·4	10·6	±0·67	8·3 13·4	74·9	±1·32	70·8 80·4
7	♀		502·0	±26·43	39·9	±3·21	30·5 53·7	13·7	±2·39	6·8 23·8	8·6	±1·78	2·1 14·1	62·2	±1·90	54·7 68·3
7	♂	Stilbœstrol 0·5 mg/day	535·0	±22·97	25·7	±2·85*	15·3 36·0	24·9	±3·41	19·2 38·5	16·8	±2·78†	10·5 32·0	67·5	±4·24	60·1 80·7
7	♀		574·0	±17·58	31·9	±2·88	22·5 43·7	12·1	±1·59	5·5 19·3	11·1	±1·84	5·3 20·4	55·2	±4·23	34·7 70·8
7	♂	Stilbœstrol 1·25 mg/day	410·0	±13·44	30·2	±2·00*	26·1 41·0	20·8	±1·63	15·5 27·6	16·8	±0·74*	14·3 20·1	68·0	±3·30	56·1 88·7
7	♀		498·0	±13·52	15·2	±2·97*	9·6 29·5	21·6	±4·50	10·8 38·9	15·5	±2·27†	8·7 22·9	47·1	±6·12†	21·6 62·2

$$S.E. = \pm\sqrt{\frac{\Sigma(x-\bar{x})^2}{n(n-1)}}$$

* Significantly different from control at 1 per cent level. † Significantly different from control at 5 per cent level.

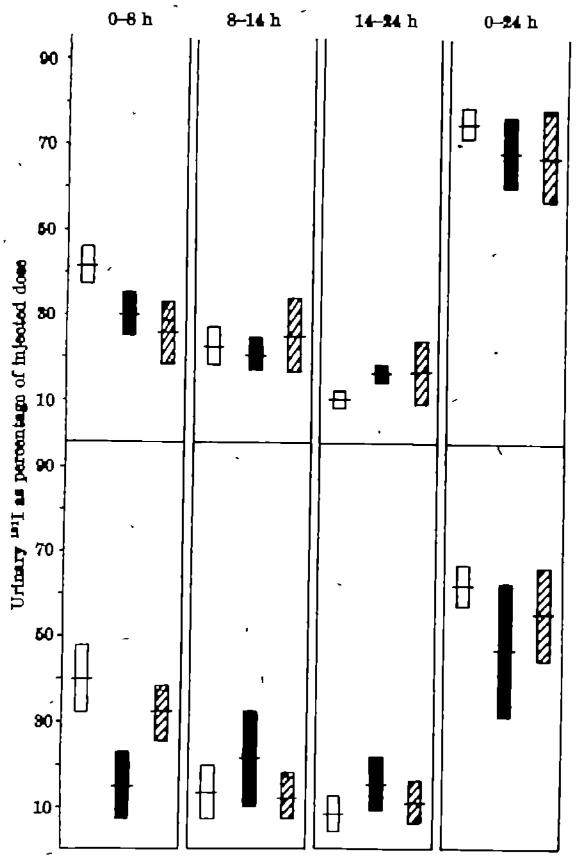

Fig. 1. Results obtained by the method of fractionated urine examination of Arnott *et al.* (ref. 6). Horizontal line and bar indicate the group mean and the limits of ± *S.D.* respectively. White, controls; shaded, stilbœstrol-treated 0 5 mg/day; black, stilbœstrol-treated 1·25 mg/day

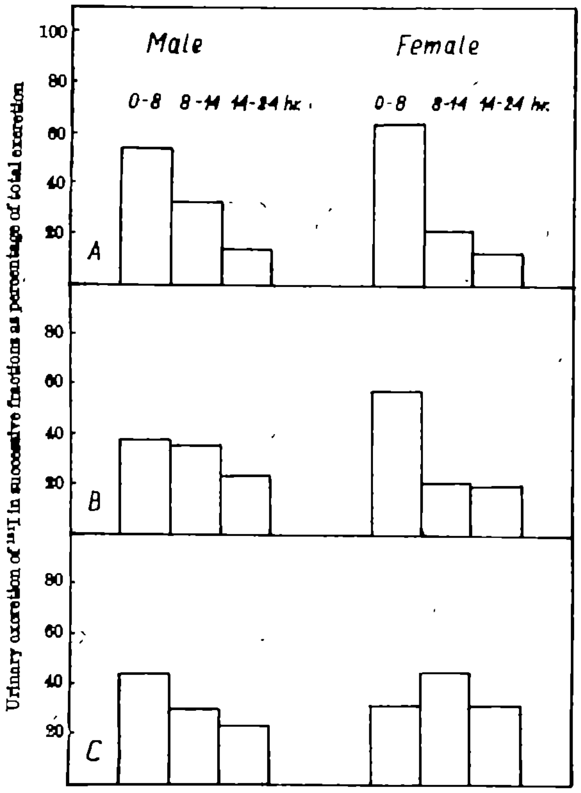

Fig. 2. Inhibitory effect of stilbœstrol on the excretion and excretion pattern of radioiodine in intact male and female guinea pigs. *A*, Controls; *B*, stilbœstrol-treated 0·5 mg/day; *C*, stilbœstrol-treated 1·25 mg/day

From Figs. 1 and 2 it can be concluded that the urinary excretion of radioiodine is greatest in the first hour after administration and thereafter gradually decreases. Stilbœstrol in a dose of 1·25 mg alters this pattern in the intact female, more than in the male, especially with respect to the first fraction. Administered at the rate of 0·5 mg per day, stilbœstrol affects the retention of radioiodine in the body; in the female, however, this effect is not statistically significant. Both the aforementioned doses give a significant change of retention of radioiodine in the male as assessed from examination of the third fraction (14–24 h).

From this evidence it appears that the female is more stimulated by large doses of stilbœstrol and returns more quickly to the normal condition than does the male. On the other hand, intact female guinea pigs are not so much affected as the male by the administration of small doses of stilbœstrol.

The volume of urine excreted per animal per day was in general significantly less after stilbœstrol administration. This was probably due in part to the decreased appetite of the experimental animals, with consequent decreased water intake, and in part to the recognized effect of œstrogens in promoting water-retention[8]. Nevertheless, the values calculated for the regression equations and coefficients of correlations show that there is no correlation between the volume of urine and the amount of radioiodine excreted in the male, but that the correlation is statistically significant at the 1·25 mg dose of stilbœstrol in the female ($P < 0.05$).

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In vitro Measurements of Oxygen Consumption of Human Heart Muscle

HYPOTHERMIA has been used extensively in medicine to depress the metabolism of the body tissues[1,2]. Frequent studies have been made of the reduction in oxygen consumption brought about by various grades of hypothermia, both in whole animals[3–5] and in isolated organs and tissues[6,7], but isolated human heart muscle has not so far been examined intensively.

We have found that small pieces of human auricular muscle, trimmed from the appendage submitted for pathological examination at mitral valvotomy, will undergo rhythmic contractions for several hours when placed in a Burn–Dale bath[8]. This observation led us to consider the possibilities of using such material for the *in vitro* examination of various aspects of human cardiac metabolism. This communication deals with the preliminary studies which have been made of human cardiac metabolism at normothermia and at various grades of hypothermia.

Small pieces of auricular muscle were received, in the operating theatre, into Krebs–Ringer phosphate solution and transported rapidly to the laboratory where tissue slices, 0·2 mm thick, were made, and these were placed in Warburg respirometer flasks each containing 2·5 ml. of Krebs–Ringer phosphate solution and 0·5 ml. of 0·02 molar succinic acid in the side-arm. It was usually possible to obtain sufficient material from each operation specimen to supply six Warburg flasks, three of which were placed

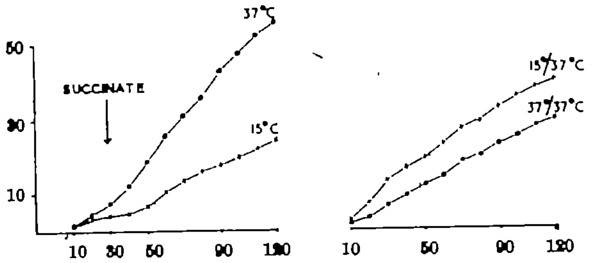

Fig. 1. Results of an experiment at 15° C, showing depression of oxygen consumption of hypothermic tissue followed by increase in oxygen consumption after rewarming.

in one Warburg apparatus, maintained constantly at 37° C, while the other three flasks were placed in a second Warburg apparatus which was at the temperature under investigation.

Endogenous metabolism was examined for 30 min and the succinate was then added from the side-arm and the oxygen uptake measured for the next 90 min. The flasks from the hypothermic bath were then placed in the bath at 37° C and after 15 min for temperature equilibrium the uptake of oxygen of all the flasks in the bath at 37° C was measured for a further 120 min. At the end of the experiment the material from each of the flasks was estimated for nitrogen by the micro-Kjeldahl technique[9], and the results finally expressed in terms of microlitres of oxygen consumed per mg nitrogen in the tissue.

At each of the temperatures selected, namely, 26° C, 15° C, and 8° C, two different specimens were examined and the results are thus from observations on auricular muscle from six different individuals.

Fig. 1 shows the results obtained from one of the experiments at 15° C, and the reduction in oxygen consumption brought about by maintaining the tissue at 15° C can be seen in the first 2-h period, against the oxygen consumption of the control tissue at 37° C. The results obtained during the second 2-h period, when all the flasks were in the bath at 37° C, show that the increased oxygen consumption of the tissue from the cold bath lies presumably in the extra supply of substrate which it has at the end of the first 2-h period compared with its control, which has been undergoing more active metabolism during the preceding 2 h.

Table 1 shows the results obtained from each of the two specimens of tissue tested at each of the temperatures under investigation. The results are expressed in terms of the percentage reduction in oxygen consumption obtained when each specimen is compared with its own control at 37° C. The oxygen consumption is reduced to 72 per cent of the value obtained at 37° C when the tissue is maintained at 26° C and to 40 and 30 per cent when maintained at 15° C and 8° C respectively. The lower part of Table 1 shows the results obtained during the second 2-h period when all the tissue was in the bath at 37° C. In all cases there is an increase in oxygen consumption of the tissue from the hypothermic bath, compared with that in the bath at 37° C throughout, and the mean value indicates an increase of some 50 per cent in oxygen consumption. Thus in all cases it was possible to metabolize succinate following the 2-h period in the hypothermic bath.

Table 1. The Oxygen Consumption at Various Temperatures when each Specimen is compared with its own Control at 37° C is shown in the Upper Part and again in the Lower Part after Rewarming

	Temperature ° C		
	26 % O_2 consumption	15 % O_2 consumption	8 % O_2 consumption
	72	38	28
	72	42	32
Mean	72	40	30
	26° C/37° C % O_2 consumption	15° C/37° C % O_2 consumption	8° C/37° C % O_2 consumption
	188	136	109
	117	147	203
Mean	153	142	156

In Table 2 can be seen the oxygen consumption in each of the experiments during the first 30 min of endogenous metabolism and then during the second 30-min period after the succinate had been added. At 37° C the addition of succinate caused the oxygen consumption to be doubled during the second 30-min period; but this stimulation is reduced as the temperature falls, being only slightly reduced at 26° C, much more at 15° C, while at 8° C it is apparent that succinate is not being metabolized at all.

Table 2. Oxygen Consumption at Various Temperatures Before and After Addition of Succinate

Time min	37° C μl. O_2		26° C μl. O_2		15° C μl. O_2		8° C μl. O_2	
	1	2	3	4	5	6	7	8
0–30	16	15	12	9	4	4	8	5
30–60	32	30	18	19	5	7	3	4

This preparation of human heart muscle appears to function in a stable and reproducible manner, and when many more specimens have been examined it should be possible to give a Q_{10} for human heart muscle. The results of the preliminary experiments show a reduction in oxygen consumption to 72 per cent of the value obtained at 37° C when the tissue is maintained at 26° C and to 40 and 30 per cent of the value obtained at 37° C when the tissue is maintained at 15° C and 8° C respectively. The ability to metabolize succinate decreases with declining temperature and has been arrested by the time 8° C is reached. Both the specimens from bath at 8° C, however, showed a rapid return of ability to metabolize succinate when placed in the bath at 37° C.

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Utilization of Proline during Healing of Fractures

Summer[1] has recently demonstrated that following oral administration of proline the level of serum proline was lower in children afflicted with osteogenesis imperfecta than in normal children. Since this effect was shown not to be due to urinary excretion, it seemed that the relative rate of proline disappearance from the blood system (proline 'tolerance') could serve as an index of connective tissue metabolism. To determine the validity of this concept, the serum proline concentration was measured by the method of Troll and Lindsey[2], at two intervals after an intravenous injection of proline into normal rabbits and into rabbits with healing fractures.

Ten New Zealand white female rabbits were fasted overnight and subjected to a proline 'tolerance' test. Blood samples were withdrawn by heart puncture, prior to and 30 and 60 min after the intravenous administration of 65 mg proline/kg body-weight. After a day's rest the same rabbits were anaesthetized with ether and one femur was snapped with the aid of a pipe wrench. The fractures were not reduced or immobilized. After 10 and 34 days the proline 'tolerance' test was repeated. The fracture site

Table 1. EFFECT OF FRACTURE HEALING ON THE REMOVAL OF PROLINE FROM THE BLOOD

Min. after intravenous injection of 65 mg proline/kg	Serum proline-level (g/mL.) 10 days of healing		Prior to fracture		30 days of healing
30	268 ± 49·5 (8)		207 ± 25·1 (10)		143 ± 13·0 (6)
		$P = <0·3$		$P = <0·05$	
60	131 ± 23·3 (9)		148 ± 19 0 (8)		81 ± 6·0 (6)
		$P = <0·7$		$P = <0·01$	

* The numbers in parentheses indicate the number of determinations; ± *S.E.*

was freely movable on the 10th day but quite firm by the 34th day. The animals in general lost weight between the first and second test but tended to regain this loss prior to the 34th day.

From the data of Table 1, it can be concluded that by the 34th day an increased rate of proline utilization accompanies callus formation; the serum proline level is abnormally low at both 30 min and 1 h after injection of proline. In contrast, after only ten days of healing when callus is small, there is no significant difference in proline 'tolerance'; 30 min after an injection of proline the average value is higher and more variable in the fracture animals than in the controls. Furthermore, the increase in proline 'tolerance' during the period of growth of callus is a relatively constant response. These observations suggest that increased proline tolerance recently reported in patients with osteogenesis imperfecta should be interpreted with caution; many patients hospitalized with this disease are recovering from fractures, both clinical and non-symptomatic microfractures. It should be emphasized, however, that fracture healing effects do not necessarily disprove the relation between osteogenesis imperfecta and increased proline 'tolerance'. But because of the potential importance of that relationship, it must be investigated further in patients having no visible fractures.

In any event, that proline disappearance from the blood is accelerated by fracture healing suggests the proline 'tolerance' test might be used as an indicator of bone formation. Whether the effect on proline 'tolerance' specifically reflects callus formation or, non-specifically, either a phase of tissue repair or an aspect of metabolism associated with a phase of recovery from injury, is a question to be settled by additional experiments. A proline 'tolerance' test would have the advantage of not involving radiation, permitting its daily repetition with minimal discomfort or hazard.

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HISTOLOGY

Hypogastric Nerve of the Dog

EXPERIMENTS attempting to distinguish between the effects of reserpine on sympathetic ganglia and on post-ganglionic fibres were carried out on the inferior mesenteric ganglia and the hypogastric nerves of the dog. Analysis of the tissues showed such large and variable amounts of both noradrenaline and adrenaline in the hypogastric 'nerves', coupled with a high degree of resistance to the depleting effect of reserpine, that it was obvious that these 'nerves' were complex in structure. Langley and Anderson's detailed analysis[1] of the hypogastric nerves of the cat and the rabbit had shown these to consist of about 90 per cent non-medullated and 10 per cent (afferent) medullated fibres. As these authors pointed out later[2], a proportion of the non-medullated fibres are preganglionic and are interrupted in ganglia situated usually near the innervated organ, such ganglia being more frequent in the rabbit than in the cat. This analysis did not, however, explain the chemical findings on catecholamines in the dog, since these strongly suggested the presence of chromaffine tissue in the nerves.

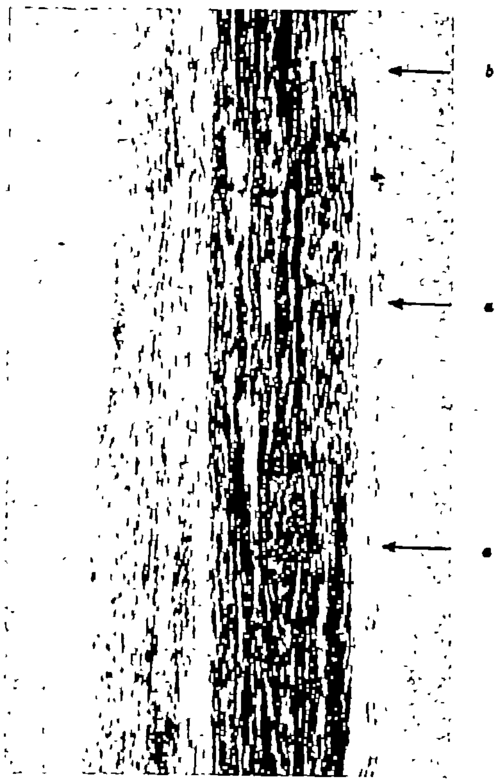

Fig. 1. Longitudinal section through dog's hypogastric nerve about midway between inferior mesenteric ganglion and pelvic plexus. Clusters of (*a*) chromaffine, and (*b*) nerve cells in the core of the nerve

The occurrence of chromaffine tissue in sympathetic ganglia, particularly those of the abdomen, and in the preaortic nerve plexus of cat and man was known since the work of Kohn[3]. However, whereas in man there is only a hypogastric plexus and no hypogastric nerve, the dog has distinct hypogastric nerves, which are 4–8 cm long and have very few branches and no macroscopic swellings. The question, therefore, arose where chromaffine tissue, if such was present, might be localized in such a nerve.

Longitudinal serial sections were made of the inferior mesenteric ganglia and the hypogastric nerves of 4 dogs, 2 adult and 2 puppies of either sex. The dogs were bled to death under anaesthesia. The tissue was stretched out and tied on a glass plate and fixed in a solution containing, per 100 ml., 10 ml. 40 per cent formaldehyde solution, 2·5 g potassium bichromate and 1·0 g sodium sulphate. After washing overnight it was embedded in paraffin, sectioned at 6μ and stained with cresyl violet. As expected, large chromaffine bodies were found inside the inferior mesenteric ganglia, but these did not extend into the hypogastric nerve. However, small elongated groups of 5–50 chromaffine cells occurred either within the very core of the nerve (Fig. 1), or along its surface underneath the epineurium. These groups were usually found in the middle part of the nerve, 2 or more cm away from either the lower pole of the mesenteric ganglion or the beginning of the pelvic plexus. They were not necessarily in the vicinity of nerve cells. Nerve cells were abundant in the meshes of the pelvic plexus, but were also present, though scarce, within the hypogastric nerve proper, and were usually arranged like a string of beads in the core of the nerve. They were found in larger clumps at the origin of the hypogastric nerves near the lower pole of the inferior mesenteric ganglion.

So far as can be judged from this small material there was no difference in the amount of chromaffine tissue in the nerves of young and of adult dogs. Similarly, Kohn[3] found that chromaffine tissue does not degenerate in adult cats and rabbits.

In view of the present interest of pharmacologists in the hypogastric nerve–vas deferens preparation of the guinea pig devised by Huković[4], the fact should be kept in mind that this 'nerve' is a complex structure and varying in composition from species to species, and not just a bundle of postganglionic fibres.

I thank Mr. B. S. C. Hollands for preparing the sections.

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PATHOLOGY

Ineffectiveness of Autotransplantation on Growth of the Methylcholanthrene Sarcoma in Rats and Mice

RECENTLY, Ishibashi *et al.*[1] reported that the subcutaneous autotransplantation of a patient's own tumour slices was effective against the growth of the original tumour and sometimes caused its regression. It seems, however, that experimental evidence on the effectiveness of autotransplantation is not sufficient to try it against human cancer. Attempts were made in this laboratory to examine whether autograft of tumour induced by methylcholanthrene could increase the survival days of the host after appearance of a tumour.

The animals consisted of 100 virgin female Wistar strain rats weighing about 105 g and of 100 mice of *A1* strain[2] of both sexes, 15–20 g in weight. They were injected subcutaneously in the interscapular region with 3 mg in rats and 1 mg in mice of 20-methylcholanthrene (California Corp. Biochem. Res., Los Angeles) in olive oil, and were fed a synthetic cube diet (Central Laboratory for Experimental Animals, Tokyo) and given tap water *ad libitum*.

Subcutaneous autotransplantation. Seventy rats in which tumours (fibrosarcoma) developed in 100–130 days after methylcholanthrene injection were subjected in this experiment. When the tumour attained a diameter of 20–25 mm, two-thirds of it was excised from 40 animals. Twenty (group 1) of them were transplanted surgically with their own tumour (2 slices, 3 × 3 × 3 mm) into the subcutaneous tissues of the dorsal surfaces apart from the indigenous tumour. Twenty other rats (group 2) were subjected to an identical procedure as the animals of group 1, without tumour implantation. Thirty animals as control (group 3) were maintained freely without surgical excision or implantation of the tumour. After surgical operation, maximum diameter and its right-angled one in all tumours were recorded at least once a week.

Growth rates of tumours in rats of each group are presented in Fig. 1*a*. The growth curve of group 1 has almost the same as that of group 2. Average survival after tumour occurrence was 50·6 ± 10·1 in group 1, 46·9 ± 7·0 in group 2 and 47·0 ± 13·1 days in group 3, respectively. Statistical analysis revealed that there were no significant differences in survival days among three groups.

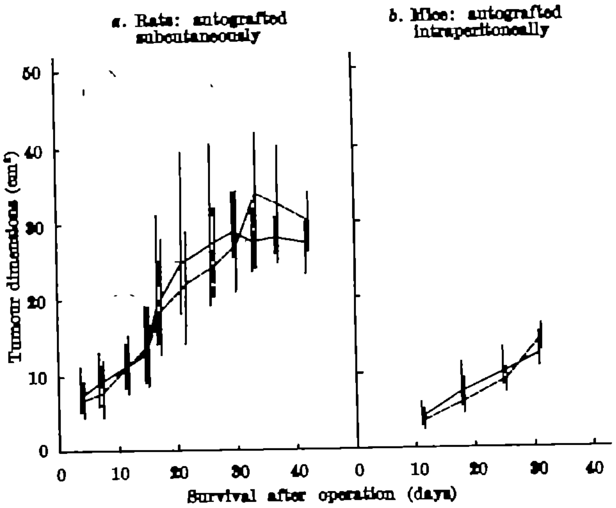

Fig. 1. Growth rate of the methylcholanthrene sarcoma in the autografted (——) or the non-autografted (- - - - -) rats (*a*) and mice (*b*). The length of the longitudinal lines denotes the range of tumour sizes

Intraperitoneal autotransplantation. When the tumour reached a size of 12–15 mm in diameter, mice were autotransplanted with tumour (2 slices, 2 × 2 × 2 mm) into the peritoneal cavity by 13-gauge trocar. Sixty mice in which tumour developed (fibrosarcoma) 70–100 days after injection of the carcinogen were subjected to the following experiment. In 20 mice, two-thirds of the tumour was excised and the tumour was inoculated intraperitoneally (group 4). The tumour in 20 other animals was excised similarly with group 4 and the abdominal wall was inserted by the trocar without tumour transplantation (group 5). The remaining 20 mice were maintained freely without any operation (group 6).

Growth rates of the tumours of each group are presented in Fig. 1*b*. The curve of group 4 has almost the same configuration as that of group 5. Average survival days after tumour occurrence was 39·3 ± 3·3 in group 4, 38·2 ± 5·5 in group 5, and 35·8 ± 5·4 in group 6, respectively, and differences among three groups were of no significance.

The results of the present experiments showed no convincing effect of the immunological treatment on malignancies, although the negative results might mean that the materials or the techniques were inadequate. After preparation of this paper, we read Rhodes's experiment[3] in which the tumour (the spontaneous tumour of the $c+$ strain) was autografted subcutaneously with Freund's adjuvant and tetanus toxoid into autochthonous mice. He found also no significant difference in the life-span between autografted and non-autografted animals.

We thank Dr. K. Sugiura, Sloan-Kettering Institute, for the gift of the trocar, and Prof. T. Nagayo and Dr. K. Kojima of this Department for their advice. This work was supported in part by research grants from the Ministry of Education and the Japan Cancer Society.

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Hepatitis in African Monkeys

INFECTIOUS hepatitis is thought to be caused by a virus which is transmissible to man[1–4] but not to monkeys or other laboratory animals[5–9].

The present communication is a preliminary report on work carried out to determine the susceptibility of West African monkeys to infectious hepatitis. For this purpose, young animals were inoculated subcutaneously with suspensions of human tissues and fæces from fatal cases of this disease. The livers of these monkeys were examined in serial biopsies taken at intervals of 2–3 days for six weeks after inoculation. All biopsies were divided into two portions; one stored in the frozen state to preserve virus, the other examined by cytological and cytochemical methods for changes similar to those occurring in the human liver[10–12].

Following inoculation, young patas monkeys (*Erythrocebus patas patas*) showed liver changes during the period 3–6 weeks after inoculation. The parenchymal changes (Fig. 1) consist of occasional small foci of liver cells showing a slight increase in nuclear and nucleolar size and a concurrent increase in nucleolar and cytoplasmic ribonucleoprotein. Occasional necrotic cells and mitotic figures also occur. Some of the portal tracts exhibit an infiltration with inflammatory cells and swelling of the connective tissue fibroblasts. The stored portions of biopsies showing such changes were inoculated into further young patas monkeys. These animals showed similar, although more pronounced, parenchymal and stromal alterations during the same period.

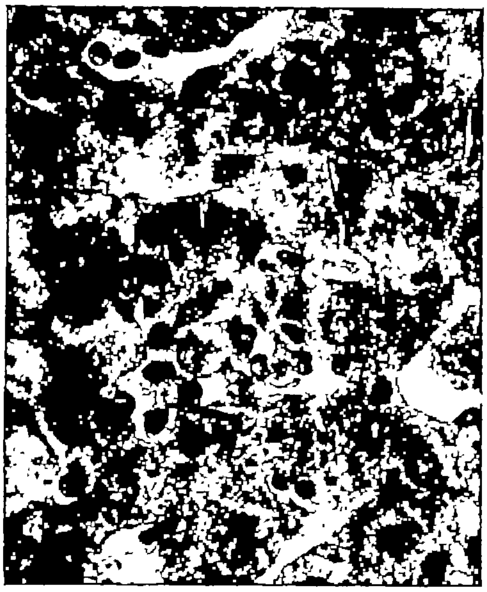

Fig. 1. Early parenchymal focus in patas monkey liver 23 days after inoculation (× 600)

Although the livers of all infected monkeys show focal parenchymal and stromal changes, in many third and later passage monkeys, these alterations afterwards spread to involve the entire liver. In these cases, parenchymal changes are diffuse throughout the lobule (Fig. 2) but necrosis is limited to a few individual or small groups of liver cells. In viable liver cells (Fig. 3) the nuclei and nucleoli are usually slightly enlarged and the latter shows an increased amount of ribonucleoprotein. The cytoplasm is partly or completely filled with ribonucleoprotein but swelling of the cells does not occur. There is no increase of lipids but glycogen is reduced or absent. The mitochondria appear increased in number in viable cells but are swollen in degenerating or necrotic cells. The sinusoids are often dilated, especially near the central veins, and contain an increased number of lymphocytes and granulocytes. The Kupffer cells are swollen. The portal tracts show a moderate to marked infiltration with inflammatory cells and a variable degree of degeneration. Occasionally complete necrosis of the portal tract occurs. The capsule and intercalated veins also show localized areas of infiltration and degeneration.

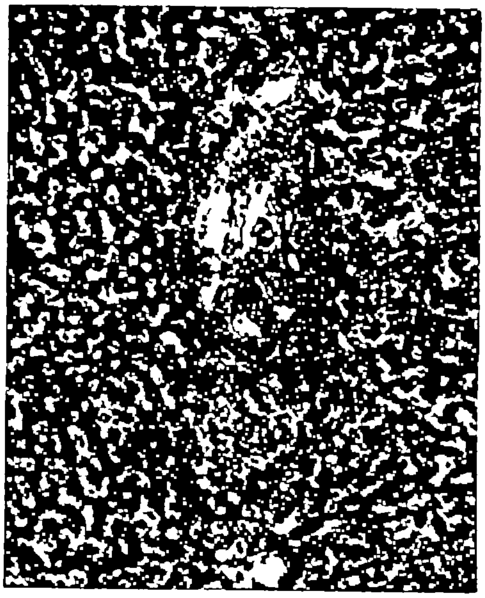

Fig. 2. Liver of patas monkey dying 29 days after inoculation (× 125)

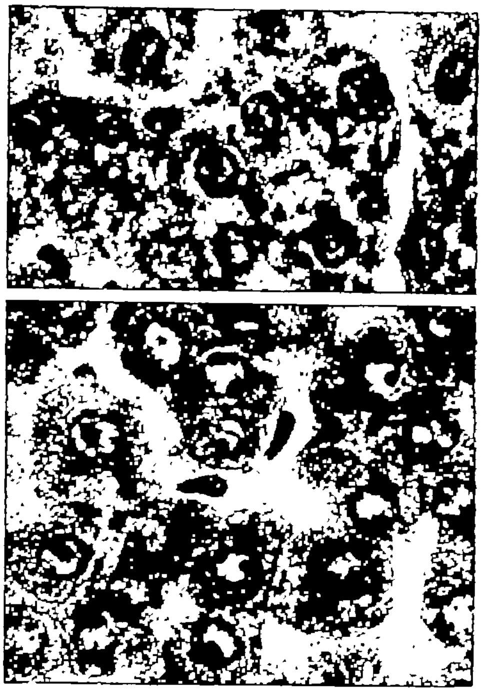

Fig. 3. Liver cells of healthy (above) and infected (below) patas monkeys (× 1,000)

Subcutaneous inoculation of a variety of African monkeys with infected patas liver suspensions has given rise to similar changes in the livers of five other species of Cercopithecoid monkeys, but not in baboons, drills or mangabeys. Though the majority of infected monkeys exhibit no clinical manifestations of disease, young animals with widespread changes in the liver usually show anorexia and become emaciated. In these instances, death frequently ensues. There is never any clinical evidence of jaundice.

Changes in the liver similar to those in infected animals have not been seen in the livers of 223 uninfected African monkeys, neither have they been induced by inoculating pooled biopsies from uninfected into healthy monkeys. Inoculation of infected patas liver suspensions into white mice and white rats has not given rise to disease or changes in the livers in these animals. No virus has been isolated in monkey kidney tissue cultures. Attempts to isolate bacteria from infected monkey livers in a variety of artificial media have been unsuccessful. No micro-organisms have been seen in stained liver sections. The agent passes a Seitz filter and withstands a temperature of 56° C for 30 min. These findings suggest that the hepatitis occurring in young patas monkeys is caused by a relatively thermostable virus. At the time of writing, this virus has been isolated on eight separate occasions following the inoculation of suspensions of liver, spleen, colon and faeces from these cases. All isolations have been maintained in serial passage through the subcutaneous inoculation of infected patas monkey liver suspensions.

The results reported here suggest that hepatitis may be induced in young patas monkeys through inoculation of material from human cases of infectious hepatitis. Although the liver changes in infected animals parallel those in the human liver in this disease, further studies are necessary to determine whether the virus causing the hepatitis in monkeys is the same as that causing human infectious hepatitis.

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IMMUNOLOGY

Properties of Phage-neutralizing Antibodies produced by New-born Rabbits

The new-born rabbit is known to show an immunological reactivity which differs in several respects from that of the mature animal. We have found that rabbits injected at birth with coliphage T_2 show, after a short delay, phage-neutralizing activity in their sera which appears to be antibody. These antibodies, however, differ in several aspects from the phage-neutralizing antibodies produced by adult animals immunized with the same bacteriophage.

For the work reported here rabbits were injected in the foot-pad, within 24 h of birth, with 2×10^9 plaque-forming units of *E. coli* T_2 bacteriophage purified by differential centrifugation[1] suspended in 0·1 ml. saline. At 7 days of age the animals were killed and their serum assayed for phage-neutralizing activity following the procedure of Barlow *et al.*[2], the titration being performed either immediately or after storing the serum for a few days at 4° C. All the sera examined showed activity ranging from 4 to 64 phage-neutralizing units (50 per cent p.n.u.) per ml.; no activity at all was found in the fresh serum of control 7-days-old rabbits not injected with phage at birth. We then examined the effect of various treatments on the phage-neutralizing activity found in the serum of immunized baby rabbits; for comparison we also examined the normal serum from a non-immunized adult rabbit and the serum from an adult rabbit repeatedly immunized with coliphage T_2. This last serum was diluted with serum from untreated 7-days-old rabbit in order to give a titre of 50 per cent p.n.u./ml. comparable with that of the immunized new-born animal. The results of a typical experiment are presented in Table 1.

Table 1. T_2 PHAGE-NEUTRALIZING ACTIVITY OF RABBIT SERA, MEASURED AS 50 PER CENT P.N.U./ML.

Treatment	Normal adult	Immunized new-born	Immunized adult
None, dilution for titration in buffer as indicated by Barlow *et al.*[2]	8	64	64
None, dilution for titration in 1:10 fresh serum from untreated 7-days-old rabbit	8	250	64
Heating at 56° C for 30 min	0	0	64
Heating at 56° C followed by dilution in 1:10 fresh serum from untreated 7-days-old rabbit	0	0	64
With 0·01 M sodium EDTA	0	0	64
Absorption with 'Zymosan' at 17° C	0	0	50
Ultracentrifugation* activity in the central part of the sector	−	−	+
Ultracentrifugation* activity in the peripheral part of the sector	+	+	+

* Spinco Model *E* analytical ultracentrifuge, cell equipped with an Yphantis–Waugh moving partition centrepiece. One central and one peripheral fraction were collected after the passage, into the peripheral part of the sector, of a peak sedimenting at about 19*S*.

The results suggested that the phage-neutralizing substances produced by the immunized new-born rabbit might be similar to those naturally present in the adult and often included in the properdin system. We tested our sera against a phage antigenically unrelated to that used for immunizing the new-born animal, namely, coliphage T_5, and we found no activity whatever.

The simplest interpretation of the results is that the phage-neutralizing activity found in the sera of rabbits immunized at birth with T_2 phage was indeed due to antibody. Thus it fulfilled the requirement of appearing as the consequence of a given antigenic stimulation and of being specifically directed against the same antigen; the phage-neutralizing activity appeared 4–5 days after the antigenic stimulation, a latent period not dissimilar from that of many primary antibody responses, and also was associated with the 19*S* globulins, a serum fraction known to include many kinds of antibodies.

On the other hand, the phage-neutralizing substance produced by immunized new-born rabbits has many of the properties once ascribed to properdin[3]. This is perhaps not so surprising in view of the possibility that properdin may represent a family of somewhat peculiar antibodies[4], arising as the consequence of natural stimulation with a number of antigens, a possibility also borne out by the recent observations[5] that the phage-neutralizing activity of normal (human) sera appears to be due to a combination of molecules with different specificity. Indirectly our results give support to the hypothesis that these are indeed antibodies elicited by 'natural' contact with minimal amount of each phage against which a neutralizing activity is found in 'normal' serum. So it would appear that heat-labile and complement dependent 19*S* antibodies are formed by the new-born rabbit as a consequence of immunization with sufficient amounts of phage, and by the adult animal as a product of basal stimulation with what might be the minimal amounts of antigen that penetrate the intestinal tract.

Perhaps our observations may be interpreted on the basis of recent results which show that the antibodies produced by different animals in the early stages of the immune response usually are 19*S* [6–8] and may be complement dependent[9] and more heat-labile than those produced later on in the course of immunization[10]. Thus the new-born rabbit would respond to immunization with phage in a more sluggish way than the adult, the 'early' antibodies being the only ones produced for a period of about two weeks (which is the age at which we first observed the production of 'classical' heat-stable anti-phage antibodies). This situation might not be valid for species other than rabbit because, for example, heat-stable anti-phage antibodies may be produced by the new-born guinea pig[11] or even by the opossum embryo[12]. In the rabbit, heat-stable antibodies (but mainly 19*S*) may be produced by 10–16 days old animals immunized at birth with some bacterial antigens[13] or with antigens plus adjuvant[14].

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ANATOMY

Periodic Acid–Schiff-positive Component of the Medullary Bone of the Laying Domestic Fowl

INVESTIGATION of avian medullary bone in this laboratory led to the observation of a substance therein which gave a very strong positive reaction with the periodic acid–Schiff technique. In some instances this took the form of branching homogeneous strands or fibrils of irregular thickness distributed in a less-strong periodic acid–Schiff-positive matrix; in other instances it was more dispersed and less distinctly fibrillar. Variation in the amount of this substance was also noted in different stages of the egg-laying cycle and, as a loss of chondroitin sulphate from the cartilage is known to precede endochondral bone formation, an attempt was made to determine the nature of the periodic acid–Schiff-positive substance in avian bone and to correlate the variations in appearance and amount with the alternate absorption and deposition known to occur in this bone during the egg-laying cycle[1].

Specimens of medullary bone were obtained from forty laying hens of the Thornber 505 hybrid strain, varying in age from 1 year to 18 months, specimens being taken from about the middle of the shaft of humerus, femur, tibia and fibula of each bird within a few minutes after death. Specimens from each block were fixed in absolute alcohol, or in Heidenhain's susa containing twice the prescribed quantity of trichloroacetic acid to initiate decalcification. Alcohol fixation tissues were decalcified in N acetate buffer at *p*H 4·5; decalcification of susa-fixed tissues was completed in 'Permutit' ion exchange resin ('Zeo-Karb 225') in 10 per cent formic acid. After decalcification the tissues were dehydrated, cleared, embedded in paraffin and sectioned at 5μ.

Sections from each specimen were stained with periodic acid–Schiff technique[2,3] either alone or following preliminary treatment by one or other of the following: (1) salivary amylase (saliva) for 30 min at 37° C; (2) bovine testicular hyaluronidase (L. Light and Co.) for more than 16 h at 37° C (ref. 4); (3) ribonuclease (L. Light and Co.) for more than 16 h at 37° C (ref. 5); (4) methanol–chloroform for 16 h at 37° C (ref. 6).

All sections were stained with nuclear fast red[7] and acriflavine[8], toluidine blue *p*H 3·8 (ref. 9), and 0·01 per cent azure *A*[10], amido black 10*b* (ref. 11), alcian blue[12] and gallocyanin chromalum[13] and hæmatoxylin eosin. The methylene blue extinction test[14] and the gentian violet reaction[15] were used to estimate basophilia.

All the long bones of the birds in this series contained medullary bone, though this was generally scanty in the humerus, due to the intrusion of the calvicular air-sac. In one case, however, larger amounts than usual were found. Variations in the amount of medullary bone were noted in different birds, but the variations appeared to be random and could not be correlated with the position of the egg in the tube. This confirms the finding of Bloom *et al.*[1]. Variations in the quantity and appearance of this intensely periodic acid–Schiff-positive material were also observed, but again no correlation between this and the position of the egg could be distinguished. It was noted, however, that the diffuse non-fibrillar phase was characteristic of the second half of the period of shell deposition, although not confined to this phase.

A strong periodic acid–Schiff-positive reaction indicates the presence of complex polysaccharides with the 1,2-glycol linkage, including glycogen, mucopolysaccharide, mucoprotein and glycolipoid and, possibly, also nucleic acids. The unlikely presence of the latter in this material is negatived by the fact that the intensity of staining is not reduced by prolonged pre-treatment with ribonuclease, which results in hydrolysis of both RNP and DNP[5] and also by the failure of the material to stain with gallocyanin chromalum. Glycolipoids and glycogen are eliminated by pre-treatment with methanol–chloroform and diastase respectively and it would seem, therefore, that this material is a mucopolysaccharide or mucoprotein. Because of its high protein content, the latter might be expected to stain with amido black, and failure to obtain a reaction with this dye suggests that the substance is a mucopolysaccharide. This receives confirmation from the finding that it exhibits metachromasia with toluidine blue and azure *a*. Under the conditions of this latter staining technique metachromasia is exhibited mainly by sulphated mucopolysaccharides but also by hyaluronic acid (through its carbonic acid radical) and by nucleic acids (through phosphoric acid radicals). The latter are eliminated by their lack of response on treatment with ribonuclease and hyaluronic acid by the continuing periodic acid–Schiff response without change after 16 h hydrolysis in hyaluronidase. Furthermore, there is a positive reaction with alcian blue, specific for acid carbohydrates[12]. There is thus evidence that the periodic acid–Schiff-positive substance in avian medullary bone is a sulphated mucopolysaccharide. The failure to respond to hyaluronidase despite prolonged incubation is interesting, in view of the report by Meyer and Rapport[16] that testicular hyaluronidase is capable of hydrolysing the chondroitin sulphate component of cartilage.

This substance stains also by the nuclear fast red and by the acriflavine techniques, reactions which are regarded as being specific for sulphated mucopolysaccharides[7,8]. Again, basophilia with methylene blue at a *p*H of less than 2·5 tends to confirm this suggestion. However, there is evidence against the view that this basophilia necessarily indicates sulphated mucopolysaccharides[17]. Further confirmation with regard to the nature of this substance is found in the fact that a reaction is obtained in alcohol-fixed material stained for 24 h in a solution of gentian violet at a dilution of 1 : 1,280,000. Harada[15] has claimed that only sulphated mucopolysaccharide will respond at this dilution, the carboxylated mucopolysaccharides failing to respond to dilutions greater than 1 : 250,000. It is interesting, however, to note that this response is obtained only with alcohol-fixed material, the susa-fixed tissue

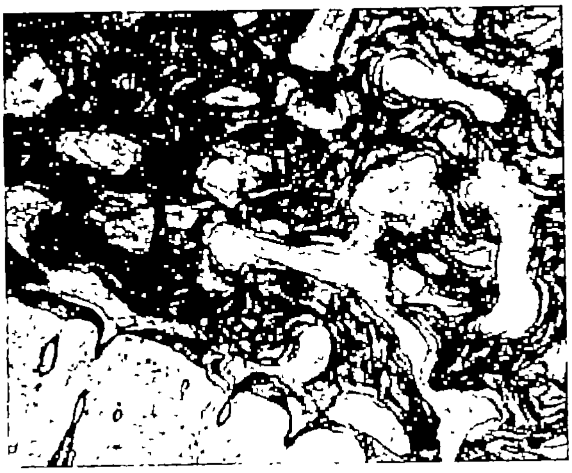

Fig. 1. Section showing cortical and medullary bone from the femur shaft of a laying hen. The specimen was taken shortly after an egg had been laid and prior to the next ovulation. Note the strongly periodic acid–Schiff-positive strands of sulphated mucopolysaccharide incorporated in the medullary bone matrix. (Hæmatoxylin and periodic acid–Schiff)

failing to react at dilutions greater than 1 : 250,000. The significance of this is not understood.

Irving[18], using sudan black following pre-treatment with pyridine, alcohol or benzol, has demonstrated the presence of a line of sudanophil material (calcification line) at the surface of developing intramembranous bone. This substance is periodic acid–Schiff-positive and metachromatic and thus appears to be an acidic mucopolysaccharide. In formalin-fixed material, however, it could not be stained with methylene blue at low *p*H values (2·5) without pre-treatment in one or other of the foregoing reagents and it is thus distinguished from the mucopolysaccharide of avian medullary bone. Moreover, the sudanophil material described by Irving[18] fails to stain with alcian blue and is, in any event, confined to surfaces at which appositional growth is occurring, being separated from the bone only by a thin layer of pre-osseous matrix. Clearly, therefore, this substance is not identical with the periodic acid–Schiff-positive material of the labile avian medullary bone and, although there is evidence that the latter substance varies in amount and appearance at different stages of the egg-laying cycle, it does not appear to be associated with calcification in the same way as the sudanophil material of developing mammalian bone.

Investigation of the cell picture in the medullary bone (predominance of osteoblasts or osteoclasts) is continuing in an attempt to correlate more accurately the changes in the appearance of the periodic acid–Schiff-positive substance with the phases of shell deposition.

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RADIOBIOLOGY

Alkaline Earths in Heparin

Heparin is widely used as a blood anticoagulant. In the determination of calcium, in plasma, for example, it is commonly assumed that heparin has no effect on the assay. We have recently found, however, that the strontium content of heparin could be a very substantial correction in the determination of the strontium content of heparinized plasma. This result stimulated us to determine the calcium, strontium and barium content of five different preparations of heparin which were available here.

The heparin for assay was taken as solid (when possible from an unopened ampoule) prepared by Boots Pure Drug Co., Ltd., or Evans Medical, Ltd. Calcium was determined directly by flame spectrophotometry. Strontium and barium were determined by neutron activation analysis. The results are shown in Table 1.

Table 1. Calcium, Strontium and Barium in Heparin in p.p.m.

Heparin batch No.	Calcium	Strontium	Barium
64373	2,930	92	4
64373/1	2,810	84	—
68424	390	5	2·5
66306	700	7·5	12
D 25200	300	9	8

It will be seen that there was a considerable range in calcium, strontium and barium depending on the particular preparation. If, for example, 20 mg of heparin (batch No. 64373) equivalent to about 2,400 units were added to 100 ml. of human blood, the adventitious calcium would be approximately 60 μg per 50 ml. plasma or 1·2 per cent. The strontium in the heparin would be nearly 2 μg and the barium 0·1 μg or about 100 per cent and 10 per cent respectively of the normal strontium and barium content of the plasma.

The results given in Table 1 are sufficient to show the importance of analysing each preparation of heparin when it is to be used as an anticoagulant for the estimation of alkaline earths in plasma and that the amount of heparin used should not be appreciably greater than that required to prevent clotting of the blood.

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BIOLOGY

Relation between Carcass Composition and Live Weight of Sheep

A re-examination of the work of Hammond[1], Wallace[2,3], Pálsson and Vergés[4,5], and Wardrop[6], suggests that the carcass composition of a sheep, in terms of dissected bone, muscle and fat, is more closely related to its live weight than to its age. Using all the available data from these papers, including ewes and wethers irrespective of breed, age, stage of pregnancy and nutritional history and ram lambs up to the age of 2 weeks, the following regression equations have been computed for estimating the body composition of sheep from empty live weight (that is, the weight of the live animal at slaughter minus the contents of its alimentary tract):

$$\text{(i)}\ \frac{\text{Weight of carcass bone } (y_1)}{\log y_1 = 0\cdot722 \log x - 0\cdot706} \tag{1}$$

$$\text{(ii)}\ \frac{\text{Weight of dissected carcass muscle } (y_2)}{\log y_2 = 1\cdot018 \log x - 0\cdot554} \tag{2}$$

$$\text{(iii)}\ \frac{\text{Weight of dissected carcass fat } (y_3)}{\log y_3 = 1\cdot539 \log x - 1\cdot843} \tag{3}$$

Equations (1), (2) and (3) account for 96·1, 98·0 and 88·3 per cent, respectively, of the variance of the dependent variable ($\log y_1$, $\log y_2$, $\log y_3$) contributed by its relation to the independent variable ($\log x$). In each case, x and y are in kg, x being empty live weight. The data on which these regression equations are based, together with the regression lines, are shown on a log–log grid in Fig. 1. Data on individual sheep have been used, where available, but otherwise it has been necessary to use group means.

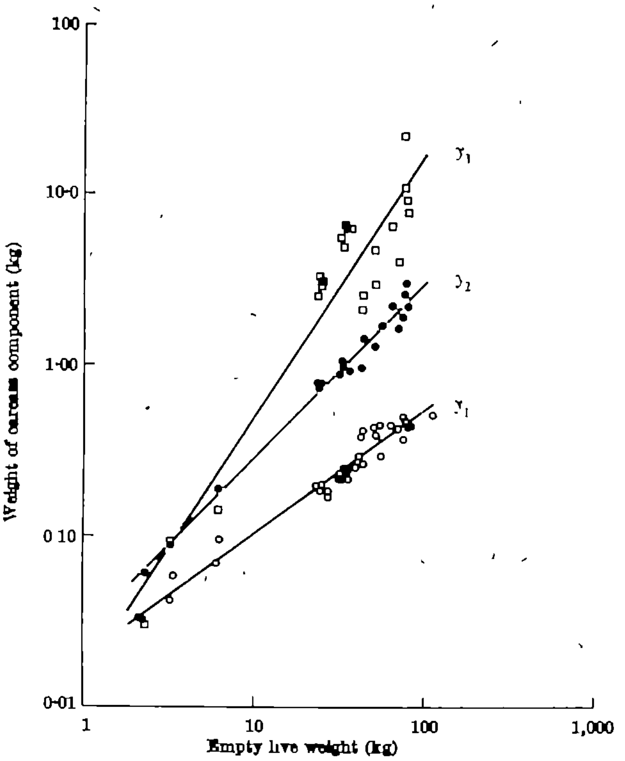

Fig. 1. Weight of dissected carcass bone (○), muscle (●) and fat (□) compared with empty live weight of sheep. The regression lines have been plotted using the equations given in the text

These equations are of the same form as Huxley's[7,8] allometric equation. They indicate that, as a sheep increases in empty live weight, each of these tissues also increases in weight. However, the slopes of these lines show that, as empty live weight increases, the percentage of carcass fat also increases, but the percentage of carcass bone decreases, whereas the percentage carcass muscle remains almost constant. The equations imply also that as a sheep loses weight, changes in body composition will result from the loss of all three tissues, the losses being given by these equations.

These equations all suggest that there is a constant differential growth ratio[7,8] between each carcass tissue and empty live weight. However, pooling of mass observations, as has been necessary here, may obscure changes of slope[9] which could occur within individual animals. In Fig. 1 the deviations from the regression lines are large enough to suggest that no equation is likely to be sufficiently accurate in its predictive value to adequately represent all sheep for any one carcass component. It will be necessary to investigate breeds and strains of sheep within various environments to establish more accurate regression equations.

By growing sheep at different rates and killing them at the same live weight, Pálsson and Vergés[4,5] were able to demonstrate the profound effects of plane of nutrition on the shape of young sheep. However, as indicated here, these various planes of nutrition had small effects on carcass composition. In addition, these treatments did not affect the weights of the commercial cuts of meat in the carcasses of these sheep. This evidence suggests that the shape of a sheep is of doubtful importance from a commercial point of view.

Similar conclusions have been reached following an examination of published data on cattle and pigs. It is proposed to publish this work in detail elsewhere.

I thank Mr. R. Seebeck for computing the regression equations.

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A Secondary Induced Thiamine Deficiency in Mink

NUMEROUS reports attest to the occurrence of thiamine deficiency in fur-bearing animals due to destruction of the dietary source of the vitamin by thiaminase activity. Fresh-water fish were first implicated as sources of the thiaminase enzyme, and their presence as major diet ingredients for foxes led to the definition of characteristic thiamine deficiency symptoms as 'Chastek paralysis'[1–3]. Ranch mink fed similar rations developed corresponding symptoms[4,5]. Extension of the diet sources used with mink led to the recognition that marine fish might also be troublesome as contributors of thiaminase activity[6] and at present a number of different fish species are listed as containing significant amounts of thiaminase. These have been assembled by Harris[7], who reports 28 fresh-water and 8 salt-water fish species as thiaminase-active.

Another distinct deficiency disease in mink, termed 'cotton-fur', has been shown by Stout *et al.*[8] to occur when two other fish species, Pacific hake (*Merluccius productus*) and Atlantic whiting (*M. bilinearis*), were fed as ingredients in an otherwise satisfactory basal mink diet. This condition has been identified as an induced iron deficiency and has been shown to respond to parenteral iron therapy[9]. Mink fed raw hake have characteristically become progressively anaemic and emaciated, but have retained a fair appetite and normal muscular activity. Hake has never been implicated as a thiaminase-containing fish species, yet there has been some controversy as to whether whiting contains thiaminase activity[10,11].

This communication describes a situation wherein mink placed on a hake diet formulated to produce the 'cotton-fur' anomaly developed typical symptoms of Chastek paralysis, and relates the thiamine deficiency to the feed consumed by the hake just prior to their being caught.

A group of 34 standard dark mink housed individually in wire pelter cages were placed at weaning in mid-July on a diet composed as shown in Table 1. This diet was formulated on the basis of past experience as causative of the 'cotton-fur' condition, due to its content of Pacific hake.

In marked contrast to previous 'cotton-fur' studies, mink in this instance evidenced early anorexia. Within 3 weeks of first receiving the test diet, they refused the major portion of their daily diet offering. Concurrently animals became extremely emaciated and loss due to death occurred. Since the ration was known to induce iron-deficiency, five mink were each injected with 25 mg of iron ('Armidexan', Armour Veterinary Laboratories) intra-

Table 1. Composition* of Experimental Ration

	%
Pacific hake	50·00
Turbot	15·00
Mixed rockfish	10·00
Horsemeat	7·00
Wheatgerm meal	4·50
Meat meal	3·24
Soybean oil meal	3·24
Ground oat groats	3·24
Alfalfa meal	2·34
Skim milk powder	1·44
	100 00

* Calculated on an 'as fed' basis.

muscularly; of these, three died indicating no therapeutic value of iron. Death losses continued and necropsy revealed complete depletion of internal fat stores and livers which appeared mottled and pale pink to bright orange in colour.

Individual mink exhibited signs of tetany when handled and showed other symptoms of Chastek paralysis. In checking the possibility of thiamine deficiency, four animals which showed varying degrees of paralysis were each given an intraperitoneal injection of 5 mg of thiamine hydrochloride. A rapid response to this treatment was noted and mink regained control of their muscles and several commenced eating within about 0·5 h of administration of thiamine.

Since Pacific hake had not previously been listed as a thiaminase-containing fish and since the remaining diet ingredients were known to be innocuous relative to production of thiamine deficiency, it was presumed that some alteration of this particular lot of hake had occurred. Examination of the viscera showed the stomachs of the hake to be distended and filled with anchovies (*Engraulis mordax*). No reference as to thiaminase activity of this latter small fish species is available; however, the striped anchovy of the Gulf of Mexico (*Anchoa hepsetus*) does contain the thiaminase factor[12]. A number of the hake stomach contents were removed, weighed and calculated to constitute approximately 14 per cent of the weight of the entire fish. As hake made up 50 per cent of the test diet, the anchovies were present to the extent of about 7 per cent of the total diet, as fed. Apparently this level was sufficient to destroy the dietary thiamine with the result that Chastek paralysis occurred. Afterwards, stomach contents of hake were removed, remaining animals were given 5 mg of thiamine hydrochloride intraperitoneally and the experiment was successfully continued to completion. Growth curves (Fig. 1) illustrate weight loss and subsequent improvement (following thiamine administration and anchovy removal) of mink fed contaminated hake rations as compared with growth of animals fed hake free of anchovies or an adequate control ration.

This experience is further confirmation of the potency of thiaminase in rendering biologically inactive the thiamine content of other dietary ingredients. More significant, however, it provides evidence of a secondary induced thiamine deficiency and demonstrates that not only the primary fish components of the diet, but also the stomach contents of such fish need to be considered as potential sources of thiaminase activity. In this regard it is notable that Neilands[13] found that stomach contents of two perch species showed considerable destruction of thiamine *in vitro* while the fish tissue *per se* was devoid of such activity.

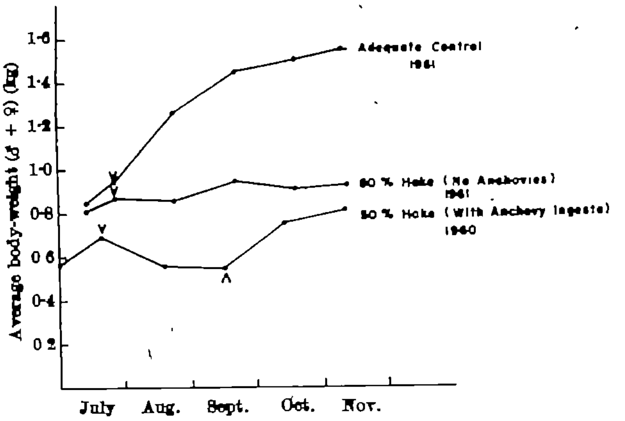

Fig. 1. Growth curves of mink fed fish diets with and without anchovy contamination. V, On test ration; Λ, injection of B_1 and removal of anchovies

This incident may help to explain the disparity between observations of thiaminase activity appearing in previous literature. Green *et al.*[14] observed that whiting caused Chastek paralysis in foxes fed this fish as part of their ration whereas others[10,11] were unable to demonstrate thiaminase activity of whiting *in vitro*. Quite possibly the whiting in the former case had ingested enough thiaminase-containing fish to be harmful while the latter fish were free of such contaminants.

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Circadian Adrenal Cycle in C Mice kept without Food and Water for a Day and a Half

CERTAIN rodents (as also is the case with man) show circadian (about 24-h; *circa, dies*[1-3]) rhythms in many body functions. Prominent among these rhythms is the adrenal cycle, gauged indirectly by observations on functions influenced by corticosteroids[4,5] and directly by determinations of corticosterone in serum[6-9] and in the adrenal gland itself[10]. Investigations of an indirect index, the blood eosinophil rhythm, suggested that the adrenal cycle persists in mice kept under conditions which gravely alter the œstrus cycle, such as the prolonged 50 per cent reduction in dietary calories[11]. This communication summarizes shorter-term work with a more direct index, that is, the level of corticosterone in serum and adrenals. Circadian periodic changes in these indices as well as in rectal temperature and pinnal mitoses continue in *C* mice deprived of all food and water for 36 h.

Two experiments were performed on a total of 192 female *C* (Bagg albino) mice, 3–4 months of age. This strain has been maintained at the University of Minnesota Medical School by brother-to-sister mating for more than 12 years. For each experiment, the mice were housed singly in temperature-controlled 'periodicity rooms', illuminated only by fluorescent lights for a 12-h period each day (0600–1800). All the animals were standardized for 6 days on this regimen and, thereafter, groups of mice were killed at 4-h intervals. At each of these time points rectal temperature was measured and immediately thereafter the mice were killed for collection of blood, adrenals and ears.

Sampling in a first experiment started at 0400 and ended 24 h later. In this experiment the animals had food and water available throughout the sampling period. In another experiment begun at 0800 of one day and completed at 2000 of the next, food and water were removed about 3 h prior to start of sampling.

The times indicated with the results are mid-points; actual sampling at any one time-point extended over a period of about 1 h. At intervals of several minutes cages were removed from the periodicity room to an adjacent laboratory in which temperature measurements by thermistor-bridge circuit and other procedures were carried out. Following decapitation by scissors, the blood was allowed to drain from the neck into a centrifuge tube. Blood from 6 animals was pooled and the serum obtained. Corticosterone in serum pools and in pairs of adrenals from individual mice was determined according to the fluorometric method of Silber, Busch and Oslapas[12]. This method has been qualified elsewhere as to specificity, and the usefulness of data transformed into a percentage of series mean (relative values) particularly for periodicity analysis also has been documented[7]. Data were subjected to analysis of variance.

In keeping with earlier work on other species[13,14], the circadian rhythm in rectal temperature stood out clearly in starving and thirsting mice, as well as in mice kept with food and water available *ad libitum*. As further anticipated in the light of earlier less-extensive unpublished work from this laboratory, the circadian rhythm of pinnal mitoses also was maintained in *C* mice deprived of all food and water for 36 h.

Fig. 1 demonstrates the extent of agreement in absolute values of serum corticosterone between the two pools from each of the groups investigated (left half), and the group means expressed as relative values, namely, as a percentage of the mean of the first 6 values, the latter being equated to 100 per cent (right half). A rhythm in animals that had food and water available *ad lib.* until death is readily seen. The peak value at 1600 agrees well with that recorded earlier for mice of the *C* strain and of other inbred stocks studied under similar conditions, notably a regimen of light from 0600 to 1800, alternating with darkness[4,7,10].

The results of starved animals show a progressive increase in level of corticosterone during the 36-h period of examination. Whether dehydration underlies this trend is not known. In any event, the trend does not obscure two peaks separated by about 20–24 h from each other, peaks that stand out more clearly in the relative values. The statistical significance of these rhythmic changes is supported by the results of an analysis of variance. Time is indeed a significant source of variation in the serum corticosterone-levels of both groups ($P < 0{\cdot}01$).

The persistence during a 36-h period of food and water deprivation of the rhythm in adrenal corticosterone, in turn, is demonstrated in Fig. 2 by the average values per gland pair and the *S.E.*, plotted against time.

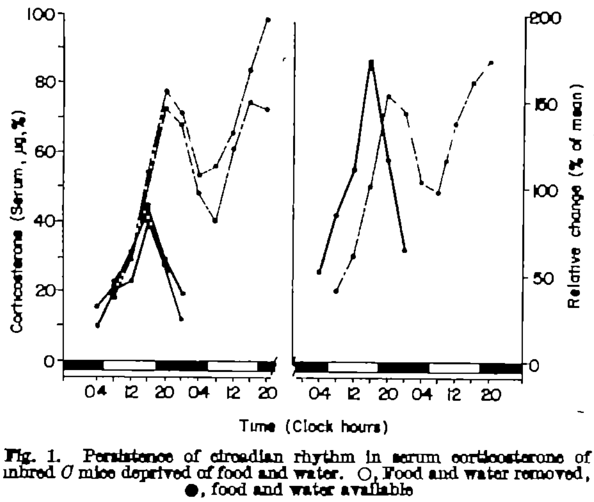

Fig. 1. Persistence of circadian rhythm in serum corticosterone of inbred *C* mice deprived of food and water. ○, Food and water removed, ●, food and water available

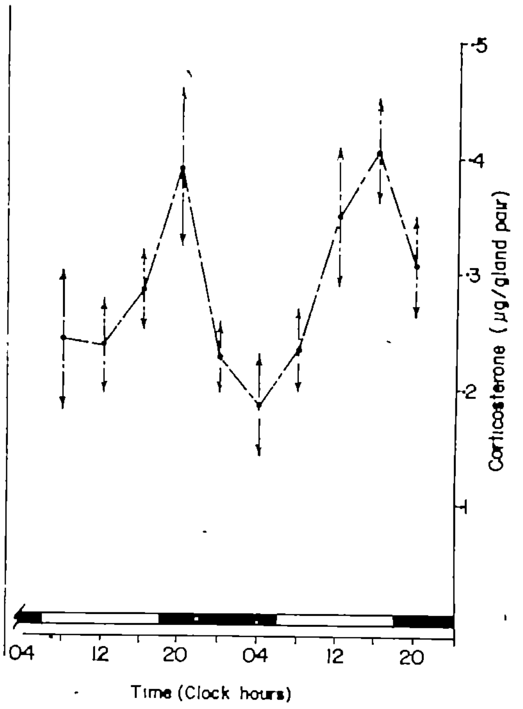

Fig. 2. Persistence of circadian rhythm in adrenal corticosterone of inbred *C* mice deprived of food and water (means and *S.E.*)

Figs. 1 and 2 as a whole, summarizing an examination of 192 mice, can be interpreted to suggest that the adrenal cycle[1,4,11], gauged by hormone contents of blood and gland, persists, at least for a while, in *C* mice subjected to starvation and water deprivation. To discuss a possible difference in the timing of circadian rhythms in the two groups of mice studied is not justified without added data over more extended periods.

Awaiting further study is also the question whether the overall serum hormone-level around which rhythmic changes recur undergoes a consistent change during longer periods of thirsting and starving.

On the practical side, the present results serve to re-emphasize[1] objections to a not uncommon assumption made in the design of many experiments, tacitly if not explicitly. In certain endocrine and metabolic investigations, among others, it has been presumed that circadian rhythms can be 'eliminated' by starvation. Common designs in these fields involve the removal of food, with or without the removal of water for a period of 12–24 h. Such designs have their uses for aims other than the 'elimination of rhythms'. For further comments on this point, the reference should be made to a study summarizing about twenty years of work by Chossat[15], awarded the prize for physiology by the French Academy of Sciences in 1843. This author demonstrated the persistence of circadian periodic behaviour in the cloacal temperature of pigeons until the day of their death from food and water deprivation. In a mammal, the mouse, a circadian susceptibility rhythm also has been shown to persist in animals deprived of food or water, at least for 36 h[16]. This point of methodological and theoretical interest must be kept in mind in work on the adrenal cycle of *C* mice as well as on their circadian rhythms in rectal temperature and pinnal mitoses. Periodicity analysis is required for a safe description of changes in periodic functions, including studies made from the point of view of starvation *per se* or with the view of other conditions studied in starved animals, as was suggested and practised already by Chossat[15]. Moreover, the basic nature of a circadian temporal co-ordination in physiological function is further supported by documentation of the persistence of rhythms at various levels of organization in a thirsting and starving mammal.

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Transmission of anti-*Brucella abortus* Agglutinins across the Gut in Young Mice

IT has been recorded[1] that antibodies from hyper-immune anti-*Salmonella pullorum* sera prepared in various species were absorbed from the gut of young rats at significantly higher rates than antibodies from hyper-immune anti-*Brucella abortus* sera. The absorption rates of these antibodies depended more on the antigen used in their production than on their species of origin. A similar conclusion was thought to apply to young mice when it was observed that the absorption from the gut of antibodies from hyper-immune anti-*Brucella abortus* serum prepared in a rabbit was markedly less than that of other rabbit γ-globulins. If the relative rates of absorption of antibodies from the gut are expressed in concentration quotients which are the ratios of the concentrations of antibodies in the serum of the animals fed and in the immune serum fed, the maximum concentration quotients obtained after feeding 11-day hybrid $C_{57}Bl \times A_2G$ mice with rabbit anti-sheep red cell agglutinins[2] or with rabbit normal γ-globulin[3] were 1/64–1/128, whereas the maximum concentration quotients obtained after feeding with rabbit anti-*Brucella abortus* agglutinins were < 1/1,024. This phenomenon was investigated further, and preliminary results are presented here.

The concentration quotients for feeding to 11-day $C_{57} \times A_2G$ mice or to 12-day albino rats 0·01 ml. of rabbit anti-*Brucella abortus* serum per gram body-weight are given in Table 1. Agglutination titres of the immune serum administered and of the sera of the experimental animals, killed 4 h after feeding, were determined by the saline dilution technique and by a modification of the Coombs test[4] using a high-titre goat anti-rabbit-serum-proteins serum. Since the latter test measures the total amount of complete and incomplete antibodies in the test sera, it is evident that the antibody in the immune serum administered is mostly of the complete type. Complete antibody could not be detected in the sera of the fed animals, whereas the incomplete antibody could be measured easily and, in the mice, gave concentration quotients equal to those for feeding rabbit normal γ-globulin or anti-sheep red cell agglutinins.

The sedimentation behaviour of the antibodies in the immune serum used, and in the pooled serum of mice fed with the immune serum, was investigated in the ultracentrifuge by the method described by Hemmings and Jones[5]. The test sera were diluted to a protein concentration of 2 per cent and layered on to a 20–12 per cent sucrose gradient in a tube of the *S.W.* 39 head of a 'Spinco' model *L* centrifuge and spun for 18 h at 30,000 r.p.m. This resulted in the separation of the heavy and light components of the test serum in the sucrose gradient, which was afterwards fractionated into a series of 40 samples. Alternate fractions were titrated against *Brucella abortus*, while the remaining fractions were used for protein determinations by the method of Lowry *et al.*[6]. The sedimentation profiles of the sera with the distribution of the anti-*Brucella* titres superimposed are shown in Fig. 1, which indicates that the distributions of the antibody titres of the test sera are all very similar with medians in the 7*S* region.

Rabbit anti-*Brucella* serum was injected intravenously at doses of 0·01 ml./g body-weight to each of four 11-day $C_{57} \times A_2G$ mice, two of which were killed 5 min later, and their sera pooled and titrated. The others were killed after 4 h and their sera pooled and titrated. The results (Table 2) show that the titre of the complete anti-

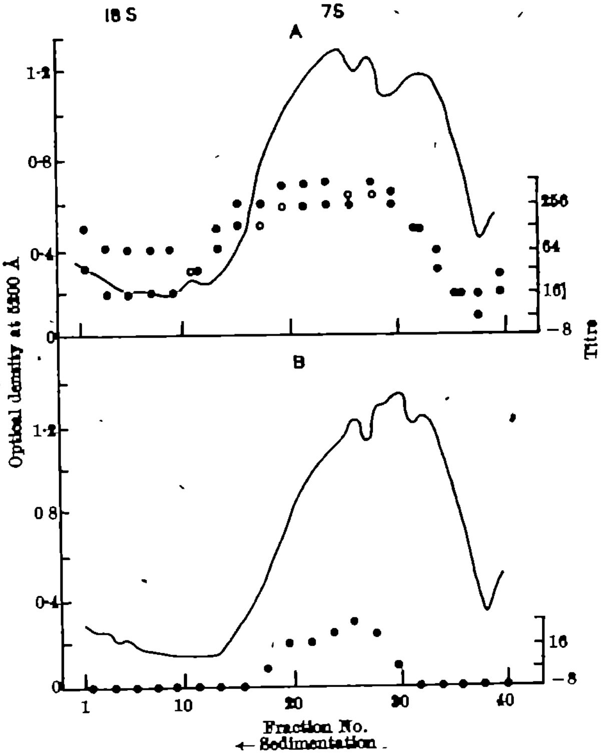

Fig. 1. Profile of sedimentation of (*A*) rabbit anti-*Brucella* immune serum and of (*B*) the serum of young mice fed with the immune serum. The continuous line represents relative protein concentrations expressed in optical density. ○, Saline agglutinating titres; ●, Coombs agglutinating titres

Table 1. CONCENTRATION QUOTIENTS FOR YOUNG MICE AND RATS FED WITH RABBIT ANTI-*Brucella* AGGLUTININS

Animals fed	Agglutinin titration	Titre of serum fed	No. of experimental animals having serum concentration quotients of:			
			1/32	1/64	1/128	1/1,024
Mice	Saline	4,096	0	0	0	6
	Coombs	4,096	1	4	1	0
Rats	Saline	4,096	0	0	0	2
	Coombs	4,096	0	2	0	0

body in the sera of young mice remains unchanged during the 4-h test period. Consequently, differences, if any, in the nature of the antibodies fed to young mice and those measured in their circulations 4 h later will have been effected in the absorptive cells of the gut rather than in the circulation.

Table 2. ANTIBODY ACTIVITY OF THE SERA OF YOUNG MICE INJECTED INTRAVENOUSLY WITH RABBIT ANTI-*Brucella* SERUM

Interval after injection	Serum titre	Dilution of antibody in serum
5 min	Saline = 512	1/8
	Coombs = 512	1/8
4 h	Coombs = 512	1/8
	Saline = 512	1/8

It has been established that the activity of the immune serum used is due primarily to 7*S* and 18*S* complete antibodies, and possibly to 7*S* and 18*S* incomplete antibodies, whereas the activity of the sera of young mice fed with the immune serum is due to 7*S* incomplete antibodies. Consequently, the transmission of rabbit anti-*Brucella* agglutinins across the gut may involve any or several of the following possibilities:

(1) During transfer across the absorptive cells the 18*S* antibodies may be degraded into the 7*S* incomplete type. An analogous change *in vitro* would be the decomposition of 18*S* saline agglutinating Rh antibody by the action of mercaptan into 7*S* Coombs sensitizing antibody[7].

(2) A configurational rather than a degradational change from the 7*S* complete antibody into a 'more acceptable' 7*S* incomplete type may be effected by the absorptive cells. A similar explanation has been suggested to account for the observation that bovine γ-globulin, which had reached the circulation from the gut of young rats, entered the circulation in relatively larger amounts when fed a second time to other young rats[8].

(3) The absorptive cells may exhibit selection between 7*S* complete and incomplete antibodies and exclude the former from the circulation. If this were the case, such a high degree of selection between two antibodies prepared in the same species would be hard to reconcile with the much lower degree of selection exhibited between antibody molecules of different antibody activities prepared in different species[8], unless the effect of variation in the configuration of the antibody reactive portions[9] of 7*S* antibody molecules on selection overrides that of the species-specific parts.

The inability to measure the incomplete antibody activity of the immune serum used, due to the higher concentration of the complete antibody, precluded hitherto any assessment of the relative importance of these possibilities. Further investigation of the problem is desirable, especially in view of the light it might throw on the mechanism of the transmission of Rh antibodies across the human placenta.

I thank Prof. F. W. R. Brambell for his advice and Mr. W. A. Hemmings for carrying out the ultracentrifugal run. We are indebted to the Rockefeller Foundation for providing the Spinco ultracentrifuge.

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An Anomalous Infection of Mice with *Schistosoma mansoni*

MICE exposed percutaneously to cercariæ of *S. mansoni* develop an infection which can be well standardized. For the past three years we have exposed albino mice, kept in identical conditions of diet and housing, to about 200 cercariæ of a Venezuelan strain[1] which led to the development of a bisexual infection in the portal and mesenteric veins and the deposition of eggs in the liver, intestinal walls and occasionally in the lungs.

In one experiment on a group of ten mice, in spite of a normal bisexual development of the trematodes, no eggs could be found in any of the organs of the hosts. In this experiment all cercariæ were shed by a single snail, *Australorbis glabratus*. The total number of cercariæ shed by the snail, which died the following day, was about 2,000, much higher than the usual number encountered in the average experiment, that is, to 600–700 a day. The snail itself was infected with 5–10 miracidia.

Eight mice were autopsied 55–140 days following infection and no ova were found in any of their organs. Perfusion of the liver and the mesenteric circulation[1] revealed that the 33–83 (average 65) schistosomes counted per mouse were of normal size, many of them copulated, with a ratio of 42–63 per cent (average 52 per cent) males. Slides of the fresh livers and intestines examined under microscope showed no eggs, nor did the trypsin–pepsin digestion of the organs followed by exposure to intense light in river water liberate miracidia[2]. The reproductive organs of the schistosomes stained with 0·005 per cent methylene blue solution were normal in size and number in the males, as were the ovaries and the vitelline glands in the females. However, in the uterus at the site where normally the ovum is located an irregular mass without shell was noted. Two of the mice of this group infected ten months ago are still alive, though animals infected with the same inoculum usually do not survive more than two months.

It has already been shown that induced nutritional deficiencies in guinea pigs[3] and in mice[4] may cause variations in the sexual organs of the schistosomes, and that in certain hosts[5–8] hermaphroditism of the male occurs; but we cannot find a precedent for complete sterility in mouse, a species allowing the development of a bisexual infection with deposition of eggs. We exclude nutritional influences in the present case and we think that the sterility, presumably due to the anomaly noted in the female schistosomes originated from cercariæ shed by the same snail, may have been determined by the miracidium. It is known that the sex of the parasite is predetermined in the miracidium, which forms the cercaria[9], and it could be assumed that the deformation resulting in sterility may depend on a genetic variation in the parent miracidium, which may have been the same individual for all the (female) cercariæ of the infection.

We thank Dr. Eugenio Gerulewicz, Ministerio de Sanidad y Asistencia Social, Caracas, for the *Australorbis glabratus* strain.

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Effect of Dietary Calcium Lactate and Lactic Acid on Fæcal *Escherichia coli* Counts in Pigs

CERTAIN strains of *E. coli* have been isolated[1] from pigs which have died from bowel œdema. Strains of the same organism are involved[2] in certain baby pig scours. It has been reported[3], in investigations of the intestinal flora of growing/fattening pigs, that the count of *E. coli* was inversely related to those of lactic acid producing bacteria, chiefly *Lactobacillus bifidus*. Significant growth increases with pigs were obtained[4] with milk inoculated with *Lactobacillus* sp. when compared with that inoculated with *Streptococcus* sp.

Two trials were conducted in the present work. The first trial was carried out during November–December 1961 over a four-week period. Three pairs of pigs from three separate litters, and weighing initially 70–80 lb. live-weight, were housed in one pen which was equipped with individual feeding accommodation. The pigs were fed the same basal diet which had the following composition: 7½ per cent fish meal, 5 per cent soya bean meal, 5 per cent bran, 15 per cent pollard, 25 per maize, 42 per cent barley, 0·5 per cent vitamin/trace mineral premix. Three pigs, one from each pair, were fed the basal diet which had 2 per cent of the barley replaced with 2 per cent calcium lactate, Bow-Calac., Ltd., Widnes. The basal diet for the three control pigs was adjusted to the same calcium content as the lactate diet by the addition of ground limestone, again in place of barley. The pigs were fed twice daily on a scale of feeding which was related to their live-weights[5]. Freshly voided fæces were collected after the morning feeding and transported as soon as possible to the laboratory. *E. coli* counts were made daily for 5 days each week, on 1-g samples of the wet fæces for a four-week period. During the third and fourth weeks dry matters and *p*H were also recorded on the fæces. The mean weekly *E. coli* colony counts/g wet fæces, with the mean percentage fæcal dry matter, and daily live-weight gains, are given in Table 1.

Table 1. MEAN *E. coli* COUNTS ($\times 10^6$) PER G OF WET FÆCES, DAILY LIVE-WEIGHT GAINS, AND MEAN PERCENTAGE FÆCAL DRY MATTER (IN BRACKETS)

	Pig No.	Week 1	Week 2	Week 3	Week 4	Daily light-weight gain (lb.)
Control	1	1·08	9·03	46·44 (25·7)	21·03 (25·5)	1·14
	3	17·06	2·32	4·36 (26·7)	1·23 (27·4)	1·54
	5	10·75	10·38	16·60 (25·8)	10·70 (28·0)	1·32 Mean 1·33
Lactate	2	3·17	0·36	29·68* (21·0)	0 44 (23·8)	1·29
	4	15·27	0·50	0·25 (28·0)	0·09 (24·9)	1·25
	6	5·19	2·29	0·23 (26·5)	0·03 (29 0)	1·54 Mean 1·36

* Pig 2 scoured during third week

A second trial was conducted, for an 8-week period, during April–June 1962, using three pairs of pigs from each of three litters and weighing initially 60–70 lb. *E. coli* counts were made as in the first trial for five days/week for the first two weeks. This was reduced to twice weekly for the remainder of the trial. The pigs were housed in the same pen and fed twice daily as in the first trial. Since small but variable amounts of lactic acid were found in the calcium lactate used, and because only one pig seemed to be responding to the lactate in this trial, lactic acid (B.P.), 2 g/lb. of diet, was given to the lactate pigs from the fifth to eighth weeks. The data are given in Table 2.

Table 2. MEAN *E. coli* COUNTS ($\times 10^6$) PER GRAM OF WET FÆCES, DAILY LIVE-WEIGHT GAINS, AND MEAN PERCENTAGE FÆCAL DRY MATTER (IN BRACKETS).

Pig	No.	Week 1*	Week 2	Week 3	Week 4	Week 5†	Week 6	Week 7	Week 8	Daily live-weight gains (lb.) Weeks 1–4	Weeks 5–8	Weeks 1–8
Control	1	48·90 (19 3)	18 62 (21·2)	21·33 (24 0)	51·15 (23·1)	27·45 (22 6)	8·37 (22·6)	26·72 (24·1)	9·24 (24·2)	1·29	1·39	1·34
	3	118·25 (25·5)	23·71 (28 9)	20·13 (28·5)	26·85 (27·8)	39·90 (24·9)	23·55 (28·6)	39 67 (29·1)	12·80 (27·3)	1·39	1·46	1·43
	5	87·85 (23·6)	79 76 (26·4)	0·57 (27 8)	5·78 (26·5)	4 99 (25·3)	2 32 (24·3)	1·15 (27·8)	5 61 (28·0)	1·43 Mean 1·37	1·54 Mean 1·46	1·48 Mean 1·42
Lactate	2	449·32 (24·7)	93·19 (24·5)	6·30 (28 9)	32·70 (26·1)	1·80 (22·3)	0·93 (25 9)	0·29 (28·4)	0·35 (28·9)	1·29	1·64	1·46
	4	57·06 (20·0)	4·38 (23 7)	0 09 (26 6)	0·39 (24·8)	0·17 (23·2)	0·036 (28·5)	0·04 (25·9)	0·067 (26·2)	1·50	1·54	1·52
	6	272 75 (23·1)	113·38 (16·8)	14·66 (19 9)	7·11 (17·7)	20·80 (22·1)	9·90 (25·9)	11·67 (25·9)	0·235 (29·8)	1·29 Mean 1·36	1·61 Mean 1·60	1·45 Mean 1·48

* Control diet fed to all pigs during the first week.
† Lactic acid, 2 g/lb. of diet, was added to the lactate diet from 5th week onwards.

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Unidirectional Response to Angular Acceleration recorded from the Middle Cristal Nerve in the Statocyst of *Octopus vulgaris*

YOUNG[1] suggests that the three orthogonally arranged sections of the crista of the statocyst of *Octopus vulgaris* could function as detectors of angular acceleration in a manner similar to the semicircular canals of vertebrates. He also suggests that the macula could provide continuous information about the position of the head. That the statocyst in cephalopods indeed participates in the control of equilibrium and posture has been shown behaviourally[2,3]. Now we have recorded from the middle cristal nerve of the statocyst the activity of single units of the longitudinal crista that respond unidirectionally to angular acceleration.

We suspended a disarmed octopus in a tank with circulating sea-water, removed the left eye and cut the cranial cartilage down to the statocyst nerves, leaving a thin layer of cartilage on top. Next, we removed mantle and viscera, leaving attached to the aorta only the cranium with the statocysts, brain and right eye, and perfused aerated sea-water through the aorta. The preparation and the micro-electrode holder were mounted on a turntable which allowed smooth rotation and tilting while recording. The statocyst was placed in the centre of rotation. The electrodes were thick-walled micropipettes filled with Wood's metal and plated with platinum black.

We recorded from the left statocyst of thirteen animals and studied more than 60 units, isolated or in groups.

In the absence of stimulation the electrode passes through the nerve without recording spontaneous activity until the surface of the sac is reached and some cells are killed. Otherwise, if one taps the table gently while penetrating the nerve, one finds units that respond with a series of 4 or 5 bursts with 3–4 spikes per burst for each tapping, at a frequency of about 30 bursts/sec. If several units are recorded simultaneously their responses appear phased. These units are highly sensitive to low-frequency vibrations transmitted through the ground but do not respond to air-borne sounds. These same units responded with a large discharge (100 spikes/sec) to a counter-clockwise rotation in the horizontal plane (as seen from above) and with a smaller discharge (45 spikes/sec) at the end of a clockwise rotation. A sinusoidal movement elicited a response that extended from the end of the clockwise through part of the counter-clockwise phase. Increasing the frequency of the sinusoidal movement caused a rise in the frequency of discharge and the recruitment of new units. Recruitment also occurs with vibrational stimulation. That different units have different thresholds is apparent from the recruitment. All this is illustrated in Fig. 1. (1) The absence of resting activity; (2) the response to the counter-clockwise rotation that subsides before the end of the swing (*B*); (3) the response to the end of the clockwise movement which begins before the stop or reversion of the swing (*A* and *C*); (4) the complete adaptation at the cessation of movement (*A*). It is apparent that the effective stimulus was angular acceleration in the counter-clockwise direction. A cinematic analysis considering the phase relation of the response to the angular displacement (θ), the angular velocity ($\dot{\theta}$) and the angular acceleration ($\ddot{\theta}$) makes this obvious. These experiments, however, do not prove that these receptors respond to angular acceleration only. They could also respond to unidirectional linear acceleration. If this were the case the two accelerations could be distinguished by comparing the activity in the two statocysts.

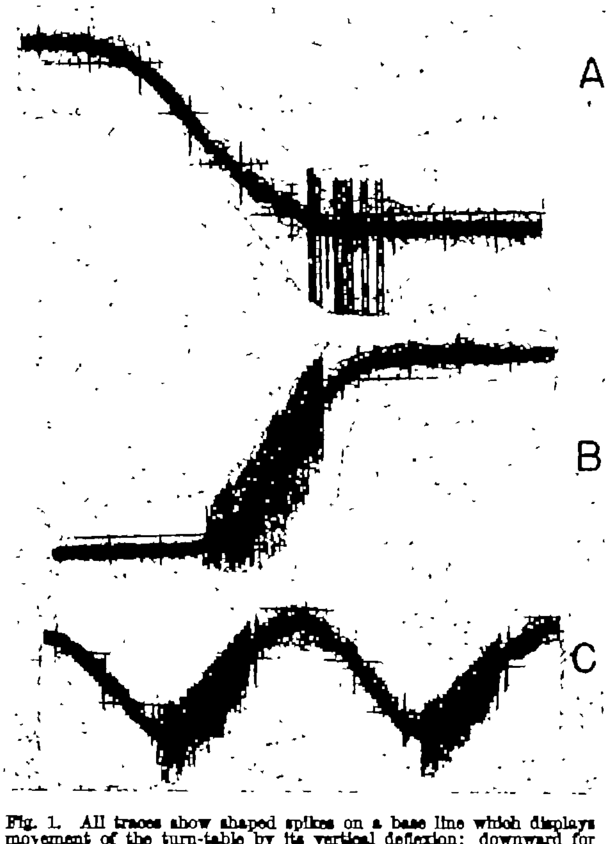

Fig. 1. All traces show shaped spikes on a base line which displays movement of the turn-table by its vertical deflexion: downward for clockwise and upward for counter-clockwise rotations

Young[1] expected the longitudinal crista to detect rotations around the longitudinal axis through a vertical deflexion of the horizontally placed cupula. Our recordings from the middle cristal nerve issuing from it indicate that this crista at least responds to rotation around the vertical axis and that these rotations can cause an adequate movement of the endolymph and cupula (whichever this movement is). However, we have not examined rotations around the other axes and we do not yet know which is the optimal stimulus for this section of the crista, nor if we can deem the responses of each section of the crista distinct enough for each of them to indicate rotations in one plane only.

We do not know which cells we recorded from, but we presume that the most likely are the large hair cells, which have the largest axons[1]. In the crista there are also some second-order neurones that send their axons to the brain[1]; but we are inclined to believe that these perform a more complex function and that we did not record from them. Whichever is the case, two facts stand out: (1) That the same unit responds to two modes of stimulation, namely, to low-frequency vibration and to angular acceleration. These differences in response pattern could be used for their central distinction. A similar duality of response is found in statocyst receptors of crustaceans[4]. (2) That there is no resting activity and that the response to angular acceleration is unidirectional. Whether this unidirectionality is due to a mechanical property of the statocyst, the cupula or the hair cells or to the interaction of the latter with local neurones, we do not yet know, but this property and the absence of resting activity make this system different from the known equilibrium systems of vertebrates[5] and crustaceans[4].

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Vectorcardiogram of *Anguilla anguilla* L. and *Pleurodeles waltlii* Mich.

PREVIOUS investigations[1–4] on the epicardial electrocardiogram of fishes (*Anguilla anguilla* L., *Ameiurus nebulosus* Raf., *Cyprinus carpio* L., *Salmo irideus* Gibb., *Acipenser sturio* L., *Scyliorhinus canicula* L. and *S. stellare* L.), of larval Urodeles (*Ambystoma tigrinum* Green) and adult Urodela (*Pleurodeles waltlii* Mich., *Triturus cristatus* Laur. and *Salamandra maculosa* Laur.) showed that the general activation front in the epicardial layers moves approximately from the heart basis towards the apex in the fishes and in *Ambystoma tigrinum*, and from the apex towards the basilar region in *Pleurodeles waltlii*, *Triturus cristatus* and *Salamandra maculosa*.

Furthermore, it was observed by the simultaneous recording of epicardial and intracardiac leads that the stimulus spreads at the same time into all layers of the cardiac muscle and not from the sub-endocardial layers outwards, as in the mammalian heart.

In order to confirm the aforementioned results an investigation of the direction of the cardiac vectors in the different planes was undertaken. For this purpose frontal and sagittal plane QRS vectorcardiograms were recorded in a fish, *Anguilla anguilla*, and in a Urodele, *Pleurodeles waltlii*; from these tracings the spatial direction of the stimulus was reconstructed.

The investigations were carried out on 12 specimens of *Anguilla anguilla* and on 4 of *Pleurodeles waltlii*. The animals were narcotized with 20 mg/kg of amobarbital sodium intramuscularly, and put into an earthed Faraday cage.

For the recording of the vectorcardiogram the cube arrangement developed by Grishman[4] and at present used for human beings was utilized; according to this system the electrodes are placed so as to represent four of the corners of a cube having the dipole centre at its centre.

The electrodes were placed as follows:

(*A*) Vertical bipolar lead: the electrodes were located cranially and caudally to the heart.

(*B*) Horizontal bipolar lead: two symmetrical plates were placed subcutaneously laterally to the heart.

(*C*) Sagittal bipolar lead: two plates were placed subcutaneously dorsally and ventrally to the heart.

The composition of *A* and *B* gave as a result the vectorcardiogram in the frontal plane, while from *A* and *C* the vectorcardiogram in the sagittal plane was obtained. Care was taken, so far as possible, to apply the statement of Gillard *et al.*[5], according to whom the electrodes are regarded as ideally placed when they are located at the same distance from the heart—the distance between each pair being the same—and the distance between heart and electrode about five times the heart's radius.

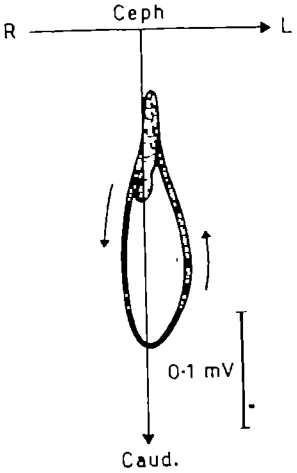

Fig. 1. QRS vectorcardiogram of *Anguilla anguilla* L. in the frontal plane. *R*, right; *L*, left; *Ceph.*, cephalic extremity; *Caud.*, caudal extremity

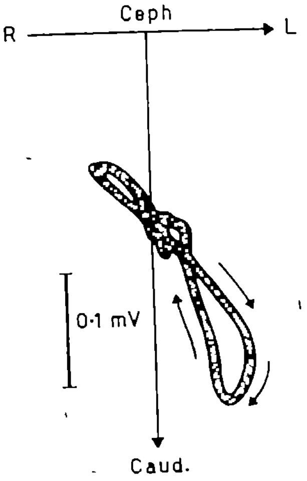

Fig. 2. QRS vectorcardiogram of *Anguilla anguilla* L. in the sagittal plane

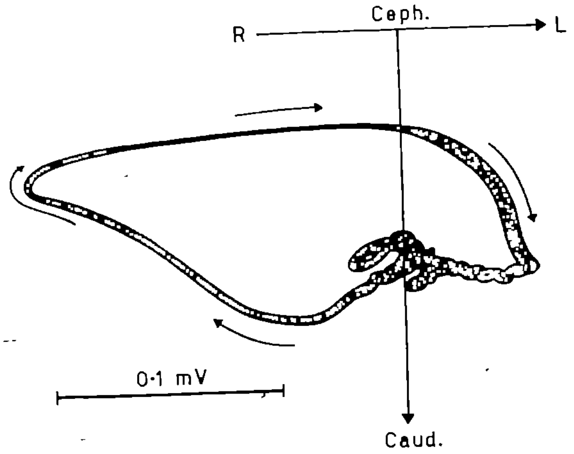

Fig. 3. QRS vectorcardiogram of *Pleurodeles waltlii* Mich. in the frontal plane

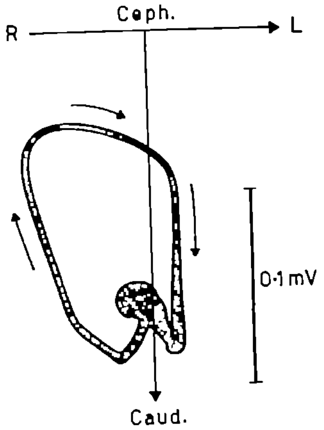

Fig. 4. QRS vectorcardiogram of *Pleurodeles waltlii* Mich. in the sagittal plane

The electrodes were made of stainless steel needles and silver plated; the diameters varied from 5 to 10 mm in conformity with the size of the animal.

All the recordings were performed by a dual-beam oscilloscope Tektronix 502.

In Figs. 1–4 the morphology of the QRS vectorial loops in the frontal and sagittal plane for each of the species examined is illustrated.

As well as the morphological characteristics of the vectorial loop itself, the spatial direction of the excitation wave, as can be ascertained by the analysis of the different tracings, must be stressed. In the case of the eel it can be seen that the stimulus starts in the base of the heart and spreads into the apical region, moving from right to left and from back to front.

In Pleurodeles, on the contrary, the excitation wave starts from the apical region, moves backwards and rightwards, spreading at last into the basal region leftwards and forwards.

Thus, these vectorcardiographical investigations confirm what has been established by scalar electrocardiography concerning the activation of the epicardial layers.

It can therefore be admitted that the general action of the fishes' heart is clearly different from that of Pleurodeles, where the activation front is approximately directed from the apex to the base, thus resembling the behaviour of several mammals.

The patterns observed in Pleurodeles is very likely the same for all the Urodela: in fact, either the epicardial electrocardiograms or the frontal and sagittal vectorcardiograms recorded on few specimens of *Triturus* and

Salamandra allow us to postulate the existence of a general apico-basilar direction of the activation front.

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ENTOMOLOGY

Incorporation of ^{14}C-Acetate into Scent Constituents by the Green Vegetable Bug

THERE is rapidly growing interest in the nature and functions of the hypodermal glands of insects, which produce not only pheromones but also chemicals which are used in defence. Short-chain aldehydes, both saturated and unsaturated, the paraffin tridecane[1-4] and possibly furans and quinones[2] have been identified in the repugnant secretion (scent) of a number of species of the hemipterous super-family Pentatomoidea. In this group the scent is believed to play a defensive part[1,4]. Nothing is known of the precursors of the scent nor whether the constituents are synthesized by the insect or merely concentrated from the sap which many species pass through their digestive tract in quantity. It has been pointed out that some of the scent substances are present in low concentration in the plants on which the bugs feed[1]. The present experiments were carried out in order to determine whether acetate, the main building unit in lipid metabolism, is involved in the production of the scent constituents.

The contents of the scent storage sac of the green vegetable bug, *Nezara viridula* var. *smaragdula* (F.), consist of a mixture of two liquid phases, one almost colourless (actually a very light yellow) and the other a distinct yellow. The phases are easily separated by centrifugation. The yellow phase has the higher density and has been shown by gas chromatography to contain some 13 volatile constituents, not all of which need be present in any one sample of scent. Since most of the constituents have not yet been identified, we have numbered them in order of increasing retention times on an 'Apiezon-*L*' column. Peak 12 is always a prominent constituent in both phases, and has been identified as *n*-tridecane[1]. Peak 10 has the same retention time as *n*-dodecane. Peak 11 is *n*-dec-2-enal[1] and peak 5 is *n*-hex-2-enal[1]. Peak 6 forms a dinitrophenylhydrazone with properties resembling those of α,β-dicarbonyl compounds[1].

In an experiment designed to detect utilization of acetate for the biosynthesis of scent, 42 adult bugs (both sexes) were each injected with 4 μc. of sodium acetate-1-^{14}C (specific activity 32 mc./mM) in 1 μl. of water. Thirty-six of these had been previously depleted of scent by electric shock stimulation (exposure for two brief periods to 50 V with 5 pulses/sec of duration 50 msec); 13 survived for 8 days. Of the 6 unshocked bugs, 4 survived. After 8 days the living bugs were dissected and scent collected by inserting a fine glass capillary into the scent storage sac which lies ventrally between the metathoracic legs. The capillaries were sealed and stored at −40° C for several months. The depleted sacs, the paired accessory glands and a little adjacent cuticle and fat body were stored in a test tube in 1 ml. pentane under the same conditions.

The capillary collected scent was transferred into 1 ml. of pentane and an aliquot counted in an Ekco scintillation counter. The total count (2,000/sec) showed that only 0·2 per cent of the injected carbon-14 had been incorporated into the scent. A 0·25-ml. aliquot of the pentane solution was evaporated at room temperature in a stream of air to a volume of about 25 μl. Counting of a 1 μl. aliquot of the concentrate showed that about 80 per cent of the carbon-14 had been lost during the evaporation. Four microlitres of *n*-tridecane and 25 μl. of the pentane solution of capillary drawn scent were added and the sample injected into a Pye Preparative Argon Gas Chromatograph run at 100–125° with a column of 10 per cent 'Apiezon-*L*' on 'Celite'.

The effluent gas from the gas chromatograph was passed through 9-mm. diameter glass U-tubes packed with about 7 cm. of 2-mm. diameter glass beads and containing 0·5 ml. of toluene at room temperature. Tests with *n*-hexanol-1-^{14}C have shown that this system is as efficient (about 50 per cent) as one cooled in dry ice and ethanol for trapping quantities of 30 μg or more, although quantities of less than 1 μg are trapped with only about 5 per cent efficiency. Each peak indicated by the gas chromatograph was collected in one or more tubes, and valleys between peaks were also sampled. All collections gave some increase in count above the background count, but peaks 6, 7 and 8 gave counts 4 × to 7 × background, and peak 12 (tridecane) was 100 × background. Two later gas chromatographic runs of the same radioactive material with the addition of several microlitres of non-radioactive scent to improve the trapping efficiency of minor constituents showed significant radioactivity in peaks 5, 6, 7, 8, 9, 10, 11, and 12.

Broadly comparable results were obtained with the pentane solution from the residual scent sacs and associated tissues. This initially had a total activity of about 5,000 counts/sec, but, when vacuum distilled into a liquid air trap, 90 per cent of the activity proved to be non-volatile. The pentane distillate was concentrated by slow evaporation at room temperature, which caused 25 per cent loss of the carbon-14 content, and several microlitres of cold scent were added to improve trapping efficiency. Significant radioactivity was found in peaks 4, 5, either or both of 6 and 7 (which did not separate clearly), 9, 10, 11, 12 and 13.

Scent was collected in a capillary from a total of five bugs which had been injected 9 days previously with 1 μc. of either sodium decanoate-1-^{14}C, sodium caproate-1-^{14}C or sodium propionate-1-^{14}C. The pooled scent gave significant counts (greater than 1·5 × background) in peaks 5, 6, 12 and 13 and barely significant counts (between 1·2 × and 1·5 × background) for peaks 7, 8, and 11.

Significant radioactivity has thus been found at one time or another in all peaks except 1, 2 and 3, which each contain so little material that significant activity would not have been detected unless these constituents had been particularly 'hot'. It is, therefore, clear that *Nezara* is capable of incorporating acetate into the major components of the scent (including hexenal, the unknown dicarbonyl, decenal and tridecane) and does not rely on concentrating unchanged, preformed materials from the sap of the various plants on which it feeds in order to produce its characteristic scent.

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MICROBIOLOGY

Effect of Chilling on Bacteria in Aqueous Suspension

CERTAIN bacteria in the logarithmic growth phase become non-viable when chilled in aqueous suspension. The phenomenon has been observed with strains of *Escherichia coli*[1], *Pseudomonas pyocyanea*[2] and *Aerobacter aerogenes*[3]. Loss of viability of logarithmic phase *A. aerogenes* suspended in chilled phosphate-saline is accompanied by the leakage of endogenous solutes, and the leakage products, in sufficient concentration, protect the bacteria against the lethal effect[3]. Thus, loss of viability is greater the sparser the population and is insignificant in chilled suspensions containing more than 5 × 10^8 organisms/ml. Correlation between loss of viability and leakage of endogenous solutes has now been obtained by observing the effects of chilling on three Gram-negative bacteria.

Escherichia coli strain Jepp, *Aerobacter aerogenes* NCTC 418 and a laboratory strain of *Serratia marcescens* were used. Cultures growing exponentially were obtained by seeding flasks (1·5 litre) containing 100 ml. of either a defined mannitol–ammonia–salts medium[4] or tryptic meat broth with 4 ml. of a 16-h culture in the same medium, and shaking for 2–3 h at 37°. Bacteria were separated from the culture and washed twice with phosphate-saline (0·11 M sodium chloride and K_2HPO_4 + KH_2PO_4 (0·02 M —PO_4), *p*H 6·5) by centrifugation, then resuspended in this diluent (about 10^{10} bacteria/ml.). The effect of chilling on viability was assessed by adding washed bacterial suspension (0·1 ml.) to cold phosphate-saline (10 ml.) held in a temperature-controlled bath at 0° ± 0·25°; samples were removed at intervals for determination of viability by slide culture[5] on tryptic meat broth agar. Denser suspensions were used to examine the leakage of substances from chilled bacteria: washed suspension (4–5 × 10^9 bacteria/ml.) was rapidly cooled to near 0° by contact with brine at −10° and placed in the bath at 0°; at intervals, samples (5 ml.) were quickly filtered through well-washed Oxoid filter membranes (grade *AP*). Filtrates were analysed for ultra-violet absorption at 260 mμ, adenosine triphosphate with the firefly luminescence technique[6] and ninhydrin-reacting substances[7]. As controls, filtrates separated from similar suspensions at 20° were analysed.

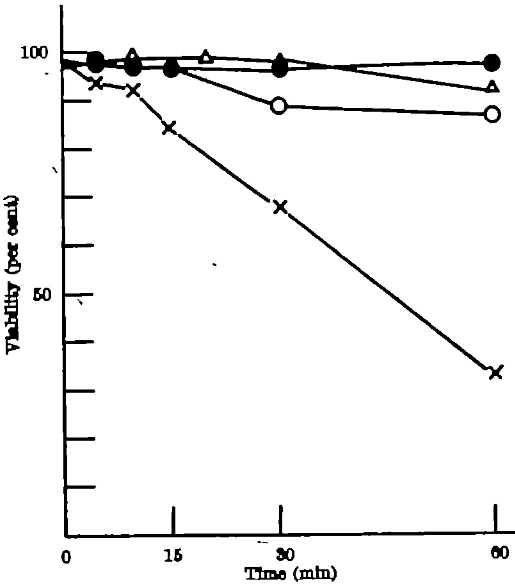

Fig. 1. Changes in viability of bacterial suspensions at 0°. Washed exponential phase bacteria were held in cold phosphate-saline, *p*H 6·5 (about 10^8/ml.). *A. aerogenes* NCTC 418 grown in a defined medium (×) and tryptic meat broth (○); *E. coli* Jepp grown in both media (●); *S. marcescens* grown in tryptic meat broth (△). Viabilities were determined by a slide culture technique (ref. 5)

Fig. 1 shows changes which occurred in the viability of suspensions containing about 10^8 exponential phase bacteria/ml. during 1 h at 0°. When grown in the defined medium, *A. aerogenes* were markedly susceptible to chilling whereas the organisms were more resistant when grown in tryptic meat broth; *E. coli* grown in either medium or *S. marcescens* grown in tryptic meat broth were almost completely resistant. The viabilities of similar suspensions of these organisms at 20° remained unchanged during 1 h.

At concentrations of about 5 × 10^9 bacteria/ml. of phosphate-saline no loss of viability of these organisms occurred during 1 h at 0°. However, when compared with similar suspensions at 20°, there was a considerable leakage of ultra-violet-absorbing substances (including adenosine triphosphate) and ninhydrin-reacting substances from *A. aerogenes* grown in the defined medium, much less from this organism grown in tryptic meat broth (Table 1). Leakage from *E. coli* at 0° was less than 20° (Table 1) and about the same at these temperatures from *S. marcescens*. Thus, on chilling bacteria in relatively dense suspension, the extent of leakage was related to the loss of viability which occurred in more dilute suspension (Fig. 1). Since the adenosine triphosphate concentration in filtrates of chilled *A. aerogenes* reached a maximum in 30 min and then decreased (Table 1), resorption, adsorption or breakdown of this substance must have occurred.

Loss of endocellular constituents on chilling bacterial suspensions may be a cause but is not an effect of bacterial death since leakage occurs from bacteria in relatively dense suspensions which remain viable. Presumably, in dilute suspension, the concentration of leakage products in the suspending fluid is too low for resorption to occur and loss of bacterial viability results. It is of interest that exponential phase *Aerobacter aerogenes* grown in a complex medium were more resistant to chilling than organisms grown in a defined medium.

Table 1. LEAKAGE OF ENDOGENOUS CONSTITUENTS FROM WASHED EXPONENTIAL PHASE *Aerobacter aerogenes* NCTC 418 AND *Escherichia coli* STRAIN JEPP HELD IN PHOSPHATE–SALINE AT 20° AND 0°

Results refer to filtrates from suspensions containing 2 mg bacterial dry weight/ml.

Organism and growth medium	Temperature of suspension	Ultra-violet absorption of filtrate (log I_0/I at 260 mμ 1 cm light path). Time (min)				ATP μg/ml. filtrate Time (min)				Ninhydrin-reacting substances as alanine (μg/ml. filtrate) Time (min)				% Viability after 60 min (slide culture, ref. 5)
		0	15	30	60	0	15	30	60	0	15	30	60	
A. aerogenes from defined medium (ref. 4)	20°	0·12	0·17	0·23	0·37	0	0	0	0	2·8	3·3	4·1	5·4	99
	0°	0·12	0·49	0·63	0·65	0	0·02	0·05	0·05	2·8	10·7	12·6	13·0	99
A. aerogenes from tryptic meat broth	20°	0·09	0·12	0·13	0·22	0	0	0	0	2·3	3·4	3·8	5·7	98
	0°	0·09	0·15	0·16	0·24	0	0·02	0·07	0	2·3	4·0	5·7	5·0	98
E. coli Jepp from defined medium (ref. 4)	20°	0·11	0·17	0·25	0·37	0	0	0	0	2·8	3·9	5·0	6·2	99
	0°	0·11	0·09	0·11	0·10	0	0	0	0	2·8	3·5	4·7	2·8	98

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Nuclear Size of Live and Dead Bovine Spermatozoa

THE size distributions of spermatozoan nuclei are of interest from two points of view. On one hand there is the question of the identification and separation of X and Y chromosome bearing spermatozoa, and on the other hand the relation of nuclear size to total content of chromatophilic substances measured by the plug or core method of microspectroscopy. We do not wish to become involved in the former question; but are interested in the latter in connexion with work on the DNA content of bovine spermatozoa being examined in this laboratory.

We have adopted the van Duijn[1] formula for determining nuclear areas. This method has been shown to yield a high correlation with areas determined by planimetry[2]. The regression of optical density of Feulgen-stained bovine spermatozoa on nuclear area is negative and highly significant[3]. Under some experimental conditions 51 per cent of the variation in optical density could be explained by this regression. Suspecting that dead cells might be an important factor in this regression, an experiment was designed to determine the size and Feulgen staining characteristics of live and dead spermatozoa. This communication reports the nuclear size relationships.

Live and dead spermatozoa were differentiated by the eosin method of Lasley, Easley and McKenzie[4] but omitting the background stain. Areas on each slide containing 10 or more live (eosin-negative) and 10 or more dead (eosin-positive) cells were mapped so that the individual cells could be relocated. The slides were then fixed, hydrolysed and stained by the Feulgen method following normal procedure[5]. The process of fixation in acetic alcohol and hydrolysis in 1 N hydrochloric acid effectively removes all the colour from the eosin-stained spermatozoa as determined by visual inspection, but there is an effect of eosin treatment on the apparent DNA content of the cell. After relocating the fields, length, maximum width and base width of the Feulgen-stained, mapped sperm heads were measured with an ocular micrometer. Twenty-eight slides were examined, representing three different semen samples, with sub-samples ranging in age from fresh to 13 days old (stored in yolk-citrate at 5° C).

The dead cells were found to be larger than the live cells in all the dimensions studied. These differences were significant at the 0·001 level of probability, except for base width which was significant only at the 0·05 level. The means and standard deviations are given in Table 1. With the exception of the standard deviations for maximum width, all the means, standard deviations and ranges were larger for the dead cells than for the live ones. Nuclear areas were likewise larger for the dead than for the live sperm. The difference in area amounted to 9·78 per cent of the area of the live cells, and was significant at the 0·001 level of probability.

The distributions of live and dead cell nuclear areas are depicted in the histograms in Fig. 1. While the modes are distinctly different, there is a large overlap of areas in the two arrays. The sampling method used does not permit any exact statements with regard to bimodality of the sperm populations studied, but if the two distributions are pooled on the assumption of a single population containing 53 per cent live cells, no evidence of bimodality can be shown.

Thus it can be seen that nuclear area of a spermatozoon cannot be taken as *prima facie* evidence that the cell was live (or dead) at time of fixation. However, the large differences between mean areas and the larger standard deviations of dead cells may account for some of the differences in DNA and the greater variability in DNA content reported in the spermatozoa of infertile as contrasted to fertile males[6,7]. Certainly such studies in the future should include some estimate of the live cells present in the sample at fixation, for such differences in fertility may be due entirely to the paucity of live spermatozoa in the semen of infertile males and not to intrinsic differences in DNA content of those live cells which are capable of penetrating the vitelline membrane.

Table 1. MEANS AND STANDARD DEVIATIONS OF NUCLEAR DIMENSIONS AND AREAS OF LIVE AND DEAD BOVINE SPERMATOZOA

	Live	Dead	Difference (%)	Significance
No. of cells	300	279		
Length	8·53 ± 0·50µ	8·93 ± 0·55µ	4·69	***
Maximum width	3·98 ± 0·32µ	4·19 ± 0·28µ	5·28	***
Base width	1·80 ± 0·24µ	1·84 ± 0·27µ	2·22	*
Area	27·51 ± 2·06µ²	30·20 ± 2·73µ²	9·78	***

* Indicates significance at the 0·05 level of probability. *** Indicates significance at the 0·001 level of probability.

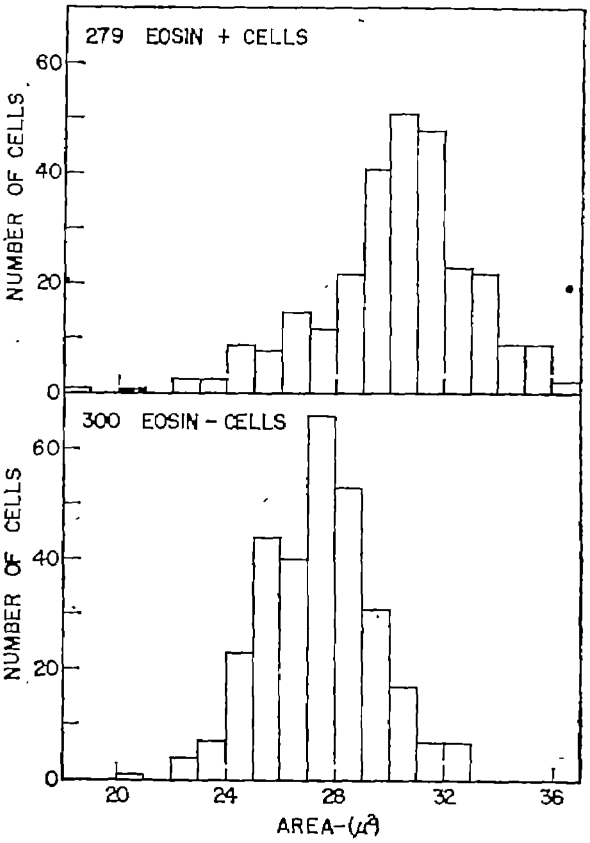

Fig. 1. Distribution of nuclear areas of dead (eosin-positive) and live (eosin-negative) sperm cells

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VIROLOGY

Measles Virus

COOPER[1] placed measles virus in the deoxyvirus group. There is now considerable evidence which is contrary to this view. The appearance of acridine-orange stained inclusions in measles virus infected cells under ultraviolet illumination suggests the presence of a ribovirus[2].

This is in agreement with the observations of Atherton[3]. Waterson *et al.*[4] reported similarity of measles virus to myxoviruses, under the electron microscope. Waterson[5] further showed that the three serologically related viruses, measles, canine distemper and rinderpest, are structurally identical with Newcastle disease virus (NDV), representative of a myxovirus sub-group. Further support for the similarity of measles virus and NDV is that these viruses undergo identical morphological changes during ether treatment, and the ability of measles virus to cause hæmagglutination.

Recent reports have shown that halogen derivatives of deoxyuridine interfere not only with normal deoxyribonucleic acid (DNA) synthesis in animal and bacterial cells but also specifically inhibit viral DNA synthesis and hence the multiplication of such deoxyviruses as herpes, vaccinia and polyoma. Cooper[1] suggested that 5-fluorodeoxyuridine may be useful in distinguishing deoxyviruses from riboviruses. In the present investigation of the nature of measles virus three derivatives of deoxyuridine, 5-fluoro-, 5-bromo- and 5-iodo-2′-deoxyuridine were used separately at a concentration of 20 μg/ml. in a study of plaque production in monolayers of *H.Ep2* cells by a known deoxyvirus (herpes strain *HFE* 253228–32), a ribovirus (arborvirus *AMM*2354) and measles virus.

Significant plaque reduction was recorded with herpes virus while no such effect was seen on plaque counts of arborvirus or measles virus, under the same conditions (Table 1). This is taken as evidence that measles virus is a ribovirus.

Table 1. PERCENTAGE PLAQUE REDUCTION IN THE PRESENCE OF HALOGEN DERIVATIVES OF DEOXYURIDINE

Inhibitor (20 μg/ml.)	Herpes virus	Arbor virus	Measles virus
5-Fluoro-2′-deoxyuridine	93	0	0
5-Bromo-2′-deoxyuridine	56	0	0
5-Iodo-2′-deoxyuridine	79	0	0

The internal component of measles virus has not been identified chemically; however, there is now a strong possibility that it is a ribonucleoprotein. If this were the case measles virus then falls into the NDV sub-group of myxoviruses.

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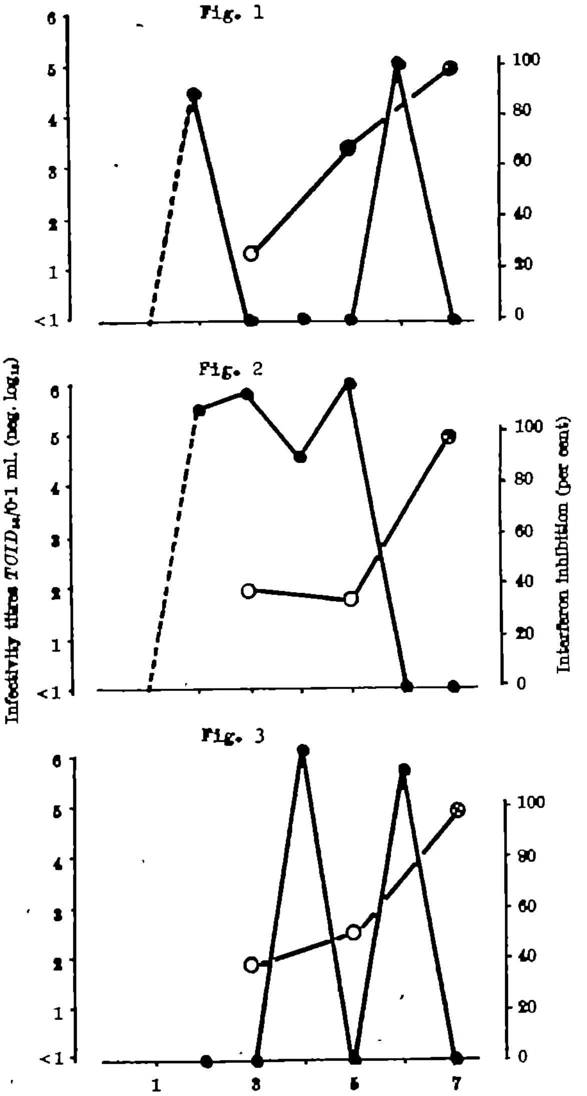
Effect of an Exogenous Interferon on Experimental Influenza Virus Infection and Interferon Production in White Mice

WE have investigated the effect of an exogenous interferon obtained from inbred albino mice (*asw* strain) after infection with a mouse-adapted influenza virus (strain *Em2*, type *A*1) on the dynamics of the influenza virus infection, in parallel with interferon production.

Exogenous interferon was obtained from lungs of mice infected intranasally with influenza virus containing allantoic fluid (50 per cent egg infective dose (EID_{50}) = 10[illegible]/0·1 ml.; dose = EID_{50} in 0·05 ml.) as described by Isaacs and Hitchcock[1]. Exogenous interferon was then concentrated according to Žemla and Vilček[2], lyophilized and resuspended in buffered broth (*p*H 7·2). Four drops of the resulting solution corresponded to yield of exogenous interferon from one mouse lung.

A control group and two test groups, each consisting of 18 inbred albino mice (*asw* strain) weighing 14–16 g, were inoculated intranasally under light ether anæsthesia. The control group was inoculated only with 2 EID_{50} of infected allantoic fluid, whereas the first test group of mice was treated 4 h prior to infection (2 EID_{50}) with four drops of exogenous interferon intranasally, and the second test group with the same dose of exogenous interferon 4 h prior to and 24 and 48 h after infection respectively. Starting with the second day after infection, 3 mice were killed from each group daily, and from their lungs 10 per cent suspensions were prepared in Horváth's[3] medium. The suspensions were clarified by centrifugation at 2,500 r.p.m. for 10 min and serial ten-fold dilutions of them inoculated into suspended chorioallantoic membrane tissue cultures according to Horváth[3]. The cultures were incubated at 35° C for 72 h and the reproduced virus detected by hæmagglutination test on plastic plates[4]. The presence of produced interferon was tested according to Isaacs and Lindenmann[5] in the corresponding lung suspensions of the third, fifth and seventh day after infection.

The results are shown in Figs. 1, 2 and 3. The titre of virus is expressed on the left in log $TCID_{50}$ values, the



Figs. 1, 2 and 3. ○, Non-significant interferon activity ($P > 0·05$), ●, significant interferon activity ($P < 0·01$)

activity of the interferon in percentage lowering of the control hæmagglutinating titres on the right scale of the diagram. It can be seen that the virus in the control group (Fig. 1) was first detected on the second day after infection. It disappeared on the third day and was not detectable up to the sixth day, in order to disappear again on the seventh day. The lack of the virus in lung suspensions on days 3, 4 and 5 is to be explained by the presence of an interferon on the fifth day[1-8].

In the first test group (Fig. 2) virus was found in the mice lungs from the second up to and including the fifth day. As an interferon could be found in the corresponding lung suspensions only on the seventh day we suggest that exogenous interferon suppressed the interferon production in comparison with the control mice and, as a consequence of it, virus multiplication could occur in the absence of interferon in the mice treated with exogenous interferon to a significantly larger extent than in controls.

In the second test group (Fig. 3) both virus multiplication and interferon production were delayed in comparison with the control group: virus was first detected in the lung suspensions on the fifth day, whereas significant interferon activity could be demonstrated only on the seventh day.

As has been seen in our experiments, exogenous interferon given in a single dose 4 h prior to infection suppressed the interferon production and, consequently, enhanced the virus multiplication, while the same dose of exogenous interferon, when given three times (that is, 4 h prior to, 24 and 48 h after, infection, respectively), suppressed not only the interferon production but also delayed the virus multiplication in the mice lungs.

There arises the question of whether exogenous interferon should have a similar effect also in human beings, and, if applied for a sufficiently long period, would be able to eliminate the virus multiplication at all.

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CYTOLOGY

Structure of Red-Cell Ghosts and the Effect of Saponin Treatment

The molecular organization of the various components of cell membranes, a frequent topic for theoretical speculation[1], is a difficult problem to approach by experimental methods. Little, if any, structural detail has been convincingly demonstrated in the plane of the membranes until the recent electron microscope investigations of Dourmashkin, Dougherty and Harris[2]. These workers discovered regular hexagonal patterns in membranes of various origin (Rous sarcoma virus, human and guinea pig erythrocytes, chicken liver cells), treated with saponin.

We have recently observed similar, and unexpected, hexagonal structures among the liquid-crystalline phases of lipid–water systems (lipids extracted from human brain)[3,4], and we have developed our own conclusions about the possible biological implications of non-lamellar organization of membrane elements. We thus welcomed the work of Dourmashkin *et al.* as a confirmation of our ideas, and thought it worth while to carry out similar experiments using X-ray diffraction techniques. Some preliminary results of this work are reported here.

Human erythrocytes, washed and suspended in phosphate buffer, pH 7·2, were hæmolysed either in hypotonic phosphate (0·01 M) or in the presence of saponin (0·05 per cent in water, 30 min at 37° C). The ghosts were then washed and centrifuged (30 min, 40,000*g*) several times and freeze-dried. We ascertained in a few experiments that freeze-drying had no drastic effect on the structures.

The X-ray diffraction experiments were carried out in a small-angle camera of the Guinier type[5] at various concentrations and at two temperatures: 22° C and 37° C. The samples were made by mixing fixed amounts of dry ghosts and water concentrations (c = weight of dry ghosts per gram of sample) were determined by dry-weight after the X-ray experiments.

With 'intact' ghosts, that is, not treated with saponin, two main concentration-ranges could be distinguished. From $c = 0·30$ to $c = 0·42$ a lamellar structure was present, characterized by 3–4 sharp reflexions, integral orders of a fundamental spacing : 170 Å at 22° C, 205 Å at 37° C. At higher concentrations, $0·42 < c < 0·70$, a second lamellar structure was found, in equilibrium with the previous one; the repeat distance was 64 Å. Samples at concentrations higher than 0·70 were not examined; at low concentration ($c < 0·30$) the scattered intensities became very weak.

In saponin-treated ghosts one type of X-ray diffraction diagram was observed, which varied very little with concentration and temperature. It was composed of a few sharp lines, the spacings of which were typical of a two-dimensional hexagonal lattice (ratios $1 : \sqrt{3} : \sqrt{4}$) (ref. 3), and of some fairly broad bands at spacings close to 58, 40 and 30 Å. The unit cell side of the hexagonal lattice was 165 Å.

The sharpness of the reflexions of the lamellar and hexagonal phases shows that the ordered regions must be fairly large in the three dimensions.

The results of the X-ray diffraction analysis were very similar to the electron microscope observations of Dourmashkin *et al.*[2]. With both techniques hexagonal structures were observed only in saponin-treated ghosts. The parameter of the hexagonal cell was almost identical : 140–160 Å in the electron microscope preparations, 165 Å in the X-ray samples.

The most exciting suggestion of the observations of Dourmashkin *et al.* is that intact membranes are hexagonal mosaics of various elements (lipids, proteins, cholesterol, water). The fact that this structure becomes visible in the electron microscope only after the action of saponin could be explained by assuming that saponin removes some of the membrane elements, leaving 'holes' accessible to negative staining. But such an interpretation can scarcely be reconciled with the X-ray observations: if a hexagonal structure were present in the 'intact' ghosts, it should be detected by the X ray diffraction techniques. We therefore find it difficult to escape the conclusion that the hexagonal structures are artefacts produced by the treatment with saponin.

It should be emphasized that our results are still preliminary. A detailed analysis of the phase diagram[3] and of the structure of 'intact' ghosts must await more complete experimental data. Furthermore, the effect of the various preparative procedures must be carefully investigated.

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France.

[1] Ponder, E., in *The Cell*, edit. by Brachet, J., and Mirsky, A. (Academic Press, New York, 1961).
[2] Dourmashkin, R. R., Dougherty, R. M., and Harris, R. J. C., *Nature*, **194**, 1116 (1962).
[3] Luzzati, V., and Husson, F., *J. Cell Biol.*, **12**, 207 (1962).
[4] Stoeckenius, W., *J. Cell Biol.*, **12**, 221 (1962).
[5] Luzzati, V., and Baro, R., *J. de Phys. et le Rad.*, **22**, 186, A (1961).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 25

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Stress and Psychosomatic Disorders".*

British Society for the History of Science (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. M. J. S. Rudwick: "The Foundation of the Geological Society of London (1807)".

Royal Institution (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. F. N. L. Poynter. "On Collecting Books for a Library of the History of Medicine".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. George Pendle: "Latin American Culture". (Second of three Cantor Lectures on "Latin America—World of Tomorrow".)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.15 p.m.—Prof. D. B. DeLoach (California): "Agricultural Technology and the Management of Surpluses".* (Fifth of eight lectures on "The United States of America".)

Institut Français du Royaume-Uni (at Queensbury Place, London, S.W.7), at 8.15 p.m.—French Scientific Films.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. W. W. Herbert: "In the Footsteps of Roald Amundsen".

Monday, February 25—Tuesday, February 26

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2)—Symposium on "Transient, Fluctuating and Distorting Loads and their Effect on Power Systems and Communications".

Tuesday, February 26

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Use of Plastics in Hydraulics".

Society for Analytical Chemistry, Physical Methods Group (in the Cuthbert Wallace Lecture Room, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2), at 6 p.m.—Meeting on "Fractionation of High Molecular Weight Compounds".

Royal Institute of Chemistry, London Section (joint meeting with University College Chemical and Physical Society, at University College, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. A. D. Walsh: "The Electronic Spectra of Small Molecules".

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. Sharples: "Recent Studies of the Crystallization of Polymers".

Society of Instrument Technology, Systems Engineering Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. A. E. Mitchell, Mr. H. H. Glaettli and Mr. H. R. Mueller: "Fluid Logic Devices and Circuits".

Royal Society of Edinburgh (in the Auditorium of the Station Hotel, Perth), at 7.30 p.m.—Mr. Arthur R. Wannop, O.B.E., F.R.S.E.: "More Food from Our Hills" illustrated by coloured lantern slides. (Meeting to inaugurate the Royal Society of Edinburgh Fund for the Promotion of Interest in Science and Technology in Scotland. Chairman: Prof. E. L. Hirst, F.R.S.)

Wednesday, February 27

Geological Society of London (joint meeting with the Institution of Water Engineers, at the Institution of Civil Engineers, Great George Street, London, S.W.1), from 2 p.m. to 7 p.m.—Symposium on "Groundwater".

Society of Chemical Industry (joint meeting with the Microbiology and Food Groups, at Queen Elizabeth College, Campden Hill Road, London, W.8), at 2 p.m.—Meeting on "Aseptic Filling".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. T. Norris: "High-Voltage Power-Transformer Insulation".

University of London (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. J. R. Anderson: "Auto-Immunity and Oral Pathology".* (Second of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. A. V. Lord: "The Present Position of the N.T.S.C. and SECAM Colour Television Systems".

Thursday, February 28

Royal Society (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "The Scientific Results of the Expedition to Tristan da Cunha, 1962", organized by Prof. W. Q. Kennedy, F.R.S.

Chemical Society (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Prof. A. R. Battersby: "The Biosynthesis of Alkaloids" (Tilden Lecture).

Institution of Mechanical Engineers, Applied Mechanics and Automatic Control Groups (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Joint Discussion on "Are Mechanical Engineers Slow in Adopting the Use of Computers ?"

Royal Institute of Chemistry, London Section (at the West Ham College of Technology, Romford Road, London, E.15), at 7 p.m.—Dr. T. S. West: "Modern Methods of Analysis—a Survey of Methods Based on Reagent Chemistry".

Friday, March 1

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Symposium on "The Experimental Taxonomy of the Pteridophytes".

Institution of the Rubber Industry (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5 p.m.—Mr. W. A. Tandy and Mr. F. Woods: "Some Aspects of Reclaim Technology". 6.15 p.m.—Dr. C. C. McCabe and Mr. R. H. Walsh: "A Sulphur Vulcanizable Ethylene Propylene Copolymer".

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. H. Beynon: "The Use of Mass Spectrography".

Society of Chemical Industry (joint meeting of the Food Group and the Manchester Section, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester), at 6.30 p.m.—Dr. E. C. Bate-Smith: "Thoughts on Meat" (Jubilee Memorial Lecture).

Royal Institute of Chemistry, London Section (joint meeting with the Brighton Technical College Chemical Society, at Brighton Technical College), at 7 p.m.—Prof. H. N. Rydon: "The Active Centres of Enzymes".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. F. A. Vick: "Harwell".

Saturday, March 2

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. Michael Sullivan: "Chinese Painting".*

Monday, March 4

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. A. Morgan Jones: "The Coronary Collateral Circulation".*

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Peter Haggett: "Coastal Deforestation in Brazil".

Institution of Electrical Engineers, Electronics Division P.G.E3 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Evaluation of Technologies for Fabricating H.F. Transistors" opened by Dr. J. R. Tillman, Mr. P. Beale, Mr. P. Charman and Dr. R. W. A. Scarr.

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. H. G. Conway and Mr. M. Hartland Thomas: "Engineering Problems of the Decimal and Metric Systems".

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Dr. P. W. Brian: "Two Biologically Active Fungal Metabolic Products. 1: Griseofulvin".

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "New Materials Which Could Play an Important Part in Railway Engineering".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. B. K. Blount: "Science in Latin America". (Last of three Cantor Lectures on "Latin America—World of Tomorrow".)

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. J. Pickthall: "About Perfumery"; Dr. B. Dudley Sully: "Perfumery Chemicals from Pinene".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturers (men or women graduates) in Physical and Inorganic Chemistry—The Assistant Secretary, Westfield College (University of London), Kidderpore Avenue, London, N.W.3 (March 1).

Assistant Lecturer in Physics; and an Assistant Lecturer in Sociology—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (March 2).

Assistant Lecturer in Zoology—The Registrar, Queen Mary College, Mile End Road, London, E.1 (March 4).

Lecturer (with research experience in nuclear physics) in Physics—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 4).

Assistant Lecturer in the Department of Psychology—The Secretary, The University, Aberdeen (March 9).

Assistant Lecturer (with a good honours degree) in Applied Mathematics—The Registrar, King's College (University of London), Strand, London, W.C.2 (March 9).

Junior Research Fellow in Physics—The Registrar, The University, Sheffield (March 9).

Lecturer in Zoology—The Secretary, The University, Aberdeen (March 9).

Chair of Veterinary Physiology—The Secretary of University Court, The University, Glasgow (March 15).

Lecturers (2) in Theoretical Chemistry—The Secretary of University Court, The University, Glasgow (March 15).

Research Assistant in Mathematical Statistics in the Department of Applied Mathematics—The Registrar, The University, Liverpool, quoting Ref. No. CV/254 (March 15).

Lecturer in Animal Health; and a Lecturer or Assistant Lecturer in Animal Physiology in the Department of Agriculture (Animal Husbandry)—The Registrar, University College of Wales, Aberystwyth (March 16).

Lecturer (preferably with Ph.D. or equivalent experience in metallurgy) in Metallurgy—The Registrar, The University, Sheffield (March 16).

Senior Lecturer in Physics; and a Senior Lecturer in Metallurgy—The Registrar, The University, Sheffield (March 16).

Lecturer or Assistant Lecturer (with a good honours degree in zoology or agricultural zoology and a special interest in agricultural entomology) in Agricultural Zoology at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 19).

Demonstrator in Inorganic Chemistry—The Secretary, Department of Inorganic Chemistry, The University, South Parks Road, Oxford (March 23).

Demonstrator in Pathology—The Registrar and Secretary, The University, Bristol (March 25).

Lecturer in Pathology (Morbid Anatomy)—The Registrar and Secretary, The University, Bristol (March 25).

Professor of Agriculture at the University of Queensland, Australia—The Secretary Association of Universities of the British Commonwealth (Branch Office), Marlborough House; Pall Mall, London, S.W.1 (Australia and London, March 29).

Lecturer in Mathematics at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 30).

Lecturer (with an honours degree in physics and an interest in some branch of optics) in the Physics Department—Prof. W. D. Wright, Physics Department, Imperial College of Science and Technology, London, S.W.7 (March 31).

Senior Lecturer or Lecturer in Metallurgy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 8).

Reader in Theoretical Physics—The Secretary of Faculties, University Registry, Oxford (April 15).

Master to teach Mathematics to Scholarship standard—The Headmaster, Ipswich School, Ipswich, Suffolk.

Postdoctoral Fellow in the Department of Physics, to investigate the acceleration of plasma to high velocities—The Registrar, University College of Wales, Aberystwyth.

Research Assistant (preferably with experience in cytology) in the Department of Genetics, to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Associated Engineering Limited. Report and Accounts 1962. Pp. 32. (Leamington Spa: Associated Engineering Limited, 1963.) [3112

Nuclear Engineering Abstracts, Vol. 2 No. 2, (April, 1961). Pp. vii+155–344. (Annual subscription covering 3 issues, post paid, £6/6/–; 18.50 dollars). (London: Silver End Documentary Publications, Ltd., 1962.) [3112

Institution of Electrical Engineers. List of Members, Part 2: Associates, Graduates and Students. Pp. 456+26. Supplement to List of Members, Part 1: Corporate Members and Companions. Pp. 11. (London: Institution of Electrical Engineers, 1962.) [3112

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. United Kingdom Fire Statistics 1961: Statistical Analysis of Reports of Fires attended by Fire Brigades in the United Kingdom during 1961. Pp. vi+129. (London: H.M. Stationery Office, 1962.) 17s. 6d. net. [3112

Ministry of Education. Inter-Library Co-operation in England and Wales: Report of the Working Party appointed by the Minister of Education in March 1961. Pp. v+34. (London: H.M. Stationery Office, 1962.) 3s. net. [81

Ministry of Education. Standards of Public Library Service in England and Wales: Report of the Working Party appointed by the Minister of Education in March 1961. Pp. vii+124. (London: H.M. Stationery Office, 1962.) 7s. net. [81

Ministry of Education. Report of the Second Commonwealth Education Conference. Pp. iii+86. (Cmnd. 1655). (London: H.M. Stationery Office, 1962.) 5s. 6d. net. [81

The Night Sky 1963. Pp. 30. (London: The Times Publishing Co., Ltd., 1962.) 4s. net. [81

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 726, Vol. 246, (3 January 1963) Area Effects in *Cepaea*. By A. J. Cain and J. D. Currey. Pp. 1–81. (London: The Royal Society, 1963.) 30s.; 4.50 dollars. [81

Annual Report of the Medical Research Council of Ireland, 1961. Pp. iv+55. (Dublin: Medical Research Council of Ireland, 1962.) [81

London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, including Management Studies and Commerce, 1962–63. Part 2: Spring and Summer Terms. Pp. 97. (London: London and Home Counties Regional Advisory Council for Technological Education, 1962.) 3s. 6d. [81

Titles of Dissertations approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academical Year 1961–1962. Pp. iv+27. (Cambridge: Secretary of the Board of Research Studies, The University, 1962.) [81

Other Countries

Commonwealth of Australia. The Australian Mineral Industry. Part 1: Quarterly Review (Containing Information to August 1962). Part 2: Quarterly Statistics (Quarter ended 31st March 1962). Pp. iv+28+iv+31. (Canberra City, A.C.T.: Bureau of Mineral Resources, Geology and Geophysics, 1962.) 6s. [2812

United States Department of the Interior: Geological Survey. Bulletin 1031–F: Geological Investigations of Proposed Powersites at Sheep Creek, Carlson Creek, and Turner Lake, Alaska. By George Plafker. Pp. iii+127–148+plates 10–12. Bulletin 1104–C: Geology of South-Central Hidalgo and Northeastern México, Mexico. By Kenneth Segerstrom. Pp. v+87–162+plate 3. Bulletin 1121–D: Geology of the Linville Quadrangle, North Carolina–Tennessee—a Preliminary Report. By Bruce Bryant. Pp. iv+30+plate 1. Bulletin 1121–L: Geology of the Littleton Quadrangle, Jefferson, Douglas, and Arapahoe Counties, Colorado. By Glenn R. Scott. Pp. iv+53+plate 1. Bulletin 1135–A: Oxidized Zinc Deposits of the United States. Part 1: General Geology. By Allen V. Heyl and C. N. Bozion. Pp. iii+52+plate 1. Bulletin 1141–G. A Pleistocene Ice Sheet in the Northern Boulder Mountains, Jefferson, Powell, and Lewis and Clark Counties, Montana. By Edward T. Ruppel. Pp. iii+22+plates 1 and 2. Bulletin 1147–A: Reconnaissance Study of Uranium Deposits in Arizona. By H. C. Granger and R. B. Raup. Pp. iv+54. Bulletin 1148 Summary of Rock Salt Deposits in the United States as Possible Storage Sites for Radioactive Waste Materials. By W. G. Pierce and E. I. Rich. Pp. v+91+6 plates. (Washington, D.C.: Government Printing Office, 1962.) [2812

United States Department of the Interior: Geological Survey. Professional Paper 364: Plant Spores and Other Microfossils from Upper Devonian and Lower Mississippian Rocks of Ohio. By Marcia R. Winslow. Pp. v+93+22 plates. Professional Paper 403–B: Marine Geology of Guam. By K. O. Emery. Pp. v+76+plate 1. Professional Paper 426: Data on Uranium and Radium in Ground Water in the United States, 1954–1957. By R. C. Scott and F. B. Barker. Pp. iii+115+plates 1 and 2. (Washington, D.C.: Government Printing Office, 1962.) [2812

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Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 98: Relations entre les Productions des Descendances Génératives et Végétatives de *Hevea brasiliensis* et Corrélations avec un Critère Morphologique (Testam). Par Dr. H. Amand. Pp. 83. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1962.) 100 francs. [3112

Administrasie van Suidwes-Afrika. *Cimbebasia.* No. 1 (June 1962): A Review of the Gecko Genus *Ptenopus* with the description of a New Species. By C. K. Brain. Pp. 18 (1 plate). No. 2, (August, 1962): Some Notes on the Vegetation of the Namib Desert, with a List of Plants Collected in the Area Visited by the Carp-Transvaal Museum Expedition during May 1959. Pp. 35. (Windhoek, S.W.A.: Staatsmuseum, 1962.) [3112

World Health Organization. Public Health Papers, No. 16: The Scope of Epidemiology in Psychiatry. By Tsung-Yi Lin and C. C. Standley. Pp. 76. 3 Swiss francs; 5s.; 1 dollar. Technical Report Series. No. 243. Committee on Malaria—Ninth Report. Pp. 43. 2 Swiss francs; 3s. 6d.; 0.60 dollars. No. 244: Dental Education—Report of an Expert Committee on Dental Health. Pp. 32. 1 Swiss franc; 1s. 9d.; 0.30 dollars. No. 245: Joint FAO/WHO Expert Committee on Nutrition—Sixth Report Pp. 65. 3 Swiss francs; 5s.; 1 dollar. Cumulative List of Proposed International Non-Proprietary Names for Pharmaceutical Preparations, Lists 1–11 published in the *WHO Chronicle*, 1953–1961. Pp. 52. 3 Swiss francs 5s.; 1 dollar. (Geneva: World Health Organization; London: H.M. Stationery Office, 1962.) [3112

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Annals of the New York Academy of Sciences. Vol. 101, Article 1 Tumor Immunity. By D. B. Amos, C. A. Stetson, Jr. and 48 other authors. Pp. 1–326. (New York: New York Academy of Sciences, 1962.) [81

New Zealand. Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March 1962. Pp. 24. (H. 21). (Wellington: Government Printer, 1962.) [81

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Chicago Natural History Museum. Fieldiana: Anthropology. Vol. 54, No. 1: An Annotated Bibliography on the Origin and Descent of Domestic Mammals, 1900–1955. By Shlomo Angress and Charles A. Reed. Pp. 143. (Chicago. Chicago Natural History Museum, 1962.) 2.75 dollars. [81

Food and Agriculture Organization of the United Nations. FAO Forestry and Forest Products Studies, No. 15: Forest Influences. Pp. xix+307. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1962.) 15s.; 3 dollars. [81

Indian Central Jute Committee. Annual Report of the Jute Agricultural Research Institute for the year 1960–61. Pp. 115. (Calcutta: Indian Central Jute Committee, 1962.) [81

Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 127, No. 8: The Genus *Tetragnatha* (Araneae, Argiopidae) in Jamaica, W.I. By Arthur M. Chickering. Pp. 423–450. Vol. 127, No. 9: The Anoles of the Eastern Caribbean (Sauria, Iguanidae) Parts 4–6. By James D. Lazell, Jr. and Ernest E. Williams. Pp. 451–478+3 plates. Vol. 127, No. 10 Jamaican and Hispaniolan Gonatodes and Allied Forms (Sauria, Gekkonidae). By Paulo E. Vanzolini and Ernest E. Williams. Pp. 479–498. Vol. 128, No. 1: A Study of the Species of the Genus *Basiliscus*. By Humberto R. Maturana. Pp. 1–34. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [81

Museum of Comparative Zoology at Harvard College. Breviora. No. 166, (September 5, 1962): Lectotypes of Species of Ogcocephalidae Selected from Syntypes in the Museum of Comparative Zoology. By Margaret G. Bradbury. Pp. 4. No. 167, (September 5, 1962): *Bathyclupea schroederi*, a New Bathyclupeid Fish from the Western Tropical Atlantic. By M. M. Dick. Pp. 4. No. 168, (September 7, 1962): Two New Species of Fossil Talpid Insectivores. By Katherine M. Reed. Pp. 6+1 plate. No. 169, (October 15, 1962): New Records of Inshore Fishes from the Atlantic Coast of Panama. By Ira Rubinoff and Roberta W. Rubinoff. Pp. 7. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [81

Food and Agriculture Organization of the United Nations. FAO Agricultural Studies, No. 57: Diseases of Free-Living Wild Animals. By Dr. A. McDiarmid. Pp. viii+119. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1962.) 7s. 6d.; 1.50 dollars. [81

The South African Institute for Medical Research. Annual Report for the year 1961. Pp. 182. (Johannesburg: The South African Institute for Medical Research, 1962.) [81

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THE ADVISORY COUNCIL ON SCIENTIFIC POLICY IN BRITAIN

THE fifteenth annual report of the Advisory Council on Scientific Policy* is on the whole a disappointing document. Its main interest lies in the discussion of international co-operation in science, and particularly the recommendations regarding an International Institute for Science and Technology, as proposed by a Working Group of the North Atlantic Treaty Organization over which Dr. J. R. Killian presided, and in its discussion of a report on biological studies from the Biological Research Committee of the Royal Society. This report is appended, as are some notes on the scope and methods of an inquiry into research and development expenditure which are also considered by the Advisory Council. Scientific libraries, however, are cursorily dismissed in a paragraph which shows no awareness of how important a contribution the Advisory Council might make, if it were so minded, to the development of the new National Reference Library of Science and Invention, or to the shaping of the national library system to which the Minister of Education is immediately committed. Further, once again the question of research into natural resources is deferred, pending the report of the Slater Committee, while that of the organization of scientific research is postponed pending the report of Sir Burke Trend's Committee reviewing existing organization for the promotion of civil science by Government agencies. Of any progress, meanwhile, in implementing the recommendations of either the Plowden Committee or the Zuckerman Committee on the management and control of research and development there is no mention.

Two other matters are briefly considered in the report. First, the arrangements described in the Advisory Council's last report for the supply of costly equipment such as computers for research appear to have proved their value. In 1961 a total of £2·1 million was allocated for computers to universities through the University Grants Committee, and when two universities applied for the very large *Atlas* computers that Committee took the view, endorsed by the Treasury, that provision must be made on a national basis. As a substantial requirement for time on such a computer was foreseen by the Atomic Energy Authority, it was decided to establish an *Atlas* computer of the most advanced design at the Rutherford Laboratory of the National Institute for Research in Nuclear Science at Harwell. This would manage the computer and allocate a defined minimum of computer time to the Atomic Energy Authority, and the rest to the Meteorological Office or other Government departments and to universities, on the advice of a committee of computer experts. Three universities have also decided to use their resources as contributions to a larger machine and propose to meet part of their computer needs by *Atlas* machines or *Atlas* components. Needs over the next five or six years should be adequately met by the arrangements now made.

Secondly, during the year the Council considered a report, endorsed by the Royal Society, from the British National Committee on Astronomy, proposing the establishment of an Institute of Theoretical Astronomy, if possible at the University of Sussex, and providing facilities generally for encouraging research in theoretical astronomy as well as a centre of teaching and for the exchange of ideas and research workers. The Advisory Council is satisfied that steps ought to be taken to strengthen, co-ordinate and stimulate such studies in the United Kingdom and to provide satisfactory facilities for collaboration with astronomers overseas. A plan of the kind and on the scale proposed would, in the Council's view, be a good way of meeting these needs, and it is understood that the University of Sussex is considering this project in conjunction with the Royal Society and the British National Committee on Astronomy. The Council also reports that, in accordance with its recommendations in their report for 1959–60, effective arrangements have now been made for expanding seismological studies at the University of Edinburgh. Conditions for seismological work at its station at Eskdalemuir are as favourable as anywhere in the United Kingdom; the Department of Scientific and Industrial Research has made a grant of £43,100 over three years for staff and equipment, while a further grant of £10,480 over three years has been made to the University of Durham for researches in seismology of the Earth's crust.

The Advisory Council was invited by the Minister for Science at the end of 1961 to consider the whole question of international scientific activities and to advise on the issues of policy which arise. While welcoming the growth of international consultation through the numerous agencies of the United Nations, the North Atlantic Treaty Organization, the European Nuclear Energy Agency and the International Atomic Energy Agency, the Advisory Council sees some danger of duplication between these activities where the Agencies have similar objectives or functions. This can be largely avoided by forethought, and accordingly the Council has invited its Sub-committee on Overseas Scientific Relations to review from time to time the various consultative international activities.

International collaborative research projects, such as the International Geophysical Year, have proved highly rewarding and the scientific results have been substantial. The Advisory Council welcomes the increase in international collaboration of this type. The expenditure involved is usually small, and programmes can be organized in a way that permits results which could not otherwise be achieved. It is an added advantage that much of the expenditure is likely to be incurred in the currencies of the countries concerned. Several of these projects have been organized under the auspices of the International Council of Scientific Unions, and the Council refers particularly to the forthcoming International Quiet Sun Year and the International Biological Programme, and to the International Cell Research Organization which it is proposed to set up under the auspices of the United Nations Educational Scientific and Cultural Organization.

The United Kingdom is also participating in four centralized international schemes, in which research facilities are provided and controlled internationally. One of these, the European Space Research Organization, to which the United Kingdom is expected to contribute £7·6 million in 1962–67, is still in the formative stage. The others are the European Organization for Nuclear Research, to which Britain's contribution is estimated at

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* Annual Report of the Advisory Council on Scientific Policy, 1961–1962. (Cmnd. 1920.) Pp. vi+45. (London: H.M. Stationery Office, 1963.) 3s. net. See also p. 851 of this issue of *Nature*

£10·4 million, the Halden reactor experiment (£140,000) and the *Dragon* reactor project (£7·2 million), all over the same period. Although these schemes differ widely in type, they all involve centralization on a regional basis and they share the calls on high-grade scientific man-power.

The Advisory Council stresses the importance of the principle that, in centralized international schemes providing facilities for fundamental research, national teams, working in their own laboratories, should have a major share in the use of the facilities provided. Proper use of very advanced equipment provided internationally is, however, only possible by member countries in which a substantial measure of activity already exists and, in the Council's view, it is important to ensure that national research teams from universities can use the opportunities in the very advanced fields of research which the European Organization for Nuclear Research and the European Space Research Organization will provide. While in schemes such as the *Dragon* reactor project, economic and technological considerations may be the determining factors in the decision to participate or not, in reaching decisions regarding participation in scientific schemes, which are usually very costly, it is important that strictly scientific considerations should be given full weight and that all should be satisfied that, from Britain's national point of view, the enterprise is worth the heavy cost involved.

While the Council agrees that the development of international co-operation in science is, in principle, desirable, it is not induced by the success which has hitherto attended the participation of the United Kingdom in such schemes to conclude that any and every international scientific collaborative arrangement has merit simply because it is international. Each scheme must be considered on its scientific merits and there are limits to the cost which a country can incur on scientific activities. Moreover, it is essential to maintain reasonable balance between national and centralized international activities, and it should be noted that the Council attaches great importance to a close relation between the two. It also suggests that one of the most useful ways of assisting international co-operation in science would be to review and improve the existing arrangements for international exchange of scientists.

This recognition that international co-operation is no substitute for national excellence in science does not lead the Advisory Council to overlook the element of assistance to smaller countries or to the 'larger objectives' —objectives such as strengthening European economy or union which may be served by participation. Due weight is given to these factors; but the Advisory Council rightly holds that it is a mistake to resort to international activities, whenever possible, simply because it is politically desirable to extend the area of co-operation. Ultimately, the political value of co-operation is proportional to the extent to which there are real scientific advantages for all the participants.

It is from this pragmatic point of view that the Advisory Council then considers the particular proposals for an International Institute for Science and Technology, recently discussed in these columns (196, 1127; 1962). While the Council is in sympathy with the basic aims and objectives which inspire the proposals for such an institute and considers the basic objectives sound, the majority of the Council believes that these ends can be attained more readily and economically by alternative methods. It questions the soundness of the analogy drawn between an international institute and international research facilities, such as are provided by the European Organization for Nuclear Research. In most fields of science it believes that the nucleus around which work of the highest quality can be developed is the exceptional individual and not a research facility, and this view is supported by the policy which the Carnegie Institution of Washington and the Nuffield Foundation have consistently followed. Moreover, it doubts whether it is wise to ignore the value of existing equipment and stimulus such as are to be found in the great existing centres of research at universities and elsewhere. Most of the Council doubts whether it would be possible to attach research leaders of the highest quality to a new multi-discipline organization to be built up from the beginnings in Europe at the present time, though others believe that, given the necessary backing of Governments, this would be possible.

The Advisory Council suggests, therefore, that in place of a new institute, international agreement should be sought on the selection in Europe of individual centres in existing universities, etc., in particular fields of science and technology which could readily and economically be developed into a nexus of the kind of international centres envisaged. It gives as examples the Imperial College of Science and Technology, which already draws a high proportion of its postgraduate students from overseas, and the National Physical Laboratory. Such international agreement would cover international recognition of the research degrees conferred by these centres as having parity with the research degrees of the participating countries. International and national support for these centres would then be channelled to develop them as rapidly as possible into inter-disciplinary postgraduate centres recognized as the leading institutions in their field in Europe. Besides provision of international research fellowships and visiting professorships, an essential element would be short-period exchanges or visits to maintain close contact and co-ordination by members of the various institutes to other similar centres.

The Advisory Council believes that the benefits of such an arrangement would ultimately spread to the whole of the universities, etc., of which the centres were part and that in this way it would be possible to go far towards truly international universities. It suggests that such proposals could suitably be put forward to the North Atlantic Treaty Organization as the basis of an alternative both to the plan for a single institute and to the proposal for an international research fund. Subsequent to these recommendations, the possibility of establishing an institute which would comprise, in one country, a central 'core' of perhaps two centres, together with a headquarters and with associated centres in other countries, has been considered and the Advisory Council does not regard such a scheme as contrary in its essential principles to its earlier advice.

It might be remarked that Dr. Killian and his colleagues on the Working Group fully recognized the value of the exceptionally gifted individual as a nucleus for research schools of outstanding merit, and the Group laid considerable stress on the importance of flexibility to cope with this situation and its implications. The Advisory Council scarcely seems to do justice to this aspect, nor does it comment on a further draft proposal for an international foundation for the development of science and technology. Since, however, Sir John Cockcroft is a member of the Advisory Council as well as of the Working Group, it may be assumed that the proposals of the Working Group were fairly considered. The broad comments of the Advisory Council are unexceptional and should receive full weight in considering any proposals for international co-operation in research and in decisions as to the organization of Britain's scientific effort generally.

On biological studies, the Advisory Council does not necessarily accept at every point the views expressed in the report of the Biological Research Committee of the Royal Society, but in its present report it is concerned essentially with the diagnosis of the state of biological studies in the United Kingdom. Consideration of the institutional changes which may be desirable to deal with the problems revealed by the diagnosis is deferred until the Committee under Sir William Slater concludes its examination of the case for a research council dealing with research into natural resources. In view of the overlap, this decision is reasonable enough. However, at the same time, it must be pointed out that here once again the Advisory Council scarcely shows the drive and sense of urgency that are desirable. Granted that rapid action is not necessarily precluded when the Slater Committee reports, this means that the Advisory Council is unlikely to contribute further to informed public discussion of an issue of immediate importance within the next twelve months.

It is such circumstances that foster doubts as to the adequacy of the existing structure and the real effectiveness of the Advisory Council and such criticism is not invalidated by the very sound discussion of the state of biological studies in the present report. In fact, that discussion, and the report on which it is based, bring out the imperative need for some further consideration of the respective functions of the University Grants Committee and the Research Councils in fostering biological research. That is a first step towards remedying on any permanent basis the relative neglect of biological research which is emphasized by the present report.

That neglect is attributed primarily to the failure within the universities to recognize the need for developing biological studies. It is generally agreed that biology at present is in a state of transition. With a continuing change of emphasis from descriptive studies to the analysis of living matter and the interactions of biological systems, traditional branches of biology are being increasingly infiltrated by other scientific disciplines: the interaction of these subjects has been responsible for many of the most promising new developments. Such interaction is at present occurring at a wide range of levels, and, as the Advisory Council for Scientific Policy frankly recognizes, implies the need for major changes in the organization of biology departments especially in universities. The plans for an International Biological Programme, concerned with biological productivity and human welfare, are designed to deal specifically with one aspect of this situation, but more generally the proposal for an International Institute of Science and Technology is intended to deal with this problem of inter-disciplinary relations and overcome the difficulties which the rigidity of departmental organization in universities often offer.

In the universities at present the situation is far from uniform. In some there are large and well-equipped centres for biological research, but in others there are small traditional departments with relatively modest equipment and insufficient space for expansion. In many branches of biology adequate progress is only possible with first-rate modern equipment and facilities and inter-disciplinary co-operation. For this co-operation to be achieved, the Advisory Council considers much greater financial support to biology should be provided. Besides this, however, leaders in research will have to face much more severe administrative and managerial problems, and in all but the newest schools recently equipped by the University Grants Committee, there is a critical shortage of space for research equiment, accentuated by the growth in new directions which cut across the traditional structure of faculties.

Special measures may be required to meet the situation, but the Advisory Council recognizes that the establishment of new universities presents special opportunities for creating biological departments embodying a multi-disciplinary approach. It is vital, it holds, for the proper evolution of biological studies in the United Kingdom that centres where the necessary conditions exist, or can be established, should be developed and provided with staff and resources comparable with the best centres in the world. It gives a broad hint, though no more than a hint, that this will not be possible with the level of support for the universities which is at present contemplated by the Government. Even with a substantially higher level of support for university research, some selection of céntres of biological research for development will be unavoidable, especially in the creation of new departments.

The Advisory Council has several times during the past ten years directed attention to weaknesses in the traditional pattern of university education in the biological sciences, pointing, for example, to the importance of basic training in mathematics, physics and chemistry. These comments are the more pertinent in the light of the new developments just noted, and the Advisory Council hopes that throughout biology departments there will continue to be developments in both established and new universities in training biologists with adequate knowledge of the physical sciences and mathematics. It suggests also that more senior and responsible posts are required particularly to meet inter-disciplinary needs if facilities are to be provided which will attract the younger men who might otherwise be tempted to emigrate to centres abroad providing such conditions. While the Advisory Council recognizes that Britain may be limited in the scale on which expensive facilities of this kind can be provided, it strongly insists that such additional resources as can be made available should be channelled with vigour and foresight into the chosen centres.

In these comments and recommendations the Advisory Council follows broadly the conclusions of the Royal Society's Committee. The latter concluded specifically that insufficient biologists are being trained even to meet present-day needs and that the present expenditure must be at least doubled if Britain is to exploit the present possibilities in biological research. Little would be gained by redistributing the funds at present available, but if additional resources are provided the means for distributing Government grants should be modified so as to permit more positive planning. The need for biologists has been underestimated and a reassessment of the need by the Committee on Scientific Manpower is recommended. The opinion is expressed that previous estimates were based largely on a survey of the needs of industry and took insufficient account of the needs of agriculture, veterinary medicine, fisheries, forestry, etc., in which biologists tend to find employment, while in borderland subjects it is necessary to match with biological recruits those coming from the physical sciences.

Reference is also made to a number of particular deficiencies. It is questioned whether the Advisory Committee on Fisheries of the Development Commission is in a sufficiently strong position to finance and expand work on many aspects of physiology, biochemistry and the physical sciences where marine and fresh-water organisms and their environment offer exceptionally favourable subjects for study. The position of the centres for fundamental research in marine and fresh-water biology in Central and East Africa and in Malaya is noted as calling for immediate investigation. It is curious, however, that neither here nor in the specific references to the role of the Research Councils is the Overseas Research Council mentioned. In terrestrial ecology and general zoology the main specific deficiency in the present organization is considered to be the lack of adequate means of financing biological expeditions. Moreover, in economic microbiology it is submitted that the Microbiological Research Establishment at Porton should be freed from Service control and expanded so as to act as a national institute of microbiology, with its own governing body, suitably attached to an appropriate Research Council and with close liaison with university departments.

The almost unlimited possibilities in the development of research on marine and fresh-water animals while the present restricted work is hampered by lack of funds is again stressed in this report, as is the inadequacy of the grants which the Nature Conservancy is at present able to make for biological or botanical research. Certainly there is firm criticism of the present departmental structure in the universities for teaching and research in biological sciences. Obviously the time is ripe for reform, apart from the opportunities for experiment which the creation of new universities offer, and there is a note of urgency which the University Grants Committee can scarcely ignore. In particular, greater flexibility is required if the universities are to be enabled to foster precious talent. In the absence of sufficient senior research posts, with security of tenure, and more post-doctoral fellowships Britain is losing too many trained scientists abroad. Whereas nine-tenths of the research personnel engaged in the earliest research in a new field were provided by the United Kingdom, this rapidly changed to a situation where three-quarters of the research workers engaged in its further exploitation were in the United States.

The reluctance of the Advisory Council to commit itself to any reassessment of the role of the Research Councils in the organization of research in advance of the report of the Trend Committee is understandable; but it is clearly of the utmost importance that the whole system should be reconsidered as a matter of urgency, but in relation, for example, to the University Grants Committee and to the universities themselves, and in respect of the scale of Britain's whole effort. In particular, it is once more apparent that the place and functions of the Overseas Research Council are of significant importance and that the Nature Conservancy requires much greater resources at its disposal. The urgency derives only in part from the opportunities provided by university expansion; it arises equally from the pace of development in Britain, the productivity campaign and the position of research in the emergent countries overseas. The report of the Royal Society's Biological Research Committee demonstrates the urgency in one particular field, but it is clear enough from the whole report of the Advisory Council how imperative it is that it and the Minister for Science should be thinking urgently and constructively about the whole scale and balance of the research effort. The present report is scarcely less tentative than that for 1960–61, and another successive report of the same character must seriously prejudice the position of the Advisory Council itself as an effective element in the national structure for advising on and planning the development of Britain's research effort.

FUTURE OF BRITAIN'S NATIONAL PARKS

THE thirteenth annual report of the National Parks Commission*, covering the year ended September 30, 1962, is noteworthy for its unequivocal criticism of Government policy in regard to the national parks. This is most clearly expressed in regard to the Government's failure to indicate when a place will be found in the legislative programme for what the Commission regards as the essential amendment of the 1949 Act. It is no less obvious, if implied rather than expressed, in the Commission's remarks on coastal preservation, the closure of railways and on development questions, such as electricity supply, water supply, aerial masts and disused Service sites.

The magnitude of these latter issues is sufficiently illustrated by the simple fact that during the year some 471 proposals for development affecting landscape beauty in national parks, in areas of outstanding natural beauty, or elsewhere were referred to the Commission by Government departments, by park and other local planning authorities, or by amenity organizations. There were, however, several encouraging features of the year which may be briefly noted before discussing the major issue. In the ten national parks a number of creative projects were proposed by the Park Planning Authorities. Some progress is reported in dealing with the litter problem, and on Dartmoor and elsewhere encouraging examples are cited of co-operation with local people in preserving and enhancing the countryside. In the Lake District and on the Pembrokeshire coast particular attention was directed to problems arising out of caravan camping and the increasing congestion of roads by motorists, while in the Exmoor National Park particularly difficult problems arose in the conflict of interests in the enclosure of open country and in the reconciliation of the need for landscape preservation with the demands of forestry. An Order designating East Hampshire an 'area of outstanding beauty' was confirmed by the Minister on September 26, 1962, and a proposal to designate an area to be known as the Forest of Bowland is to be submitted for confirmation shortly, but there was a disappointing further delay in the confirmation of an Order designating the Sussex Downs such an area. However, some progress was made towards completing proposals for the South Downs Long Distance Route, the first such route to be proposed as a bridle way, and for a route in Yorkshire from near Filey northwards along the coast to Saltburn and thence inland along the escarpment to Helmsley. Proposals for solving the problem of the route of the Pennine Way in Northumberland were submitted to the Minister, who has now approved proposals submitted for the variation of three other sections of the Pennine Way.

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* National Parks and Access to the Countryside Act, 1949 Thirteenth Report of the National Parks Commission for the year ended September 30, 1962. Pp. vi+80+8 plates. (London: H.M.S.O., 1962.) 6s. 6d. net.

Turning now to the major issue of development, the Commission views with grave apprehension the large-scale plans which the Electricity Board has in mind, although it fully appreciates that the Board is giving increasing attention to its statutory duty to pay due regard to the impact of its schemes on natural beauty. For the Commission's part, it recognizes the need for more generating stations and transmission networks, but nevertheless maintains its objection to particular schemes such as those for for Edern in Lleyn in North Wales and Hamstead in the Isle of Wight. It again points to the dangers of a piecemeal approach to these problems and has urged both the Board and the Minister of Power to consider as a whole the proposals for extending a new high-voltage transmission line westwards as far as Fraddon in Cornwall. While it welcomes evidence that water supply is likely to receive more deliberate consideration, it is disturbed at the absence of a central body to ensure that there is no unnecessary multiplication of aerial masts and that any new proposal utilizes an existing tower or mast if possible. At present, any one of eight Government departments may satisfy itself that no existing mast can be used, and it is understood that unless it issues a certificate to this effect the Government will not clear the proposal.

The National Parks Commission suggests that the Planning Authority should likewise be in a position to require, in respect of any new proposal, a certificate from the appropriate sponsoring department that no existing mast, tower or building can be made to serve. The point has been put to the Ministry for consideration, but a reply is awaited on this and also on the further submission that it should be accepted as Government policy that a site for a new mast within a national park, or in any area of special scenic value, should be considered only when a site outside such areas is not capable of serving the purpose. The Commission considers that the sponsoring department responsible for examining the project should at the same time consider alternative sites outside the special scenic areas; such departments should refuse to put forward for planning approval any project involving a site in a national park or in any area of special scenic value unless it can certify on Government authority that sites outside such areas cannot be used for the project in question.

The National Parks Commission has already provided Government departments with maps showing the areas which, in its view, need special protection. Clearly the argument applies with no less force to the scientific interests of nature reserves, and the Nature Conservancy and the National Parks Commission are equally affected by this whole development problem. Meanwhile, however, the National Parks Commission is alarmed at the prospects implied in the Government's statement on the development of broadcasting as to the need for many more transmitting stations. This again is not entirely removed by the Government statement that all new ultra-high-frequency programmes for the same area will be transmitted from the same mast and site, and the Commission is seeking further information from the Ministry.

Government indifference or negligence appears also over the disused Service sites and in regard to fresh proposals for training areas. Once again the Commission directs attention to the way in which land and buildings acquired in good faith for what seemed to be essential defence requirements have afterwards become redundant and have been left derelict, and to a tendency to disregard the duty to clear up before disposing of a site. Often the development was only allowed in the particular area because it was imposed on the planning authority as being in the national interest. Sometimes attempts have been made to dispose of the sites for private development—sites which the planning authorities would never have sanctioned in such areas—and hence to saddle others with the costs of clearance or restoration. The Commission rightly argues that it is indefensible that one Government department should refuse to carry out clearance operations when, under other legislation, in fulfilment of a statutory duty laid down by Parliament, another department will be meeting most of the cost.

The Commission is constantly urging Government departments to take what is manifestly the right and reasonable action, but it has become necessary for it to raise the whole subject with the Minister, as it may be necessary to do also over new proposals concerning training areas. The Government's whole 'policy' here appears to be based on entirely wrong priorities and values, and in particular on a refusal to accept Mr. Harold Macmillan's own view, in 1952, that national parks are areas in which amenity and access are to be given an overriding position. That is persistently refused. There is no evidence of determined effort to find military training areas outside national parks. Requests for impartial inquiry into the persistent retention or misuse of areas, such as Dartmoor, which should have been the first to be relinquished, are refused and the Services are never forced to consider either the pooling of requirements or to recognize that if a particular request is granted, further requests elsewhere are *ipso facto* excluded.

Coastal preservation and the closure of railways provide further examples of Government indifference to the essential issues. While it is true that the National Parks Commission has made some positive contribution to coastal preservation in that about 30 per cent of the coastline of England and Wales is included within the areas established as national parks or as areas of outstanding natural beauty, it follows from what has already been said that the effectiveness of this protection is still in doubt. The Coastal Preservation Committee of the Council for the Preservation of Rural England suggested the appointment of a Committee, similar to the Minister of Transport's Committee on Landscaping of Trunk Roads, to advise him on problems relating to the development of land around the coast. The Minister was unable to agree to this suggestion, and referred to the general duty of the National Parks Commission to advise him on all questions relating to natural beauty. The Commission believes that the Minister of Housing and Local Government recognizes the need for a proper coastal preservation policy, but it is disappointed that he has not yet issued a promised circular to local planning authorities on the review of Development Plans, and asking them to make special surveys of the coast with the view of deciding where facilities for holiday-makers should be concentrated while safeguarding the rest of the coast so that its attractions might be enjoyed to the full.

As regards railways, the national parks lie in the rural areas where drastic curtailment of services has occurred or is most likely. Proposals to withdraw services from all railway lines into Brecon were strongly opposed locally, and the Commission believes that if the purposes for which the Brecon Beacons National Park was established are to be achieved, access by train from the heavily populated industrial centres of South Wales is important. The Newport to Brecon and Neath to Brecon lines are regarded as worthy of special treatment because of the exceptional beauty of the landscape which can be seen from them. These lines have now been closed, however, and the Commission is equally disturbed about the implications of this policy of closure for the Exmoor National Park, and generally the Commission insists that railways provide an important means of public access to national parks and are in themselves facilities from which the scenery can be enjoyed.

Apart from this, of course, the withdrawal of passenger railway services from national park areas is bound to intensify the difficulties which are already being experienced in places with regard to road transport. Nevertheless, there is as yet no sign that the Government is aware of the implications of the present policy, if indeed it is sufficiently interested in the national parks to be concerned one way or another with their future. There, in fact, the deepest note of concern of the whole report is struck. The Commission records its profound disappointment that there is still no sign from the Government that an early place in the legislation programme may be expected for amendment to the 1949 Act. Lord Jellicoe's remarks as Parliamentary Secretary to the Ministry of Housing and Local Government, in addressing the Conference of Park Planning Authorities at Moretonhampstead last May, indicated that prospects of early legislation are not brighter than a year ago and it must now be remembered that the present Parliament reaches the end of its five years in the autumn of 1964. If legislation is not introduced during the next two sessions, prospects of early introduction in a new Parliament are unlikely to be good, particularly if a new Government assumes office.

While it is agreed that more could be done within the framework of the Act as it now stands, the Commission is firmly of the opinion that legislation giving effect to the relatively modest amendments it has recommended would provide much greater scope for the Park Planning Authorities to undertake more positive work because of the increased rate and range of Exchequer grants. In particular, grant for administration would encourage the appointment of staff to deal solely with work concerning the national parks. The Commission considers this to be vital to the successful operation of the national park system and that it cannot press too strongly the real need for the changes suggested. Meanwhile, it is discouraging, both to the Commission and to Park Planning Authorities, that the Government does not care sufficiently about national parks to devote a small part of Parliamentary time to a relatively simple, and politically uncontroversial, piece of legislation.

Lord Strang, the chairman of the Commission, in addressing the Ramblers' Association Conference last September, noted that Parliament can be refreshingly conscious of the interests of national parks, and of this a debate in the House of Commons when a motion was adopted calling for the introduction of amending legislation to the National Parks and Access to the Countryside Act, 1949, is encouraging evidence. From the Opposition Front Bench, Mr. C. Hughes, in a sound but constructive as well as critical speech, supported the motion. However, in accepting the motion on behalf of the Government, Mr. F. V. Corfield, Parliamentary Secretary to the Ministry of Housing and Local Government, declined to give any undertaking about time for amending legislation, although he admitted that the Government had recognized for some time that the Act required amendment. In view, however, of the Parliamentary situation, might not this latest report of the National Parks Commission prompt some determined effort to secure agreement between Government and Opposition to provide Parliamentary time for the introduction of the relatively simple and uncontroversial amending legislation that is now so urgently required?

FIFTY YEARS OF *DROSOPHILA*

Genetics and Evolution

Selected papers of A. H. Sturtevant. Selected and edited by E. B. Lewis. Pp. x + 334. (San Francisco and London: W. H. Freeman and Company, 1961.) 54*s*.

IT is more than fifty years since the results of experiments in breeding the fruit fly began to issue from the small laboratory (it was only 30 ft. by 27 ft.) of T. H. Morgan in Columbia University. The work began in 1910 with Morgan's conversion to the chromosome theory, the theory of Weismann, Boveri and his colleague Wilson. It reached its climax in December 1921 when he in turn tried to convert the reluctant Bateson to the new doctrine. In the intervening period a revolution had been accomplished and the foundations of a new genetics had been laid by Morgan and his pupils.

Now we have a part of the record of this revolution in the selected papers of the youngest of the three chief collaborators, Alfred Sturtevant. When Sturtevant published his first paper (on heredity in horses) he was eighteen years old. Three years later, in 1913, he wrote his most notable work, the account of linkage between "six sex-linked factors" in *Drosophila ampelophila*. The result was a chromosome map which within a few years became the focus and the symbol of the new science. Its discovery constituted the first unmistakable step from the biological side towards the union of the physical and the biological sciences.

After this paper, the first in the present selection, there followed the series of investigations described, or perhaps we should say reported, in this book. Four main lines should be noted.

(1) The re-arrangement of genes, discovered as 'crossing-over-suppressors' in 1917 led to a new view of the different kinds of change in chromosomes in 1921, and later to the conception of different kinds of hybridity and to the theory of interchanges in hybrid species (as in *Œnothera*).

(2) The recognition of the bar-eye mutation as due to a duplication of a bit of chromosome widened this view still further in 1925. It also introduced the idea of the position effect as yet another mode of genetic change in the chromosome materials.

(3) Side by side with these analytical studies, evolutionary and synthetic hypotheses were continually being applied. The discovery of *Drosophila simulans* was the first part of the story of parallel variation combined with divergent changes in related species.

(4) Sex reversal, sex ratio control, sexual selection, intersexes and the origin of gynandromorphs: all these were used to solve ancient problems and to raise important new issues. Thus gynandromorphs, considered develop-

mentally, led on to the study of mosaics, to the principles of gene action and of the diffusion of gene products. Whence, as he points out in an introductory note, Beadle and his colleagues derived a new beginning in biochemical genetics.

These seem to be Sturtevant's main contributions, and they are well represented in the selected papers. Represented, but not, alas, expounded. These papers were written for a circle of *illuminati*. A small circle, it was, but quite sufficient it seemed to its members. Each paper began with the briefest introduction or, more often, with references to one or two earlier papers on *Drosophila*. Here is the first catch. These earlier papers are not usually in the present selection.

But there are still other catches. The whole theory of linkage and crossing-over relies on observations of chiasmata between chromosomes. But these observations were not made on *Drosophila*. There is no reference to them. Again, the importance of the bar mutation is that it confirms, in a limited sense, Bateson's theory of 'Presence and Absence'. Sturtevant indeed explains the mutation as due to 'Presence and Absence'. But he contradicts Bateson's theory: and he does so without mentioning Bateson. This is an extreme example.

In exposition parallel difficulties arise. It often happened that everything turned in a complex linkage experiment on which end of the chromosome was supposed to be left or right and where the 'spindle attachment' was supposed to be. Many more students in remote places must have struggled with these esoteric problems: and some, like myself, may have struggled in vain.

Thus, these papers will be useless without the explanatory volumes published jointly with Morgan and the other collaborators and now unfortunately inaccessible. For the historian of genetics equipped with these volumes, however, they will reveal several matters of interest.

First, these papers show that the driving force in Sturtevant's work has been observation and experiment and not generalization. Not only the driving force but also the continuity. That first sentence with the reference which joins each paper to its predecessors genuinely and without pretence represents the intellectual link: and no summary is usually needed, for the whole is the briefest conceivable abstract. Secondly, these papers show the parts played by the several collaborators. Wilson used to say that Morgan's three greatest discoveries were Bridges, Sturtevant, and Muller. But we can now see that the discoveries were reciprocal. Morgan gave literary coherence as well as scientific perspective to this powerful combination. He could pause to look back and look around while they looked almost entirely forward. Thirdly, we now see the different way they looked forward. The chromosome theory had answered so many questions. The chromosome map had asked so many more. On one hand there was Muller, bent on relentless analysis, pursuing the determinant to its smallest atomic element which he reached by the induction of mutation in the gene. On the other hand there was Sturtevant, turning aside to those awkward problems, biological problems, intermediate between the gene and the chromosome and between the cell and the organism: and both methods we see having consequences which have been reaped by many others. In this contrast, over-simplified, we also see what will continue for some time to be one of the chief polarities of biological inquiry.

The problems that concerned the early 'Drosophilists' interest to-day a widening public in the most practical sense. To-day, one after another, the situations that revealed themselves in the fly—to be confirmed and elaborated soon afterwards by the chromosomes of plants—are revealing themselves in man. There has been a gap of fifty years: and for the benefit of those who are interested in man it is to be regretted that there is still no worthy account of the great *Drosophila* discoveries. Sturtevant's papers are therefore a timely reminder of a still unrecorded passage in the history of science. C. D. DARLINGTON

RADIATION HAZARDS

Irradiation of Mice and Men

By Dr. John F. Loutit. Pp. xii+154. (Chicago and London: University of Chicago Press, 1962.) 5 dollars; 40*s*.

WHILE the threat of nuclear weapons of gigantic destructive power hangs over mankind, interest in the hazards of radiation will be kept alive. It is now generally accepted that all doses of radiation, however small, have a cumulative effect which may be to the disadvantage of individuals exposed to them. The big question is how to balance the disadvantage with the benefit which may be derived from radiation in medicine and industry. Many articles, reports and books are written to explain the immediate danger and to forecast the possible consequences of radiation in the near or distant future. In view of the many diverse opinions expressed by the various authors, under the influence of national, financial, moral or political considerations, this book, though limited in its scope, will be welcomed by all who want unbiased information about the hazards of radiation. The subject of the book is a series of lectures given to the Section of Nuclear Medicine at the University of Chicago in late 1961 by Dr. Loutit, who is director of the Radiobiological Research Unit at Harwell and a member of the International Commission on Radiological Protection.

The reader can thus expect a critical study of the problem and guidance in areas where so far no general agreement has been reached. Dr. Loutit has made extensive use of reports by the United Nations Scientific Committee, the Medical Research Council, the Committee on Radiological Hazards to Patients, the Agricultural Research Council, the International Committee on Radiological Protection and of various other surveys. He has also drawn on his own experience and that of his colleagues at Harwell to provide the sort of first-hand information that only the experimenter can supply.

After an introduction in which the biological targets of radiation are briefly described, the information given in the book centres around two topics: the effect of radiation on the life-span and the incidence of malignant diseases, particularly leukaemia, which might be affected by radiation. Past experience in medical radiology and industry has shown that persons exposed in their occupation to radiation often die prematurely, due mainly to cancer. The mortality pattern of the uranium miners at Schneeberg exemplifies this point. The question remaining is, whether the cumulative effect of low dose chronic irradiation can shorten the life-span. The author marshals the statistical data and the pertinent experimental findings to comfort his reader that so far no evidence has yet been obtained which could suggest any non-specific reduction in life-expectancy, when the exposure is below the officially accepted 'permissible' dose, that is, five rads per annum. In this section the problem of the nature of premature or accelerated ageing, which can be brought about in mice by radiation in specially designed experiments, is discussed in some detail. The author explains the various difficulties which are involved in the interpretation of the experimental findings and indicates their essential strengths and weaknesses, mostly weaknesses, when they are extrapolated to man.

In the second part of the book, Dr. Loutit deals with the possible effect of radiation on the incidence of leukaemia. He summarizes our recent knowledge concerning spontaneous, as well as radiation-induced, leukaemogenesis in mice then proceeds to examine the findings of various surveys carried out on the leukaemia incidence in man. These surveys, one is retrospective the other prospective, arrived at different conclusions; the former revealed an association between leukaemia and radiation; the latter did not. Dr. Loutit critically examines the facts in which some writers on radiation hazards have based their prediction of the shortening of life-span and of the increase in leukæmia incidence which might follow the increase in fall-out or other source of radiation and his conclusion is not flattering to most of the speculators.

A large part of the book is taken up with the thorny problem of strontium-90, the product of nuclear fission which is liberated into the atmosphere by atom bomb explosions. It represents a serious radiation hazard because it is absorbed and retained in the human bones, has a 30-year half-life and, like radium, it does produce anæmia, bone destruction and not infrequently bone cancer. The Radiological Research Unit at Harwell devoted much effort to the examination of strontium metabolism, and the reader will find in the book a great amount of factual data obtained by the group. Perhaps this part will be heavy going to many non-expert readers; but on the other hand this section of the book illustrates very clearly the fact that extensive and painstaking scientific investigation is needed to obtain reliable data for estimating the hazard of radiation to man.

The aim of the author in his series of lectures was to disseminate unbiased information. This aim was well fulfilled, and by publishing the lectures he has made the valuable information available to a much larger public. Let us hope that those who are seriously concerned with the administration of our future will be interested in the information provided. P. C. KOLLER

ANALYTICAL CHEMISTRY IN THEORY AND PRACTICE

Treatise on Analytical Chemistry

Part 2, Vol. 7. Edited by I. M. Kolthoff and Philip J. Elving, with the assistance of Ernest B. Sandell. Pp. xxiii + 567. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1961.) 139*s*.

IN a previous review of the earlier volumes of this *Treatise*, it was remarked that the various volumes were appearing haphazardly. At that time Volumes 1 and 3 of Part 2 had appeared, but not Volume 2. Since that time, so far as is known, Volume 7 is the only further section which has appeared.

In Volume 7 the systematic analytical chemistry of the elements is continued. It consists of chapters on the following elements: sulphur (B. J. Heinrich, M. D. Grimes and J. E. Puckett), selenium and tellurium (T. E. Green and M. Turley), fluorine (C. A. Horton), the halogens (G. W. Armstrong, H. H. Gill and R. F. Rolf), manganese (M. D. Cooper and P. K. Winter) and rhenium (C. L. Rulfs).

All these chapters are set out according to a standard pattern and are well classified. It is possible by reference to the contents page for each chapter to find readily any special information regarding the particular element. Each section describes the occurrence, manufacture and uses, the toxicology and industrial hazards, and the general properties of the various compounds. There are sections on sampling and methods of decomposition, dissolution and preliminary treatment of samples. Reference is made to almost every method available for the determination of a particular element, whether it be instrumental or purely chemical. Each section concludes with a selection of recommended procedures. The high standard of the previous volumes has been maintained, and the weaknesses which are inevitable in such a massive compilation are remarkably few.

A more extensive treatment of barium sulphate precipitation would have enhanced the value of the section on sulphate determination. It is surprising to find this procedure covered in two pages when it has been the subject of so many extensive investigations. There are no references to methods for the purification of barium sulphate, nor to alternative precipitants. It is also somewhat surprising to find the use of tetrahydroxyquinone and rhodizonic acid as indicators still recommended. When these indicators first came into use, there were no satisfactory titrimetric methods for the determination of sulphate ion. Much better procedures are now available.

The chapter on the analytical chemistry of fluorine is the most comprehensive that I have seen, but one would not altogether agree, however, with the methods recommended for the decomposition of organic fluorine compounds, nor the method for titrating fluoride with thorium nitrate.

The chapter on the other halogens is valuable because of the extent of the treatment; nevertheless, there are a few omissions. The section on rhenium is particularly to be welcomed. This metal was not discovered until 1925, hence its analytical chemistry is not as extensive as, and is less well known than, the other elements considered in this volume.

Part 2, Volume 7, is a worthy successor to the earlier volumes, and the editors of the *Treatise* must again be congratulated on their general approach to the problems involved in this type of compilation.

R. BELCHER

THE MYCOPLASMATACEAE

Pleuropneumonia-Like Organisms (PPLO) Mycoplasmataceae

By E. Kleineberger-Nobel. Pp. ix + 157. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1962.) 40*s*.

THE Mycoplasmataceae are now known to be ubiquitous organisms occurring as commensals and pathogens in many animal species and also as unwelcome fellow travellers in tissue-cultured cells. Ignorance of their existence in these situations may lead to serious misinterpretations. By collecting in one small volume much information previously dispersed in the scientific literature, this work should be a valuable source of reference for medical and veterinary diagnosticians and research workers.

Two short chapters describe the causative organisms of the major epizootics and other members of the group which have received most attention in research. They also provide a historical background to development of knowledge of pleuropneumonia-like organisms (PPLO). Chapters on morphology, isolation and metabolism (the latter by S. Razin) form the major portion of the book and present the data clearly and concisely. The large allocation to these topics is justified since technical problems have been the main limitations to progress in the examination of this group of organisms. The chapter on morphology is well illustrated and should be of particular value to those unacquainted with PPLO. Other chapters deal with the susceptibility of PPLO to inhibitory agents, the relationship of PPLO to chicken coccobacilliform bodies and also to the controversial relationship of PPLO to *L*-forms of bacteria. The chapter on serology, immunology and pathogenicity suffers from an important omission since publication preceded recent applications of fluorescent antibody techniques in the elucidation of the association of a PPLO with human atypical pneumonia and in the detection of PPLO in cell cultures. Investigations of the growth of PPLO in tissue cultured cells and the problem of contamination of cell cultures with PPLO receive a small amount of attention considering their practical importance.

With these reservations the volume is authoritative and fills a gap in microbiological literature.

IAN MACPHERSON

BENZENE EXTRACTS OF THE ORGUEIL METEORITE

By Dr. W. G. MEINSCHEIN
Esso Research and Engineering Co., Linden, New Jersey

LIFE cannot be defined in a quantitative manner[1,2]. Living things vary in size, function and composition, and these variations and the instabilities of organic molecules limit the precision with which the existence of prehistoric organisms can be established. Very few remnants of life are preserved in sediments over geological periods of time, and the compositions of these remnants in most cases do not reflect accurately the compositions of their counterparts or precursors in living plants and animals[3]. Nevertheless, the fossil remains of extinct organisms have been recognized as reliable indicators that strange organisms, unlike any seen by man, have existed on Earth. These indicators have been accepted because, so far as is known, only a living thing could have formed these fossils or the templates that gave the fossils their shapes.

Organisms also use templates to synthesize the organic molecules which make life possible[1,4]. Plants and animals are remarkably selective of the compounds they make and use. The precise structures of organic molecules may be equally as indicative of a biological source as are the features of fossilized skeletal remains[3,4]. Based on the established stabilities and the analyses of carbon compounds in sediments and in organisms[3,5-11] certain hydrocarbons appear to be the best-preserved remnants of former life on Earth. Hydrocarbons in association with some organic non-hydrocarbons can be extracted by benzene from most terrestrial sediments. This article will establish that benzene-extractable constituents of the Orgueil meteorite resemble chromatographically and spectroscopically those of some terrestrial sediments. In addition, it will be shown that the mass spectroscopic type analyses of alkanes from Orgueil fragments, terrestrial sediments and organisms are similar.

Reagents, apparatuses, instruments, techniques and controls used in this investigation have been described[3]. Trace metal and mineralogical determinations[12] have demonstrated the authenticity of the Orgueil fragments used in this investigation. Interpretations of mineralogical data suggest that the Orgueil meteorite originated in an aqueous environment[12,13]. Deuterium analyses[14] and data presented here strongly indicate that the benzene extract of these Orgueil fragments were not significantly contaminated by terrestrial organic matter.

Abiotic[15-17] and specifically Fischer–Tropsch[18] processes have been proposed as sources of carbonaceous substances in meteorites. Fischer–Tropsch products are commonly recognized as the best synthetic substitutes for petroleum. Active catalysts are used in making Fischer–Tropsch oils, and the oils are normally hydrogenated and fractionated to obtain petroleum-like products. Although the Orgueil fragments contain elemental sulphur which poisons most Fischer–Tropsch catalysts, a selected Fischer–Tropsch oil is included among the terrestrial samples to be discussed because this oil resembles natural lipids more so than did a variety of other abiotic products which have been analysed in these laboratories.

Infra-red spectra of a crude oil, a Fischer–Tropsch primary oil, and the benzene extracts of terrestrial sediments and the Orgueil chondrite are shown in Fig. 1. Variations are observed in the infra-red absorptions of different natural samples; but certain absorption bands usually appear in the infra-red spectra of the benzene extracts of former biospheres on Earth. In addition to the carbon to hydrogen (2,775–3,000 and 1,330–1,430 cm^{-1}) bands, sediment extracts and crude oils normally absorb near 3,300 (OH or NH); 1,030, 815, and 745 (aromatics), and 720 (*n*-alkyls) cm^{-1}. Recent sediment extracts also show complex carbonyl absorptions with maxima near 1,730 (ester carbonyl) cm^{-1}, whereas the carbonyl absorptions in extracts of ancient sediments are frequently greatest at slightly above 1,665 (organic acid salts) cm^{-1}.

Comparison of the infra-red spectra in Fig. 1 indicates the Orgueil extract absorbs at all the wave-lengths which are generally common to terrestrial sediment extracts and crude oils. Maxima in the carbonyl absorptions of the Orgueil and recent marine sediment extracts both appear at the same wave-length, near 1,730 cm^{-1}. The infra-red spectrum of the Fischer–Tropsch product suggests that it is a mixture of hydrocarbons and fatty acid esters which contains some terminally unsaturated or olefinic compounds (910 cm^{-1} absorption band).

Chromatographic data in Table 1 provide a measurement of the relative abundances of compounds of different types or polarities in the mixtures of compounds from the Orgueil meteorite and various terrestrial sources. Some of the values in Table 1 are average values. The numbers in parentheses, which appear beside the sample designations in Tables 1 and 2, indicate the number of samples averaged to obtain the values given. Standard deviations (σ) are shown for some of the analyses in Tables 1 and 2.

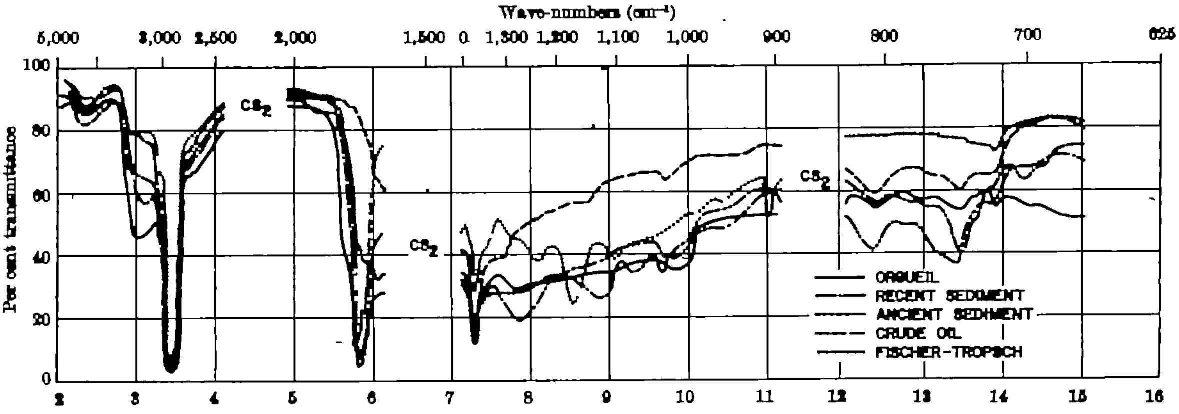

Fig. 1. Infra-red absorption spectra of the benzene-extractable fractions of the Orgueil meteorite, terrestrial sediments, crude oil, and Fischer–Tropsch (abiotic) product. Carbon disulphide was used as spectral solvent

Table 1. PERCENTAGES OF THE BENZENE EXTRACTS OF THE ORGUEIL METEORITE AND VARIOUS TERRESTRIAL SAMPLES THAT ARE ELUTED SUCCESSIVELY BY EQUAL VOLUMES OF n-HEPTANE (n-C_7), CARBON TETRACHLORIDE (CCl_4), BENZENE (C_6H_6), AND METHANOL (CH_3OH) FROM A SILICA-GEL CHROMATOGRAPHIC COLUMN

Percentages given for terrestrial samples are average for the number of samples shown by the numbers in parentheses to the right of the sample designations. Standard deviations for the analyses of the benzene extracts of terrestrial sediments are presented to the right of these analyses as σ values

Sample	Per cent of extracts eluted by			
	n-C_7	CCl_4	C_6C_6	CH_3OH
Meteorite:				
Orgueil	10·0	5·4	3 2	63·1
Sediments:				
Recent marine (12)	5·5 (σ= 5·9)	15·7* (σ=9·0)	7·3 (σ=7·5)	59·8 (σ= 8·7)
Soils (49)	5·5 (σ= 2·9)	4·8 (σ=4·1)	6·5 (σ=2·1)	64·0 (σ= 7·8)
Ancient (32)	26·3 (σ=21·6)	12·7* (σ=6·8)	24·8 (σ=7·8)	80 6 (σ=16 6)
Crude oils (103)	41·5	10·8	26·6	7·4
Abiotic:				
Fischer–Tropsch	32·6	10·4	13·0	40·3

* Contain elemental sulphur. Suggested values for sulphur-free eluates are stated in the discussion of Table 1.

Table 2. MASS SPECTROMETRIC TYPE ANALYSES OF THE SATURATED HYDROCARBONS (n-C_7 ELUATES FROM SILICA GEL COLUMNS) FROM THE ORGUEIL METEORITE, FISCHER–TROPSCH (ABIOTIC) PRODUCT, CRUDE OILS, LIVING THINGS, AND RECENT AND ANCIENT TERRESTRIAL SEDIMENTS

Percentages given for natural terrestrial samples are averages for the number of samples shown by the numbers in parentheses to the right of the sample designations. Standard deviations in the different types of hydrocarbons for various terrestrial samples are presented to the right of the type and values

Sample	Per cent of saturated hydrocarbon types						
		Cycloalkanes					
	Paraffins	1-ring	2-ring	3-ring	4-ring	5-ring	6-ring
Meteorite:							
Orgueil	17·6	28·9	17·2	11·8	11·3	6·4	6 8
Living things (11)	24·9 (6·2)	27·5 (2·8)	14·8 (1·4)	11·1 (2·5)	10·0 (2·1)	5·8 (2·6)	5·9 (2·6)
Sediments:							
Recent marine (12)	21·6 (6·2)	23 6 (2·5)	17·3 (2·4)	16 7 (1·1)	11·6 (1·5)	5·9 (1·0)	3·3 (0·6)
Ancient (19)	20·6 (6·0)	22·2 (3·6)	18·5 (2·9)	14·7 (3·2)	11·8 (3·2)	6·2 (1·2)	6·1 (2·7)
Crude oils (5)	26·1	28·5	20 2	13·0	7·6	3·5	2·8
Abiotic:							
Fischer–Tropsch	30·3	55·9	10·1	2·3	0·6	0·2	0·7

All the terrestrial sediment samples in Table 1 were fractionated prior to the beginning of the meteoritic investigations[11]. The recent marine and some ancient sediment, but none of the soil extracts, contained elemental sulphur. As shown in Table 1, the sulphur was concentrated in the carbon tetrachloride eluates of the sulphur-containing samples. Mass spectral analyses showed sulphur in only 3 of the ancient sediments, and the concentration of sulphur in the eluates of 4 recent sediment extracts were determined. Benzene extracts of the Orgueil also contained free sulphur. It was removed immediately from these extracts to eliminate the possibility of subsequent reaction between sulphur and the meteoritic carbon compounds[18]. All data in Table 1 may be placed on a sulphur-free basis by averaging the ancient sediments which did not contain sulphur and by correcting the eluates of the recent marine sediments for their determined sulphur contents. The sulphur-free data for the recent marine and ancient sediment samples are (per cent): recent marine sediments; n-heptane (n-C_7)–6·0, CCl_4–8·0, benzene (C_6H_6)–5·0, methanol (CH_3OH)–63·0: ancient sediments; n-C_7–30·5, CCl_4–10·3, C_6H_6–23·7, CH_3OH–30·2.

On the basis of sulphur content and the silica-gel chromatographic analyses, the benzene extracts of the Orgueil fragments resemble those of recent marine sediments more than of the other terrestrial samples reported in Table 1. Interestingly the terrestrial samples, with exception of the Fischer–Tropsch product, show a systematic variation in chromatographic compositions. Soils and recent sediment extracts contain lower concentrations of saturated (n-C_7 + CCl_4 eluates) and aromatic (C_6H_6 eluates) hydrocarbons than do ancient sediment extracts and crude oils. Preferential preservation of hydrocarbons in ancient sediments in general[11] and selective accumulation[19–21] of hydrocarbons in petroleum deposits may explain the gradational changes in biologically derived substances from different terrestrial sources[3,11,21]. But, of direct interest to the meteoritic investigation, these differences indicate how to differentiate between the remnants of living things that are initially deposited and those that are eventually preserved or redistributed in ancient sediments or former biospheres.

The similarity between the chromatographic analyses of the benzene extracts of recent terrestrial sediments and the Orgueil fragments affords evidence that the meteoritic fragments were not contaminated appreciably by either crude oils or other carbonaceous materials from ancient terrestrial sediments; whereas the high concentrations of benzene-extractable materials in the Orgueil

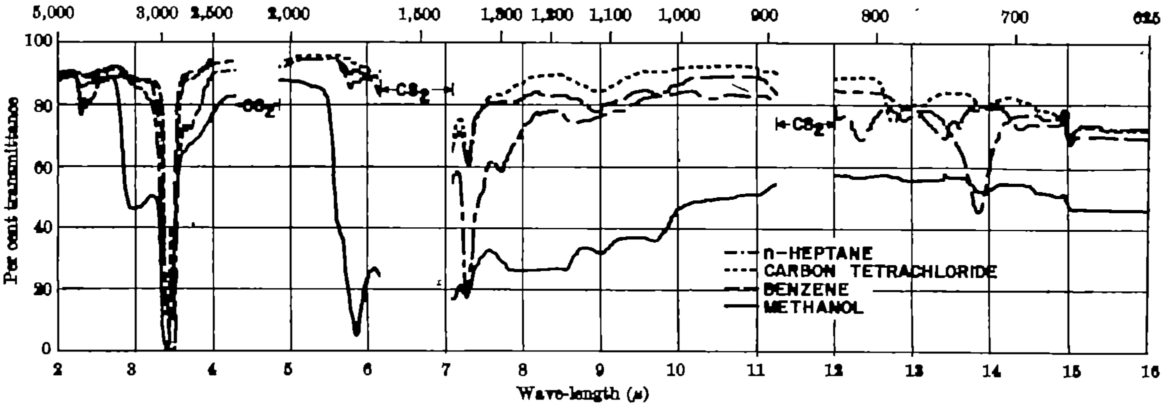

Fig. 2. Infra-red absorption spectra of the silica-gel chromatographic fractions of the benzene extracts of the Orgueil meteorite. Carbon disulphide was used as spectral solvent

chondrite make it unlikely that the meteorite could have acquired these materials from soils which contain on the average less than 1/50 of the concentration of extracts found in this meteorite[3,21].

Infra-red spectra of the silica-gel chromatographic fractions of the Orgueil extracts are presented in Fig. 2. These spectra show only carbon to carbon and carbon to hydrogen bond absorptions for the n-C_7 and CCl_4 eluates. Minor concentrations of aromatic hydrocarbons are suggested by the 795 cm^{-1} absorption band in the CCl_4 eluate spectrum, and the 720 cm^{-1} band in the n-C_7 eluate indicates the presence of n-paraffins in this fraction. These results show that the n-C_7 eluate and a major portion of the CCl_4 eluate are composed of saturated hydrocarbons. Aromatic hydrocarbons are concentrated in the C_6H_6 eluate, and the bands at 1,030, 815 and 745 cm^{-1} in the spectrum of this fraction are typical of the absorptions observed for aromatic mixtures isolated from terrestrial sediments and crude oils[3,23]. These bands have been referred to as 'oil bands' because they appear in the infra-red spectra of all aromatic fractions of petroleum[23]. The spectrum of the CH_3OH eluate in Fig. 2 has the OH or NH absorption at 3,300 cm^{-1} and the carbonyl absorption near 1,730 cm^{-1} which are observed in the infra-red spectra of most recent marine sediment extracts (Fig. 1).

Additional confirmation of the interpretations of the infra-red spectra of the Orgueil eluates is provided by the ultra-violet spectra in Fig. 3. Negligible ultra-violet absorptions by the n-C_7 eluate are indicative of a saturated hydrocarbon fraction, while the small absorptions by the CCl_4 eluate suggest that it consists mainly of saturated hydrocarbons containing minor concentrations of aromatics or conjugated olefines. Aromatic hydrocarbons from the meteoritic extracts are concentrated in the strongly absorbing C_6H_6 eluate, and the maxima in the ultra-violet and mass spectra of this eluate can be attributed to phenanthrenes, pyrenes and chrysenes[3]. Fluorenone has a spectrum resembling the CH_3OH eluate spectrum in Fig. 3.

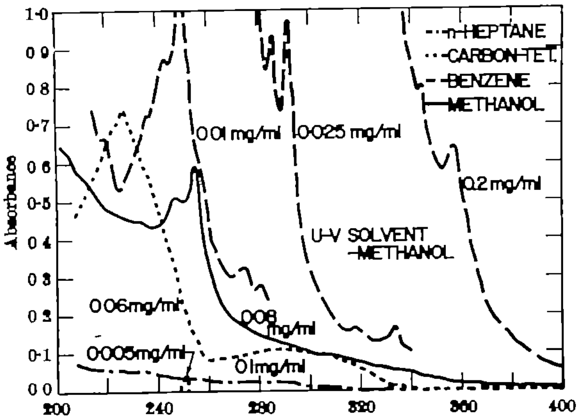

Fig. 3. Ultra-violet absorption spectra of the silica-gel chromatographic fractions of the benzene extracts of the Orgueil meteorite. Iso-octane was used as spectral solvent

Previously reported results show that some recent marine sediments[21] and soils[21,24] contain relatively simple aromatic hydrocarbon fractions. The simplicity of the aromatic mixtures in recent sediments and the structures of aromatic compounds in sediments and petroleum have been cited as evidence that aromatic hydrocarbons are derived from plant and animal steroids, isoprenoids and related compounds[10,11,21]. It has been postulated on the basis of theoretical considerations and the distributions of aromatics in sediments that these compounds are products of the catabolic activity of anaerobes[11].

Mixtures of aromatic hydrocarbons in extracts of ancient terrestrial sediments and in crude oil are much more complex than the aromatic fractions in recent sediments[11,21,25]. The complexity of the aromatic mixtures in ancient sediments has been attributed to the mixing of hydrocarbons from many different sedimental environments that occur during the redistributions of hydrocarbons which take place over geological periods of time and which lead in some instances to the formation of petroleum deposits[11,21,25]. The complexity of the aromatics in ancient sediments also may result partially from chemical alterations that over long periods of time may change the structures of some of the aromatic hydrocarbons in sediments.

As in the case of the chromatographic analyses of the terrestrial samples in Table 1, gradational changes can be observed in the compositions of the aromatic fractions from recent sediments, ancient sediments, and crude oils. These changes may be deduced from the ultra-violet spectra in Fig. 4, and the results in Fig. 4 may be interpreted as supporting the interpretation of the data in Table 1.

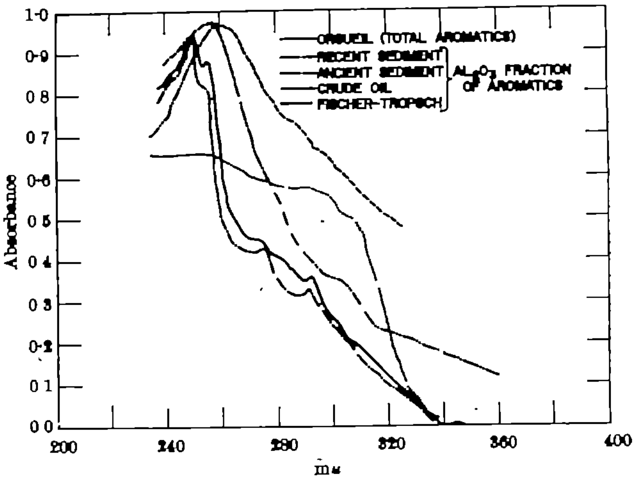

Fig. 4. Ultra-violet absorption spectra of aromatic fractions from the Orgueil meteorite and terrestrial samples. Iso-octane was used as spectral solvent

Relatively simple mixtures of aromatics give sharper absorption bands in the ultra-violet region than do complex aromatic fractions. Although none of the terrestrial samples available for this investigation contained aromatic fractions as simple as those previously reported[21,24], or as simple as the aromatics (C_6H_6 eluate from silica gel) isolated from the Orgueil meteorite, an additional fractionation of the C_6H_6 eluate of the recent marine sediment did yield a fraction as shown in Fig. 4, that had an absorption spectrum similar to the spectrum of the meteoritic aromatics.

Alumina chromatography was used to simplify the mixtures of aromatics from all the terrestrial samples. The ultra-violet spectra presented in Fig. 4 for the terrestrial samples were obtained on equivalent alumina fractions that had the same adsorption properties on alumina as do phenanthrenes, chrysenes, and pyrenes[21]. These alumina fractions were selected because the aromatic fraction of the Orgueil meteorite is composed predominantly of phenanthrenes, chrysenes and pyrenes[3]. These same aromatics are frequently the most abundant aromatics in recent terrestrial sediments[21,24].

It is noteworthy that the sharpness of the absorption bands or detail of the spectra in Fig. 4 decrease from the Orgueil or recent marine sediment fraction spectra to the ancient sediment fraction spectrum to the crude oil fraction spectrum. The ultra-violet spectrum of the Fischer–Tropsch fraction does not resemble the spectra of equivalent alumina fractions from other terrestrial samples. The simplicity of the aromatic hydrocarbons from the Orgueil effectively eliminates the possibility that these compounds were obtained by chance contamination of the meteorite by crude oil or organic matter from ancient terrestrial sediments. Extensive distillations and chromatographic fractionations of a crude oil followed by a careful recombination of selected fractions would be required to obtain a fraction from oil that has ultra-violet and mass spectra[3,26] similar to the aromatics from the Orgueil. Only rarely do recent marine sediments contain so simple a mixture of aromatics as do these Orgueil fragments, and the concentration of aromatics in these fragments are many times the average aromatic concentration in recent terrestrial sediments. Extracts of living things contain negligible concentrations of aromatic hydrocarbons. Neither terrestrial organisms nor organic substances are likely sources of a significant portion of the aromatics in these Orgueil fragments.

Alkane hydrocarbon type analyses[26] are given for the saturated hydrocarbon fractions (n-C_7 eluates) of meteoritic, sedimental, biological and Fischer–Tropsch (abiotic) samples in Table 2. These mass spectrometric analyses indicate the types of alkanes in the various fractions. All the type analyses of the saturated hydrocarbons from the Orgueil meteorite fall within the ranges of values found for biological and sedimental hydrocarbons, whereas the Fischer–Tropsch's saturates contain far higher concentrations of mono*cyclo*alkanes (or perhaps olefines) and much lower concentrations of poly*cyclo*alkanes than do any of the terrestrial samples included in the average analyses reported in Table 2.

Reactants and reaction conditions that existed throughout the evolution, development and destruction of a meteoritic parent body are not accurately known. However, extensive investigations[5–11,21,22] of the composition and origins of carbonaceous materials in terrestrial sediments indicate that living things have exerted a detectable control on the composition of these materials. Although unknown reactants under undefinable conditions may have made the carbon compounds in the Orgueil meteorite, a general dissimilarity between an abiotic (Fischer–Tropsch) product and the benzene extracts of terrestrial sediments has been demonstrated. The analyses in this article show statistically significant agreements between the benzene-extractable fractions of Orgueil fragments and some terrestrial sediments as well as between meteoritic and biological alkanes.

This investigation was suggested by Dr. Bartholomew Nagy of Fordham University, and the extractions of the Orgueil fragments were conducted by Dr. D. J. Hennessy of Fordham University. I thank Drs. Hennessy and Nagy for their helpful discussions of this work, and Drs. Brian Mason, R. F. Folinsbee, Lincoln LaPaz, and E. P. Henderson for providing the meteorite samples. Mr. T. C. Menzel, G. G. Wanless and J. J. Waters assisted in some analyses of meteoritic extracts. A 500-year-old butter sample was contributed by the late Dr. Werner Bergmann of Yale University.

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MAGNETIC AND CHEMICAL INVESTIGATIONS OF IRON IN TEKTITES

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THE ferric-to-ferrous iron ratio (hereafter designated simply as the ratio) in glasses is dependent on three important factors: (*a*) the thermal history; (*b*) the oxidizing or reducing conditions of the glass melt; (*c*) the time of heating. Abou-El-Azm[1] has shown that the reduction of Fe_2O_3 added to high-silica glass increased rapidly above 1,400° C. Banerjee[2] has discussed the effects of a reducing atmosphere and the time of heating on the ferrous iron in glasses, and Bamford[3] has examined the state of the iron in some reduced glasses.

Friedman, Thorpe and Senftle[4] have compared the chemical composition with the magnetic properties of tektites and some glasses formed by the fusion of terrestrial rocks. They found that the ratios for six rocks fused at 2,500° C in a solar furnace under atmospheric conditions with one exception approached a limiting value of about 0·4. This value is somewhat higher than that of the average ratio (~ 0·13) calculated for tektites from data in the literature[5]. These calculated ratios varied considerably and ranged from 0·03 to 0·31 (ref. 4). The larger ratio (~ 0·4) for the fused rocks was attributed to fusion under normal atmospheric conditions ($P_{O_2} = 1/5$ atm.). The variation of the ratio reported for tektites may be due in part to some scattered questionable analyses in the literature. On the other hand, if these differences were real, it suggests that the partial pressure of oxygen in the melting atmosphere was substantially different for the various tektite falls, and/or that there may have been considerable differences in melting temperatures.

To solve this problem, more, and also more accurate, analyses of the ferric-to-ferrous ratio in tektites from different strewn fields is desirable. To this end, monitored, high-precision chemical analyses have been made on twenty-one bediasites. Analysis of the results shows the ratio to be constant (0·053) within narrow limits for bediasites.

Table 1. CHEMICAL ANALYSES OF ELEMENTAL TITANIUM, MANGANESE, AND IRON IN BEDIASITES*

Sample No.	Titanium (%)	Manganese (%)	Total iron (%)	Ferrous iron (%)	Ferric iron (%)	Fe^{+3}/Fe^{+2}
Bediasites						
BM-1	0·39	0·015	2·25	2·23	0·02	0·008$_9$
BM-14	0·40	0·014	2·19	2·12	0·08	0·037$_7$
30775–77	0·36	0·027	2·32	2·19	0·13	0·059$_4$
B–140	0·41	0·023	2·62	2·59	0·03	0·011$_6$
B–20	0·35	0·039	2·21	2·18	0·03	0·013$_8$
B–17	0·37	0·039	2·61	2·57	0·04	0·015$_6$
B–50	0·57	0·021	2·73	2·56	0·16	0·062$_5$
B–74	0·44	0·023	3·03	2·88	0·15	0·052$_1$
BM–18	0·39	0·018	2·76	2·76	0·00	0·000$_0$
B–80	0·48	0·023	3·26	3·09	0·16	0·051$_8$
B–6	0·44	0·020	3·10	2·91	0·19	0·065$_3$
30247–38	0·46	0·020	3·22	2·99	0·23	0·076$_9$
B–84	0·44	0·023	3·08	2·94	0·14	0·047$_6$
B–85	0·43	0·033	3·09	2·92	0·15	0·051$_4$
B–86	0·47	0·024	3·40	3·14	0·22	0·070$_1$
B–87	0·45	0·023	3·39	3·15	0·24	0·076$_2$
30780–23	0·54	0·020	3·51	3·43	0·12	0·035$_0$
B–76	0·51	0·015	3·73	3·64	0·09	0·024$_7$
B–105	0·52	0·023	3·96	3·74	0·22	0·058$_8$
30763–10	0·60	0·016	4·11	4·01	0·22	0·054$_9$
B–90	0·63	0·015	4·28	4·09	0·19	0·046$_4$

* Cuttitta, F., Carron, M. K., Fletcher, J. D., and Chao, E. C. T., 1962, Chemical composition of bediasites and philippinites: *U.S. Geol. Surv. Astrogeologic Studies Semiann. Prog. Rep. to N.A.S.A.*, Feb. 26, 1961—Aug. 24, 1961. Nickel content is less than 50 p.p.m. (Cuttitta, Carron, Fletcher and Chao, 1962).

Magnetic susceptibility measurements have been made as part of an investigation of the physical properties and chemical composition of tektites. To supplement the chemical analyses, a magnetic susceptibility method was developed for the quantitative determination of total and ferrous iron. The proposed susceptibility method is less tedious and time-consuming than chemical analysis, is non-destructive, can be made on milligramme amounts of sample, and provides results comparable with those obtained by chemical analyses. Both chemical and susceptibility techniques have been used to determine ferrous and total iron in selected tektite specimens from other strewn fields. Similar to bediasites, our results show that the ratio is nearly constant (≈0·05) within narrow limits for these specimens.

Twenty-one bediasites were carefully selected from a collection of about four hundred specimens[6]. The selection was made so as to represent the range of the indices of refraction and specific gravities exhibited by the tektite collection. End members of this suite are *BM*-1, with an index of refraction of 1·4828 ± 0·0004 and specimen *B*-90 with an index of refraction of 1·5076 ± 0·0004. The specimens are listed in Table 1 in increasing order of index of refraction. The bediasites were analysed for manganese, titanium, nickel, ferrous iron and total iron as Fe_2O_3, and with the exception of nickel the results are shown in Table 1. (Nickel analyses are all less than 50 p.p.m. Individual quantitative analyses were made spectrographically by J. D. Fletcher (personal communication).) In addition, the total iron as Fe_2O_3 was determined for 15 other selected tektites (Table 3). These results are averages of duplicate determinations by the various methods, the coefficient of variation of the average being 3 per cent for ferrous iron and total iron, 5 per cent for titanium, and 10 per cent for manganese and nickel. Although spectrophotometric, volumetric, spectrographic, and X-ray fluorescence techniques were used, the average obtained by these methods will be referred to simply as the chemical determination throughout this article. Using these different techniques, the determinations were closely monitored by similar determinations in granite *G*-1, diabase *W*-1, and selected National Bureau of Standards certified samples.

In the spectrophotometric method[7,8] ferrous iron reacts with *o*-phenanthroline in the *p*H range of 2–9 to form a red complex having an absorption maximum at 512 mμ. The complex is stable and conforms to Beer's Law up to a concentration limit of 10 p.p.m. Ferric iron must be reduced to ferrous iron initially with hydroxylamine hydrochloride, hydroquinone, ascorbic acid, or *N,N'*-dimethylformamide. These analyses were made at *p*H 4·5 using acetic acid–sodium acetate as a buffer.

The X-ray fluorescence determination of total iron, titanium, and manganese required 250 mg of sample which was fused with 250 mg of La_2O_3 and 1·000 g of $Li_2B_4O_7$ in a graphite crucible at 1,000° C for 8–10 min[9]. The cooled bead was ground in a mixer-grinder to pass a 325-mesh sieve. The sample was double pelleted with a boric acid backing for additional strength. The analyses were made with a single channel fluorescent vacuum X-ray spectrometer using a lithium fluoride-analysing crystal and a krypton–halogen-filled Geiger counter. X-rays were produced with a tungsten target tube operated at 30 kV and 10 m.amp. The time required to accumulate 10,000 counts for the iron $K\alpha$ line at 57·45° 2θ, titanium $K\alpha$ line at 86·07° 2θ, and manganese $K\alpha$ line at 62·91° 2θ was measured. The X-ray results were obtained by counting each standard and sample twice at random.

The ferrous iron content of the tektites was determined by spectrophotometric and volumetric methods. In the volumetric method, the tektite sample was decomposed with hydrofluoric and sulphuric acids in the absence of air. The ferrous iron in the resulting solution was titrated with $K_2Cr_2O_7$ using sodium diphenylamine sulphonate as an internal indicator.

The spectrophotometric determination of ferrous iron[10] was made on 5–15-mg amounts of sample. Decomposition of the tektite was effected at room temperature by hydrofluoric acid containing pentavalent vanadium to oxidize ferrous iron as it dissolves. The spectrophotometric method utilizes the reversibility of the reaction:

$$V^{5+} + Fe^{2+} \rightleftarrows V^{4+} + Fe^{3+}$$

The direction of this reaction is dependent on *p*H. In strongly acid solution, the reaction proceeds quantitatively from left to right. When the *p*H is increased to greater than 2, ferric iron is quantitatively reduced to the divalent state by quadrivalent vanadium. The re-formed ferrous iron is determined by using *o*-phenanthroline as the chromogenic agent[7,8].

Manganese and titanium were also determined spectrophotometrically. Disodium-1,2-dihydroxy-benzene-3,5-disulphonate (tiron) yields a strong yellow colour with titanium (IV) in the *p*H range 4·3–9·6 (ref. 11). The interference of iron was overcome by reduction with ascorbic acid at *p*H 4·7, and the absorbance of the resulting solution was measured at 410 mμ. The purple permanganate colour was utilized for the determination of manganese. The oxidation was effected with ammonium persulphate (peroxydisulphate) at the boiling point in a phosphoric–nitric acid medium in the presence of silver nitrate. The absorbance of the solution was measured at 525 mμ.

Nickel and manganese were determined by a quantitative spectrographic method similar to that described by Bastron, Barnett and Murata[12].

The total iron plotted against the ferrous iron in Fig. 1 shows a good linear relationship which passes through the origin. Thus:

$$C_{Fe^{t}} = kC_{Fe^{++}} = C_{Fe^{++}} + C_{Fe^{+++}} \qquad (1)$$

where k is the slope of the line, and C is the concentration of the iron. If $C_{Fe^{+++}}/C_{Fe^{++}} = R$, then from equation (1) the ratio $R = k - 1$. From a least-squares analysis of the data, k was determined, and $R = 0{\cdot}053$. As shown in Table 1, the ratio ranges from 0·01 to 0·08 and has a median value of $R = 0{\cdot}051$. This is in good agreement with the value, $R = 0{\cdot}053$, calculated from the data of Fig. 1. It is thus evident that the ferric-to-ferrous ratio in bediasites is constant within relatively narrow limits.

The magnetic susceptibility measurements were made on a quartz helical spring balance developed by Senftle *et al.*[13], and the procedure followed has been described in a previous paper[14]. Care was taken to clean the specimens in hydrochloric acid and to measure the susceptibility at several field strengths to ensure against possible con-

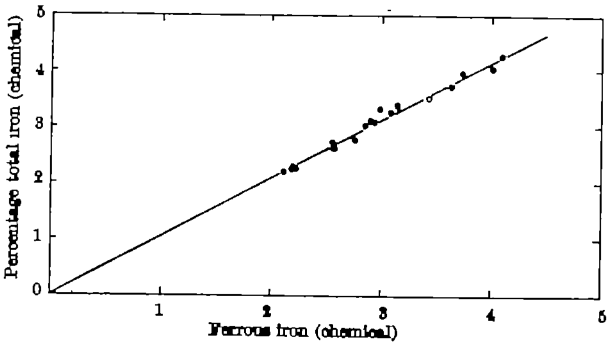

Fig 1 Variation of ferrous iron with total iron in bediasites as determined chemically

tamination with ferromagnetic impurity. All measurements were made at room temperature (29° C).

Tektites exhibit mild paramagnetic but no significant ferromagnetic properties[14]. From the elemental composition of tektites, it is apparent that, to a first approximation, the paramagnetism is a function of the iron content. Nickel, manganese and titanium concentrations are low, and their combined contribution to the paramagnetism is probably less than 2 per cent. Thus, the magnetic susceptibility of a tektite, at a given temperature, can be described as:

$$\chi_T = \chi_{Fe^{++}}C_{Fe^{++}} + \chi_{Fe^{+++}}C_{Fe^{+++}} + [1 - (C_{Fe^{++}} + C_{Fe^{+++}})]\chi_g \quad (2)$$

where $\chi_{Fe^{++}}$ and $\chi_{Fe^{+++}}$ are the specific atomic susceptibilities of ferrous and ferric iron calculated from equation (10) here, $C_{Fe^{++}}$ and $C_{Fe^{+++}}$ are the concentrations of ferrous and ferric iron, and χ_g is the residual magnetic susceptibility of the base glass, including the small amounts of manganese, titanium and nickel. Owing to the almost uniform composition of tektites, χ_g can be considered to be constant for all practical purposes.

Table 2. COMPARISON OF THE MAGNETIC AND CHEMICALLY DETERMINED TOTAL IRON AND FERROUS IRON IN BEDIASITES

Sample No.	Magnetic Susceptibility E.M.U./g(×10⁶)	% Total iron Calculated from eq. 8	% Total iron Determined chemically	% Ferrous iron Calculated from eq. 6	% Ferrous iron Determined chemically
BM–1	4·00	2·25	2·25	2·17	2·23
BM–14	3·68	2·09	2·19	2·02	2·12
30775–77	4·25	2·38	2·29	2·30	2·19
B–20	4·00	2·25	2·22	2·17	2·18
B–17	4·53	2·53	2·61	2·43	2·57
B–50	5·15	2·85	2·73	2·74	2·56
B–74	5·35	2·95	3·03	2·82	2·88
BM–12	5·18	2·86	2·76	2·75	2·76
B–80	5·84	3·20	3·26	3·07	3·09
B–6	5·73	3·15	3·10	3·02	2·91
30247–38	5·92	3·24	3·22	3·11	2·99
B–84	5·55	3·05	3·08	2·93	2·94
B–85	5·64	3·10	3·09	2·98	2·92
B–86	6·43	3·51	3·40	3·36	3·14
B–87	5·90	3·23	3·39	3·10	3·15
30780–23	6·78	3·69	3·51	3·53	3·43
B–76	6·51	3·55	3·73	3·40	3·64
B–105	7·30	3·96	3·96	3·79	3·74
30763–10	7·83	4·23	4·11	4·05	4·01
B–90	7·88	4·25	4·26	4·07	4·09

The magnetic susceptibilities and a comparison of the magnetically and chemically determined total iron and ferrous iron of the bediasites are shown in Table 2. The susceptibilities are also plotted against the total iron determined chemically in Fig. 2. The relation is remarkably linear, and a least-squares analysis of the data leads to the equation:

$$\chi_T = 193{\cdot}6 \times 10^{-6}\, C_{Fe^{t}} - 0{\cdot}3607 \times 10^{-6} \quad (3)$$

Although the actual points are not shown, to avoid confusion, the curve of susceptibility as a function of the ferrous iron also is a good linear relation and is represented by the broken line slightly above the solid total iron line in Fig. 2. A least-squares treatment of the data yields the following equation:

$$\chi_T = 204{\cdot}5 \times 10^{-6}\, C_{Fe^{++}} - 0{\cdot}414 \times 10^{-6} \quad (4)$$

Actually the susceptibility of the tektite glass without the iron (*y*-intercept) should be identical in both equations, but due to the scatter of the experimental points they are slightly different. However, assuming both are the same, it can be shown algebraically that the ferric-to-ferrous ratio, *R*, is a constant and equal to:

$$R = b - a/a \quad (5)$$

where a is the coefficient of $C_{Fe^{t}}$ ($193{\cdot}6 \times 10^{-6}$) and b is the coefficient of $C_{Fe^{++}}$ ($204{\cdot}5 \times 10^{-6}$) in the foregoing equations. Substituting with numerical values, one obtains an approximate value for the ratio, $R = 0{\cdot}056$. Again, this is in close agreement with the least squares chemically determined ratio (0·053).

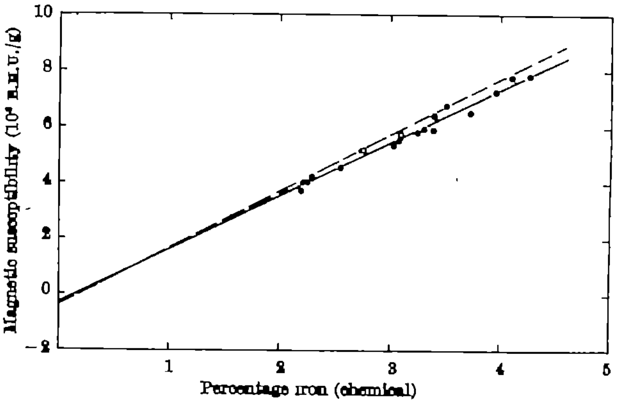

Fig. 2. Variation of the magnetic susceptibility with total iron content in bediasites (solid line). Dashed line is the susceptibility as a function of ferrous iron (to avoid confusion, actual points are not shown) $\chi_T = 193{\cdot}6\, C_{Fe^{t}} - 0{\cdot}3607$

The magnetic susceptibility measurements are good to better than 2 per cent, and hence, assuming no large effects due to other transition elements, the total iron as given by the magnetic measurements and equation (3) should be accurate to slightly more than 1 per cent. The susceptibility measurements are made relatively easy and fast, and thus this technique for total iron analysis proved very convenient for bediasite investigations. As the ratio is nearly constant, it also provided a practical method to obtain the ferrous iron content. Likewise, the ferric iron could be obtained, but due to its relatively low concentration it was not nearly as accurate. (The ferrous and ferric iron contents can be calculated from the expressions:

$$C_{Fe^{++}} = 0{\cdot}489\, \chi_T + 0{\cdot}219 \quad (6)$$

$$C_{Fe^{+++}} = 0{\cdot}0285\, \chi_T - 0{\cdot}031 \quad (7)$$

where C_{Fe} is in percentage and χ_T is the magnetic susceptibility in 10^{-6} E.M.U./g.)

Having established the ferric-to-ferrous iron ratio for bediasites as approximately constant, one now asks whether or not the same ratio holds for tektites from other strewn fields. Ultimately, chemical analyses of total ferrous and ferric iron made on a reasonable sampling from each strewn field would be desirable to answer this question. In place of detailed chemical analyses, however, the magnetic susceptibility technique offers a method of determining this information with only a total iron chemical analysis being required.

If for convenience one expresses equation (1) in terms of the concentration of total iron, $C_{Fe^{t}} = C_{Fe^{++}} + C_{Fe^{+++}}$, then:

$$\chi_T = \left(\frac{\chi_{Fe^{++}} + R\chi_{Fe^{+++}}}{1 + R}\right) C_{Fe^{t}} + (1 - C_{Fe^{t}})\chi_g \quad (8)$$

As $(1 - C_{Fe^{t}})$ is approximately one, and as χ_g is small, one can approximate the last term in the equation by a constant without incurring a large error. Thus:

$$\chi_T = \left(\frac{\chi_{Fe^{++}} + R\chi_{Fe^{+++}}}{1 + R}\right) C_{Fe'} + G \qquad (9)$$

where $G = (1 - C_{Fe'})\chi_g$. This is the analytical expression for the experimentally determined relation, equation (3). In so far as the composition of all tektites is quite uniform, it is reasonable to assume that G will be essentially single valued for all tektites. Thus, if equation (3) can be shown to hold for tektites from other strewn fields one may conclude that the ferric-to-ferrous iron ratio is approximately the same for these tektites.

A comparison is made in Table 3 of the total iron determined from equation (3) with those determined chemically for tektites from several strewn fields. The comparison is very good indicating that within narrow limits the median $C_{Fe^{+++}}/C_{Fe^{++}}$ ratio is nearly the same for all the tektites studied, and essentially the same as that for bediasites, namely, about 0·053. Before a definite conclusion can be made, however, a detailed analysis similar to that made on bediasite should be made on tektites from other strewn fields.

Table 3. COMPARISON OF THE MAGNETIC AND CHEMICALLY DETERMINED TOTAL IRON IN SELECTED TEKTITES FROM OTHER LOCATIONS

Sample No.	Location	Mag. suscept. ($\times 10^6$) E.M.U./g	% Total iron From eq. (3)	% Total iron Chem
I_D–1	Indochina	7·76	4·19	4·25
I_D–8		7·11	3·86	3·88
I_D–13		6·85	3·72	3·81
I_D–23		7·53	4·07	4·14
J_S–7	Java	7·68	4·15	4·13
J_S–18		7·03	3·82	4 06
1771–6	Australia	6·17	3·37	3 36
77611		5·87	3·22	3·22
TT–11–7	Thailand	6·66	3·63	3·78
TT–18–1		6·23	3·46	3·46
P_I–73	Philippine Islands	8·57	4·61	4·61
P_I–94		7·24	3·93	3·83
P_I–33		6·76	3·68	3·31
P_S–30		6·16	3·37	3·55
P_S–65		6·62	3·61	3·69
P_G–12		7·47	4·04	4·24
P_G–25		6·47	3·53	3·74
P_P–163		6·80	3·70	3·85
P_P–170		6·79	3·69	3·69
P_P–172		6·55	3·57	3·73
P_P–177		7·78	4·20	4·15
P_P–187		6·97	3·79	3·78
P_P–216		6·99	3 80	3·89
T–13	Ivory Coast	8·64	4 65	4·85

Our results indicate that the ferric-to-ferrous iron ratio for all tektites is relatively constant (approximately 0·05) and low. These results do not concur with ratios calculated from data reported in the literature[4,5,14], which show a considerable variation within a strewn field and also between different tektite families. For example, both the average analyses given for australites by Barnes[4] and the more recent analyses reported by Taylor[14] indicate an average ratio of about 0·12. Taylor mentions that some of his samples were crushed in a steel mortar and mechanically ground in an agate mortar before chemical analysis. It is conceivable that such a preparation in air may have oxidized a significant amount of iron to the ferric state.

As indicated in the introduction, tektites show a relatively uniform composition and history of formation. Unlike other natural glasses (volcanic glasses and fulgarites) and synthetic glasses, the tektites from different strewn fields have one interesting chemical characteristic, namely, a low ferric-to-ferrous iron ratio indicating a relatively uniform thermal history under reducing conditions.

Synthetic glasses of tektite composition prepared under reducing conditions and high temperature should exhibit similar characteristics. It should be possible to calculate their total and ferrous iron content from a magnetic susceptibility measurement and equations (3) and (6). In contrast, synthetic glass melts prepared under normal atmospheric conditions should undergo a slight oxidation of the iron to Fe_3O_4, and hence the calculated total and ferrous iron will be high compared with the chemical values.

Magnetic and chemical analyses on several synthetic glasses of tektite composition prepared in a normal atmosphere of air are shown in Table 4. The total and ferrous iron as calculated from the magnetic susceptibility data is high as compared with the chemical values in almost every case. In two cases, so much Fe_3O_4 was formed that the samples were ferromagnetic and could not be measured.

Table 4. FERRIC AND FERROUS IRON IN SYNTHETIC GLASSES OF TEKTITIC COMPOSITION AS DETERMINED BY MAGNETIC AND CHEMICAL METHODS

No.	Simulated composition, firing temperature and time	Mag. suscept. ($\times 10^6$ E.M.U./g)	Total iron Calc. from eq. (3)	Total iron Chem.	Ferrous iron Calc. from eq. (6)	Ferrous iron Chem.
STG–2	Australite, 1,800° C 16 h.	7·52	4·07	3·74	3·90	3·48
ST–65	Australite, 1,800° C 16 h	*	*	5·20	*	4·43
ST–72	Australite, 1,800° C 16 h	8·29	4·47	4·23	4·27	3·73
ST–80	Australite, 1,800° C 16 h	*	*	3·17	*	2·80
ST–1	Bediasite, 1,410° C 16 h	3·31	1·90	1·74	1 84	1·36
ST–2	Bediasite, 1,410° C 16 h	3·21	1·84	1·75	1·57	1·60

* Specimens ferromagnetic. Reliable measurements could not be made

These data indirectly support the concept that tektites, unlike other natural glasses, have experienced a thermal history under reducing conditions. Further, the method of calculating total and ferrous iron is valid only for tektites, and not generally applicable to other glasses. The foregoing results lead to some interesting aspects of the magnetic properties of iron in tektites. The paramagnetism of the iron arises from the electrons circulating in orbit around the nucleus. The total magnetic moment of such an electron may be divided into two parts: that due to the spin of the electron, and that due to its orbital motion. While the presence of electrons in neighbouring atoms does not generally affect the spin, the electric fields of nearby electrons can significantly alter the orbit of an electron. In fact, the ligand fields are often strong enough completely to immobilize the orientation of an electron orbit against the effect of an outside magnetic field. The orbital magnetic moment is then said to be quenched, and the resulting orbital magnetic moment is small or zero. For the case of a free ion removed from all external fields, the magnetic susceptibility in electromagnetic units per gramme due to both spin and orbital moments is given by:

$$\chi = \frac{N\beta^2\mu^2}{3kTM} = \frac{N\beta^2}{3kTM}\,[4S(S+1) + L(L+1)] \quad (10)$$

where N is Avagadro's number, β is the unit Bohr magneton, k is Boltzmann's constant, T is the absolute temperature, and M is the atomic weight. μ is the total magnetic moment for the transition elements and is equal to $\sqrt{4S(S+1) + L(L+1)}$, where S and L are the spin and orbital quantum numbers, respectively. In the case of a ligand field which is sufficiently strong to quench the orbital moment, $L = 0$, and the 'spin only' magnetic moment is responsible for the magnetic susceptibility.

Theoretically, $\chi_{Fe^{++}}$ and $\chi_{Fe^{+++}}$, the specific susceptibilities of ferrous and ferric iron, can be determined from equation (10) by inserting the proper spin and orbital quantum number. These can be obtained from consideration of the atomic energy-levels. For ferric iron $S = 5/2$ and $L = 0$, and $\chi_{Fe^{+++}}$ is simply $259{\cdot}4 \times 10^{-6}$ E.M.U./g at 29° C. For ferrous iron, however, the calculation is not so simple. The quantum numbers are $S = 2$ and $L = 2$, and hence $\chi_{Fe^{++}}$ should equal $222{\cdot}4 \times 10^{-6}$ E.M.U./g at 29° C for the free ion. From the rather extensive work on the magnetic properties of synthetic glasses, ferrous iron in glass had indeed been shown[6,7] to have a susceptibility of about 218×10^{-6} E.M.U./g at 29° C, corresponding to an orbital magnetic moment which is only very slightly quenched. However, the normal ferrous iron imparts a blue coloration to the glass. A blue coloration is not evident in tektites which range from a light sea-green to a brownish black in colour. Moreover, previous work[14] on the magnetic properties of tektites has shown that the orbital moment of ferrous iron is almost completely quenched. Thus, one cannot accept this value (218×10^{-6} E.M.U./g at 29° C) for the specific magnetic susceptibility of ferrous iron in tektites.

The fact that the $C_{Fe^{+++}}/C_{Fe^{++}}$ ratio is relatively constant for tektites will allow one to make an approximate calculation of the specific susceptibility of the ferrous iron. By equating the bracketed coefficient in equation (9) with the experimental slope (equation 3) and using the chemical values of the ratio, $R = 0{\cdot}053$, and $\chi_{Fe^{+++}} = 259{\cdot}4 \times 10^{-6}$ E.M.U./g, $\chi_{Fe^{++}}$ is calculated to be $190{\cdot}2 \times 10^{-6}$ E.M.U./g. This corresponds to an almost completely quenched orbital magnetic moment. While this is only an approximation consistent with this value of R, it is sufficiently accurate to show that the moment is almost completely quenched. The significance of this relative to the structure of the tektite glass will be discussed in another paper.

A magnetic susceptibility technique has been devised to give a non-destructive, micro-analysis of the total and ferrous iron content of tektites. The measurements are much easier and faster to make providing an accuracy comparable with monitored, high-precision chemical methods.

The ferric-to-ferrous ratio has been found to be constant within relatively narrow limits for bediasites. The results also suggest that the median ratio is approximately the same for all tektite families, indicating a reasonably uniform thermal history of formation and melting atmosphere. The low ratio (≈ 0·05) implies an atmosphere with a very low partial pressure of oxygen. The constancy of the ratio in comparison with the wider range of ratios reported in the literature indicates that some of the reported ferric and ferrous analyses may be in error by a small but significant amount. The specific magnetic susceptibility of ferrous iron in tektites as calculated from the foregoing ratio shows that the orbital magnetic moment is almost completely quenched.

We thank Dr. E. Chao of the U.S. Geological Survey and E. Henderson of the U.S. National Museum for many of the tektite specimens. We also thank Sherman White. H. Rose and M. K. Carron of the U.S. Geological Survey for their assistance with the measurements and chemical analyses, Dr. Dean Chapman, National Aeronautics and Space Administration, and Dr. T. P. Kohman, Carnegie Institution of Technology, for generously providing specimens for our work, and Dr. H. R. Branson of Howard University and Dr. S. S. Goldich of the U.S. Geological Survey for their interest in this work.

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MODEL EXPERIMENTS FOR VORTEX COLUMNS IN THE ATMOSPHERE

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ORGANIZED columns of vorticity are observed in many parts of the Earth's atmosphere, in such forms as dust whirls and dust devils, tornadoes, waterspouts, fire storms and hurricanes. These columnar flows cover a very wide range of horizontal length and velocity scales, say, 10 m–2000 km in length and 10–50 m.p.s. or more in velocity, and they exhibit a wide range of behaviour. However, each may be regarded as a rotating column within which the mean angular velocity (or vorticity) of the fluid is several orders of magnitude greater than ambient. In each case thermal convection plays a fundamental part in the flow development.

It seems to be accepted generally that convergence into convection columns or thermals may produce amplification of existing ambient rotation about the vertical, and that this provides a mechanism for the generation of dust devils in a locally unstable environment. But in spite of the obvious interest and meteorological importance of the growth of rotating columns in the atmosphere, there have been relatively few attempts to investigate the amplification of ambient vorticity through the action of turbulent thermal convection. Recently, Dessens[1] has reported the formation of 'tornadoes' near the base of a large experimental convection column in the atmosphere; and Byram and Martin[2] have reported 'fire whirl' experiments in which a pan of alcohol is burned at the bottom of a vertical cylinder into which air is drawn tangentially. These are both interesting experiments, but they involve a variety of effects and have neither the degree of simplicity nor of experimental control which is needed for a basic study of the convective amplification of vorticity. As a stage in such an investigation, I have been carrying out model experiments on the behaviour of turbulent buoyant jets of alcohol and salt solution in a uniformly rotating tank of water. (The plume from a simple source of buoyancy represents a limiting case of this wider range of source conditions.)

The experiments are carried out in a 'Perspex' tank 75 cm in diameter and 150 cm deep, which can be part filled with water and set rotating about a vertical axis at steady speeds between 0·3 and 4·5 radians per sec. After the water has reached a state of sensibly uniform rotation, a stream of dyed liquid is injected as a vertical jet along the axis, either from a nozzle at the centre of the base or from one just below the centre of the free surface. An upward jet of alcohol/water or a downward jet of salt solution may be termed positively buoyant, a water jet neutral, and an upward jet of salt or downward jet of alcohol negatively buoyant. The inflow is maintained for long enough to generate a jet rather than a 'cloud' of buoyant fluid, and then turned off to avoid significant effects due to the walls and free surface. The development of the rotating plume can be recorded photographically by still or ciné cameras directed: (1) horizontally, from a fixed station, or (2) vertically downward, from a station rotating with the tank. The horizontal records illustrate the development of the rotating column, while vertical streak-photographs of the aluminium-powdered free surface may be used for obtaining angular velocities in the columns.

In terms of jet diameter and mean vertical velocity at the nozzle, the operating range of Reynolds numbers for the issuing jet in the first set of experiments was about 5×10^3–5×10^4; while the range of Rossby numbers based on

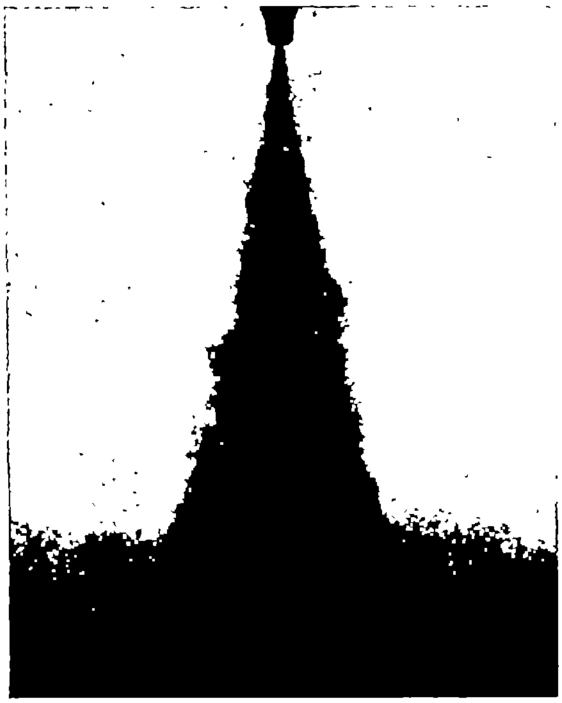

Fig. 1 A positively buoyant downward jet of salt solution in a stationary tank

diameter and mean inflow velocity at the nozzle was $2 \times 10^2 - 2 \times 10^3$, or if based on averaged values over the height of the jet was about two orders of magnitude smaller.

A feature of the experimental arrangement is the fact that small disturbances to the velocity of a rotating fluid propagate in a two-dimensional fashion. This has only a moderate effect on the vigorous initial motion of the jet, but constrains the subsequent pattern of flow quite strongly into a column: hence the flow observed at the free surface relatively quickly adopts a profile appropriate to an average over the greater part of the water depth. This effect may be observed in Fig. 2.

The experimental observations fall into three groups according to whether the jets are positively buoyant, neutral or negatively buoyant. There are only minor differences between the upward and downward jets of specified buoyancy, and these arise from the character of the bounding surface near the nozzle.

When a positively buoyant downward jet of dyed salt solution is released in a rotating tank the outer layers of the jet are swept into vigorous rotation at an angular velocity greater than that of the tank and ambient water. This vorticity diffuses rapidly through the jet to produce a rotating column with typical angular velocity about 2–10 times that of the tank (the precise level depending on the source conditions of the buoyant jet). The rotating column usually remains centred on the nozzle so long as the inflow continues, though it often shows signs of sub-division; but when the jet is stopped the column wanders slowly about the tank and usually subdivides into two or three columns of comparable strength, and occasionally more. These vortex columns persist for a long time.

Fig. 1 shows a downward jet of salt solution in a stationary tank, and is included for purposes of comparison. The development of a rotating plume is illustrated in Fig. 2*a*, *b* and *c*. In this case the angular velocity of the tank was 1·23 radians/sec; the density of the salt solution was 1·18 g/ml.; the jet ran for 15·2 sec; and the photograph reproduced in Fig. 2*a* was taken at 5·2 sec relative to the time of starting the jet, Fig. 2*b* at 20·8 sec, and Fig. 2*c* at 40·2 sec. The vertical scale marks which can be seen are 10 cm apart. The developing jet is much less clearly straight-sided and at first shows a large intermittent variation in width; but shortly after the nozzle flow has ceased (Fig. 2*b*) the decaying jet is roughly straight-sided and occupies a much larger volume than it would in a stationary environment (Fig. 1); the weaker outside disturbances are already taking up a two-dimensional form (Fig. 2*b*). Later (Fig. 2*c*) the jet has separated into two rotating columns with almost vertical edges. Fig. 3 shows a streak photograph of the free surface dusted with aluminium powder taken with a camera fixed relative to the rotating tank. This is a composite shot showing two major vortex columns and one additional weak one which have been generated from an earlier jet emission, together with a vigorous starting column about a second jet emission from the nozzle.

A neutral jet in a rotating tank produces no change in the state of uniform rotation of the ambient fluid (apart from some initial disturbance which soon decays).

A negatively buoyant downward jet of alcohol in a rotating tank of water generates a number of rotating columns (which are now, of course, clustered towards the free surface) in which the angular velocity is smaller than that of the surrounding water.

These effects can be explained quite simply in terms of the distortion of mean vortex tubes by the buoyant jet. When a vortex tube is stretched the associated angular

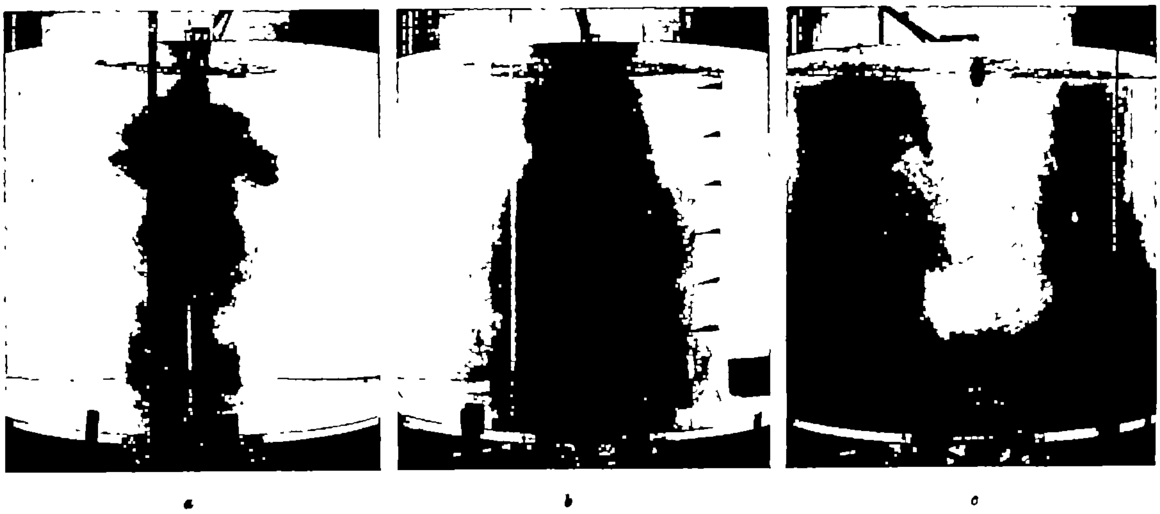

Fig. 2 Development of a positively buoyant downward jet of salt solution in a rotating tank

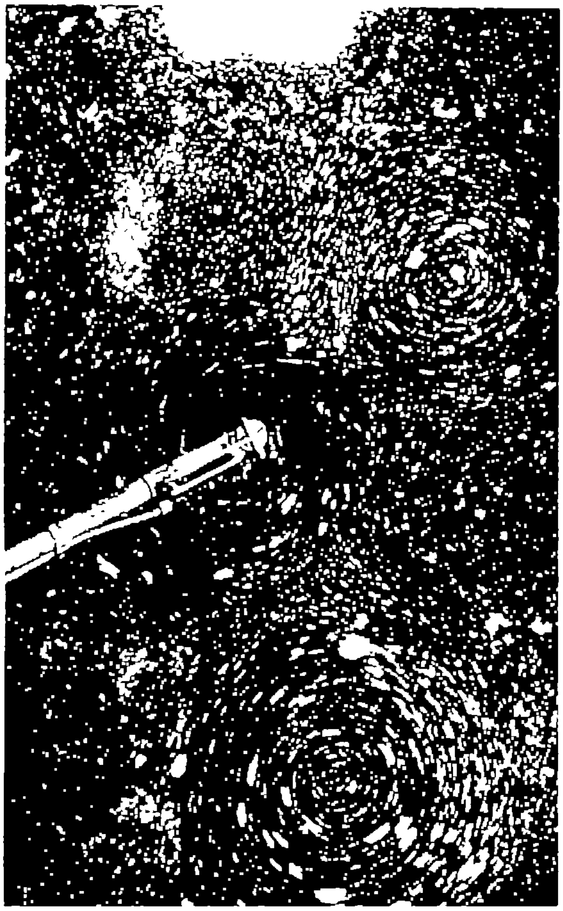

Fig. 3. A surface streak photograph showing the flow relative to the rotating tank. In this case there are two free vortex columns from an earlier discharge of the jet, and a third column anchored around a continuing inflow from the nozzle

velocity increases in proportion to the relative extension; when it is shortened there is a corresponding reduction in angular velocity. All jets entrain ambient fluid and share with it their linear momentum, so that the mean axial velocity in a buoyant jet always decreases with increasing distance from the source (except in short, buoyancy-dominated regions of very buoyant jets). It follows that in the interior of a jet (buoyant or otherwise) there will always be a continuous shortening of mean vortex tubes parallel to the axis. On the other hand, the entrained fluid enters the jet in a slow radially inward stream that will convect ambient vortex tubes into the jet; sections of vortex tube crossing the inclined outer parts of the jet are at first stretched as they are drawn into the faster parts of the flow. Thus, if we were to ignore turbulent diffusion, there would be a net stretching of mean vortex tubes in the outer parts of the buoyant jet, and a net shortening in the interior. Turbulent diffusion rapidly averages these regions of increased and decreased angular velocity, and in a neutral jet the two cancel and there is no modification of the ambient vorticity. But in a positively buoyant jet there is a slower decrease of mean axial velocity with increasing distance from the source, and consequently a net stretching of mean vortex tubes over the jet as a whole, so that the angular velocity in the jet is increased above ambient, while in a negatively buoyant jet there is an overall shortening of mean vortex tubes with a corresponding decrease in angular velocity across the jet as a whole.

This phenomenon is in fact a familiar one and can be demonstrated easily. If a cup of hot black coffee (or tea) is stirred with a rotary motion so as to leave the coffee (or tea) swirling smoothly, and a stream of cold milk is poured into the centre of the cup a small vortex will be formed, and this vortex will often be strong enough to dimple the surface noticeably. Hot milk will not produce such a vortex. In fairness to the experimenter it should be noted that it is relatively easy to mask the effect, and some practice may be needed.

The material of this article was reported at the Boulder Conference on Fluid Mechanics in Geophysics (September 1962) sponsored by the American Physical Society and the American Meteorological Society, and a theoretical analysis and detailed quantitative results will be published elsewhere in due course. I thank the Meteorological Research Council for a grant towards the cost of this work.

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RUTHERFORD AND NUCLEAR POWER

By DAVID A. KEYS

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THE completion of the first six-week trial run of the first Canadian Nuclear Power Generating Station at Rolphton, Ontario, brings to mind the predictions made by the late Lord Rutherford and his colleague Frederick Soddy in the early days of this century. During Rutherford's Macdonald professorship in McGill University, Montreal (1898–1907), many of the fundamental researches were performed on which the ultimate release of energy from the atom was attained. In his *Radioactivity*, written in 1902, he realized the atom as a source of large amounts of energy as the following quotations indicate[1]: (The significant predictions made by Rutherford are indicated by italics in this article.)

"*There is thus reason to believe that there is an enormous store of latent energy resident in the atoms of the radio-elements.* This store of energy could not have been recognized if the atoms had not been undergoing a slow process of disintegration. *The energy emitted in radio-active changes is derived from the internal energy of the atoms.*

"The existence of this store of latent energy does not ordinarily manifest itself, since the atoms cannot be broken up into simpler forms by the physical or chemical agencies at our disposal. Its existence at once explains the failure of chemistry to transform the atoms and also accounts for the rate of change of the radioactive processes being independent of all external agencies. . . . *If it should ever be found possible to control at will the rate of disintegration of the radio-elements, an enormous amount of energy could be obtained from a small quantity of matter.*"

The means of controlling at will the rate of disintegration has been found, and so the Nuclear Power Generating Station was able to deliver on its first trial an average power of 14,000 kW of electricity to the Ontario Hydro-Electric Power Commission's system.

It is of further interest to note the following account which appeared in the London *Daily Mail*[2], which may have been the result of some interview the reporter had with Rutherford at the Cavendish Laboratory. The account was headed in bold print:

"THE MIGHTY ATOM"

"Scientific struggle to harness it"

"Leading men of science in Britain, the United States and even Russia are to-day all corresponding over the question of minute advances in their several laboratories on the structure and behaviour of the atom. They believe that they are on the edge of discoveries that may largely, in the sequel, end the struggle for life—at least so far as light and power are concerned.

"The finest instrument in the world is probably that in a Cambridge laboratory and it is opening up new secrets, and Professor Sir Ernest Rutherford, who has some remarkable fellow-workers, such as Mr. Aston, is accepted as one of the world's high priests of the new alchemy. He and others have as good as proved *that each atom, though it is quite incommensurable, has in it the power of a thousand horses. If we can harness this power almost all our material difficulties are at an end.*

"This is still a dream, still a sort of laboratory theory; but little side discoveries—such as that supposed elements (chlorine, for example) are not really elements—are being made continually; and in the course of this co-operation of the world's best brains, all intensely eager in the same hunt, we may expect discoveries greater than the discovery of radium and of more general application.

"*Infinite material power is at our finger-tips, waiting release by finer and finer instruments. Nothing is more likely to benefit humanity than help to the making of such machines.* The best are costly, and this form of science is ill-endowed."

The morning this appeared, the late Dr. G. F. C. Searle directed my attention to the article, making a few characteristic caustic remarks about "the thousand horses".

Soddy in his book[3] spoke of explosive power which the sudden release of energy from radium would produce in the following words:

"In only two days radium gives out more energy, weight for weight, than the most powerful explosive known liberates during its explosion. If the energy liberated in a thousand years could be released instantaneously, a single milligramme of radium would equal in its effect a ton of any known explosive."

Soddy also realized the energy available in the atom as the following statement shows:

"These considerations force us to the conclusion that there is associated with the internal structure of the atom an enormous store of energy which, in the majority of cases, remains latent and unknowable."

Rutherford was responsible for the idea of the possibility of energy being derived from the atom due perhaps to statements made in such popular addresses as that delivered when he was at McGill University. In his address to the Royal Institution[4] in 1904, he stated:

"A few pounds (of radium emanation) would suffice to provide enough power to drive a liner across the Atlantic."

I remember, more than half a century ago, the late Prof. (later Sir John) MacLennan holding up a glass of water and stating that there was enough energy in the atoms of this water to run the *Lusitania* across the Atlantic.

The fulfilment of these early predictions by the new nuclear power reactor in Canada, the country where some of them were first recorded, is of interest to those who knew Rutherford when he performed his experiments which inspired the search for nuclear energy "to benefit humanity".

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ASSIMILATION OF MINERALS BY HIGHER PLANTS*

By Dr. GEORGE J. FRITZ

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At the present time there are sixteen elements which are known to be required for the growth and development of higher green plants: carbon, hydrogen, oxygen, potassium, calcium, magnesium, nitrogen, phosphorus, sulphur, boron, chlorine, copper, iron, manganese, molybdenum and zinc. The first nine are required in relatively large amounts and are called macroelements; the last seven are required in much smaller amounts and are known as microelements. The elements cobalt, sodium and vanadium are essential to certain micro-organisms, but their essentiality for higher green plants has not been definitely established yet; but if these three elements are shown in the future to be essential to higher plants, then it can be predicted that their requirements will be only in ultramicro amounts.

The essential elements are supplied as inorganic plant nutrients by the three media of the external environment: atmosphere, water and soil. Thus, the bulk of the carbon and oxygen in the tissues of higher green plants is derived from the carbon dioxide of the atmosphere, and probably all the hydrogen comes from water: in the photosynthetic process, carbon dioxide of the atmosphere is reduced in the leaves by the hydrogen from water to the level of carbohydrates; in this process, the oxygen of water escapes from the plant as oxygen gas, which is a by-product of photosynthesis. Water also is probably utilized in some metabolic processes other than photosynthesis, and these activities might account for the incorporation of small amounts of hydrogen and oxygen into organic matter. Another possible source of small amounts of carbon, oxygen and hydrogen in plant tissue is bicarbonate ion in the soil, which is known to be absorbed and incorporated into cellular constituents such as malic and aspartic acids by plant roots[14]; whether this process contributes a significant part of the total carbon, oxygen and hydrogen in plant organic matter, and if so under what conditions, is still not completely clarified. In summary, these three elements, carbon, hydrogen and oxygen, supplied primarily by the atmosphere and water, are the principal constituents of organic matter; in fact they contribute 90 per cent or more of the total dry weight of the average plant. Since all the other essential elements are supplied to higher plants primarily by the soil (except in those cases in which nitrogen is supplied as a gas), they may properly be called mineral elements.

If the oxygen in organic matter is derived from carbon dioxide, then what part does gaseous oxygen play? Higher green plants carry on aerobic respiration; in this process, gaseous oxygen acts as an electron and hydrogen acceptor and is thereby reduced to water, which mixes with the water already present in plant tissues. Therefore the oxygen gas consumed in respiration does not become organic matter directly, but may become organic matter indirectly if it is incorporated via some metabolic step which utilizes water. Another process is now known which consumes small amounts of gaseous oxygen; plant tissues perform a process[7,11] which may be called 'oxygen fixation', in which a small portion of the oxygen gas which is applied to tissues is added directly to organic substrates. The products of 'oxygen fixation' have not been studied intensively, but probably include organic peroxides.

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* Substance of a lecture given before the Soil and Crop Science Society of Florida during the twenty-second annual meeting, at Winter Park, Florida, on November 13, 1962.

In a consideration of the transformation of inorganic nutrients of the external environment to cellular constituents of plants, it is difficult completely to divorce the subject from the functions which elements perform, and it is useful to review the general roles in which elements may participate in cellular metabolic processes. Metabolism is a general term which refers to the chemical reactions which occur in a living cell. Except for the metabolism of nitrogen and phosphorus, relatively little is known about the chemical transformations which mineral elements undergo after they are incorporated into the living material of the cell. Assimilation is a term which refers to the chemical events associated with the transformations accompanying the incorporation of inorganic nutrients into organic matter; it is this part of metabolism, especially with respect to mineral nutrients, which is considered here.

The sequence of events by which inorganic mineral nutrients may be assimilated differs from element to element, and in fact some mineral nutrients which have been absorbed by a plant may not be converted to organic matter at all. For example, nitrate ion might be stored in the vacuole as such without undergoing chemical change, or nitrate might be converted first to ammonia and then incorporated into amino-acids or proteins. An element like potassium may be bound by weak electrostatic forces to organic anions produced by cellular metabolism, and thus potassium may serve to maintain cellular electroneutrality. In other cases a nutrient such as iron might exist in an organic form in the external environment even before it is presented to the plant, and might even be absorbed in an organic form by the roots. But in spite of these variations in behaviour of mineral nutrients, the major roles which elements have within a plant may be summarized in a few categories.

An element may be essential to plant growth and development for one or more of the following reasons: (1) it may be a component of a vital protoplasmic constituent; (2) it may participate in a vital biochemical or biophysical event; (3) it may exert a significant effect on an enzyme reaction; (4) it may contribute to the maintenance of protoplasmic organization and integrity; (5) it may function as a free ion. In view of the complexity of higher green plants, it is not surprising to find that some elements function in more than one of these categories; thus magnesium, for example, probably plays a part in all five categories: (1) magnesium is a component of chlorophyll, a vital protoplasmic constituent; (2) magnesium participates in a vital biophysical event, that is, the absorption of light quanta by the chlorophyll molecule; (3) magnesium is well known as an activator of many enzymes which are completely inactive *in vitro* in the absence of magnesium ion; (4) magnesium is known to stabilize water-in-oil emulsions as do other divalent ions and thus probably contributes to the maintenance of protoplasmic organization; (5) magnesium may act as a free ion, for example, by balancing anions, and may serve to maintain cellular electroneutrality.

Assimilation of Nitrogen, Phosphorus and Sulphur

Nitrogen, phosphorus and sulphur are considered together because they are usually supplied as anions. Nitrogen is quantitatively the most important of these: 99 per cent of the living parts of living organisms are made of the four elements carbon, hydrogen, oxygen and nitrogen. Nitrogen is usually supplied to the plant as nitrate ion, although plants can also utilize other sources of nitrogen, such as ammonia; gaseous nitrogen may also be assimilated in some cases in which the higher green plant lives in symbiotic relation with specific micro-organisms. Whether nitrate or nitrogen is utilized, each of these is probably first reduced by the plant to ammonium ion before being incorporated into organic matter. The sequence of events in the reduction of nitrogen from the +5 oxidation state in nitrate ion to the −3 oxidation state in ammonia has been the subject of considerable research in recent years, and has been reviewed periodically[8,14]. While some of the details of the reduction process have not been completely elucidated, nevertheless the outlines are reasonably well known. The following scheme[14] presents probable steps in the reduction, nitrate being reduced successively to nitrite, nitric oxide, hyponitrite, hydroxylamine and ammonium ion:

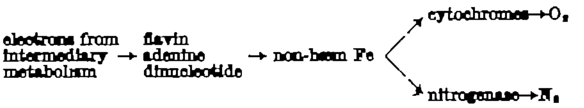
$$\mathrm{NO_3^-} \xrightarrow{(\mathrm{Mo})} \mathrm{NO_2^-} \xrightarrow{(\mathrm{Fe,\ Cu})} \mathrm{NO} \xrightarrow{(?)} \mathrm{N_2O_2^{=}} \xrightarrow{(\mathrm{Fe,\ Cu})} \mathrm{NH_2OH} \xrightarrow{(\mathrm{Mn})} \mathrm{NH_4^+}$$

Some of the indicated molecular species may have only a transient existence during the reduction process. Each step in the sequence requires the participation of a different enzyme system; for example, the reduction of nitrate to nitrite requires a molybdo-flavoprotein enzyme; the reducing power which is required for the reduction is supplied by coenzymes derived from other metabolic processes. The subsequent steps in the sequence also require other enzymes and associated metals as well as reduced coenzymes, and the known micronutrient requirements for each enzyme system are also indicated in the scheme. The indispensability of these particular micronutrients for nitrate reduction is apparent.

Our understanding of the incorporation of nitrogen gas into organic matter by higher green plants which exist in symbiotic relation with certain micro-organisms, as well as the nitrogen fixation by other free-living micro-organisms, has advanced considerably in recent years. It now appears that in nitrogen fixation, nitrogen acts as an electron and hydrogen acceptor and is reduced to ammonia[15,17]. The intermediates may never be isolated in sufficient quantities to be identified with certainty; the reduction probably occurs in a stepwise fashion as follows:

$$\underset{\text{nitrogen}}{\mathrm{N{=}N}} \xrightarrow{+2\mathrm{H}} \underset{\text{diimide}}{\mathrm{NH{-}NH}} \xrightarrow{+2\mathrm{H}} \underset{\text{diamine}}{\mathrm{NH_2{-}NH_2}} \xrightarrow{+2\mathrm{H}} \underset{\text{ammonia}}{\mathrm{2NH_3}}$$

Nicholas[15,17] has recently shown for the micro-organism *Azotobacter vinelandii*, a free-living aerobic organism capable of nitrogen fixation, that gaseous nitrogen and gaseous oxygen used in respiration compete with one another for electrons supplied by other metabolic activities of the cell. The competing pathways, and the probable electron carriers, based on evidence presented by Nicholas[15], may be shown schematically as follows (the enzyme system which activates gaseous nitrogen is called nitrogenase):

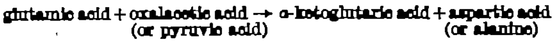

electrons from intermediary metabolism
flavin adenine dinucleotide
non-hæm Fe
cytochromes→O_2
nitrogenase→N_2

The microelement requirements for nitrogen fixation by *A. vinelandii* include at least iron, molybdenum and cobalt; the iron is needed in the complex which contains iron in a non-hæm form; molybdenum and probably also cobalt may be a part of the nitrogenase system[16]. Cobalt is required for nitrogen fixation by *A. vinelandii*[16], and it is also required when certain leguminous species grown under symbiotic conditions are actively fixing nitrogen gas[1]; however, this latter requirement may reflect a need for cobalt by the infecting bacteria rather than by the root cells themselves.

Whether nitrogen is absorbed as ammonia, or is reduced to ammonia from nitrogen or nitrate ion, the ammonia is rapidly incorporated into organic matter. The assimilation of ammonia has been reviewed recently by Webster[19]; to summarize briefly a process which may have further complications, it appears that initial ammonia assimilation probably proceeds to a large extent in the formation of glutamic acid by a process called reductive amination, catalysed by the enzyme called glutamic acid dehydrogenase:

$$\alpha\text{-ketoglutaric acid} + NH_3 + NADH_2 \longrightarrow \text{glutamic acid} + NAD + H_2O$$

Aspartic acid and alanine are also found when labelled ammonia is applied to plant tissues, but in lesser amounts than glutamic acid; they are probably formed by transamination from glutamic acid as follows:

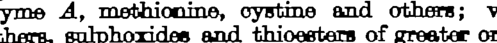


$$\text{glutamic acid} + \underset{\text{(or pyruvic acid)}}{\text{oxalacetic acid}} \rightarrow \alpha\text{-ketoglutaric acid} + \underset{\text{(or alanine)}}{\text{aspartic acid}}$$

These three amino-acids, glutamic acid, aspartic acid and alanine, serve as a pool from which most of the other amino-acids can be formed, as well as amides and proteins and other products of nitrogen metabolism. (Over-simplification of this subject might possibly lead to misunderstanding, and Webster[19] should be consulted for details.)

The assimilation of phosphorus probably occurs mostly if not entirely by esterification of phosphate ions. Inorganic phosphate is absorbed by the plant from the external environment as $H_2PO_4^-$ or HPO_4^{--} ions, depending on the *p*H, and is then esterified into one or more of several classes of organic phosphate compounds which are found in plants[9]. These groups may be listed as: (1) carbohydrate phosphate esters and carbonyl-phosphate esters; (2) nucleic acids, that is, ribose and deoxyribose phosphate diesters which unite nitrogen bases; (3) phosphoproteins, including hydroxyamino-acid phosphate esters and guanidino phosphates; (4) polyphosphates in biologically important coenzymes such as flavin adenine dinucleotide, adenosine triphosphate, nicotinamide adenine dinucleotide, etc.; (5) phospholipids such as glycerol phosphate esters and diesters of nitrogenous bases; (6) inositol phosphate esters such as phytic acid and phytin.

Assimilation of sulphur in higher green plants has been studied to a much lesser extent than the assimilation of nitrogen and phosphorus. The form in which sulphur is usually supplied to the plant is the sulphate ion; the reduction of sulphur from an oxidation state of +6 in sulphate ion to −2 in sulphydryl (—SH) has been studied in some micro-organisms, and the subject has been recently reviewed by Thomas[18] and by Wilson[21]. Tracer experiments with higher green plants have also demonstrated that inorganic sulphate is found especially in the amino-acid cysteine ($HS—CH_2—CH(NH_2)—COOH$). By assuming that the reduction pathway in higher green plants is similar to that in micro-organisms and goes through sulphite and thiosulphate to sulphydryl, and also by assuming in analogy with pathways demonstrated for micro-organisms that sulphydryl groups react with the amino-acid serine to produce cysteine, then a pathway of sulphate assimilation in higher plants which is consistent with our present-day knowledge may be outlined as follows:



$$SO_4^{=} \rightarrow SO_3^{=} \rightarrow S_2O_3^{=} \rightarrow \text{—SH} \xrightarrow{\text{serine}} \text{cysteine}$$

Sulphur is a component of many molecules of great biological interest, including thiamine, biotin, glutathione, coenzyme *A*, methionine, cystine and others; various thioethers, sulphoxides and thioesters of greater or lesser complexity also occur in plant tissues. The investigation of the synthesis, function and degradation of these compounds has only begun.

Assimilation of Potassium, Calcium and Magnesium

Even though it is probable that some anions absorbed by a plant may not be metabolized at all, and may perhaps serve only to neutralize cations, yet it is evident from the discussion so far that assimilation of the bulk of nitrogen, phosphorus and sulphur is accompanied by an active and intense metabolism; the chemical transformations which these nutrients undergo in their conversion from inorganic to organic forms is drastic. In comparison with this behaviour, potassium, calcium and magnesium may be considered to be metabolized to a lesser extent. Substances like organic acids and proteins probably exist in the cellular milieu as anions; neutral substances like carbon dioxide are also converted by photosynthesis into organic anions; these anions are neutralized either by hydrogen ions produced metabolically or by the ions K^+, Ca^{++} and Mg^{++}. Thus, one of the important functions of cations is their action as free ions in the maintenance of electroneutrality; relatively weak electrostatic forces bind the inorganic ions to the organic anions. Cations like potassium, calcium and magnesium probably support the maintenance of protoplasmic organization and integrity in the cell. Protoplasm is a complex colloidal system of an emulsion-like nature, and requires stabilization *in vivo*. Our present knowledge of this phase of cell biology is very scanty, but it is widely recognized[9] from studies of colloidal systems *in vitro*, that monovalent-metal soaps, being soluble in water, act as stabilizers of oil-in-water emulsions, whereas divalent-metal soaps, being water-insoluble and oil-soluble, serve as efficient stabilizers for water-in-oil emulsions. Probably both these kinds of emulsions are present in protoplasm, and monovalent and divalent cations may thus play important parts.

Not only can potassium, calcium and magnesium function as simple charged particles, but these substances may also have physiological importance as insoluble salts. The middle lamella of the cell wall consists largely of insoluble pectic compounds which are mixtures of calcium and magnesium pectates; phytin, the calcium–magnesium salt of inositol, is found in large amounts in seeds and seedlings. Whether physiologically important potassium salts of low solubility occur in plant tissues is still an open question, but their existence may be suspected because of the wide range of known potassium salts which have low solubilities[16].

Other more complicated forms of potassium, calcium and magnesium also exist in plant tissue. Metal ions form complex or co-ordination compounds with organic groups which donate electrons to the metal. The complexes which result when electron-donor atoms are attached to each other as well as to the metal are called chelate or sequestered compounds. Donor atoms which undergo combination with the metal in the formation of these complexes are usually nitrogen, oxygen and sulphur. Complexes and chelates are formed by nearly all metals, including alkali and alkaline earth metals[4,12]. Among potassium, calcium and magnesium, the best-known naturally occurring chelate is chlorophyll: the magnesium atom is present in the centre of the porphin ring, and is bound to the four pyrrole nitrogen atoms much as the iron atom in iron porphyrin. Organic anions such as citrate, tartrate, malate and various amino-acids also form stable chelates with metal ions, including calcium and magnesium. Less well known is the fact that alkali metals like sodium and potassium can also form chelate compounds[4,12]. Many cases of non-specific activation of enzyme systems by univalent and divalent cations may possibly find their explanations in part in this phenomenon. Without doubt the common observation that sodium can replace potassium as a nutrient stems from the action of potassium as a free ion in the plant, bound ionogenically by neutral polar valences to organic anions and functioning as a charge neutralizer or as a stabilizer of protoplasmic emulsions. But the fact that sodium cannot replace potassium completely—potassium is an essential element to higher green plants whereas sodium is not—leads to the conclusion that potassium performs a function which sodium cannot perform; but this special function of potassium has not yet been discovered. It is suggested here that organic complexing agents exist in plant tissue which have higher affinities for potassium than for sodium; the resulting co-ordination of chelate compounds of potassium, functioning perhaps as an unknown essential cellular constituent, may account for the essentiality of potassium.

In summary, the cations potassium, calcium and magnesium may exist in plant tissues in one or more of the following forms: (1) simple charged particles, bound ionogenically to particles of opposite charge by normal polar valences; (2) co-ordinated compounds (complexes, chelates) bound by non-ionogenic auxiliary valences; (3) substances of low solubility (for example, phytin, calcium-oxalate, calcium-pectate, etc.).

Assimilation of Micro-elements

Our knowledge of the roles of some of the micro-elements has increased considerably during the past decade or so. Elements like copper, iron, manganese, molybdenum and zinc have been shown to act as nuclei of metalloprotein enzymes or as enzyme activators, and their roles have been reviewed[13,14]. Chloride ion has been shown to be required in the photo-oxidation of water by isolated chloroplasts[3]. Boron probably forms sugar borate complexes which are associated with the translocation of sugars[8]. So far, major research emphasis has been placed on the roles of micro-elements in enzyme systems; other roles not now investigated, such as possible effects on membrane structure, cell organization, etc., should also be considered[14]. But in spite of increasing awareness of the functions of micro-elements, remarkably little detailed information is available concerning the processes of assimilation of the micronutrients.

With the possible exception of Cl$^-$, probably very low concentrations of free micro-element ions are present in soils and in plant tissues. The elements copper, iron, manganese and zinc occur in the soil as cations which form insoluble salts with systems such as sulphate, chloride, carbonate, phosphate and many others; boron and molybdenum usually occur as oxy-anions which form insoluble salts of other metals. These metals in soils are locked in the mineral portion of the soil in the form of insoluble inorganic salts; the metals are converted to soluble form by weathering processes, perhaps first involving conversion to bicarbonates of low or intermediate solubility by the action of carbon dioxide dissolved in rain-water, or by attack by natural organic chelating agents produced by micro-organisms or by plant roots, which secrete small amounts of organic anions which tend to solubilize the micro-elements[6]. Inside the plant, micronutrient cations like copper, iron, manganese and zinc may be incorporated into chelate compounds by reaction with cellular metabolites; molybdenum also may form co-ordinated or chelate compounds of biological interest, perhaps involving the binding of the molybdyl ion (MoO$_2$) (ref. 20). It is becoming increasingly clear that organic substances of various kinds bind metals with greater or lesser tightness. The story of the assimilation of the micro-elements involves numerous interactions of metals with metabolites of various kinds, ranging from those which bind metals so tightly that the metal must be considered not in equilibrium with other substances present which might otherwise interact with the metal, to other binding agents which have low affinities for the metal and in which the metal bonds are labile. The porphyrins bind iron and vitamin B_{12} binds cobalt with extreme tightness; other substances like organic acids, amino-acids, peptides and proteins bind metals less tightly so that the metals remain exchangeable. Purines, pteridines, nucleotides and nucleic acids also have been shown to bind metals, but little work has been done to measure the affinities of these substances for individual metals[2]. Thus, a metal may be carried within the plant as a chelate or co-ordination compound, and may undergo exchanges with other metabolites which form complexes of greater stability. The process of absorption of micronutrients from the external environment by plant roots may involve in part simple replacement at the root surface of one complexing agent for another. Considerable efforts by biochemists and physiologists will be needed to attain detailed knowledge of assimilation of micronutrients.

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OBITUARIES

Prof. A. G. Ginetzinsky

PROF. A. G. GINETZINSKY, head of the Laboratory of the Institute of Evolutionary Physiology in Leningrad and corresponding member of the Academy of Medical Sciences of the U.S.S.R., died on October 20, 1962, at the age of sixty-seven. To many of his colleagues abroad, Ginetzinsky's contributions to physiology became known only comparatively recently, despite the fact that he was the author of a series of important discoveries over the past forty years. He was born at Vologda in 1895. After graduating at the Medical Institute in Leningrad, he joined the laboratory of the late Academician L. A. Orbeli, whose influence as a teacher continued to guide him in the pursuit of problems in nerve and muscle physiology. After holding for many years the chair of physiology in the Medical Pediatric Institute, Leningrad, Ginetzinsky moved to the Sechenov Institute of Evolutionary Physiology.

Of his many scientific studies, I shall refer to four which have gained international interest and recognition (though some very belatedly and not until his findings had been rediscovered in other places).

In December 1922, Ginetzinsky gave an important communication at the thirty-seventh Physiological Congress in Petrograd, describing the effect of sympathetic nerve fibres on fatigued muscle. He showed that stimulation of sympathetic nerves can influence and, under certain conditions, restore the force of muscle contraction evoked by motor nerve impulses. The work was done, I believe, at the suggestion of Ginetzinsky's teacher; but there is no doubt from Orbeli's writings that his pupil had played a very prominent part in this discovery, which for some reason is commonly referred to in the non-Russian literature as the 'Orbeli phenomenon'.

In 1935, Ginetzinsky and N. I. Michelson reported that in muscles which had been completely paralysed by curare, a nerve impulse still evokes a small electric response in the region of the nerve-muscle junction. This was the first description of a phenomenon which became known, and intensively studied, several years later under the name of the 'end-plate potential'.

In 1942, Ginetzinsky and N. M. Shamarina published a most interesting paper the main findings of which were rediscovered seventeen years later in Sweden and in Great Britain. It confirmed the well-known fact that 'adult' skeletal muscle fibres are not very sensitive to acetylcholine except in a narrow region around their nerve-muscle junctions. The authors proceeded to show that, a few days after birth, the whole length of the muscle fibre reacts to low concentrations of acetylcholine, and that the final state, of a locally restricted sensitivity, is not attained until later. Furthermore, when adult muscles are deprived of their nerve supply, their acetylcholine sensitivity again begins to spread, from the nerve-muscle junction along the whole length of the muscle fibres.

During the last years of his life, Ginetzinsky's principal interests had turned to problems of kidney physiology, especially the control of water excretion and the mechanism of action of the anti-diuretic hormone. In 1958, he published a note in *Nature* (**182**, 1218; 1958) in which he put forward a new theory, namely that the re-absorption of water in the renal tubules is controlled by a local release of hyaluronidase which causes a temporary breakdown of the intercellular 'cement substance' in the wall of the distal and collecting tubules and so opens up channels for osmotic water flow. His evidence indicated that a hyaluronidase is released from the kidney cells under the influence of anti-diuretic hormone, and that the action of the free enzyme raises the water permeability of the tubular wall. This novel hypothesis has aroused much interest and some controversy; but its main features have been greatly strengthened by subsequent work done in Britain and by Ginetzinsky himself.

I had the pleasure and privilege of meeting Prof. Ginetzinsky in Moscow in 1961 and, like many of our colleagues, I was greatly looking forward to his intended visit to Britain in 1963, when he was to have given a series of lectures in the University of London. Ginetzinsky's contributions to physiology took a considerable time to cross the language barrier: if recognition outside his own country was belated, it fortunately did not come too late, and I like to think that he derived a good deal of satisfaction from it during the last years of his life.

BERNARD KATZ

Academician A. V. Topchiev

ACADEMICIAN A. V. TOPCHIEV, a noted organic chemist, a skilful administrator and a clever '*cultur*' diplomat, died in Moscow on December 27.

Born in 1907, Alexander Vasilievich Topchiev graduated at the Moscow Institute of Chemical Technology in 1930. After his graduation he made rapid progress and soon achieved eminence as a scientist and as a specialist in the field of petroleum chemistry. His numerous papers, a number of them dealing with petroleum hydrocarbons, earned him world-wide recognition. He also played an important part in the creation and development of the Soviet petroleum, chemical and polymer industry. Among his publications one may single out a few of the more outstanding ones, such as one on the nitration of hydrocarbons, another on boric fluoride as a catalyst and one on radioactive isotopes, the last paper being presented at the Congress on the Peaceful Uses of Atomic Energy held in Geneva in 1958.

In 1947–49 he acted as deputy Minister of Higher Education. In 1949 he was elected a member of the Soviet Academy of Sciences, and eventually he was entrusted with the key post of scientific secretary of the Academy. He was also director of the Institute of Petroleum–Chemical Synthesis. He received the Stalin Prize and the Order of Lenin for his work. As a very active member of the Communist Party, he was a deputy in the Supreme Soviet of the Russian Federation. His abilities as an administrator and a diplomat ensured his election to a number of committees and he was often sent abroad.

Topchiev was a frequent visitor to England, since his first visit in 1955 when he came to London as the leader of a group of Russian scientists attending a conference on nuclear energy. At this meeting he spoke with much feeling of the desire of the Russian scientists to restrict nuclear energy to peaceful uses. In 1956 he again led a party of Russian scientists attending the opening of Calder Hall. He was known as a most active fighter for peace and international scientific co-operation, and he was one of the founder members of the Pugwash peace movement at its first conference in 1957. At a scientific gathering in the United States in 1961, Topchiev again spoke eloquently about problems of peace and international co-operation. He was also a frequent and eloquent expositor of the Russian space programme, a circumstance which suggests that he had an important part to play in this.

To sum up, one may say that Topchiev was an outstanding scientist, an outstanding administrator, an organizer of international scientific and cultural co-operation and a most popular ambassador of his country, greatly admired by his fellow delegates and by his hosts for his frankness of speech, sincerity of purpose and good humour.

S. I. TOMKEIEFF

Dr. J. Howard Dellinger

DR. J. HOWARD DELLINGER, formerly chief of the Central Radio Propagation Laboratory at the National Bureau of Standards, U.S. Department of Commerce, died on December 28.

Dr. Dellinger, who initiated radio research at the Bureau in 1911, was also known for his work at national and international conferences on radio problems. He is credited with the discovery of the simultaneous occurrence of solar eruptions and radio effects (the Dellinger effect). He also investigated the ionosphere and high-frequency radio propagation and supervised the development of many basic radio aids to air navigation, and the initiation of the U.S. standard frequency broadcast service (*WWV*).

After retiring from the Bureau in 1948, Dr. Dellinger continued his work as a radio consultant and adviser.

Dr. Dellinger joined the staff of the National Bureau of Standards in 1907. He began working on the determination of the conductivity of copper for a few years. He was appointed chief of the Radio Section in 1919.

During the Second World War, Dr. Dellinger was selected by the Armed Services Joint Communications Board to direct the work of the Interservice Radio Propagation Laboratory. It was the task of this group to draw together accumulated research knowledge and data from which to make radio propagation predictions for the Armed Services. In 1946 this organization became the Central Radio Propagation Laboratory, and the prediction service was made available for civilian as well as military use. Dr. Dellinger continued as chief until his retirement from the Bureau in 1948.

Dr. Dellinger was born in Cleveland, Ohio, in 1886. He attended Western Reserve University during 1903–7 and received his bachelor of arts degree from George Washington University in 1908. He was a fellow at Princeton during 1912–13, receiving his Ph.D. there in 1913. He was elected to Phi Beta Kappa at Western Reserve and was presented with a D.Sc. degree by George Washington University in 1932.

He was author of more than 200 papers in his fields, and was radio editor for *Webster's Dictionary*. He was a Fellow of the Institute of Radio Engineers (vice-president in 1924, president in 1925, Medal of Honor in 1938), a member of the U.S. Academy of Sciences (vice-president in 1935, 1939 and 1941), and a member of the International Scientific Radio Union (honorary member for life of the U.S. National Committee, honorary president for life).

NEWS and VIEWS

The National Research Council of Canada :

Dr. B. G. Ballard, O.B.E.

Dr. B. G. Ballard, vice-president (scientific) of the National Research Council of Canada since 1954, and acting president since the death last August of Dr. E. W. R. Steacie (*Nature* Obituary, 196, 110; 1962), has been appointed president of the Council. Dr. Ballard was born in 1902 at Fort Stewart, Ontario, and graduated from Queen's University, Kingston. Following a Westinghouse graduate course in electrical engineering in 1924–25 he joined the staff of the Westinghouse Electric and Manufacturing Co., East Pittsburg, Pennsylvania. In 1930 he was appointed to the National Research Council's Division of Physics, and throughout the following ten years built up the electrical engineering section of that Division. In 1946 he was appointed assistant director of the Division of Physics and Electrical Engineering, and two years later, when a full Division of Radio and Electrical Engineering was established, he became director. He was awarded an O.B.E. for his war-time work on the development of mine-sweepers, and the Ross Medal of the Engineering Institute of Canada in 1949 for his publication entitled *Recent Canadian Radar*; in 1953 he received the Coronation Medal. Dr. Ballard is president of the Canadian Standards Association. He is an honorary member and former president of the Engineering Institute of Canada, a Fellow of the Institute of Radio Engineers, and of the American Institute of Electrical Engineers, of which he was a former vice-president.

Dr. L. Marion, F.R.S.

Dr. Léo Marion, senior director of the National Research Council of Canada, and director of the Division of Pure Chemistry, has been appointed vice-president (scientific) of the Council in succession to Dr. B. G. Ballard. Dr. Marion was born in Ottawa in 1899. He graduated with honours from Queen's University in 1926 and gained his Ph.D. degree from McGill University in 1929. The same year he began his career with the National Research Council of Canada, continuing in the field of organic chemistry, principally alkaloids, on which he did postdoctoral work at the University of Vienna in 1934–35. Since 1947, Dr. Marion has served as editor-in-chief of the Canadian journals of research and since 1952 as editor of the *Canadian Journal of Chemistry*. He also serves on the editorial board of three other scientific journals. He became head of the organic chemistry section of the Division of Chemistry in 1943, director of the Division of Pure Chemistry in 1952, and senior director of the National Research Council in 1960. Dr. Marion was elected a Fellow of the Royal Society of London in 1961. He is also a Fellow of the Royal Society of Canada and a past-president of Section III; a Fellow of the Chemical Institute of Canada and immediate past-president of the Institute; past-president of l'Association Canadienne-Française pour l'Avancement des Sciences; a member of the American Chemical Society, and a Fellow of the Chemical Society of Great Britain. He has received many awards for his contributions to the field of alkaloid chemistry, including the Médaille Léo Pariseau of l'Association Canadienne-Française pour l'Avancement des Sciences, the Palladium Medal of the Chemical Institute of Canada, and the Gold Medal of the Professional Institute of the Public Service of Canada.

The Commonwealth Scholarships (Amendment) Bill

The debate in the House of Lords on February 5 on the second reading of the Commonwealth Scholarships (Amendment) Bill added little to the points stressed in the House of Commons (see *Nature*, 197, 731, 749; 1963). The Earl of Lucan referred to the need of the overseas universities for large numbers of expatriated teaching staff and said that the African universities alone were estimated to need something like 5,000 teachers in the next twenty years. He urged that the Government and leaders of the professions could not be too emphatic in their assurances that those who took posts overseas for limited periods to help in higher education would not prejudice their careers but rather improve their prospects. Lord Peddie stressed the importance of non-academic students expanding educational facilities, but Lord Ogmore strongly urged that postgraduate study was most advantageously pursued in one of the more mature countries, and suggested some of the British graduates should hold their Commonwealth fellowships in African and Asian universities. In replying on the debate for the Government, the Duke of Devonshire pointed out that the 500 places provided for under the scheme should be considered against the 35,000 students from Commonwealth countries who were already studying in Great Britain. He gave no indication that the £6 million provided over the period 1960–65 would be increased, but added that he understood that under the scheme £200 a year maintenance was allowed for a scholar's wife.

The Diploma in Technology

In a written answer in the House of Commons on February 8, the Minister of Education, Sir Edward Boyle, stated that of 2,697 first-year students for the Diploma in Technology, 2,097 qualified by the General Certificate of Education and 538 by Ordinary National Certificate. Of these first-year students, 1,234 were college-based and 1,463 works-based; for second-, third- and fourth-year students the corresponding figures were given as 666 and 1,367; 375 and 1,019; 310 and 856. Of first-year students at January 31, 1963, 1,718 were in engineering (including 122 in chemical engineering, 596 in electrical, and 517 in mechanical engineering); 445 in applied chemistry and 225 in applied physics. For the second-, third- and fourth-year students the corresponding figures are: 1,328 (85, 515 and 443), 299 and 179; 954 (40, 377 and 308), 187 and 136; and 847 (45, 300 and 282), 132 and 87.

The Expanding Universities

In a written answer in the House of Commons on February 12, the Financial Secretary to the Treasury, Mr. A. Barber, stated that Exchequer grants to the universities for recurrent and non-recurrent expenditure would rise from £84·1 million in 1961–62 to an estimated minimum of about £130 million in 1966–67. Student numbers would increase from 111,385 to 150,000 in the same period. The universities had estimated that the entry in 1966 should be more than 10,000 higher and would represent about the same percentage of the much larger age-group.

British Medical Aid to the Developing Countries

In opening an adjournment debate on medical aid to the developing countries in the House of Commons on February 11, Mr. J. Boyden concentrated on the report of Sir Arthur Porritt and his Working Party, endorsing an earlier tribute of the Secretary for Technical Co-operation to the work of Sir Arthur and his colleagues. Mr. Boyden emphasized the urgency of the problem, pointing out that the Working Party itself had noted a further decline in British influence in the medical field overseas during the four months it was at work. Replying for the Government in the absence of Mr. D. Vosper through illness, the Joint Parliamentary Secretary to the Ministry of Health, Mr. B. Braine, said that the Government's policy in providing technical assistance was to meet the requests of developing countries in whatever form they appeared. The delay in publishing the report was due to the new financial provision involved which could be considered only in the context of the estimates of the Department of Technical Co-operation for 1963–64. Mr. Vosper would not have been able to make a statement had he been present, but would do so at the earliest opportunity, and meanwhile, Mr. Braine said that the Government accepted the greater part of the recommendations in the report.

To-day, 900 undergraduate students from overseas are studying in Britain's medical schools and there are 9,000 student nurses from overseas in Britain's hospitals. Almost half the doctors at registrar level in the hospitals are from overseas, and in the past two years the Department for Technical Co-operation has arranged and paid for postgraduate training for 265 doctors and 170 nursing sisters from overseas, in addition to 170 doctors from the dependent territories for postgraduate training at the expense of their own Governments. Under the Overseas Assistance Scheme, 1,385 medical staff have been designated for the coming year at a cost of £1·6 million. The Department also recruited 131 doctors during the past two years for service overseas, and many medical appointments were made under the Colombo Plan and the Special Commonwealth African Assistance Plan, while medical equipment to the value of £68,000 was provided under these plans in 1961–62, and £262,000 is at present being expended on medical research for the benefit of developing countries.

Mr. Braine admitted to some disappointment in the response to serve overseas. The Government proposed to create 25 bursaries for postgraduates from the developing countries, and these would be administered on lines similar to those provided under the Colombo Plan. The supervision of postgraduate education in non-teaching hospitals, he said, was now in hand with the aid of £250,000 provided by the Nuffield Provincial Hospitals Trust. The Government was aiming at establishing 12 senior and 6 junior lectureships in 1963–64 at the two schools of tropical medicine as a contribution to preventive medicine overseas. If all the 235 vacancies at present existing were to be filled, a climate of opinion must be created in the medical profession and the hospital boards as favourable to service overseas as that already created in the teaching profession, and to that end Mr. Braine welcomed the publicity afforded by the debate.

Reactor at Full Power at Buchanan, New York

Full power operation has been achieved at Con Edison's Indian Point atomic–electric generating station in Buchanan, New York, thirty-five miles from the centre of New York City. At full power, Indian Point generates 275,000 kW for Con Edison's six million kW system serving the area of New York City. The steam which drives the turbine at Indian Point receives approximately two-thirds of its heat from the atomic reactor and one-third from oil-fired superheaters. The reactor, according to its designer and builder, the Babcock and Wilcox Co., has operated smoothly since it achieved criticality. The core of the reactor, the first power reactor to use thorium as fuel, will last approximately two years without re-fuelling.

The Imperial College of Science and Technology

A brochure, *A Decade of Expansion: 1953–1963, Imperial College*, describes, with diagrams and illustrations, the progress made since January 1953 with the expansion of the Imperial College of Science and Technology from about 1,650 full-time students to 3,000 (Pp. 36. London: Imperial College, 1963). This target has almost been achieved. Academic work has at no stage been interrupted, while standards have been maintained and even improved, and many pioneering academic ventures have been started. In South Kensington the area has grown from 9 to 25 acres and the floor space from 800,000 to almost 1·8 million sq. ft. gross, and many semi-obsolete buildings have been replaced. While these developments have been achieved basically through national funds made available through the University Grants Committee, substantial help has come from charitable foundations, industry, and private individuals. Capital expenditure on buildings of more than £17 million was involved. The academic staff of 491 includes 51 professors and 75 readers, and there are some 1,100 postgraduate students, and the total of 2,900 students includes 659 from overseas, while the annual income for supported research exceeds £500,000, half of which comes from the Department of Scientific and Industrial Research. Despite this encouraging picture when the brochure looks to the future, it points out that the grants announced for the new quinquennium 1962–67 are so low that the College will be unable to man completely the large, and sometimes unique, new buildings which have been or are being completed. This will have a serious effect on the admission of students and the quality of the specialist postgraduate education provided; the Departments of Mathematics and of Chemistry would be special sufferers. Remedial action is urgently needed if the full benefits of the expansion are to be realized, and, in its absence, it is anticipated that the number of students will not exceed 3,100, whereas on the recurrent grant applied for a total of 3,700 was visualized. Reference is also made to the social needs of students. By the end of 1962–63 residence should be provided for 22 per cent of the students, perhaps about one-third of the demand, but unless finance permits the expansion of the College to be resumed these figures will have deteriorated by the end of the quinquennium.

Hazards to Health due to Radiation

The first edition of the *Code of Practice against Radiation Hazards* for the guidance of members of the Imperial College of Science and Technology, University of London, was issued in June 1957. The latest edition, the fourth (October 1962), supersedes former editions and gives the regulations applicable to the use of equipment producing ionizing radiation, to accelerators, and to the use of sealed and unsealed radioactive isotopes. The first eight pages of the booklet contain the *Code*, which is mandatory. The purpose of the *Code* is to control the hazards to health arising from radiation, to ensure the safe disposal of waste material and effluent, and to avoid contamination of laboratories and their surroundings, and it deals mainly with administrative procedure and instructions. The remainder of the booklet consists of eleven appendixes, in which detailed information, interesting physical data, and extremely valuable advice on practice are given. Maximum permissible levels of radiation; protection against ionizing radiation particularly from X-ray apparatus; classification of radioisotopes; requirements for radiochemical laboratories; monitoring; storage and disposal of active materials and waste; emergency procedures and fire precautions are some of the topics discussed. An extensive bibliography is also provided. For other university departments which have yet to establish their own radiation safety committee and produce their own regulations, the *Code* should prove an excellent model to copy.

U.K. Library Telex Directory

The second edition of the *Library Telex Directory*, compiled from 700 questionnaires issued to organizations known to have access to telex and reported to possess library or information services, contains entries for 400 libraries compared with 131 in the first edition in 1960 (Pp. 24. London: The Library Association, 1962. 8*s*.; L.A. members 6*s*.). Some entries are qualified by restrictions. Some notes on the use of telex in libraries which preface the *Directory* stress the importance of stating the urgency of a request, of not economizing in words at the expense of clarity, of sending brief acknowledgments of action taken or to be taken, and of spacing loan requests on the paper roll and including name and address with each separate item.

The Quarter-Inch Map of Great Britain

February 1 saw the completion of the Ordnance Survey's Fifth Series of the *Quarter-Inch Map of Great Britain* when the last two sheets were published. Hence the whole of Britain is now covered by this post-war series begun in 1957. The quarter-inch 'travellers' map, as it could be called—for here lies its greatest utility—owes its beginning to the War Office and the Ordnance Survey. It was begun in 1859, but its publication was delayed for almost twenty-five years owing to the all-out effort of the engravers of the day to complete the one-inch map then on hand; moreover, its sale to the public was not until 1887. In order to conform with continental practice and the requirements of air navigation the scale of the *Quarter-Inch Map of Great Britain* is, in fact, now 1 : 250,000 and not 1 : 253,440. As with previous editions, the Fifth Series has been derived from the one-inch map, in this case the post-war Seventh Series. Compared with the *Quarter-Inch Fourth Edition*, the amount of detail on the Fifth Series has been reduced in the knowledge that there exists an up-to-date one-inch map for more detailed reference. Relief is shown by hypsometrical or layer tints and contour lines, with the addition of hill shading to give a more readable picture of the ground. Special effort has been made to make the map useful to the motorist by clearly defining Motorways, Class I and II roads and dual carriageways.

The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme

Awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Dr. Madge G. Adam, university demonstrator in astronomy, Oxford, to assist her to visit Mount Stromlo Observatory for a year from August in order to use the 74-in. telescope there for a study of magnetic stars in the southern sky; Dr. U. Aswathanarayana, reader in geology, Andhra University, to enable him to familiarize himself with recent advances in methodology and experimental techniques of radioactivity methods of dating, at Oxford, from April until September; Dr. N. Collis-George, associate professor of soil science, University of Sydney, to enable him to become acquainted at first-hand with the theoretical and experimental methods of describing the movement of water through soils, at Cambridge, from July until December; Dr. P. J. Grubb, university demonstrator in botany, Cambridge, to enable him to investigate the sodium/potassium relations of the *Atriplex* spp. growing in the arid areas near Adelaide, from April until October; Dr. J. R. Richards, Fellow in the Department of Geophysics, Australian National University, to assist him to gain further experience in the measurement of lead isotope ratios and to extend his experience in the arts of isotope geology and geochronology at the University of British Columbia and the University of Toronto, for about eight months from March; Dr. C. G. Winder, associate professor of geology, University of Western Ontario, to enable him to gain experience of studying conodonts at the University College of Swansea and to study the type exposures of the Ordovician, Silurian and Devonian systems of rocks which occur in Wales and Devon, from April until September 1963.

Announcements

Mr. J. West has been awarded the recently instituted City and Guilds of London Institute's Prince Philip Medal. This award, which is made annually, is restricted to persons under 40 years of age who have "travelled the City and Guilds path" and passed one of the Institute's examinations. Mr. West, when assistant manager of P. and O. Orient Management, Ltd., was the designer of the 45,000-ton passenger liner *Canberra*.

A members' day incorporating the annual general meeting, arranged by the National Vegetable Research Station Association, will be held on March 12. Further information can be obtained from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick.

The fourteenth annual meeting of the Tissue Culture Association will be held at the Somerset Hotel, Boston, during May 27–30. Three of the six sessions will be devoted to a symposium on "Metabolic Control Mechanisms in Animal Cells". Further information can be obtained from Prof. P. Goldhaber, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, Mass.

A conference on "Semiconductor Power Controls", arranged by Texas Instruments, Ltd., will be held in Bedford on March 18. Subjects under discussion will include: basic SCR circuit considerations; gate-controlled switches; proportional power controls; motor-control circuits; d.c. motor-control circuits; a.c. motor-control circuits; power supplies. Further information can be obtained from Mr. W. A. Yates, Texas Instruments, Ltd., Manton Lane, Bedford, England.

An international symposium on "Aqueous Reprocessing Chemistry of Nuclear Fuels", organized by the European Company for Chemical Processing of Irradiated Fuels and the Organization for Economic Co-operation and Development, European Nuclear Energy Agency, is to be held in Brussels during April 23–26. Further information can be obtained from the Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 38 Boulevard Suchet, Paris 16 ème.

The jubilee meeting of the British Ecological Society will be held at Queen Elizabeth College, London, during March 28–31. The programme has been divided into six sessions: ecology and conservation; Quaternary ecology; the history of ecology in Britain; experimental and autecological studies; production ecology; the community concept. Prof. W. H. Pearsall will give the Jubilee Address, entitled "The Development of Ecology in Britain", on March 29. Further information can be obtained from A. Macfadyen, Department of Zoology, University College, Swansea, or from Dr. P. J. Newbould, Department of Botany, University College, Gower Street, London, W.C.1.

EXPENDITURE ON RESEARCH AND DEVELOPMENT IN BRITAIN

IN the final section of its annual report for 1961–62*, the Advisory Council on Scientific Policy again reviews the expenditure on scientific and technical research and development, which in 1961–62 is estimated at £634 million or about 2·7 per cent of the gross national product, of which about £67 million was capital expenditure. Private industry carried out about 58 per cent of total research and development, universities and technical colleges being responsible for 5·1 per cent, research associations for 1·3 per cent, Research Councils for 3·6 per cent, and public corporations for 3·4 per cent. On the other hand, the Government provided 60·7 per cent (£385 million) of the finance, of which 38·7 per cent was from defence departments and 4·6 per cent from the research councils, while private industry provided 33·6 per cent (£213 million), only 0·2 per cent coming from the universities and technical colleges. Of current expenditure by the Government, £86·4 million was by defence departments, £45·7 million on civil research and development, and £19·4 million on the research councils. Technical colleges spent £1·8 million, universities £24·8 million, research associations £6·5 million, public corporations £15·4 million, and private industry £340·8 million.

The proportion of the gross national product expended on research and development continues to rise, from 1·7 per cent in 1955–56 and 2·3 per cent in 1958–59. Expenditure by universities and technical colleges has risen from £14·4 million in 1955–56 to £32·4 million, by research associations from £4·9 million to £8·1 million, by public corporations from £5·8 million to £21·4 million, by research councils from £10 million to £23 million, and by private industry from £185 million to £367·7 million, while that by defence departments has only risen from £65·7 million to £93·2 million. Government expenditure in work carried out in private industry was nearly £177 million, and most of this was in the electronics, electrical engineering, and especially the aircraft industry. Total expenditure in the United States in 1961–62 is estimated at about £5,360 million, or 2·89 per cent of the gross national product, excluding capital expenditure.

A note on the scope and methods used in this inquiry into research and development expenditure appended to the report gives the qualified manpower employed on research and development in private industry as about 31,000 and the number of science mathematics graduate teachers in grant-aided schools in England and Wales as 20,055 and in Scotland 5,109, increases of 968 and 85 respectively, on the previous year. The additional scientific manpower is computed from 6,844 first degrees and diplomas awarded in pure science in 1960–61, 3,982 in technology, 620 diplomas in technology, 158 associateships of educational institutions, and 5,885 new members of scientific and technological professional institutions. Government expenditure on civil research in 1960–61 was £18·19 million and is estimated at £21·5 million in 1961–62 and £27·2 million in 1962–63. For agriculture, forestry, fisheries and food the figures are £10·66 million, £12·7 million and £12·9 million, respectively; for industry and communications, £20 million, £19·2 million and £23 million; medical and health, £5,255 million, £6·3 million and £6·66 million; overseas research, £1·52 million, £1·5 million and £1·6 million; universities and learned societies, £19·37 million, £21·4 million and £23·9 million; and for nuclear science, £54·1 million in 1961–62 and £56·7 million in 1962–63. Of the expenditure on Research Councils, estimated at £36·4 million in 1962–63 compared with £31·6 million in 1961–62 and £27·7 million in 1960–61, £17 million was for current and £5·1 million for capital expenditure on the Department of Scientific and Industrial Research with a scientific staff of 3,650. For the Agricultural Research Council the corresponding figures are £6·8 million, £954,000 and 2,672; for the Medical Research Council, £5·4 million, £441,000 and 1,883; and for the Nature Conservancy, £534,000, £56,000 and 121.

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* Annual Report of the Advisory Council on Scientific Policy, 1961–1962. (Cmnd. 1920.) Pp. vi+45. (London: H.M. Stationery Office, 1963.) 3s. net. See also p. 825 of this issue of *Nature*.

TRAINING OF GRADUATE SCIENCE TEACHERS IN BRITAIN

A REPORT on the training of graduate science teachers in England, Wales and Scotland, issued as the first section of Part 2 of the Science and Education Report of the Science Masters' Association and the Association of Women Science Teachers*, is based largely on information supplied by the twenty-three university departments of education in England and Wales in answer to a questionnaire. Some seventy senior science teachers were also consulted and twenty-seven others with less than five years' experience, and a joint meeting was held with the Standing Conference of Heads of University Departments of Education in December 1960.

The report points out that the present shortage of science teachers and consequent rapid promotion to senior posts may quickly cause the young graduate to be faced with the general supervision and maintenance of a laboratory as well as to be asked to produce new teaching syllabuses and to devise new courses. It is hoped that he will wish to keep himself informed of developments within and on the fringe of his own subject as well as in the field of education. This clearly requires a comprehensive course of study, not merely coaching in practical techniques, and the sub-committee responsible for the report submits that a successful teacher is one who can convey clearly and accurately both the established knowledge of science and the spirit of scientific inquiry and can indicate the part which science plays in the wide field of human achievement, practical and intellectual. The Associations believe that all intending science teachers should undergo an effective training course, but they also believe that the potential value of present courses is often not realized.

Their inquiries revealed that eight out of twenty-three departments of education had no practical facilities, four had a small room or laboratory, seven had one laboratory, and four had two or more laboratories. Many of the small rooms were for one subject only, and some departments used facilities elsewhere in the university. Only eleven departments held laboratory courses, ranging from five to sixty hours per year, and the money spent on apparatus averaged £2 8s. per student per year; in fitting out new laboratories the capital expenditure in 1959–60 was £9 per student. The sub-committee concluded that the facilities provided for practical work were quite inadequate both in time and in accommodation. If practical work is to be given adequate time, science students must be prepared either to devote more hours weekly to their training, or to have their training extended beyond the present university terms.

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* *The Training of Graduate Science Teachers in England, Wales and Scotland.* (A section of Part II of the Science and Education report issued by the Science Masters' Association and the Association of Women Science Teachers.) Pp. 32. (London: John Murray. Published for the S.M.A. and A.W.S.T., 1962.) 2s. net.

To overcome the inadequacy of laboratory accommodation, the report recommends the establishment of Science Teaching Centres in all universities, in the departments or institutes of education or elsewhere. These centres should have adequate laboratory accommodation, be organized by a director and have a panel of visiting school teachers. It is suggested that the establishment of such centres would help to meet the present need for refresher courses for established teachers and for part-time courses leading to a higher professional qualification, such as university diploma in the teaching of science. It is also recommended that the training course to be taken by all intending science teachers should pay adequate attention to general principles of education and to studies in the philosophy, method and social consequences of science. School practice should comprise a complete school term and only in schools where adequate supervision and help are provided. There should be more liaison between school supervisor and departmental tutor both at the personal and professional level. Departments of Education should be asked to consider the merits of continuous assessment and the time spent on method and practical work, and Department of Education staffs and local branches of the Science Masters' Association and the Association of Women Science Teachers should ensure that effective and continuous contact exists between each Department and the science teachers in its area. Conditions in Scotland are regarded as in many ways in advance of those in England and Wales; but there is considerable dissatisfaction with the student/staff ratio and the inadequate provision of rooms and equipment in the colleges.

CHALLENGES OF SCIENCE IN SOUTH AFRICA

IN his presidential address to the Diamond Jubilee Congress of the South African Association for the Advancement of Science at Cape Town, July 1962, Prof. J. P. Duminy, under the title "The Challenges of Science To-day", discussed more particularly the universal shortage of manpower which, he suggested, arises out of the unplanned and unexpected advances in technology in the past decade—out of what can be described as an explosion in the field of scientific knowledge. But for this, South Africa might have been able to cope with its internal post-war demands for trained personnel. However, extreme difficulty is now being experienced in attracting men from abroad to institutions there and in training South Africans in their own universities to meet the manpower needs.

South Africa has never enjoyed the means of providing facilities at its universities necessary for training men to the levels of teaching and research in science and technology which are essential. Many of those who go overseas in order to obtain qualifications never return. Some mobility and interchange are desirable, but for several years the movement has been consistently one way. Investigations into the sources of trained manpower have shown that the universities will be powerless to play their full part unless the interest of youth is aroused and larger numbers are stimulated to take up careers in science and technology. The Manpower Development Foundation, created under the auspices of the Association and in collaboration with the Federation of Science Teachers' Associations, seeks to improve the teaching of science in the schools. Publication of a periodical dealing with methods and material for teaching physics, chemistry, mathematics and biology is planned. A significant contribution is also being made by the Permanent Exhibition of Modern Science.

In conclusion, Prof. Dunning, observing that science is not independent of philosophy and that philosophical thinking is not incompatible with scientific concepts, urged that the schism between the humanities and the sciences must be healed and science enabled to make its full contribution in our thinking about the realities of human existence and the relations between human beings.

PROGRESS AND INNOVATION IN SCIENTIFIC RESEARCH

IN his Peter Le Neve Lecture to the Royal Society of Arts on January 9, on "Progress and Innovation", Mr. J. C. Duckworth, managing director of the National Research Development Corporation, said that in these days of the large development and design team it is often difficult to decide what is truly invention and what is purely the logical outcome of applied research or development work. The actual invention of a new device or process can occur very soon after the requisite knowledge is available, and indeed almost as part of the process of acquiring it, or the invention may come long after the necessary knowledge is available. On the other hand, the inventor may be too far ahead of the 'frontiers of knowledge' for his ideas to be practicable.

Mr. Duckworth suggested that over the past two centuries the nature of invention has changed. The principle of designing new equipment to meet a need is now well established and such design should not be considered invention unless it incorporates some novel feature or concept beyond that which the ordinary design engineer can be expected to provide. The fact that ideas such as the hovercraft can be conceived by an individual should encourage those who believe that inventions may be possible by those outside the large research and development teams. Moreover, it is probable that individual inventors will have most success where a combination of different disciplines is required to produce the new idea.

After emphasizing that an invention only makes its impact if the economic and social climate is ready for its acceptance, in addition to the need for technical development to reach a sufficiently advanced stage, Mr. Duckworth observed that within the team the creative genius of invention is still required to make more than humdrum progress. The National Research Development Corporation was set up partly due to the belief that inventions of obvious merit were being suppressed because their development jeopardized the position of established products; however, the Corporation found no reliable evidence of this. Development contracts involve an appreciable proportion of the effort and expenditure of the Corporation which operates its development activities in an area in which it is particularly difficult for the individual inventor to succeed. In many other industrialized nations industry is more receptive to new ideas and will generally take up innovations more quickly and enthusiastically than in Britain, or is prepared to take greater risks where final commercial viability is less easy to assess. The Corporation generally becomes directly involved where the technical possibility is more in doubt, the commercial prospects are difficult to assess, or where it appears that there may be a benefit to the community as a whole, and where the benefit to one branch of industry may be marginal.

There is no doubt that the individual inventor now has a difficult task and that in the scientific field the inventor is increasingly a highly qualified man working with adequate resources. True inventiveness should always be encouraged and in spite of the fact that the lone inventor now has a smaller chance of making significant scientific advances, if he does succeed in doing so he has a better chance than ever before of getting his ideas adopted, followed by fair treatment and financial reward.

THE EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

THE seventh annual report of the European Organization for Nuclear Research (CERN) * covers the period to the end of 1961 and gives a detailed account of the activities of the Organization during a year which Prof. V. F. Weisskopf, the director-general of the Organization, describes in his introduction to the report as "an experimental year". It was the first year in which the research programme got into something approaching its full stride and in which tentative solutions were tried out to the problems of internal organization, of the shaping and management of the programme itself, and of relationships with other research organizations and research physicists.

The staff on December 31, 1961, numbered 1,091, of whom 187 were scientists and engineers, 573 technicians, 149 administrative and 182 ancillary members. They were organized in twelve divisions under a directorate of three and the director-general. The total expenditure during 1961 amounted to 70·2 million Swiss francs, of which 36·2 million was devoted to capital expenditure, mainly on buildings and on the accelerators and experimental equipment; 8·4 million on general expenditure; and 25·6 million on staff salaries and expenses.

The mean intensity of the proton synchrotron remained at about $2{\cdot}5 \times 10^{11}$ protons/pulse with a trapping efficiency of 50 per cent. The machine time allocated to nuclear physics experiments increased by about 50 per cent, from 62 h per week during January to 93 h per week during October. About 15 per cent of the total machine time was lost through various breakdowns. The commissioning during August of a new experimental hall (North Hall) made room for two extra beams to be set up, making a total of five beams in use round the machine at the end of 1961.

Two CERN-designed electrostatic separators came into operation during the year. A voltage of 750 kV could be held over a gap of 14 cm in a 3–4 GeV/c. antiproton beam and of 500 kV over a gap of 8·5 cm in a 1·5 GeV/c.K⁻ beam. Other new separators, including a radio-frequency separator, are under construction.

The research performed by members of the Nuclear Physics Division with the proton synchrotron was very fruitful. In the section of the report dealing with the activities of this Division, 16 experiments with the proton synchrotron and 11 with the synchro-cyclotron are described in some detail. For the proton synchrotron these include elastic and near-elastic scattering of protons on protons; total cross-section measurements of high-energy pions on hydrogen; strange particle physics; the measurement of resonances in the two-pion system using a spark chamber; the determination of the lifetime of the neutral pion, about 2×10^{-16} sec; muon electron scattering; and the search for magnetic monopoles. Long experiments were performed with the help of two large French bubble chambers. 300,000 photographs were taken with the 81-cm Saclay hydrogen bubble chamber in a beam of slow antiprotons and 80,000 pictures by the same chamber in a fast (3·0–4·0 GeV) antiproton beam. (It was in the latter series of pictures that for the first time, early in 1962, a charged anti-*Xi* particle was found.) Interesting results were also obtained in the study of photographs taken with the 30-cm hydrogen bubble chamber.

The experiments with the synchro-cyclotron included the outstanding experiment to measure the magnetic moment of the muon to an accuracy of 1 in 200,000, thus establishing that the muon is accurately described as a Dirac particle with no interaction other than the electromagnetic and the weak kinds—that is, that it behaves exactly as a heavy electron. During 1961 an experiment on the scattering of negative μ-mesons on *C* was started, and the reaction $\mu^- + p \rightarrow n + \nu$ was investigated directly without nuclear complications in the Italian 20-cm hydrogen bubble chamber. The energies of μ-mesic X-ray transitions (2*p*–1*s* and 3*d*–2*p*) were measured for many nuclei throughout the Periodic Table, and the search for the neutrino-less conversion of a muon into an electron using an improved μ^- beam intensity and a spark chamber has continued.

Using a modular system of construction, the Electronics Workshop has produced a comprehensive range of transistorized nucleonic instruments for use in the nuclear physics experiments. The range includes a fast triple coincidence circuit, fast discriminator, 5-way splits, delays and triggers, 100-Mc/s pre-scaler, fast-pulse amplifier with a gain of 10 and rise of 2 nsec, and pulse burst generators. Some work has been initiated in the field of image intensifiers and scintillation chambers.

During the year under review it became clear that more attention would have to be given to data handling and analysis, and that more and more machine time would be required from computers with faster operation and larger memories. The introduction of a 709 computer has greatly increased the computing facilities; but the absence of a link between the 709 and the *Mercury* computer has been the main bottleneck. A number of general-purpose programmes have been added and an improved compiler has been introduced to simplify the testing of *Autocode* programmes.

The staff of the Theoretical Study Division at the end of 1961 comprised ten scientists, one computer-programmer and two secretaries. In addition, about thirty theoreticians under the CERN and Ford Foundation fellowship programmes were working at any one time in the Division. Various problems characteristic of the very-high-energy region opened up by the CERN proton synchrotron were given special attention. Near-elastic scattering of nucleons on nuclei, inelastic nucleon–nucleon scattering with very small energy loss, and the interactions of high-energy neutrinos with complex nuclei, were the main subjects of study. A variety of problems relating to the physics of pions and nucleons and of strange particles have also been investigated. The Theoretical

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* CERN, European Organization for Nuclear Research. Annual Report for 1961. Pp. 153. (Geneva: CERN, 1962.)

Study Division organized an international conference at CERN on the theoretical aspects of very-high-energy phenomena during June 5–9, 1961. The proceedings were published as *CERN Publication 61–22.*

The Film-badge Service of the Health Physics Group monitored 1,069 people for γ-radiation, and in addition about 140 people were also under neutron film-badge control. Only 78 people received a dose exceeding 1 rem for the year. In seven cases the maximum permissible annual exposure of 5 rems was approached. Arrangements for making blood tests of people exposed to radiation were improved and extended. A monitoring system for all waste leaving the CERN site has been set up. Routine weekly surveys of the radiation-level from the proton synchrotron and from the synchro-cyclotron were made.

Careful consideration was given during the year to the question of the rate of growth of the Organization in the future. The Council agreed on a budget for 1962 which would permit a moderate rate of growth and decided that during 1962 a more profound study should be undertaken by a special working party of the basic questions on which any policy for the future should be founded. Provision was made in the budget for 1962 for an intra-European study-group to produce design studies of two possible future projects, a storage ring device to be fed from the present proton synchrotron and a 300-GeV high-intensity proton synchrotron. The device is likely to consist of two storage rings of the same size as the proton synchrotron, but with d.c. magnets with split focusing and bending. The r.f. system would also be smaller and more delicate. The cost is estimated to be less than twice the cost of the proton synchrotron and the construction time about five to six years.

Because of a serious shortage of staff in the Public Information Office the production of the *CERN Courier* had to be practically suspended and many other activities were greatly handicapped. Four television programmes and ten radio programmes were arranged. English, French and Italian versions now exist of the CERN film ***Matter in Question***, which was awarded the Grand Prix in its class at the International Festival of technical and scientific films in Budapest. A German version is to follow during 1962. A total of 5,867 people in 206 conducted parties were shown around the establishment, and a new scheme of visits is being worked out to allow visitors still to see as much as possible although the accelerators are now practically inaccessible. The separate report of each Division concludes with a list of publications by members of the Division, and in two appendixes to the annual report are listed the 1961 CERN publications; reprints of publications during 1961 in other periodicals by members of the staff of CERN; and details of the lectures, seminars and colloquia held at CERN.

S. WEINTROUB

RECENT DEVELOPMENTS IN ASTRONOMIC AND SPECTRONOMIC INSTRUMENTS

WORK undertaken during 1962 by Sir Howard Grubb Parsons and Co., Ltd., has included a number of interesting and unusual projects. A 48-in. reflecting telescope has been erected at the Victoria Observatory, British Columbia, and a rather uncommon type of telescope, a twin 16-in. reflector, has been manufactured for the Royal Observatory at Edinburgh. Each tube of the twin reflector contains a 16-in. primary mirror and a Cassegrain mirror giving an overall focal ratio of $f/15$. One of the tubes can be off-set from the other in any direction up to 5° and simultaneous photometry can be carried out on two stars. The operation of the telescope can be made fully automatic.

Because of difficulty in obtaining acceptable glass blanks for the components of the complex optical system for the 40-in. reflector for the Cape Observatory, the completion of the telescope has been held up. The optical system uses a parabolic mirror and a lens system affording a wide field of good definition at the prime focus. A large correcting plate is located at about the prime focus and a correcting lens is inserted in the beam just before it comes to a focus. When the optical system is assembled it will be necessary to mount it in the telescope tube and test it in the tube. The test will be carried out in the test tower at the works. All the heavy items and fabrications for the 98-in. Isaac Newton reflecting telescope have been received and many of the machining operations have been completed.

Newly developed instruments include the 'Spectromaster', an infra-red spectrometer, covering a wavelength range of 0·6–25μ, and an interferometric spectrometer based on the work of Dr. H. A. Gebbie at the National Physical Laboratory. New techniques have been developed to produce very narrow band interference filters for the visible and selective filters for the infra-red. For the U.K. Atomic Energy Authority establishment at Harwell, two remotely controlled gas analysers to monitor carbon monoxide and carbon dioxide in a helium test loop for *Pluto* have been made, and four glass windows have been polished for use in a liquid hydrogen bubble chamber. Four oval windows for a similar purpose are to be ground and polished for use by the European Organization for Nuclear Research (CERN). Measuring 70 × 25 × 6 in. and weighing approximately 1,300 lb. each, they will constitute the largest pieces of optical glass ever to be manufactured anywhere in the world.

PALÆONTOLOGY IN THE U.S.S.R.

CONSIDERABLE advance has been made in palæontological research in the U.S.S.R. during the past half-century, following on the great development in this field of scientific activity in Russia during the nineteenth century. Characteristic of the present-day approach is the re-orientation of palæontology so that it is no longer treated as a mere annex of stratigraphy but is linked more closely with biology (theory of evolution, ecology, geological facies and conditions of fossilization). The Palæontological Society, founded in 1916, has actively sponsored this research and much has been done by the numerous geological institutes and the universities. Publication has been covered by various scientific periodicals, more particularly the four series—*Palæontology of the U.S.S.R.*, *Problems of Palæontology*, *Palæontological Journal* and *Transactions of the Palæontological Institute.*

The old-type description of fossils, collected by overworked geologists, is replaced by planned systematic work, specially organized to combine field and laboratory investigations. Examples of such work can be seen in recent expeditions to investigate a large accumulation of fossil bones of reptiles of Permian age, discovered in 1949 near the village of Eschovo, south of the town of Ochera on the River Kama. The systematic excavation of this fossil field was begun by members of the Palæontological Institute in 1957 and is still in progress. The results so far obtained are summarized by P. K. Chudinov (*Priroda*, 4, 78; 1962). The majority of fossils belong to the group of *Deinocephalia*, genera *Estemmenosuchus* and *Eotitanosuchus*. The name *Ocheria* is given to the remains of a new genus. There are also a few remains of *Amphibia*, such as *Melanosaurus*. The fauna, as a whole, is considered to be intermediate between those of North American and South Africa. Another important ossuary, discovered by members of the East Siberian Geological Institute in a locality 15 km south-west of Ulan-Ude Transbaikal region, and reported by L. N. Ivaniev (*Priroda*, 6, 78; 1962), consists of fossil bones of Tertiary and Quaternary mammals. They include well-preserved remains of Hipparion and other genera belonging to the same faunistic group.

The following is a very brief guide to some of the work covered during the past decade:

Protozoa, especially Foraminifera—by a number of research workers, especially micropalæontologists.

Archaeocyathidae, a group of rather problematic fossils intermediate between corals and sponges, found in the deposits of the Lower and Middle Cambrian, especially in the Asiatic part of the U.S.S.R. A. G. Vologdin (*Priroda*, 10, 185; 1962) describes a large amount of work on the investigation of this group since the expert summary by A. P. Borisyak (*Priroda*, 7–9, 315; 1919). More than one thousand species have been described. Cœlenterata, B. S. Sokolov; Bryozoa, M. I. Shulga-Nesterenko and A. I. Nikiforov; Brachyopoda, Chernyshev *et al.*; Trilobita, V. V. Weber and E. V. Lermontova; Quadrupeda, P. P. Shushkin; fossil plants, I. M. Pokrovskaya and S. M. Naumova.

The only way of dealing with the abundant palæontological literature published during the past three years is to give the titles of the recent volumes of the *Transactions of Palæontological Institute*:

80, The Spiriferidae of the Kazan stage of the Russian platform and the conditions of their co-existence, by A. D. Slyusareva (1960); 81, The Devonian bivalve Phyllopoda, by N. Novozhilov (1961); 82, The late Devonian sea of the Kuznetsk depression, history of its development, population and deposits, by T. N. Bel'skaya (1960); 83, The principles of systematics, system and phylogeny of Palæozoic Ammonoidea, by V. E. Ruzhentzev (1960); 85, Palæozoic insects of Kuznetsk basin, by E. E. Beckerr-Migdasova, O. M. Martynova and A. G. Sharov (1961); 86, Devonian Bryozoa of the Minusinsk and Kuznetsk basins, by I. P. Morozova (1961); 87, Artinskian Ammonoidea of the Middle Urals, by M. F. Bogoslovskaya (1962); 88, Ecology and the development of Silurian and Devonian brachiopoda of the Kuznetsk, Minusinsk and Tuva basins, by E. A. Ivanova; 89, Cambrian Algae of the south-eastern part of the Siberian platform, by K. B. Korde (1961); 90, Fundamental problems in the systematics of Nautilidea and related groups, by V. N. D. Shimansky and F. A. Zhuravleva (1961); 91, Saline-water Pliocene Cardiatidae of the U.S.S.R., Part 4, by A. G. Eberzin (1962).

But the most spectacular and certainly the most important publication in the Soviet Union is the projected 15-volume *Principles of Palæontology* written by two hundred specialists and containing not only accounts of recently discovered fossils in the Soviet Union but also a systematic account of the world's fossils, illustrated by numerous photo-reproductions and line drawings, and accompanied by a critical discussion of their systematics, nomenclature and distribution in space and time.

The titles of the fifteen (non-numbered) volumes of the *Principles of Palæontology* (*Osnovy Paleontologii*) are: General part, Protozoa; Porifera, Archaeocyathidea. Cœlenterata; Mollusca: Loricata, Bivalva, Scaphopoda Supplement: Vermes; Mollusca: Gastropoda; Mollusca: Cephalopoda. I, Nautiloidea, etc.; Mollusca: Cephalopoda. II, Ammonoidea, etc.; Bryozoa. Brachiopoda, Supplement: Phonoronidea; Arthropoda: Trilobita, Decapoda; Arthropoda: Trachaean, Chelicerata; Echinoderma, Hemichordata; Astomata, Pisces; Amphibia, Reptilia, Aves; Mammalia; Algae, Fungi, Bryophyta, Pteridophyta; Gymnospermae. Angiospermae.

One can get more information by looking at the particulars of the two most recently published volumes in this series:

Bryozoa. Brachiopoda. Suppl.: *Phonoridea*, edited by T. G. Sarycheva, written by twenty authors. 343 pp. 82 plates, 485 text-figs. 1960. Price 3 r. 82 k.

Mammalia, edit. by V. I. Gromova. Written by thirteen authors. 421 pp. 543 text-figs. 1962. Price 2 r. 61 k.

The *Principles of Palæontology* (*Osnovy Paleontologii*) is produced by the Palæontological Institute, Moscow Academy of Sciences (Lenin Prospect, 33, Moscow B-71) in collaboration with a number of other institutes.

S. I. TOMKEIEFF

BIOLOGICAL SIGNALS AND NOISE

By DR. L. MOLYNEUX

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THE word 'noise' in a scientific context is usually used to describe random fluctuations of potential; however, the definition can be usefully expanded to mean 'extraneous information of the same kind as that which is of interest in a particular situation'. For example, if the biological phenomenon is the movement of some part of the anatomy with respect of its surrounding tissue (say, the pulsation of an artery), then 'noise' will include any movement of the artery with respect to the pick-up device which is not directly due to arterial expansion.

Or again, if the biological signal is the potential difference between two points on the skin (say, the electro-encephalogram) then the 'noise' will include any potential difference appearing across the input electrodes, regardless of whether the source of the disturbance is external (alternating current mains supply) or internal (muscle potentials).

Anyone who has tuned a radio from station to station will have heard between the stations fairly 'white' noise. The expression 'white' here means that the frequency spectrum of the noise is spread over most of the audible band-width, and that it is not concentrated at a particular frequency. It is noticeable that a weak station can be better understood in the presence of 'white' noise than if the interference is coming from another station broadcasting in the same language. This is because, in the case of the interfering station, the 'noise' more closely resembles the signal than do the random background disturbances. The difficulties of separating two signals consisting of the spoken word in the same language may be demonstrated by imagining the definition of the necessary filter. It would certainly be elaborate, depending, for example, on the timbre of the voices or perhaps (with the help of a fast electronic computer) the content of the two messages.

The use of the word 'definition' to mean a functional account in everyday language of the characteristics of a device can form a bridge between the doctor and the engineer, which each can interpret in his own terms. For example, suppose it was necessary to have some reliable indication of each time that the heart pumped blood into the aorta. The phenomenon, the pumping of blood, is not directly detectable in the intact body and the definition of the signal must be based on some aspect of it. Each of these definitions will include 'noise' and the first consideration in the design of a suitable instrument must be the selection by definition of the aspect that gives the best signal-to-noise ratio. Suppose that the problem outlined here was being discussed by interested parties. The blood rushing around the aortic arch causes the whole body to jerk a little, though this aspect of the phenomenon, while directly related to it, is clearly mixed with much 'noise', since the movement of the body due to this cause is very small and is easily confused by incidental movement of the subject. It would, therefore, be rejected as a signal source. Pulsations of an artery would probably also be rejected because of movement of the subject, though this would depend to some extent on the conditions of use and, in some circumstances, anaesthesia, for example, might provide a useful signal.

The heart is a complicated muscle, and each time it beats a small potential difference, the electrocardiogram, may be detected on the surface of the skin. The 'noise' mixed with this wave-form consists of any other potentials of comparable or greater magnitude and, for reliable results, it is not sufficient to rely on detecting this wave-form in its entirety; some aspect of it must be chosen. Discussion would reveal that the R wave is reasonably constant in form and is always present when the heart contracts. The definition of the heart pumping blood into the aorta can therefore be narrowed to only the R wave of the electrocardiogram.

At this point the preliminary discussion is at an end; the doctor knows that the instrument will respond to each R wave, but he also knows that some abortive beats of the heart which pump no blood (extrasystoles) will appear as a beat, since the phenomenon is accompanied by an R wave. He will therefore have to take this aspect of the signal-to-noise ratio into account when interpreting the results. The engineer is left with the problem of re-defining the R wave in electrical terms. This is most difficult, since there is at present very little basic information on the technical nature of the various physiological phenomena. What, for example, is the typical frequency of the R wave? At the moment, it is only possible to make a guess and then test the apparatus embodying the guess on as many subjects as possible. This is a lengthy and tiresome procedure and adds little to the knowledge of the basic phenomenon.

There is at the present time a need for some means of collecting and sorting information of this kind; perhaps an appropriate body might take an interest in the work.

VISCO-ELASTIC PROPERTIES AND THE FRICTION OF SOLIDS

Friction of Polymers: Influence of Speed and Temperature

THE frictional behaviour of polymers as a function of speed has not been systematically studied, and much of the published data are contradictory. In our own laboratory such studies have been limited to very low speeds (10^{-2} cm/sec) where frictional heating is trivial or at very high speeds (10^{4} cm/sec) where frictional heating and its effect on surface layers is dominant. This article describes briefly experiments on the friction of polymers at speeds up to a few 100 cm/sec (20–30 f.p.s.) and at ambient temperatures from − 60° C to 300° C (ref. 1). The polymers range from amorphous and crystalline thermoplastics, where the molecular chains have considerable freedom of motion to highly cross-linked polymers which retain their strength and rigidity up to relatively elevated temperatures.

The friction was measured between a flat rotating disk and a cylindrical pin which was loaded with its axis normal to the plane of the disk. Dead loading was used, and the frictional force was measured with strain gauges attached to suitably mounted spring strips. Heating coils were incorporated so that the ambient temperature could be raised from room temperature to 300° C and the friction chamber could be evacuated to about 10^{-4} mm mercury. The polymer specimens were generally prepared by abrasion under water on silicon carbide papers. Except where otherwise stated, the friction was measured at a pressure of 10^{-4} mm mercury.

Friction of Polytetrafluoroethylene: effect of speed and temperature. Because frictional heating at speeds of a few 100 cm/sec might cause chemical degradation, the first polymer chosen was P.T.F.E., which has high melting and decomposition temperatures and is chemically very stable.

The first marked observation is that the friction rises with increasing speed (Fig. 1). With specimens containing a higher degree of crystallinity the increase is more gradual.

If the rise in friction is due to thermal softening produced by frictional heating, the rise should be observed at lower sliding speeds if the specimen starts off at a higher temperature. Experiments on these lines (see Fig. 1) show that the exact opposite occurs.

Friction and deformation properties. Earlier work[2,3] suggested that the friction of polymers could be expressed mainly in terms of the shear-strength of the material and the area of contact during sliding. Experiments were, therefore, carried out on the shear-strength s and the indentation hardness p as a function of rate of deformation. The ratio s/p did not, however, correlate particularly well with the observed dependence of friction on speed and further investigations on these lines are desirable[4]. It was also considered that energy might be dissipated by the impact of surface asperities, especially during high-speed sliding; but again there was poor correlation, between the observed friction and the impact strength of

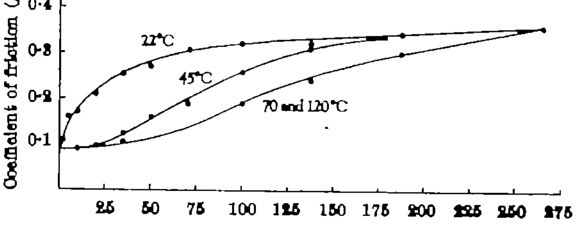

Fig. 1. Variation of friction with speed for P.T.F.E. sliding on P.T.F.E. (40 per cent crystalline). Environment 10^{-4} mm mercury. Load 100 gm. At room temperature the friction rises from about $\mu = 0.07$ to $\mu = 0.3$ when the sliding speed increases to about 100 cm/sec. As the ambient temperature is increased the rise in friction is shifted into the higher speed-range

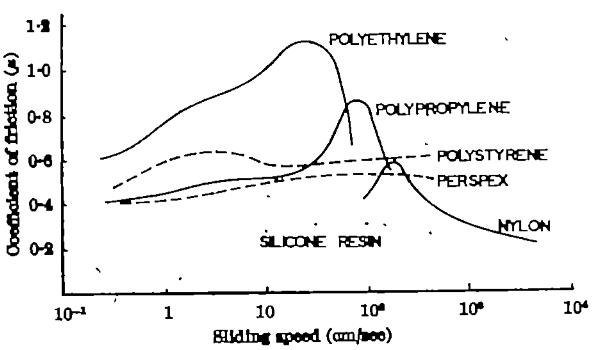

Fig. 2. Variation of friction with speed at room temperature for thermoplastic crystalline polymers (polyethylene, polypropylene, 6,6-nylon), for thermoplastic amorphous polymers (polystyrene, 'Perspex') and for a typical thermo-setting polymer (silicone-resin). With the first group there is a marked rise in friction and a drop when the speed exceeds about 100 cm/sec. The resin shows the least effect of speed on friction

the polymer as a function of temperature and speed of deformation. Finally, experiments were carried out in which a hard steel ball was rolled over the polymers at various speeds. This reproduces approximately the bulk deformation losses occurring during sliding[4]. It was found that under the conditions of our experiments this deformation component constituted a small fraction of the observed sliding friction, a result in close agreement with the work of Flom[5].

Frictional behaviour of other polymers. Similar experiments were carried out on thermoplastic crystalline polymers (polyethylene, polypropylene, 6,6-nylon) on thermoplastic amorphous polymers (polystyrene, polymethyl-methacrylate) and on thermo-setting polymers such as silicone-, polyester- and formaldehyde-resins. Some typical results are shown in Fig. 2. It is seen that with the crystalline polymers there is a marked increase in friction with speed and a drop above about 100 cm/sec. At higher ambient temperatures, not shown in Fig. 2, the curves are again displaced towards higher speeds. With the amorphous polymers the variation of friction with speed (and temperature) is less marked, while with the cross-linked polymers there is practically no change of friction with speed. It is evident that the friction becomes less dependent on speed and temperature as the polymer structure becomes more rigid. This may be seen from Table 1, in which the physical properties of the polymers are compared with their friction–velocity dependence. In general, a high impact-strength and a large elongation-to-fracture are associated with marked flexibility of the polymer chain. It is apparent that polymers with these properties have a large friction–velocity dependence.

Table 1. PHYSICAL PROPERTIES OF SOME POLYMERS AT ROOM TEMPERATURE

Polymer	Character	Impact strength notched Izod (ft. lb.)	Tensile strength lb./in.^{2}	Elongation at tensile break (per cent)	Friction–velocity dependence
I. *Thermoplastic crystalline*	Flexible chains				
P.T.F.E.		4	2,500	50–400	Marked
Polythylene		3	2,500	450	Marked
6,6 Nylon		2·5	11,500	90	Marked
Polypropylene		1	5,000	220	Marked
II *Thermoplastic amorphous*	Some flexibility: 'glassy' at room temperature				
Polymethylmethacylate		0·4	8,000	5	Moderate
Polystyrene		0·2	4,500	1	Moderate
III. *Thermosetting cross-linked*	Rigid structures				
Epoxy resin		0·2	10,000	1·7	Very little
Polyester		0·2	5,000	2	Very little
Melamine		0·3	10,000	0·7	Very little

Mechanism of polymer friction. These results show that under the conditions of our experiments the major part of the friction does not arise from bulk deformation losses, in contrast to results obtained with well-lubricated surfaces[7]. Nor does it appear to arise from impact with surface asperities. The greater part must arise from some type of adhesion mechanism.

Experimentally the most striking observation is that the friction increases with speed, and that if the temperature is raised the increase in friction occurs at a higher sliding speed. The speed effect cannot, therefore, be due primarily to frictional heating. It is useful to plot the friction as a function of speed, using a linear scale for the friction and a logarithmic scale for the speed. Results for P.T.F.E. are given in Fig. 3, the results for speeds greater than 1,000 cm/sec being taken from the work of Bowden and Persson[8]. The friction rises to a maximum and then falls. At higher temperatures the curve is displaced into the region of higher speeds.

These curves closely resemble the dynamic loss characteristics of a polymer as a function of frequency of deformation. A schematic description is inserted in the top left-hand corner of Fig. 3. The damping loss rises to a maximum at a characteristic frequency and then falls as the frequency increases. Raising the temperature shifts the curve into the region of higher frequency.

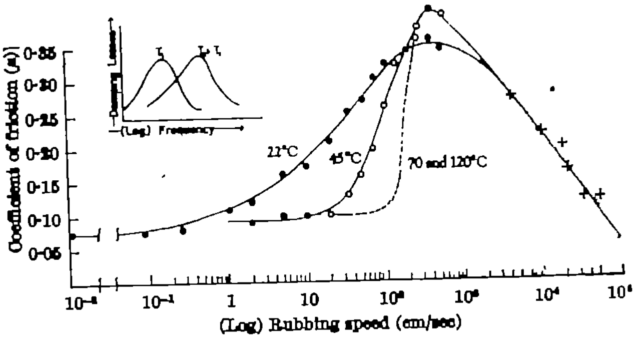

Fig. 3. Friction of P.T.F.E. on P.T.F.E. (40 per cent crystalline) as a function of sliding speed. Speeds greater than 1,000 cm/sec are from Bowden and Persson (ref. 8). The insert shows damping losses as a function of frequency. If the temperature is raised the peak is shifted to a higher frequency

This suggests that the frictional behaviour involves time- and temperature-dependent properties which are closely connected with the visco-elastic properties of the polymer. McCrum[9] has found a marked damping peak for P.T.F.E. at 176° K at a frequency of 1 c/s. By using these data and assuming an activation energy of 18 kcal/gm mol (ref. 9) we may apply an Arrhenius shift to calculate the position of the peak at, say, 22° C. It occurs at a frequency of about 10^9 c/s. In the frictional experiments at this temperature the maximum friction occurs at a speed of about 500 cm/sec. Without specifying a detailed mechanism we may regard the sliding at the interface as resembling the shear process in a visco-elastic medium. Then to obtain a frequency of 10^9 this implies a displacement of about 50×10^{-8} cm, that is, 50 Å. This is of molecular dimensions. Similar results are observed with the other polymers studied, displacements of the order of 10–100 Å being deduced. This therefore indicates an adhesion process on a molecular scale rather than a process involving asperity encounters or sub-surface deformation. This is supported by the observation that the deformation component of friction constitutes only a small part of the observed friction.

There is, of course, considerable uncertainty involved in applying the damping-loss characteristics to explain the frictional behaviour. This arises primarily from the effect of frictional heating. The damping-loss characteristics describe the properties of the material at well-defined temperatures. In the friction experiments the frictional process itself produces surface heating so that the exact temperature of the material at the sliding interface is not known with any precision. (The same uncertainty applies to the results of Bulgin, Hubbard and Walters[10] in their work on the friction of rubber.) For speeds less than, say, 100 cm/sec the temperature rise is probably of the order of tens of degrees C. At higher speeds it can be very much greater. For example, the very low friction shown on the right-hand portion of Fig. 3 obtained at speeds of 10⁴ cm/sec is almost certainly due to surface melting[8]; sliding occurs on a thin molten surface layer of relatively low viscosity. However, many of the polymers show a rise and fall in friction within a speed range of 100 cm/sec (Fig. 2). Under these conditions surface melting cannot be an important factor.

The most striking confirmation for this connexion between the frictional behaviour and the visco-elastic properties of the sliding materials is provided by the experiments of Grosch on rubber (following communication). He carried out friction measurements over a wide range of temperatures and sliding speeds, but the sliding speeds never exceeded 3 cm/sec, so that frictional heating was always trivial. His results show that the whole of the frictional behaviour can be described by a single 'master-curve' if the data are subjected to the Williams, Landel, Ferry[11] transform. This transform applies to the damping properties of polymers above their glass transition temperature. The new point brought out in the present work is that a similar correlation is probably valid for polymers below the glass-transition temperature. Here, of course, the transform involves an Arrhenius shift rather than the Williams, Landel, Ferry transform.

In a qualitative way this broad correlation is supported by the comparative behaviour of polymers of different types, the main factor being the flexibility of the polymer chains. Linear crystalline polymers which have the greatest impact strength and the largest elongation-to-fracture in tension are polymers with the greatest chain-flexibility. They show the most marked dependence of friction on speed. Linear amorphous polymers are less flexible and exhibit a smaller variation of friction with speed. (However, they show a marked increase in friction when they reach the glass–rubber transition temperature.) Finally, cross-linked thermo-setting polymers which have the most rigid structures and are relatively brittle show the smallest dependence of friction on speed.

Earlier work showed that with polymeric materials the deformation component of friction is a function of the visco-elastic or hysteresial properties of the polymer. The results described here show (in agreement with the work of Grosch (following communication) and of Bulgin, Hubbard and Waters[10]) that the adhesion component involves speed and temperature-dependent properties which also reflect the visco-elastic properties of the sliding materials.

We thank Dr. F. P. Bowden for his continued encouragement, Messrs. Small and Parkes for a grant to the Laboratory, and especially to Dr. A. Schallamach and Mr. K. A. Grosch for the free discussion of their frictional work on rubber prior to publication.

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Relation between the Friction and Visco-elastic Properties of Rubber

IT has recently become increasingly apparent that friction of rubber on hard surfaces is due to two causes: (1) the adhesion of rubber to the surface; (2) to deformation occurring in the rubber when it slides on rough surfaces. Schallamach[1] and Bartenev[2] showed that the adhesional friction could be considered as an activated rate process with activation energies similar in magnitude to those associated with the visco-elastic behaviour of the rubber, and Tabor and others explained lubricated and rolling friction of rubber in terms of deformation losses[3-5].

Experiments have now been carried out, sliding several rubbers of widely differing visco-elastic properties on smooth and rough surfaces over a wide range of temperatures and sliding velocities. The highest value of the sliding velocity was kept below 3 cm/sec so that rises in temperature in the interface were small and could be neglected.

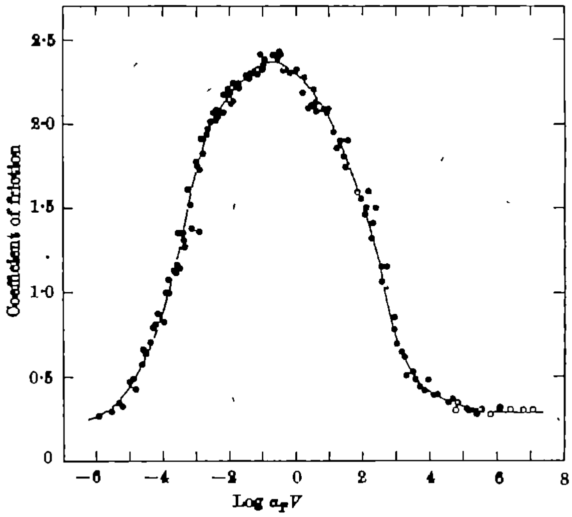

Fig. 1. Composite master curve for the coefficient of friction of an acrylonitrile–butadiene gum vulcanizate sliding on glass; reference temperature: 20° C

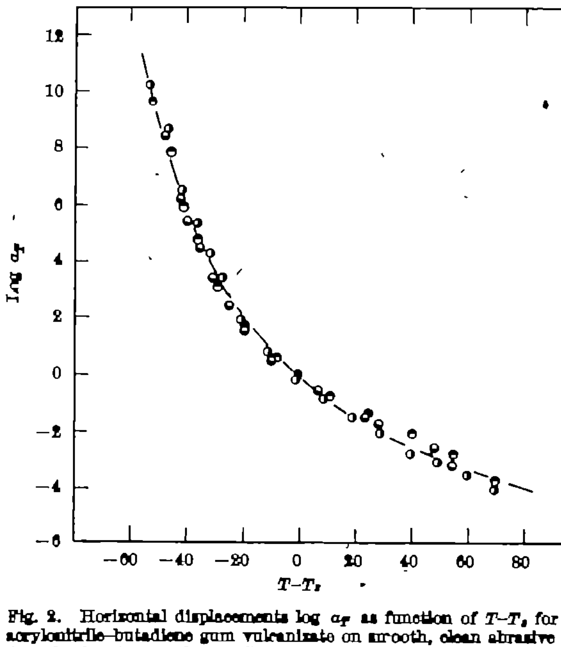

Fig. 2. Horizontal displacements log a_T as function of $T-T_s$ for the acrylonitrile–butadiene gum vulcanizate on smooth, clean abrasive and dusted abrasive surfaces. Solid line represents W.L.F. equation, $T_s = +32°$ C

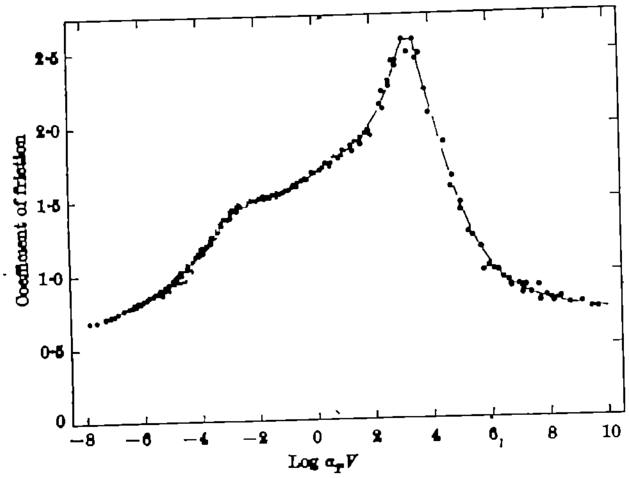

Fig. 3. Composite master curve for the coefficient of friction of an acrylonitrile–butadiene gum vulcanizate sliding on clean silicon carbide paper; reference temperature: 20° C

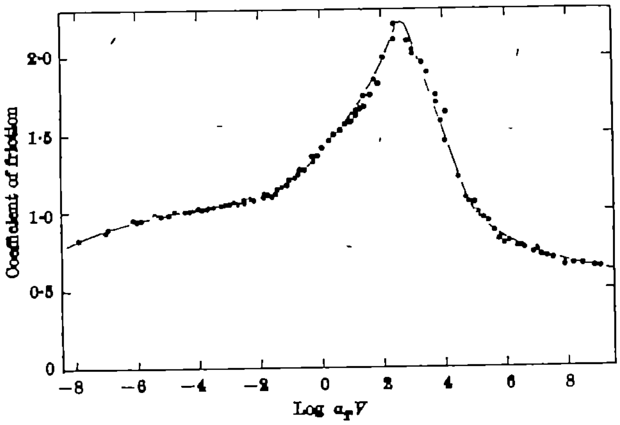

Fig. 4. Composite master curve for the coefficient of friction of an acrylonitrile–butadiene gum vulcanizate sliding on a silicon carbide abrasive, dusted with magnesium oxide powder; reference temperature: 20° C

It is found that curves giving the coefficient of friction as a function of the sliding velocity at different temperatures are segments of a single 'master curve' which describes the coefficient of friction over an extended range of sliding velocities at a selected reference temperature T_0. The master curve can be constructed from the individual segments by displacements parallel to the logarithmic velocity axis until they superpose with the curve at the selected reference temperature. An example of such a master curve is shown in Fig. 1 for an acrylonitrile-butadiene rubber vulcanizate sliding on glass. Similar curves have been obtained with other non-crystallizing rubbers on smooth and rough surfaces. This close connexion between rate and temperature dependence is common to the visco-elastic properties of a large number of polymers[6–8]. Williams, Landel and Ferry (W.L.F.)[9] have shown that the horizontal displacements necessary to construct the master curve can be expressed by a single relation, the W.L.F. equation, independent of the material and the type of visco-elastic property in question if a standard reference temperature T_s is chosen instead of the arbitrary reference temperature T_0. As Fig. 2 shows, friction conforms very closely to this general visco-elastic behaviour, the displacement functions being in excellent agreement with the W.L.F. equation. The standard reference temperatures are material constants simply related to their glass transition temperatures T_g, that is, $T_s \simeq T_g + 50$, and here, too, there is close agreement between T_s values derived from friction experiments and this requirement. The friction of a rubber on a particular surface is therefore described completely by its master curve and the glass transition temperature of the rubber.

Master curves obtained on a smooth surface are similar for all rubbers in that they are almost symmetrical around a single maximum. If direct contact between rubber and glass is prevented by dusting the surface with a fine powder (magnesium oxide was used in the present case), the friction remains almost constant over the whole range of velocities and temperatures, demonstrating that friction on a smooth track is due only to interfacial adhesion between rubber and surface. The velocity of maximum friction, V_s, increases as the glass transition temperature of the rubber decreases. This can be related quantitatively to the frequency $f_{E''}$ at which the loss modulus is a maximum if compared at the same temperature, thus:

$$\frac{V_s}{f_{E''}} = \lambda = 6 \times 10^{-7} \text{ cm}$$

irrespective of the rubber. As the loss modulus reflects the relaxation spectrum of the rubber[10] maximum adhesional friction of rubber appears to be linked to the relaxation time τ_m for which the spectrum is a maximum:

$$V_s \tau_m = \lambda$$

Friction curves on a clean rough surface, such as an abrasive paper, are always asymmetrical and the maximum usually occurs at a higher velocity than on the smooth surface, with a distinct hump appearing on the curve near the velocity for which the friction on the smooth surface is a maximum (Fig. 3). As the rubbers investigated had only a single relaxation peak, the maximum and the hump on the friction curve must be associated with the two friction mechanisms. When magnesium oxide powder is applied to the rough surface, the hump disappears while the maximum remains almost unaltered in magnitude and position as shown in Fig. 4. The maximum on the dusted rough surface appears therefore to be due to deformation losses and a correlation of the frictional behaviour with the visco-elastic properties of the rubber shows that the velocity of maximum friction on the rough track V_R bears a constant relation to the frequency f_T for which the loss factor has its maximum, both being compared at the same temperature, that is:

$$\frac{V_R}{f_T} = \alpha = 1{\cdot}5 \times 10^{-2} \text{ cm}$$

The distance α agrees closely with the spacings of the abrasive particles on the track surface used.

These experiments thus demonstrate clearly the existence of the two friction mechanisms, that is, adhesion occurring along the slopes of the asperities and cyclic deformation occurring in the rubber as it passes over them, both being visco-elastic in nature.

I thank Drs. D. Tabor and A. Schallamach for their interest.

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NATURE OF ORIENTATIONAL DEFECTS IN ICE

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Each oxygen atom in ice is surrounded tetrahedrally by four others at a distance of 2·76 Å (ref. 1). Bernal and Fowler[2] proposed that water molecules are preserved in the crystal and that there is one hydrogen atom between every pair of oxygen atoms, which are thus linked by hydrogen bonds. According to Pauling[3], all configurations compatible with these conditions occur with equal probability, giving a disordered structure which does not become ordered on cooling. This description of the hydrogen atom arrangement can be regarded as confirmed by the more recent results obtained by neutron diffraction[4].

In order to account for the electrical properties of ice[5], the existence of two kinds of defects, ionic and orientational, has been postulated by Bjerrum[6]. In ionic defects, protons are supposed to have migrated from one oxygen atom to another, thus giving rise to an equal number of H_3O^+ cations and HO^- anions. In orientational or valence defects (Fig. 1), water molecules are supposed to have rotated through 120° about an O—H . . . O axis. Such a rotation generates a vacant O . . . O separation (L-defect) and a doubly occupied O—H . . . H—O one (D-defect). A subsequent or coupled rotation of the adjacent water molecule into the vacant site has the effect of separating the D- and L-defects, which are then supposed to migrate through the crystal, thus providing a mechanism for the rotation of water molecules.

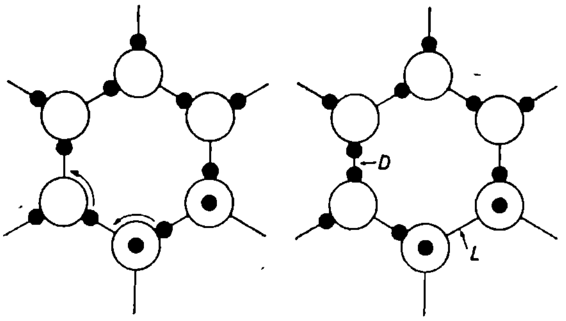

Fig. 1. Formation of a DL-pair of orientational defects in ice according to Bjerrum

The main objective of this article is to direct attention to the unlikelihood that D-defects, as already defined, can exist. Another type of defect, also complementary to the L-defect, will be proposed as an alternative to the D-defect. Finally, it will be shown that rotation of water molecules is more likely to occur by diffusion of L-defects than of the complementary type of defects. The discussion will be based on the assumption that the structures of the defects can be related to their energies; but, to anticipate certain quite legitimate criticisms, it must be emphasized that the present state of theory permits this to be done only in a rather crude fashion. We shall frequently have to be content with rough order of magnitude estimates, little better than guesses, of the energy quantities involved.

The formation of a pair of D- and L-defects corresponds to the reaction 2 $N \rightarrow$ D + L, where N represents a hydrogen bond. If E_N is the energy associated with the formation of a hydrogen bond from separated water molecules and E_D and E_L the energies associated with the formation of D- and L-defects respectively, also from separated water molecules, then the energy of the reaction is:

$$E_{DL} = E_D + E_L - 2E_N$$

The experimental value[7] of E_{DL} is 15·7 kcal mole^{-1} (0·68 ± 0·04 eV), that of $-E_N$ is between 3 and 7 kcal mole^{-1} (ref. 8). Experimental values of E_D and E_L separately are not available.

Bjerrum estimated the energy of a fault site on the basis of an electrostatic point charge model in which water molecules are represented as regular tetrahedra of radius 0·99 Å with charges of ± 0·171 e (e being the electronic charge) at the corners. According to this model, $E_D = E_L$, since both types of defect are regarded as identical apart from reversal of all charges. Bjerrum obtained values of 6–7 kcal mole^{-1} for E_D, E_L and $-E_N$, corresponding to about 24 kcal mole^{-1} for E_{DL}. The agreement with the experimental values of E_N and E_{DL} is satisfactory but, nevertheless, the values for E_D and E_L are easily shown to be unreasonable.

The energy of a L-defect is almost certainly much lower than that of a D-defect. The L-defect consists of two water molecules separated by about 2·76 Å and not linked by a hydrogen bond. Hence $E_L - E_N \sim 3–7$ kca lmole^{-1}, the energy required to break a hydrogen bond, and $E_L \sim 0$. It may be remembered that the van der Waals radius of oxygen and the crystal radius of O^{-2} are both given[9] as 1·40 Å and that many crystal structures are known in which oxide ions are separated by distances of 2·5–2·8 Å.

On the other hand, Bjerrum's estimate for the energy of a D-defect seems much too low. Since the oxygen–oxygen distance in ice is 2·76 Å and the O—H distance is 0·96 Å in water[10] and probably close to this value in ice[11], Bjerrum's model of "two protons placed between two adjacent oxygen atoms" implies that the two hydrogen atoms in the doubly occupied bond would only be about 0·8 Å apart. At this distance they would certainly exert a very strong repulsion on one another. No structure in which two hydrogen atoms are separated by such a short distance is known. The shortest reported distances between hydrogen atoms not bonded to a common atom occur in derivatives of *cyclo*nonane[12] and *cyclo*decane[13] and lie in the range 1·8–2·0 Å. It is probable that the strain energies of these *cyclo*alkanes, amounting to some 12 kcal mole^{-1} (ref. 14), are derived largely from the occurrence of such short hydrogen–hydrogen distances which can be regarded as already corresponding to repulsion energies of the order of a kcal mole^{-1} or so. The hydrogen–hydrogen distance in the supposed D-defect would thus be less than the smallest reported distances by a factor of about 2·25, corresponding to a tremendous increase in the mutual repulsion energy for any reasonable assumption for the form of the potential energy curve in this region.

Abandoning the point-charge model, we can most simply regard a D-defect as a pair of water molecules oriented so that two of the O—H bonds point directly at one another. This arrangement is not conducive to the formation of a hydrogen bond, and the energy of the system, E_D, is predominantly the interaction energy of two non-bonded hydrogen atoms separated by about 0·8 Å.

A number of different potential energy functions for the interaction of two non-bonded hydrogen atoms have been proposed by several authors and used to estimate hydrogen–hydrogen interaction energies in organic molecules. Three such functions are:

$$E(r) = 10{,}000\exp(-4{\cdot}6\,r) - 49{\cdot}2/r^6 \text{ (ref. 15)} \qquad (1)$$

$$E(r) = 6{,}600\exp(-4{\cdot}08\,r) - 49{\cdot}2/r^6 \text{ (ref. 16)} \qquad (2)$$

$$E(r) = (166/r + 366 + 23{\cdot}1\,r + 580\,r^2 + 1{,}828\,r^3)\exp(-4{\cdot}35\,r) \text{ (ref. 17)} \qquad (3)$$

where E is expressed in kcal mole^{-1} and r in Å. The first two functions are derived semi-empiricially from data pertaining to large values of r and are evidently quite incorrect for small r values when the negative attraction term in the formulæ dominates over the positive repulsion one. This can be corrected for by inclusion of an additional repulsive term, say, $1{\cdot}0 \times r^{-12}$. The third function is based on a theoretical approximation[18] to the triplet state energy of the hydrogen molecule; it is probably the most reliable for r values of less than about 1 Å. For r = 0·8 Å all three functions lead to an interaction energy of about 60 kcal mole^{-1}, a value that is much higher than Bjerrum's estimate for E_D and quite incompatible with the experimental value for E_{DL}.

Apart from this discrepancy, there is another unsatisfactory feature of the D-defect proposed by Bjerrum. Since repulsion between the two non-bonded hydrogen atoms is the dominating force, any movement of these hydrogen atoms from the oxygen–oxygen line must lower the energy of the system. The situation represented in Fig. 1, whatever its energy may be, must correspond to an energy maximum with respect to rotation of one or both water molecules, and although it might conceivably correspond to a transition state for some reaction it cannot correspond to a stable arrangement of water molecules.

That the collinear O—H . . . H—O situation corresponds to an energy maximum has recently been recognized by Cohan, Cotti, Iribarne and Weissmann[19], who used a more elaborate point charge model based on certain quantum mechanical approximations to estimate the various energy quantities involved. These authors give a value of 50 kcal mole^{-1} (2·12 eV) for E_{DL}, assuming the D-defect to be collinear. As the two water molecules involved in the D-defect are rotated as rigid bodies out of their head-on position, E_{DL} decreases, reaching a minimum of about 25 kcal mole^{-1} (1·06 eV at a rotation angle of 20°–25°), when allowance is made for the concomitant variation in the energies of the six surrounding hydrogen bonds. A 22·5° rotation of both water molecules increases the hydrogen–hydrogen distance to about 1·3 Å, corresponding to an interaction energy of some 15–23 kcal mole^{-1} according to the potential functions (1)–(3). Thus the foregoing estimate of 25 kcal mole^{-1} for E_{DL} seems fairly reasonable. These authors consider the model with both water molecules rotated out of the head-on position to be a satisfactory representation of a D-defect, but they did not, apparently, examine other possibilities. In particular, the variation in the energy for rotation angles of more than 30° was not examined, nor the effect of rotating only one molecule of the pair.

These considerations have shown that the Bjerrum model of the D-defect, at least in its collinear form, corresponds to a too high energy. The question then arises: Are there other, energetically more reasonable models? Examination of the structure of ice shows that there is indeed such a model, which is illustrated in Fig. 2 and described here.

If a water molecule in the normal ice structure is rotated about an O—H . . . O axis by 60° instead of by 120°, its second hydrogen atom is brought into a position midway between an O . . . H—O and an empty O . . . O (L-defect) direction. This situation is clearly unstable, for there will be an uncompensated force tending to restore the original situation in which all O—H bonds pointed along oxygen–oxygen directions. However, a subsequent or coupled rotation of the adjacent water molecule through 120° to bring one of its hydrogen atoms into the empty O . . . O direction is all that is required to make a stable arrangement.

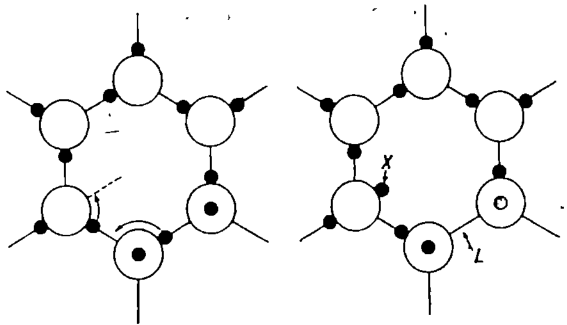

Fig. 2. Formation of an XL-pair of orientational defects in ice

The 'extra' hydrogen atom is now symmetrically situated between two neighbouring O . . . H—O bonds (to prevent confusion with the Bjerrum D-defect we shall refer to this type of situation as an X-defect) and is separated from the L-defect, which cannot spontaneously diffuse back. Once the defect pair has been formed and separated, both kinds of defects can diffuse through the crystal by jumps that involve rotation of water molecules. Each jump of the X-defect involves a coupled rotation of two adjacent water molecules, each through 60°; each jump of the L-defect involves rotation of one water molecule through 120°. In the Bjerrum model, each jump of either kind of defect involves rotation of one molecule through 120°. This appear to be the only formal difference between the two types of mechanism and the only question is: which is the more reasonable from an energetic point of view?

As in the case of the Bjerrum model, an exact calculation of the energy of an X-defect is out of the question, but again a crude estimate can be made by considering the non-bonded interactions. The extra hydrogen atom associated with an X-defect is about 1·5 Å from each of its two closest hydrogen neighbours on other molecules, assuming best possible hydrogen bonds and tetrahedral HOH angles. This distance is much longer than the hydrogen–hydrogen distance in the collinear D-defect (0·8 Å) and appreciably longer than in the D-defect with 22·5° rotation of both water molecules from collinearity (1·3 Å). But it is still too short, compared with the shortest known non-bonded hydrogen–hydrogen distance of 1·8–2·0 Å. It may, however, be easily increased to about 1·7 Å by appropriate 20° deviations of the three O—H bonds involved from their ideal directions. Such deviations can be achieved by rotating the water molecules as rigid bodies, thus weakening the local hydrogen bonding system, or by distortion of the HOH angles from the tetrahedral value. Both modes are relatively cheap in energy for small deformations and the energy increment for these 20° deviations, shared between both modes, is not expected to exceed a few kcal mole^{-1}. The interaction energy of two non-bonded hydrogen atoms separated by 1·7 Å is estimated to be 2·0, 4·4 and 6·9 kcal mole^{-1} from functions (1), (2) and (3) respectively. Equation (3) is probably the least reliable in this region. A value of about 10 kcal mole^{-1} for the energy of two such interactions is thus at least of the correct order of magnitude. We then have $E_X \sim 10$, $E_L \sim 0$ and $-E_N \sim 5$ kcal mole^{-1} and the experimental value of 16 kcal mole^{-1} for E_{XL} seems quite in keeping with these estimates. The X-defect is therefore regarded as more reasonable, on energetic grounds, than the D-defect as proposed by Bjerrum.

It remains now to consider the activation energy for diffusion of such defects. The experimental value has been given[5] as 5·4 kcal mole^{-1} (0·235 eV). Now, each jump of an L-defect involves a rotation of a single water molecule through 120°. The half-way position, with the rotating O—H bond at an angle of 60° to both O . . . O directions, is probably close to the transition state. Since no short non-bonded interactions are present in this position, the activation energy cannot be more than the energy of the hydrogen bond that is broken in the process, and it is unlikely to be much less. Thus a value of about 5 kcal mole^{-1} seems reasonable as the activation energy for diffusion of an L-defect.

Diffusion of an X-defect involves rotation of two molecules, each through 60°. The activation energy for this process is more difficult to estimate but the transition state is probably rather similar to that proposed by Cohan, Cotti, Iribarne and Weissmann[19] for the D-defect, but with both molecules rotated out of the head-on position by about 30°. From their results, this state appears to be stabilized by about 25 kcal mole^{-1} (the potential minimum at 20°–25° is quite shallow) with respect to the collinear situation. If this and our previous energy estimates are roughly correct, then the activation energy is 15–25 kcal mole^{-1}, that is, appreciably larger than for diffusion of an L-defect. It seems reasonable that the activation energy for diffusion of an X-defect should be larger than for an L-defect. The forces holding the X-defect in its equilibrium position depend on hydrogen–hydrogen repulsions, deformations of HOH angles and rotations of water molecules from preferred hydrogen bond directions. At the equilibrium position, these forces balance out but all three components, taken separately, are strained so that any movement of the system from equilibrium must be associated with a fairly steep increase in the potential energy. On the other hand, the force holding the L-defect in equilibrium depends on only one of these factors, namely, the dependence of the hydrogen bond energy on the O—H . . . O angle.

These considerations suggest that the a.c. conductivity of ice arises almost exclusively from molecular rotations associated with diffusion of L-defects. In pure ice, the active L-defects can only be formed together with an equal number of inactive X-defects, but if the latter have only a minor influence on the electrical properties, this should be apparent from measurements on ice doped with small amounts of ammonia and hydrofluoric acid. Addition of HF molecules should increase the number of active sites and decrease the number of inactive ones, whereas addition of NH_3 molecules should have just the opposite effect.

I thank Prof. H. Gränicher for several useful discussions.

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SYNTHESIS OF ORGANIC COMPOUNDS BY ELECTRIC DISCHARGES

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THE use of electric discharges for the synthesis of organic compounds under possible primitive Earth conditions was initiated by Miller[1–4]. Miller obtained some amino-acids, hydroxy acids and a few other organic compounds by the prolonged action of spark discharges on mixtures consisting essentially of methane, ammonia and water. These results have been confirmed by other investigators under identical or slightly different conditions[5–10].

Further investigations on this synthesis were carried out in this laboratory with the hope of obtaining additional amino-acids and perhaps also some ultra-violet-absorbing compounds.

In order to increase the probability of formation of new compounds, ethane was also included in the reaction mixtures. The use of ethane in these experiments is justifiable since the C_2 species has been detected spectroscopically in star atmospheres, comets and interstellar space[11,12]. In order to determine the over-all pattern of the compounds formed, ^{14}C-hydrocarbons, mainly ^{14}C-methane, were used as tracers. Furthermore, it was thought that the patterns obtained by autoradiography of two-dimensional chromatograms of the products may help in establishing a correlation between these and other experiments[13–15].

The results obtained show that an appreciable number of organic compounds are formed by the action of electric discharges on the foregoing mixtures. The products obtained include glycine, alanine, aspartic acid, asparagine, isoleucine (or isoasparagine), glycinamide and possibly proline; a yellowish oil, a highly insoluble polymer, and several ultra-violet-absorbing compounds. The formation of other products which give purple, green and brown derivatives with ninhydrin, suggesting the presence of amines, amino-nitriles, amino-amides and peptides, has also been observed. The ^{14}C-patterns produced by autoradiography of two-dimensional chromatograms of the products are reasonably simple. They are not much more complex than the patterns produced by spraying the same chromatograms with ninhydrin.

Conditions of the reaction. Fig. 1 shows the design of the apparatus used, which consists of a 3-l. flask provided with removable tungsten electrodes, and with inlets and outlets for reactants and products. Temperature and pressure of the system were measured by means of a thermometer and a vacuum gauge, respectively. The liquid phase was agitated by means of a magnetic stirrer. The spark gap of the electrodes was usually 1·5 cm; but in later experiments it was increased to 3 cm. The current was supplied by a transformer (Thordarson Electric Manufacturing Co.: V.P. 110, Sec. 25,000, V × A 1,000, cycle 50–60) from an X-ray apparatus connected to a 110-V line through a 'Variac' which was used to regulate the voltage. The discharge voltage was approximately 10,000 V when the spark gap was 1·5 cm and 15,000 V when the electrodes were separated 3 cm.

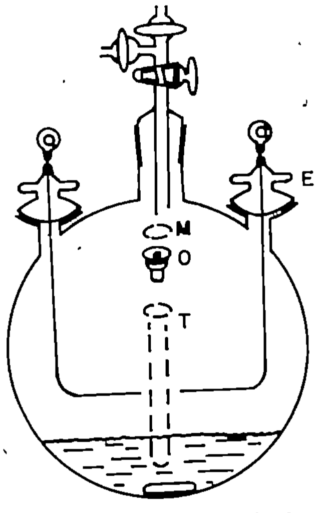

Fig. 1. Diagram of apparatus for the synthesis of organic compounds in an electric discharge. *E*, removable electrodes; *M*, manometer connexion; *O*, gas-sampling orifice; *T*, thermometer well. Dotted lines are used to represent parts on the back of the reaction vessel

After complete evacuation of the apparatus, 300 ml. of boiled distilled water were admitted into it. Then, methane, ethane and concentrated ammonium hydroxide (15 M) were allowed into the system. Pressure readings were obtained after the introduction of each component. Whenever ^{14}C-hydrocarbons (1 mc. or less) were used, they were admitted into the evacuated apparatus before anything else (water, non-radioactive hydrocarbons and ammonium hydroxide). All the reactants used were C.P. reagents. Care was exercised to prevent any infiltration of air into the system. Apart from this, no special treatment was followed to eliminate traces of air possibly present in the reagents. Table 1 gives the results for each experiment.

Table 1. COMPOSITION OF GAS MIXTURES AND OTHER DATA OF THE EXPERIMENTS WITH ELECTRIC DISCHARGES

	Exp. No.						
	S–1	*S*–2	*S*–3	*S*–4	*S*–5	*S*–6	*S*–7
CH_4 pressure (cm Hg)	12·7	7·6	10·1	10·1	7·6	10·6	22·8
$^{14}CH_4$ activity (µc.)	—	—	—	—	960	660	—
C_2H_6 pressure (cm Hg)	12·7	7·6	10·1	10·6	7·6	(31·2*)	—
H_2O (ml. liquid)	310	300	346	346	300	346	392
NH_4OH (moles/l.)	0·16	1·1	2·0	2·0	2·2	2·0	3·8
Average temperature (°C)	30	30	30	28	28	28	28
Duration of discharge (h)	1	6	5	5	7	5	5
Spark gap (cm)	1·5	1·5	1·5	1·5	1·5–3	3	3
Weight of residue (mg)	7	40	77	73	50	78	130
Activity of residue (µc.)					36	22	
Activity of distillate (µc.)					260	100	
Conversion of ^{14}C-methane to non-volatile products (per cent)					3·7	3·0	
Conversion of ^{14}C-methane to volatile water-soluble products (per cent)					28	15	

*Residual gaseous products from *S*–5, containing probably a large amount of hydrogen.

Course of the reaction. The reaction time was only 7 h or less, instead of the usual one week. This was done, in part, to make possible the detection of some of the presumed amino-acid intermediates such as amino-nitriles and amino-amides, and in part, to have reaction times comparable with those of similar experiments carried out with high-energy electrons[13–15].

The course of the reaction in the gas phase was followed by measuring the increase with time of the pressure of the system (Fig. 2). It can be seen that under the conditions used, the increase in pressure levels off after 7 h or less, depending on the concentration of the reactants. The temperature oscillation between the start and end of the experiment was only 2° or 3°, usually from 27° to 30° C, and the pressure values were not corrected for the resulting small variations in pressure.

By comparison of different experiments it can be seen that the curves are quite reproducible, and their shape is mainly determined, as might have been expected, by the initial reactant concentration in the gas phase and by the energy put into the system (spark gap). Analyses of the gas phase were not made, and therefore the nature of the gases responsible for the increased pressure was not determined. However, on the basis of previous results and knowing that dehydrogenation takes place with electric discharges, it is considered that hydrogen was one of the main gases formed. Thus, the levelling off would simply indicate that equilibrium of the dehydrogenation reaction in the gas phase at a given discharge voltage had been reached. By increasing this voltage the equilibrium would be displaced to higher hydrogen pressures. Such a change of equilibrium can be seen in curve *S*–5 of Fig. 2.

The curves of Fig. 2 should not only give an indication of the formation of hydrogen but also of the resulting dehydrogenation products, that is, alkenes, alkynes, aldehydes, hydrogen cyanide, nitriles, hydrazine, etc., which are supposed to be precursors of organic and biochemical compounds. These curves, however, do not give any information on the processes which go on in the liquid phase. It is obvious that condensations, and mainly hydrolytic and ammonolytic reactions, will continue in the liquid phase even after the gas phase appears to have reached equilibrium.

Analysis of products. In general, the analysis was undertaken as soon as the electric discharge was discontinued. The gas phase was not analysed. The total volume of the liquid phase (about 350 ml.) was filtered and then concentrated in a vacuum rotating evaporator at 40°–45° C. When the volume had been reduced to less than 20 ml. it was transferred to a tared 50-ml. flask and the evaporation was continued to dryness. The distillate was collected in a very large trap immersed in liquid nitrogen. The distillates were set aside. The flasks with the dry residues were first weighed (Table 1) and the residues were then dissolved in 5 ml. 0·1 N hydrochloric

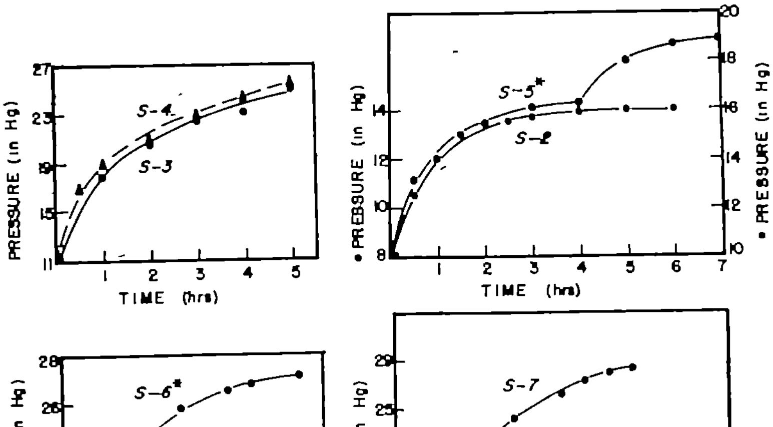

Fig. 2. Course of the reaction in the gas phase of the experiments with electric discharges The increase in pressure is supposed to be due mainly to the hydrogen formed by dehydrogenation reactions. The experiments marked with an asterisk were carried out with ^{14}C-reactants. At the fourth hour of experiment *S*–5, the spark gap was increased from 1·5 to 3 cm.

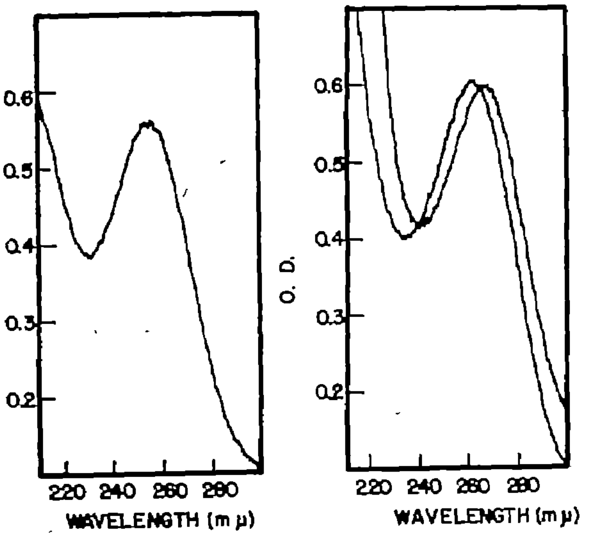

Fig. 3. Ultra-violet spectra of two compounds eluted from two-dimensional paper chromatograms of experiment *S*–3. The spectrum of *A* (left) was obtained in 1 *N* hydrochloric acid and the spectra of *B* (right) in 0·1 *N* hydrochloric acid and in 0·12 *N* sodium hydroxide

acid. The solutions thus obtained were used in the subsequent analyses which were carried out by two-dimensional paper chromatography, ion exchange chromatography, autoradiography and spectrophotometry.

Ultra-violet-absorbing compounds. The solutions from the first three experiments, after appropriate dilution with 0·1 N hydrochloric acid, were directly examined for ultra-violet-absorbing compounds. They gave somewhat similar spectra. An aliquot of *S*-1 diluted 25 times showed a sharp absorption band at about 236 mμ (O.D. ≃ 0·85). An aliquot of *S*-2 diluted 100 times and also an aliquot of *S*-3 diluted 200 times, showed a shoulder at about 236 mμ (O.D. ≃ 0·6) and a broad absorption band between 250 and 300 mμ.

Aliquots of the same solutions were then analysed by two-dimensional chromatography by the ascending technique, using Whatman No. 1 paper and *n*-propanol/1 N ammonium hydroxide, 3 : 1 (PA) and *n*-butanol/acetic acid/water, 4 : 1 : 1 (BAW) as solvents. The dry chromatograms were scanned with a short wave-length (2537 Å) ultra-violet-light lamp. Two major ultra-violet-absorbing compounds were detected in the three chromatograms. One of these two compounds (*A*) had R_F 0·80 in PA and 0·40 in BAW. The other (*B*) had R_F 0·42 in PA and 0·17 in BAW. By the intensity of the spots the formation of both these compounds followed in this quantitative order in the three experiments: *S*-3 > *S*-2 > *S*-1.

The areas corresponding to *A* and *B* from experiment *S*-3 were cut from the paper and the compounds were eluted with hydrochloric acid. Their ultra-violet spectra are shown in Fig. 3. As can be seen, compound *A* shows a maximum at 257 mμ and a minimum at 232 mμ. For comparison, isocytosine shows a maximum at 257 mμ and a minimum at 235 mμ at *p*H 1. Compound *B* shows a maximum at 262 mμ and minimum at 234 mμ in 0·1 N hydrochloric acid. This maximum shifts to 267 mμ in 0·12 N sodium hydroxide. For comparison, under the same conditions, adenine shifts from 262 to 269 mμ and purine from 261 to 273 mμ.

Although a certain similarity of compounds *A* and *B* with purines and pyrimidines is apparent, no definite statements in this respect can be made until these compounds are isolated and characterized more fully. The formation of ultra-violet-absorbing compounds in experiments with electrical discharges has also been observed recently by Knight[16].

Amino-acids and other ninhydrin-positive compounds. The reaction product was next analysed for amino-acids by paper chromatography and by ion-exchange chromatography. Fig. 4 shows the reproduction of two-dimensional chromatograms (Whatman No. 3 *MM*, ascending) of *S*-1, *S*-2 and *S*-3 after spraying with ninhydrin. There are three chromatograms for each reaction product. The first corresponds to the reaction product before ion-exchange treatment, the second to the water eluate (W) from 'Dowex 2', and the third to the acid eluate (A) from the same resin. Several ninhydrin-positive compounds, which are likely amino-acids, peptides or other derivatives of amino-acids can be observed in the chromatograms of the acid eluates. At least 8 spots were observed in *S*-1-A, 16 in *S*-2-A and 20 in *S*-3-A. The ninhydrin-positive compounds of the water eluates are presumed to be amines, amino-amides and amino-nitriles. Some of the compounds in the acid or water eluates gave a green (G) or a yellow (Y) coloration with ninhydrin. Other compounds gave first a tan or yellow-brown coloration which eventually changed into purple. Some of the amides of amino-acids are known to give the foregoing colours with ninhydrin[17].

The amino-acids present in the acid eluate from experiment *S*-3 were identified by ion exchange chromatography using a Beckman–Spinco automatic analyser. (These analyses were carried out in Dr. F. H. Carpenter's laboratory, University of California, Berkeley.) The results are given in Fig. 5. The amino-acids which were identified by their positions in the eluate from a long column include aspartic acid, asparagine, glycine, alanine and isoleucine (or isoasparagine). Traces of other amino-acids can also be observed. One of these corresponds to proline by its position in the eluate and the colour given with ninhydrin.

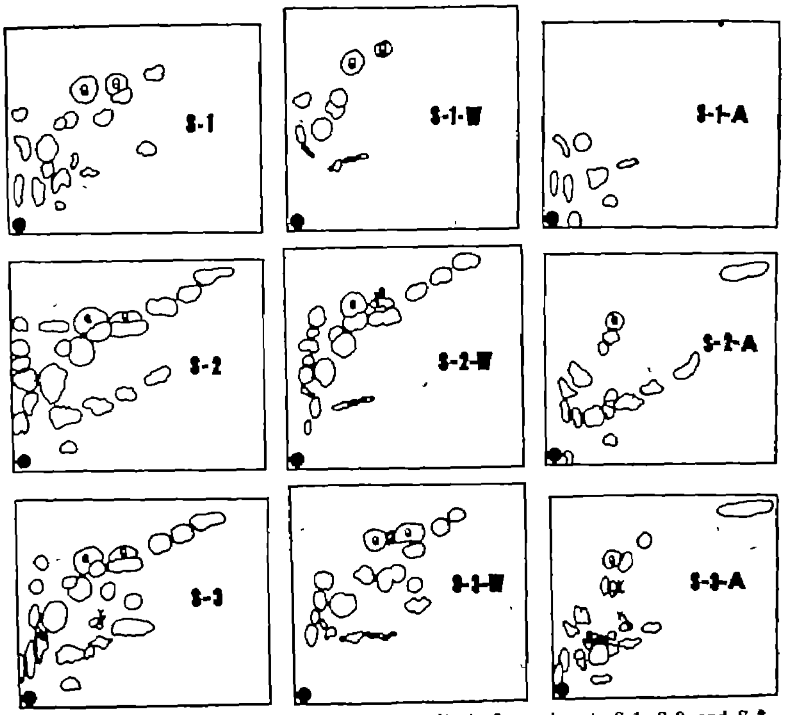

Fig. 4. Two-dimensional chromatograms (ascending) of experiments *S*–1, *S*–2, and *S*–3 sprayed with ninhydrin. The dark spot represents the origin. The development was first in *n*-propanol/1 N ammonium hydroxide, 3.1 (ordinate) and then in *n*-butanol/acetic acid/water, 4 1.1 (abscissa). The unlettered spots were purple or brown-purple in colour. To make chromatograms less complex the ultra-violet-absorbing spots are not shown here

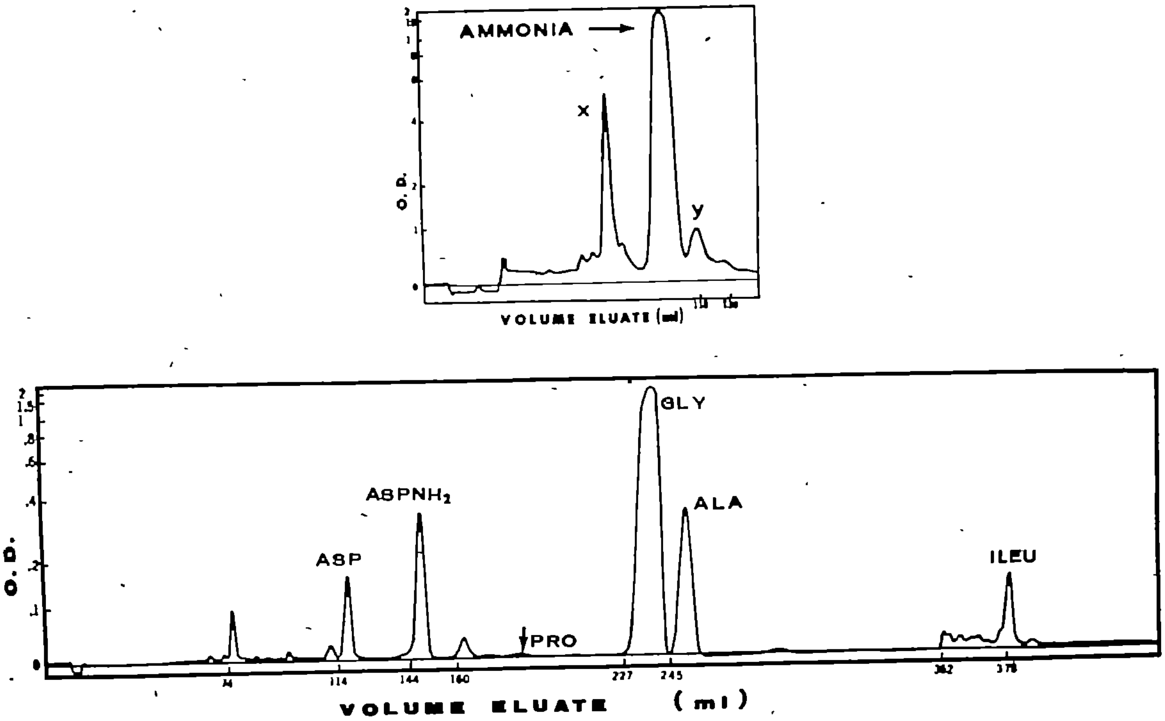

Fig. 5. Elution diagrams of the amino-acids of S-8-A separated by two ion exchange columns. A short column (top) was used for basic amino-acids and long one (bottom) for acidic and neutral amino-acids (Beckman-Spinco model 120 analyser)

Several unidentified peaks (X, Y, etc.) can also be observed in the eluate from the short column which is usually run for basic amino-acids. The large ammonia peak shown here is no doubt the result of hydrolysis of amino-acid amides such as asparagine.

Evidence for the formation of asparagine was also obtained in experiment S-7 by comparing a two-dimensional chromatogram of the reaction product of this experiment (Fig. 6) with that of a standard mixture of amino-acids containing asparagine (Fig. 7). (These chromatograms were obtained by descending development, using oxalic acid-washed Whatman No. 4 paper and n-propanol/15 M ammonium hydroxide/water, 6 : 3 : 1 (PAW) and n-butanol/propionic acid/water, 14 : 9 : 10 (BPW), as the first and second solvents, respectively). A spot with R_F 0·49 in PAW and 0·36 in BPW can be observed in Fig. 6. This spot showed a change in colour from brown to purple (Br → P). Authentic asparagine has R_F 0·53 in PAW and 0·39 in BPW (Fig. 7) and showed an identical change in colour (Br → P). The small differences in R_F were probably caused by the higher ionic strength of S-7 relative to that of the standard mixture of amino-acids.

To the right of asparagine (Fig. 6) a more intense spot which underwent the same colour change (Br → P) can be observed. By its R_F in the two solvents this compound corresponds to glycinamide. The characterization of this amide and other possible amino-amides which are suggested by the spots with similar colour changes (Br → P) in the upper right corner of the chromatogram (Fig. 6) was not carried out further because ion-exchange column chromatographic data have not yet been reported for amino-amides. It may be added that no identification of the green spots (G) of S-7 was attempted at this time. Tentatively these spots are presumed to be amino-amides and amino-nitriles since aspartic acid diamide[17] and β-aminopropionitrile[18] form green derivatives with ninhydrin.

The detection of asparagine and glycinamide is significant in two respects. First, it confirms earlier observations[19] made in our laboratory that the amino-amides are the immediate precursors of the amino-acids formed in these experiments. Secondly, it adds experimental support to the hypothesis that amino-amides may have played a part in the synthesis of polypeptides under possible primitive Earth conditions[20], since it has been shown that polypeptides can be obtained from glycinamide[20] and asparagine[21] in aqueous systems.

The compound labelled as isoleucine (Fig. 5) may very well be isoasparagine, since the latter compound has an almost identical elution time to that of isoleucine in polystyrene sulphonic acid resins[22]. It should be pointed out, however, that valine, leucine and isoleucine have been identified recently in our laboratory[18] in experiments where the action of electric discharges on mixtures con-

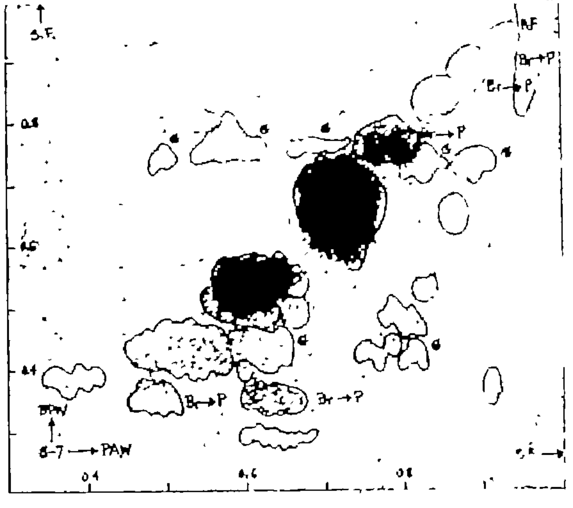

Fig. 6. Photograph of a two-dimensional paper chromatogram of S-7, sprayed with ninhydrin. Descending development in the BPW and PAW solvents (see text). Letters to the right or above the spots indicate colour or colour changes, That is, G, green ; Br→P, colour first brown, then turned into purple. Unlettered spots are purple

taining C$_2$ and C$_3$ hydrocarbons was allowed to proceed for a period of 24 h or longer. It appears that under these conditions the probability of formation of C$_4$ and C$_5$ aldehydes which are presumed to be the precursors of valine and the leucines increases significantly.

With regard to the formation of proline it should be stated that a small peak was obtained at the corresponding elution place for this compound (Fig. 5). As in the case of proline, the ninhydrin derivative of the eluted substance showed an optical density at 440; 100 per cent higher than at 570. If this peak corresponds to proline the small peak in front of proline should probably correspond to glutamic acid.

Carbon-14 *compounds*. Two experiments were carried out with carbon-14 reactants. In the first of these experiments (*S*-5) one millicurie of ^{14}C-methane (8 mg) was used. The unreacted ^{14}C-methane and other gaseous ^{14}C-components from *S*-5 were recovered and used in a subsequent experiment (*S*-6). This was done as follows: At the end of the experiment, the gases of the reaction product were trapped into two cold traps immersed in liquid nitrogen which were connected in series. The trap farther away from the flask contained 50 g of molecular sieve (Linde type 4*A*, 1/16-in. pellets) for the absorption of methane. In less than 1 h the pressure in the reaction flask fell to about 1 in. mercury, which is approximately the vapour pressure of water at 27° C. This indicated that essentially all the gases and volatile compounds had been condensed in the cold traps. After removing the main reaction product (yellowish solution) from the flask, the latter was evacuated and connected again to the traps, which were allowed to warm to room temperature. In this manner the bulk of the gases from the traps diffused back into the reaction flask. These gases, together with additional non-radioactive methane, ammonia and water, were used as reactants for the second experiment (*S*-6).

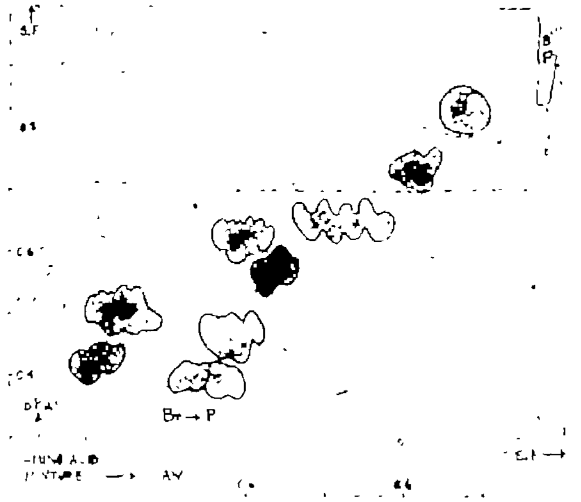

Fig. 7. Photograph of a two-dimensional chromatogram of a standard amino-acid mixture. From top to bottom following approximately the diagonal line the spots correspond to: leucine + isoleucine, valine, α-*n*-butyric acid, β-alanine, alanine, glycine + serine, asparagine (Br→P) and lysine. The two spots on the left correspond to glutamic acid (upper) and aspartic acid

The concentrated reaction products from *S*-5 and *S*-6 were then individually chromatographed in the PAW–BPW solvent system described previously. Autoradiographs of these two chromatograms are shown in Figs. 8 and 9. By comparing the autoradiograms of *S*-5 and *S*-6 with the ninhydrin-sprayed chromatogram of *S*-7, a general resemblance of distribution of spots in the three chromatograms can be observed even though different methods of detection were used and the three chromatograms correspond to three different experiments.

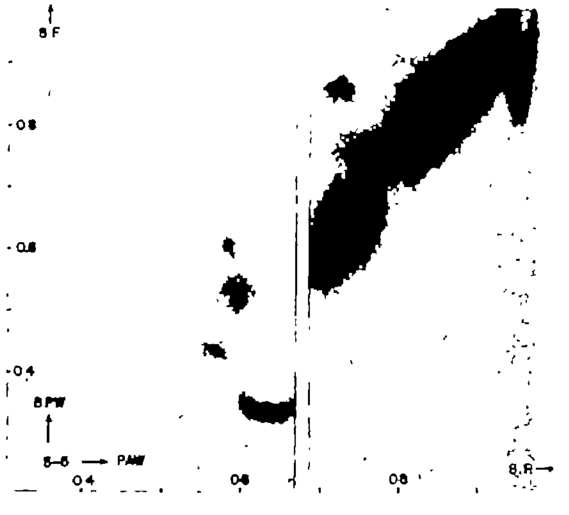

Fig. 8. Autoradiograph of a two-dimensional chromatogram of *S*–5. Because of their size this and other chromatograms had to be folded to expose two film plates which were placed back to back in the same holder. The white band corresponds exactly to the bent portion of the paper chromatogram

The first three spots on the diagonal line of *S*-6 (Fig. 9) correspond, very likely, to asparagine, glycine and alanine, as shown by comparing this chromatogram with that of *S*-7 (Fig. 6) and with the chromatogram of a standard mixture of amino-acids (Fig. 7). Radioactive and ninhydrin-positive spots can also be detected in the areas corresponding to valine and the leucines. However, since no separation of bases from amphoteric substances was carried out before chromatography, some of these spots may correspond to the amides of the foregoing amino-acids, instead of the amino-acids as such. The ^{14}C-spot to the right of asparagine (Fig. 9) corresponds, most likely to glycinamide.

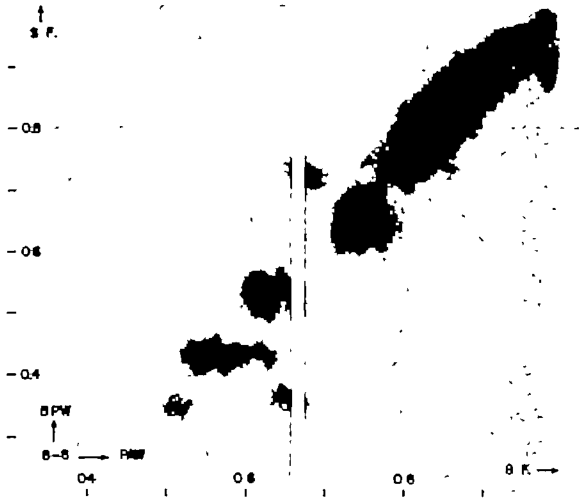

Fig. 9. Autoradiograph of a two-dimensional chromatogram of *S*–6. See Fig. 8 for explanation of white band

The largest and most diffused ninhydrin-positive compound of *S*-7 (Fig. 6) is probably caused by an aliphatic amine. (Aliphatic amines are known to produce diffused spots.) A radioactive spot corresponding to this compound can be observed clearly in *S*-5 (Fig. 8).

Aside from amino-acids, amino-amides and aliphatic amines, it is of interest that other radioactive spots appear in the area of purines and pyrimidines[11]. It should be possible to identify these compounds by eluting them from the chromatogram and co-chromatographing them individually with the suspected authentic compounds. However, a more systematic approach would be to obtain a sample sufficiently large and submit it to analytical ion exchange chromatography. The eluate could be automatically scanned with a short wave-length ultraviolet lamp and with a radioactive scintillation counter. Coincidence of ultra-violet-absorption with radioactivity would exclude any possibility of contamination.

Polymers and other compounds. In a number of experiments a transparent solid material was observed to be formed on the glass surface of the apparatus close to the electrodes. It extended in the form of a thin layer, around each electrode, to about one-fourth of the total area of the flask.

This product was insoluble in ordinary organic solvents and water, and could not be dissolved, degraded, or hydrolysed by boiling in 6 N hydrochloric acid overnight. Only after long standing in acid could it be separated in the form of thin flakes from the glass surface. This material is presumed to be similar to that obtained by Wilson[24] in analogous experiments with electric discharges. Because of its nature, which is supposed to be a highly cross-linked polyethylene-type polymer with some functional groups, and its peculiar way of formation (surface or interphase electrodic phenomenon), it remains uncertain at this moment the part that this or similar polymers may have played in pre-biochemical evolution.

Preliminary evidence for the formation of small-molecular-weight peptides was given by some of the two-dimensional paper chromatograms of Fig. 4. In particular *S*-3 and *S*-3-A showed two elongated spots close to the origin, which gave a brown colour on spraying with ninhydrin. This brown coloration changed very slowly into purple. Because of their low rate of migration and their characteristic way of reacting with ninhydrin, these compounds are probably peptides or polymers of amino-acids. Almost identical spots were observed in the reaction product from hydrogen cyanide–ammonia–water reaction mixtures[25], and it has been definitely shown by Lowe *et al.*[26] that polymers of amino-acids are formed in these experiments. The aforementioned ninhydrin-positive spots shown in chromatograms of *S*-3-A are supposed to correspond to the compounds *X* and *Y* which were observed to be eluted from a short ion-exchange column (Fig. 5).

Finally, it will be recalled that the gaseous and volatile products of experiments *S*-5 were condensed in two traps immersed in liquid nitrogen. After allowing the gases to diffuse back to the reaction flask, several droplets of a transparent pale yellow oil and a white viscous material were observed to remain in the trap adjacent to the flask. Both substances were radioactive and insoluble in water. The yellow oil was soluble in carbon tetrachloride. These materials were not analysed, but they suggest that the formation of lipophilic substances is possible in experiments with electric discharges.

I thank Prof. M. Calvin for providing the stimulus and laboratory facilities to carry out these experiments, and Dr. R. Lemmon for his advice.

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ATTEMPTS TO ISOLATE INFECTIOUS NUCLEIC ACID FROM VACCINIA VIRUS AND VIRUS-INFECTED CELLS

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NUMEROUS plant and animal viruses yield infectious ribonucleic acid (RNA) on treatment by a variety of reagents and techniques[1–4]. Furthermore, a deoxyribonuclease-sensitive unit capable of infecting *E. coli* protoplasts was isolated by degradation of phage T_2 with concentrated urea[5]. The question now arose whether an infectious deoxyribonucleic acid (DNA) could be recovered from a DNA animal virus. Vaccinia virus was selected for investigation since it is known to contain DNA (ref. 6), is readily purified[7], can be accurately titrated and identified in tissue culture systems. It is the purpose of this article to describe attempts to isolate an infectious DNA from vaccinia virus or virus-infected cells, and direct attention to the possible biological significance of our findings.

Vaccinia virus (Lederle Laboratories strain) was propagated on a *KB* cell line (obtained from the Southern Research Institute, Birmingham, Alabama) grown in Eagle's basal medium (BME) with 10 per cent calf serum. Crude virus material for DNA extraction was prepared by treating 48-h infected *KB* cells and tissue culture fluids for 5 min in a 10 *KC* Raytheon sonic oscillator. Purification and concentration of vaccinia virus was effected by fluorocarbon (Genetron 113) treatment[7] and sedimentation at $37,000g$ for 1 h. Sedimented virus particles were dispersed by brief sonoration in an appropriate diluent for DNA extraction. Virus titres of these preparations ranged from 1×10^7 to 1×10^9 plaque forming units per ml.

Most of the DNA extraction procedures adopted in this investigation used phenol treatment as one step in the extraction procedure. The cold[1] and hot[8] phenol methods were used extensively, as were modifications of the former method which consisted of adding selected anions[9] and a surfactive agent[10] to the extraction mixture. *KB* cell monolayers for testing DNA extracts were grown in

250-ml. square dilution bottles and washed three times with solutions of either 0·5 M sodium chloride, 0·7 M sucrose, or versene buffer[11], *p*H 7·1, prior to adding DNA extracts. These washings materially reduced extracellular deoxyribonuclease (DNase) activity and the concentration of divalent cations necessary for DNase activity. A hypertonic environment as well as the presence of versene has been shown to increase the sensitivity of cells to infectious nucleic acids[11,12]. DNA extracts were diluted 1 : 1 in either BME without calf serum, 0·5 M sodium chloride, or 0·7 M sucrose solutions, and 1–2 ml. of the mixture incubated with *KB* cell monolayers 15–40 min at 37°. Fresh tissue culture medium was then added and incubation continued for 48 h. At this time the cells were disrupted by sonoration and at least one 'blind passage' made on fresh *KB* cell monolayers.

In some cases DNA extracts produced an observable cytopathogenic effect in the initial test cultures. To distinguish this effect from the cytopathogenic effect resulting from vaccinia infection, 2 ml. of a 1 per cent suspension of chicken red blood cells in 0·15 M sodium chloride were added to each test culture drained free of culture fluid. Susceptible fowl cells are rapidly adsorbed to *KB* cells infected with vaccinia virus, but not to cells showing non-specific cytopathogenic effect arising from cytotoxic materials in DNA extracts.

The inability of these procedures to release an infectious nucleic acid from either crude or purified virus materials prompted consideration of DNA extraction procedures relative to the structural complexity of vaccinia virus. Enzymatic digestion of vaccinia virus has been correlated with observable changes in the structural organization of the virus particle[13,14]. Consequently, purified virus material was pretreated 1–3 h at 37° with pepsin, trypsin or ficin prior to phenol extraction. Such procedures did not yield biologically active nucleic acid. A summary of typical experiments is given in Table 1.

Table 1. NON-INFECTIVITY OF VACCINIA VIRUS DNA EXTRACTS

Exp.	Virus material	Extraction procedure	Transmittable CPE on *KB* cells	CRBC hæm-adsorption to *KB* cells
1	CV, FPV	80–88% phenol, 0°	0	0
2	CV, FPV	80–88% phenol, 40–50°	0	0
3	CV, FPV	88% phenol, 0·15 M *p*-amino-salicylate, 0°	0	0
4	CV, FPV	88% phenol, 0·15 M tri-chloroacetate, 0°	0	0
5	CV, FPV	0·2–2% sodium dodecyl-sulphate, 88% phenol, 0°	0	0
6	FPV	0·02–0·2% pepsin, 0·1 M cysteine HCl, *p*H 3·5 37°; 88% phenol, 0°	0	0
7	FPV	0·001–0·1% ficin, 37°; 88% phenol, 0°	0	0
8	CV, FPV	0·25% trypsin, *p*H 7·2, 37°; 88% phenol, 0°	0	0

Abbreviations: CV, crude virus; FPV, fluorocarbon purified virus; CRBC, chicken red blood cells; CPE, cytopathogenic effect.

The failure to recover infectious nucleic acid from crude or purified vaccinia virus suggested the alternative approach that viral DNA might be recovered from virus-infected cells prior to its incorporation into virus particles. Saltzman[15] has shown that the majority of vaccinia virus DNA is synthesized by infected HeLa cells within 6 h after initiation of infection, and before the appearance of newly formed virus. This finding suggested that infectious DNA might be isolated from *KB* cells during the early stages of virus synthesis. In Table 2 are shown data from experiments with vaccinia virus-infected *KB* cells and homogenates of vaccinia-infected rabbit testicular tissue. In the latter instance the infecting virus was a testicularly adapted strain which could be propagated equally well in *KB* cells.

The extraction or homogenization of virus-infected cells entails the hazard of releasing cellular nucleases which rapidly inactivate infectious nucleic acid[16]. Therefore, in some of the extraction procedures for infected cells either a chelating agent (versene) was used to sequester magnesium ions, or an adsorbing agent (bentonite) which has been shown to inactivate nucleases during phenol extraction of tobacco mosaic virus (TMV) and contribute to the stability of TMV infectious RNA[17].

Table 2. DNA EXTRACTION OF VACCINIA-INFECTED *KB* CELLS AND TISSUES

Exp.	Material extracted	Extraction procedure	Transmittable CPE on *KB* cells	CRBC hæm-adsorption *KB* cells
1	*KB* cells, 6–8 h post-infection	88% phenol, 0°	0	0
2	*KB* cells, 6–11 h post-infection	88% phenol, 1 × 10^{-3} M versene, 22°, 0°	0	0
3	*KB* cells, 6–8 h post-infection	0·02–2% sodium dodecyl-sulphate, 88% phenol, 0°, 22°	0	0
4	*KB* cells, 6–8 h post-infection	0·15 M *p*-aminosalicylate, 88% phenol, 0°	0	0
5	*KB* cells, 6–8 h post-infection	0·15 M trichloroacetate, 88% phenol, 0°	0	0
6	*KB* cells, 6–8 h post-infection	0·15 M trichloroacetate, 0·02–2% sodium dodecylsulphate, 88% phenol, 0°	0	0
7	Homogenate, 3–5 day infected rabbit testicular tissue	0·15 M trichloroacetate, 0·02–2% sodium dodecylsulphate, 88% phenol, 0°	0	0

None of the extracts of infected *KB* cells contained biologically active DNA. The initial extraction of infected rabbit testes yielded a DNA preparation which caused a transmittable cytopathogenic effect on *KB* cells; this effect was shown positively to be caused by vaccinia virus. Since numerous attempts to repeat this finding were unsuccessful, it was impossible to apply strict criteria for the presence of an infectious DNA in testicular extracts. The results of the initial experiment may be explained by a carry-over of infectious vaccinia virus through the extraction procedure.

It appears, therefore, that neither purified vaccinia viruses nor virus-infected cells yield infectious DNA by the methods applied in this work. Suggested explanations for these results are: (1) the methods used in DNA extraction yielded a degraded, inactive product, or did not adequately release DNA from the virus particle; (2) the inability of extracted vaccinia virus DNA to initiate cellular infection because of some unique biological relationship with the host cell; (3) that some other component of the virus, in addition to DNA, may be involved in the initiation of cell infection.

It is noteworthy that the DNA extraction methods of Experiments 3 and 4 (Table 1) have released infectious DNA from the polyoma virus[18], as has cold phenol extraction of the Shope virus[19] and the SV_{40} agent[20]. The fact that infectious DNA can be recovered from these tumour agents and not vaccinia virus suggests a significant difference in the structural organization of the tumour viruses or perhaps the interaction of their nucleic acids with the host cell. It is also significant that the recovery of infectious DNA from such extensively studied, non-tumour agents as the adenovirus, herpes and pseudo-rabies viruses has not been reported.

Pertinent to this work are the results of Pfau and McCrea, who found that single-stranded DNA was released from vaccinia virus by phenol extraction[21], and that treatment of the virus with a protease and 2-methoxy-ethanol[22] yielded double-stranded DNA, both types being non-infectious. It is apparent, therefore, that DNA of strikingly varied physical properties may be derived from vaccinia virus, and it is reasonable to assume that the physical state of the extracted DNA would have some bearing on the biological activity of this material. Unfortunately there is no information available which might clarify the relationship between the physical state of animal virus DNA and the capacity of such DNA to infect cells.

That infectious DNA could not be recovered from virus-infected cells in the early stages of virus synthesis would suggest inactivation by cellular DNases released during extractions, although precautions were taken against this eventuality. Cairns[23] and others[24] have shown that vaccinia virus antigens appear in the infected cell simultaneously with viral DNA. It is conceivable that under such conditions viral proteins and DNA may form complexes or viral sub-units which cannot be artificially degraded to yield biologically active nucleic acid. The early-appearing nucleo-protein antigen of vaccinia virus may well represent such a complex.

Finally, consideration must be given to the possibility that the larger and more complex animal viruses such as vaccinia do not possess an infectious nucleic acid *per se*. Such an assumption predicates the existence of one or more viral components other than DNA which participate in the initiation of virus synthesis. That components of the pox viruses other than DNA play a part in the interaction of virus and host cell is indicated by the experiments of Joklik *et al.*[25], who showed that protein denaturants which do not harm DNA, completely destroy the reactivability of rabbit pox virus. Preliminary experiments with ultra-violet-inactivated vaccinia virus and DNA added to *KB* cell cultures simultaneously or at different intervals have not resulted in cell infection.

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INTERRELATIONSHIP BETWEEN GLUCOSE UTILIZATION OF BRAIN AND HEART

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IT has been demonstrated[1] that brain can accumulate carbon-14 and retain it for a considerable period after the introduction of glucose-U-^{14}C into the circulation of the rat. The carbon-14 from glucose carbon which accumulates in the brain is rapidly converted into glutamic and aspartic acids[1]. Twenty minutes after subcutaneous injection of glucose-U-^{14}C, more than 70 per cent of the carbon-14 in the acid-soluble fraction from brain had already been converted into α-amino-acid carbon, but in liver only 10–15 per cent of the radioactivity was found in the α-amino-acid fraction[2]. The corresponding figure for heart (33–38 per cent) was intermediate between those for brain and liver, whereas in leg muscle it was lower than in any of the eight tissues investigated.

Heart has now been found to resemble brain in its ability to accumulate carbon-14 from labelled glucose *in vivo*. After the injection of glucose-U-^{14}C, the radioactivity in the brain-soluble fraction rapidly increases, despite the continuous decrease of radioactivity in the blood, and reaches levels much higher than that of blood[1]. As demonstrated in Fig. 1*A*, heart shows a similar increase. Leg muscle, on the other hand, does not appear to share this ability to concentrate the carbon-14 from blood glucose to values higher than the actual concentration of blood carbon-14 during the first 2 h after injection of glucose-U-^{14}C. However, after this period a retention of carbon-14 above blood-levels can be observed. The ability of the heart to accumulate carbon-14 from blood glucose has been considerably reduced after fasting (Fig. 1*B*).

The brain is known to be particularly sensitive to acute hypoglycaemia[3,4]. In cases of decreased availability of glucose, the possibility had to be considered that brain might utilize administered glucose (apart from glycogen reserves) at the expense of other organs. The following experiments were performed as part of a more extensive investigation which will be published in detail elsewhere.

Sixteen male Wistar rats of approximately the same weight were divided randomly into two groups. One group was deprived of food, the other was fed *ad libitum* on the normal laboratory diet (No. 41 *B*, J. Rank, Ltd.). Water was available *ad libitum* to both groups. After 24 h, all were injected subcutaneously with glucose-U-^{14}C (15 μc. in 0·3 ml. water in the presence of 0·037 mg of carrier glucose as supplied by the Radiochemical Centre, Amersham). The average weight (and *S.E.*) of the fed animals was 109 ± 1·4 g, and of the fasting animals was 92 ± 2·1. The concentration of blood glucose, as estimated with glucose oxidase[5], was 131 ± 6·2 mg/100 ml. for the fed group, and 77 ± 1·7 mg/100 ml. for the fasting group.

At various times (4 min–2 h) after glucose injection, the animals were killed by decapitation, and the blood (1 ml.) was immediately collected and deproteinized by the addition of 80 per cent (v/v) ethanol (8 ml.). Brain (whole, except for cerebellum), heart, liver and a sample of the gastrocnemius muscle were rapidly removed and frozen in liquid nitrogen. The time from decapitation to fixation of the tissues by freezing did not exceed 2 min. The tissues were stored in the frozen state at – 20° C until homogenization, which was done at 0° C in 80 per cent (v/v) ethanol (8 ml.) in a 'Teflon'-pestle homogenizer (A. H. Thomas Co.). Ethanol was used instead of perchloric or trichloroacetic acids because it extracts glucose, amino-acids, Krebs cycle intermediates and other substances, but not glycogen.

The homogenates were centrifuged at 0° C for 15 min at 2,500*g* (M.S.E., 'Major') and the residue was twice rehomogenized in 80 per cent ethanol (8 ml.). The combined supernatants from each sample were made up to 25 ml. with 80 per cent ethanol and plated in duplicate aliquots of 0·2 ml. and 0·4 ml. Counting was carried out at infinite thinness in a 'Nuclear Chicago' automatic Geiger counter. Duplicate counts of 1,000 were taken for each sample and activities were usually 10–100 times that of the background, for which corrections were applied. The results are shown in Table 1.

It may be seen that the carbon-14 content of the blood of all the fasting animals was considerably higher than in the fed animals. This indicates that the elimination

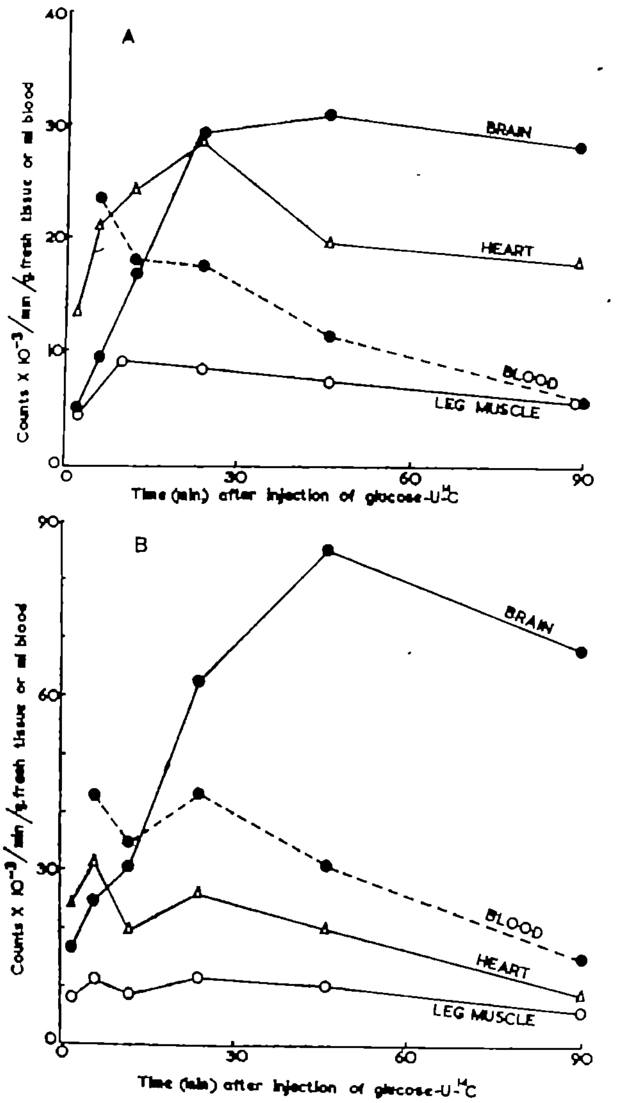

Fig. 1. Accumulation and retention of carbon-14 in ethanol-soluble fraction of rat organs after injection of glucose-U-^{14}C. Each point is the average of 2 experiments. *A*, rats were fed *ad libitum* until decapitation: *B*, food withdrawn 24 h before injection of glucose-U-^{14}C. For all other conditions, see Table 1

of glucose-carbon from fasting animals as carbon dioxide is slowed down, in accordance with the reports of similar investigations (for relevant literature, see ref. 6). As a consequence of the higher blood carbon-14 content in the fasting animals, the total amount of radioactivity in each of the tissues examined was increased (Fig. 1).

Table 1. ACCUMULATION AND RETENTION OF CARBON-14 IN ETHANOL-SOLUBLE FRACTIONS OF ORGANS OF FED AND FASTING RATS AFTER INJECTION OF GLUCOSE-U-^{14}C

Tissue	Nutrition	Time after injection of glucose-U-^{14}C 4 min	8 min	16 min	32 min	1 h
R.S.A. = 100						
Blood (counts/min/ml.)	Fed	30,875	16,504	19,504	15,625	7,569
	Fasting	60,781	24,250	44,968	41,468	20,343
R.S.A.						
Brain	Fed	34	50	130	215	376
	Fasting	54	66	99	197	425
Liver	Fed	197	73	137	128	164
	Fasting	87	87	98	146	205
Heart	Fed	88	88	174	161	207
	Fasting	79	60	54	67	62
Muscle	Fed	81	—	46	51	90
	Fasting	26	27	22	32	38

Male rats were injected subcutaneously over the left flank with 0·2 ml. of glucose-U-^{14}C (15 μc. in presence of 0 037 mg carrier glucose). Fed animals = animals fed *ad libitum* until decapitation. Fasted animals = food withdrawn 24 h before injection of glucose-U-^{14}C. Tissues were extracted with 80 per cent (v/v) ethanol as described in the text, and results expressed as relative specific activities (R.S.A.).

$$\text{R.S.A.} = \frac{\text{Counts/min/gm fresh weight of organ (ethanol-soluble fraction)}}{\text{Counts/min/ml. of blood (ethanol-soluble fraction)}} \times 100$$

Compared with the levels in the fed group, the relative specific activity in the brain and liver of the fasting group was almost unimpaired, whereas a considerable diminution of the relative specific activity was observed in the heart. The accumulation of carbon-14 in skeletal muscle, which in both groups was much lower than in heart, was even further decreased in the fasting group. Thus it appears that, under certain conditions of hypoglycaemia, the brain may accumulate and retain carbon from administered glucose at the expense of other organs.

It is conceivable that this mechanism, which seems to protect the vital functions of brain, may be, in suitable circumstances, detrimental to the functions of other organs, for example, heart. This could be relevant to the aetiology of heart disease. In this connexion, it is of interest that the tranquilliser, reserpine, 18 h after injection, has been observed in rats to cause a 55 per cent decrease in the uptake by heart of carbon-14 from blood glucose[7], while no effect on the ability of the brain or liver to accumulate carbon-14 from blood glucose could be demonstrated. Structural damage to cat heart after reserpine injection has been previously reported[8].

This work was carried out during the tenure by two of us (R. V. and H. S. B.) of Rockefeller Foundation grants, and by the third (J. K.) of a personal grant from the Polish Ministry of Health. We wish to thank Dr. Derek Richter for his interest and support.

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POLIOVIRUS NEUTRALIZING PROPERTIES OF EQUINE AND OTHER SERA

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THE neutralizing or inhibitory action of some bovine sera to one or more of the three serological types of poliovirus has been described by a number of workers[1–4]. Bartell and Klein[3] found that this property was also possessed by the sera of certain horses, pigs and chickens, and that the activity was associated with the globulin fraction of the sera. McFerran[4] demonstrated that the bovine poliovirus inhibitors had much the same stability as poliovirus antibodies in human sera, and concluded, as did Bartell and Klein, that they were probably antibodies, the origin of which was obscure. In marked contrast were the conclusions of Takemoto and Habel[5] with regard to equine serum poliovirus inhibitors; they regarded the action of the serum as being not neutralization but a

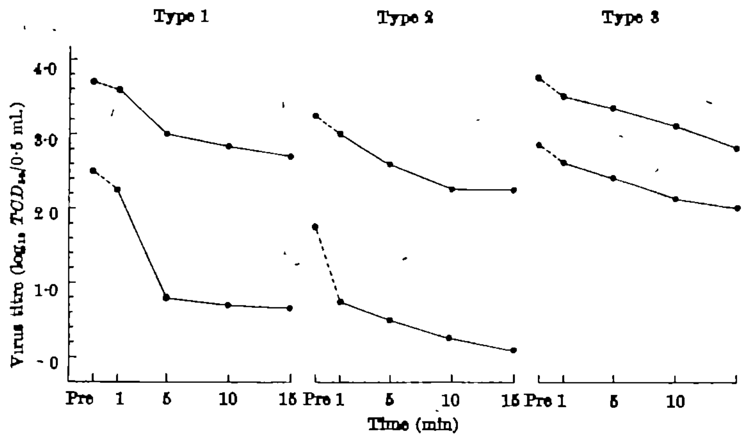

Fig. 1. Neutralization of polioviruses by equine inhibitory sera

reduction in the rate of adsorption and release of virus from the cells of the tissue culture system. Fractionation on the cellulose column, however, indicated that the activity was associated with the γ-globulin.

In this article, studies of poliovirus inhibitors of equine sera are presented and the frequencies of their occurrence analysed. A survey of inhibitors of sheep and monkey sera is also included, and suggestions as to the origin of poliovirus inhibitors are made. Monolayer monkey kidney tissue cultures were used for this work and were maintained on Earle's salt solution containing 0·5 per cent liver digest, 0·22 per cent sodium bicarbonate and penicillin and streptomycin. Poliovirus strains Brunenders, *MEF*-1 and Saukett were used unless otherwise indicated.

Adult horse, sheep and monkey sera were tested for inhibitors by mixing doubling serum dilutions with poliovirus (100 TCD_{50}/0·5 ml. of mixture), incubating 1½ h at 37° C, inoculating 0·5 ml. of each reaction mixture into four monkey kidney tube cultures and examining for cytopathic effect six days later. The percentages of sera showing inhibition are given in Table 1. In each case the type II inhibitor titres tended to be the highest and type III the lowest. Thus, in the equine sera the type II inhibitory action was sometimes detectable up to a serum dilution of 1/38; type I titres rarely exceeded 1/12 and type III 1/4. There was a statistically significant association between the presence in equine sera of inhibitors to polioviruses types I and II and between I and III, but no such marked correlation between types II and III. Monkeys are known to be to some extent susceptible to poliovirus infection; therefore, the findings for the monkey sera are presented with some reservation, particularly as the animals concerned had been held in contact with man for short periods.

Table 1. PERCENTAGE SERA FROM THREE ANIMAL SPECIES CONTAINING POLIOVIRUS INHIBITORS

Animal	No. of sera tested	Per cent sera containing inhibitors to polioviruses		
		Type I	Type II	Type III
Horse	203	17·2	33	4·9
Sheep	46	17	19	0
Monkey (*Cynomolgus* and rhesus)	699	1·1	2·0	0

The mode of action of equine poliovirus inhibitors was investigated in the following experiments. Undiluted serum was mixed with an equal volume of poliovirus suspension and incubated at 37° C, the virus being titrated before, immediately after, and at short intervals after, mixing. The titration dilutions were made in cooled medium immediately after each sample was taken. Such a test was done with two poliovirus concentrations of each of the three types. The results, which are given in Fig. 1, demonstrate a fall in virus titre during incubation, indicating that actual neutralization has taken place. This was confirmed by further experiments in which doubling dilutions of inhibitory serum were made. Part of each dilution was mixed with an equal volume of poliovirus suspension (100 TCD_{50}/0·5 ml. of mixture), incubated 1½ h at 37° C and inoculated into tube cultures, 0·5 ml./tube. Other cultures were inoculated with 0·25 ml. serum dilution and 100 TCD_{50} poliovirus contained in 0·25 ml. of medium without prior mixing and incubation. The inhibitor titres were 2–3-fold lower when prior mixing and incubation were omitted.

The heat sensitivity of the equine inhibitors is much the same as poliovirus antibodies prepared by hyperimmunization of horses, each type of inhibitor being stable up to 63° C. γ-Globulin precipitation by the method of Peterson *et al.*[7] brought about a complete removal of inhibitory activity from the supernatant; the resuspended γ-globulin neutralized poliovirus, although to a titre about two-fold lower than the original serum. The physical properties, therefore, of the equine inhibitors are rather similar to those of antibodies.

The susceptibility of the horse to poliovirus was of interest in view of the similarity of the inhibitors to antibody. No poliovirus has been detected in faecal samples and nasal swabs taken from 616 and 201 horses, respectively, over a period of 15 months; each sample was tested in monkey kidney tissue cultures. Further pertinent information was obtained during the hyperimmunization of horses with attenuated poliovirus. Two horses, the sera of which were free of type I poliovirus inhibitor, were each inoculated intramuscularly with 11·3 $\log_{10}$ $TCID_{50}$ Sabin attenuated type I poliovirus. Faecal samples were taken daily from each. Small quantities of type I poliovirus were found in the faeces taken from each animal 24 h after inoculation. No virus was detected in the subsequent faecal samples. Neither horse developed pyrexia or clinical reactions. Further type I poliovirus inoculations were given and antibody developed, but more sluggishly than might be expected if the virus were reproducing in the equine tissues (Table 2). Slow antibody responses were also obtained in other horses receiving a similar course of inoculations of Sabin attenuated type II and III poliovirus.

Table 2. ANTIBODY RESPONSE BY TWO HORSES INOCULATED WITH TYPE I POLIOVIRUS

Day of inoculation or taking serum	Log_{10} TCD_{50} poliovirus inoculated	Antibody titre
0	11·3	<1/4
14	11·0	Not tested
29	11·2	1/128
35	—	1/512

The properties of the equine poliovirus inhibitors are, therefore, very similar to those of antibody. They are apparently associated with the γ-globulin fraction and, like poliovirus antibodies prepared in horses, are stable to 63° C. The inhibitors neutralize the virus. This is contrary to the findings of Takemoto and Habel[8], who concluded that the type I poliovirus inhibitors they studied were not actually neutralizing the virus but reducing the absorption and release of virus from the cells. In their experiment inhibitory serum was mixed with high titre virus suspension (5·4 P.F.U./1 ml.), samples being removed and titrated at intervals after mixing. Little or no reduction in titre was demonstrable. However, equine poliovirus inhibitors usually have only low titres and in order to demonstrate neutralization in the type of test utilized by Takemoto and Habel it has been necessary to use poliovirus suspension of low titre. In preliminary experiments using between 5·0 and 7·0

$\log_{10}$ TCD_{50} per 0·5 ml. of serum/virus mixture, no neutralization could be detected. This is not surprising when the neutralization curves obtained with lower concentrations of virus are considered. Thus, in one of the type I neutralization curves the virus titre was reduced from 3·7 $\log_{10}$ to 2·7 $\log_{10}$, that is, 4,509 TCD_{50} were neutralized. Even allowing for a considerable elasticity in the neutralizing capacity of a serum containing antibody, the number of TCD_{50} which this serum sample would neutralize when mixed with 5·5–6·0 $\log_{10}$ TCD_{50}/0·5 ml. of poliovirus would not be readily detectable by ordinary titration techniques.

If these are antibodies, what are the antigens which stimulate their formation ? It is unlikely that polioviruses themselves form the stimulant, because: (*a*) the inhibitors reach only low titres; (*b*) poliovirus has never been isolated from horses; (*c*) horses seem to be insusceptible to poliovirus infection. Sabin and Fieldsteel[2] were also unsuccessful in their attempts to infect calves with polioviruses. It is therefore likely that the antigens are to be found in other viruses or micro-organisms. If this is so, there will probably be several, as it is unlikely that one single virus or organism can infect horses, cattle, sheep, swine, monkeys and chickens. Much evidence has accumulated for antigenic relationships between the three types of poliovirus[8–14] and between polioviruses and the various enteroviruses[10,15–18]. Among the polioviruses type II has antigens in common with type I and to a lesser extent with type III. These relationships can be emphasized by heating the antigens[9], by treating with formaldehyde[19] or by irradiating with ultra-violet light[20]. Such treatment probably alters the surface structure to reveal other antigens. Mayer *et al.*[21] and Hummeler and Hamparian[9] have demonstrated two distinct complement fixing antigens in poliovirus, one of which is 'exposed' by heating. The ability of type II poliovirus to stimulate antibodies common to the other serological types, particularly type I, could be explained by its deep antigens being more exposed. It is perhaps significant that in bovine, equine, sheep and monkey sera the type II inhibitors are most common and type III least common. It is suggested that these inhibitors are antibodies to the more deeply situated antigens common to enteroviruses, and are stimulated by various enterovirus infections experienced by the host during its life. The viruses in which these antigens are least concealed will most readily stimulate the formation of inhibitors and will be most sensitive to them when formed.

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CONCEPT OF MULTIPLE MOLECULAR FORMS OF LACTIC DEHYDROGENASE

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EXPERIMENTS which have recently been reported by Conklin, Dewey and May[1] suggest that treatment with butanol may convert the various lactic dehydrogenase (LDH) isozymes in a tissue homogenate into a single form. For example, after starch-gel electrophoresis, seven LDH isozymes were detected in untreated rat kidney homogenate, but only a single band could be observed in a sample after butanol treatment. The explanation proposed by these authors is that the enzyme is associated with various lipoproteins or lipids which alter the electrophoretic mobility. The dissociation of these complexes by means of butanol would then permit all the LDH to migrate as a single band. Further corroboration, however, was felt to be necessary.

Since this theory questions the validity of the concept of multimolecular forms of enzymes, at least in regard to LDH, the effect of butanol on LDH activity was investigated further. The results, described here, demonstrate that increasing butanol treatment results in a progressive inactivation of the enzyme. Electrophoresis of purified isozymes provides no indication that butanol converts one isozyme into the form of another before complete inactivation.

Heart, liver and kidney tissue homogenates were prepared from both human and rat sources. Approximately 0·5 g of tissue was used per ml. of 0·25 M sucrose. All samples were subjected to both starch-gel[2] and agar[3] electrophoresis, since some proteins migrate differently in the two media. Preparative electrophoresis was conducted in agar. LDH activity was detected after electrophoresis by the nitro-blue tetrazolium-substrate solution described by Allen[4]. The butanol treatment was conducted by adding various amounts of butanol to a series of tubes. The same amount of tissue homogenate or isozyme was then added to each tube and the contents mixed. The butanol addition was thus varied, while the enzyme addition was constant. Total LDH activity was determined by the method of Cabaud and Wroblewski[5].

The results obtained with human tissues are shown in Fig. 1. Increasing amounts of butanol caused a progressive inactivation of the enzyme. Measurement of total enzyme-levels in corresponding samples, after butanol treatment, demonstrated a decrease in activity to approximately 10 per cent of the pre-treatment values.

Fig. 2 shows the results obtained with isozymes isolated from human liver. Since the fourth and fifth bands showed considerable overlapping, they were taken together as a single fraction. Sufficient butanol treatment caused an almost complete loss of activity of all the isozymes.

Fig. 3 presents the results after electrophoresis of rat tissues. The butanol inactivation, apparent from this figure, was also confirmed by the measurement of total levels in corresponding samples. The untreated liver tissue exhibited a band migrating towards the anode in the starch gel. This fraction had been observed by Conklin, Dewey and May only in the treated sample. The difference

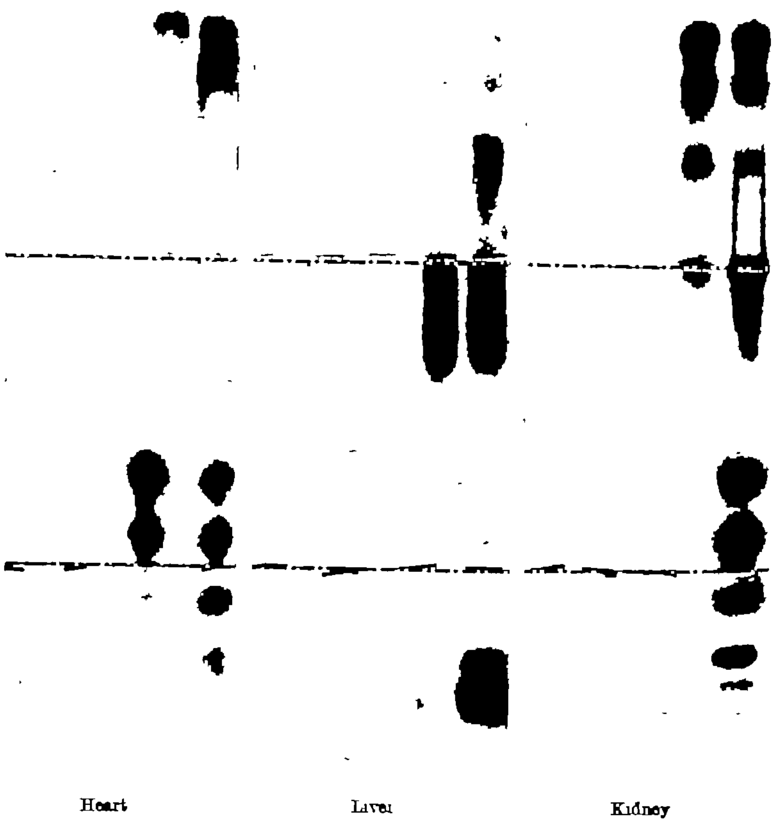

Fig. 1. Electrophoretic separation of LDH isozymes in human tissues. Migration towards the anode is upward. The origins are indicated by dashed lines. Starch-gel separations are above. The butanol additions per ml. of homogenate were, left to right, 0·50, 0·20, 0·10, 0·05 and 0·00 ml. Agar separations are below. The butanol additions per ml. of homogenate were, left to right, 0·50, 0·20, 0·10 and 0·00

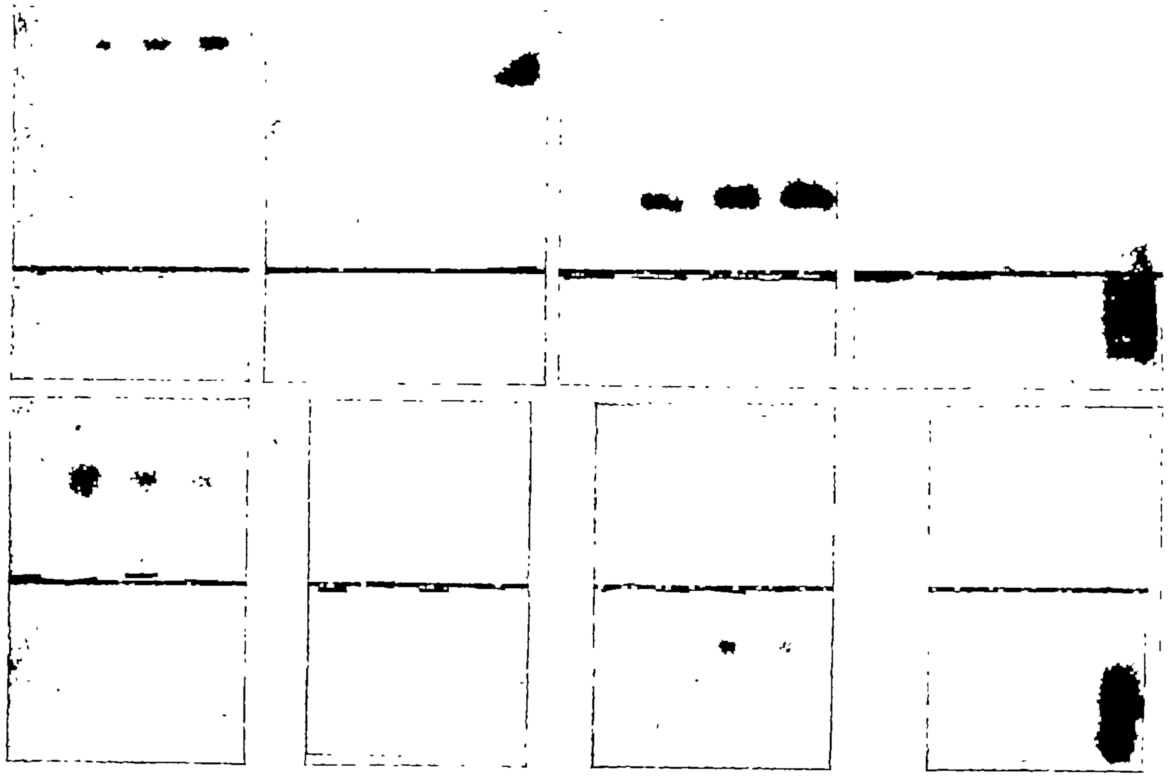

Fig. 2. Electrophoretic separation of isozymes isolated from human liver. The isozyme which migrates most rapidly towards the anode is on the left. Starch-gel separations are above, and agar separations are below. In all cases, the butanol additions per ml. of isozyme were, left to right, 0·09, 0·06, 0·04 and 0·00 ml.

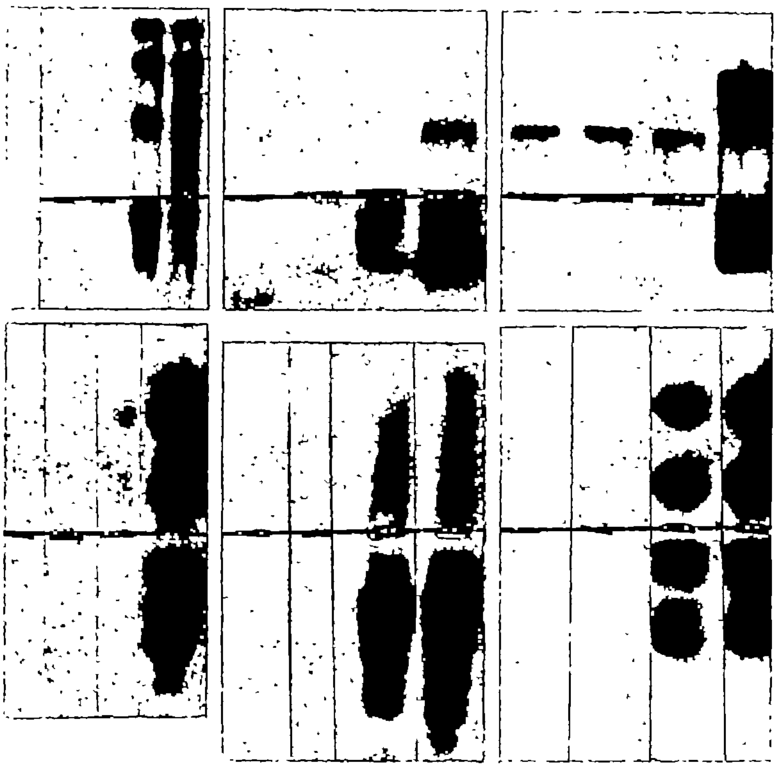

Fig. 1. Electrophoretic separation of LDH isozymes in rat tissues. Starch-gel separations are above, and agar separations are below. In each case, the sample on the left was an aqueous extract of a portion of the tissue which had been homogenized in butanol. The succeeding samples had been homogenized in sucrose. The additions of butanol per ml. of homogenate were, left to right, 0·45, 0·20 and 0·00 ml.

may be due to different quantities of enzyme applied to the gel. The corresponding band obtained after starch-gel electrophoresis of kidney tissue appears to be the only one remaining after butanol treatment. This is in agreement with the results of Conklin *et al.* The electrophoresis of the same samples in agar, however, revealed two bands in the treated kidney, corresponding in position to those of the untreated tissue. This discrepancy may possibly be related to an alteration of mobility by the starch gel.

Attempts were also made to detect specific lipids associated with the human isozymes by staining techniques, but none could be demonstrated. The conclusion which would appear most consistent with these experiments is that butanol treatment provides no reason to question the concept that LDH exists in different molecular forms.

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SOURCE OF THE GASTRIC MUCOSAL POTENTIAL DIFFERENCE

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IT has been shown recently that the potential difference across frog skin is apparently the algebraic sum of sodium and potassium diffusion potentials on the outer and inner cell membranes in the cells of the stratum germinativum[1]. However, there is no agreement as to the source of the electric potential difference across the stomach wall which has been known since 1834 (ref. 2). The following experiments were designed to investigate this problem.

Bullfrog (*Rana catesbiana*) gastric mucosae were mounted between two chambers containing the following solutions. Nutrient side: sodium chloride, 85·3 mM/l.; potassium chloride, 3·4 mM/l.; calcium chloride, 1·8 mM/l.; potassium dihydrogen phosphate, 0·9 mM/l.; magnesium sulphate, 0·9 mM/l.; sodium bicarbonate, 17·6 mM/l. Secretory side: a similar solution with the sodium bicarbonate replaced by sodium chloride. Both solutions contained 0·2 per cent (w/v) glucose and were gassed with 5 per cent carbon dioxide and 95 per cent oxygen. In fourteen experiments the average transmucosal potential difference

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* Postdoctoral Fellow of the Pennsylvania Plan to Develop Scientists in Medical Research.

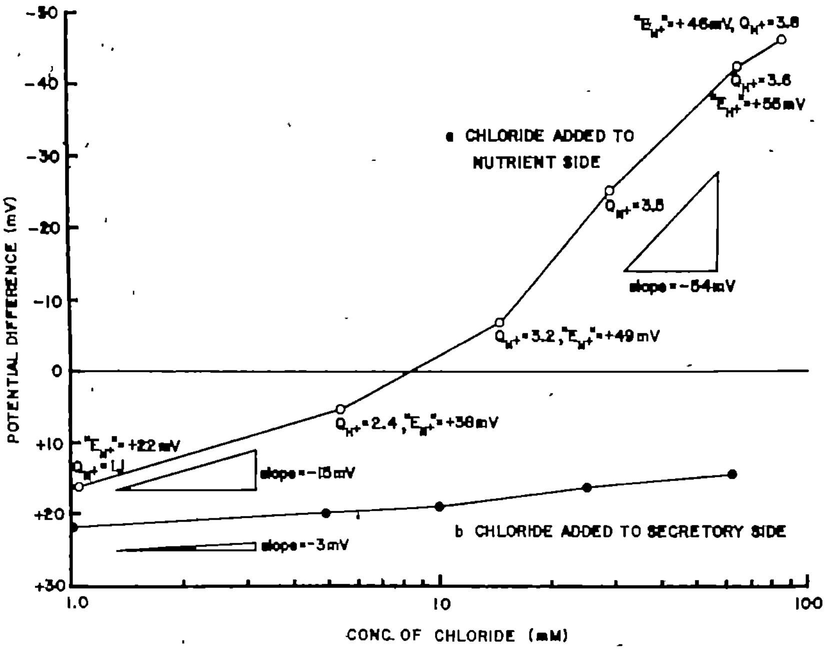

Fig. 1. Typical effects of serial addition of chloride to the nutrient side (*a*) ○; and to the secretory side (*b*) ● of a gastric mucosa bathed in isethionate Ringer's solution. The potential difference with no added chloride in the bathing solutions for the mucosa in (*a*) was + 24 mV and the rate of acid secretion (Q_{H^+}) was 1·0 µequiv./cm^2/h. After the chloride concentration was raised to 84 mM/l. on the nutrient side, the introduction of a similar chloride solution to the secretory side caused the potential difference to change from − 46 mV to a steady state value of − 33 mV. 'E_{H^+}' was calculated from the observed current of hydrogen ions times the mucosal resistance. For the mucosa in (*b*) the potential difference in the absence of chloride was + 27 mV and the Q_{H^+} was 1·1 and did not change significantly while chloride was raised in the secretory solution. Values for the slope are given as mV per ten-fold change in the concentration of chloride

at approximately 22° C was − 30·7 ± 6·6 mV (standard deviation). All values of the potential difference are given for the secretory side with respect to the nutrient side in an external circuit. The mucosal conductance was 2·9 ± 0·5 mmhos/cm^2, and the rate of acid secretion (Q_{H^+}) was 4·8 ± 1·1 µequiv./cm^2/h (1 cm^2 = 12 mg dry wt.). When all the chloride ions in the salt solutions were replaced by 2-hydroxy-ethylsulphonate (isethionate), the transmucosal potential difference changed from negative to positive and within a half-hour achieved a steady state value of + 22·2 ± 4·6 mV, while the conductance became 1·0 ± 0·4 mmhos/cm^2 and the rate of hydrogen ion secretion 1·0 ± 0·2 µequiv./cm^2/h. The electrical effects were similar in direction but much greater in magnitude than those observed when the chloride in the bathing media is replaced by sulphate[2-4] (which is less permeable than chloride and very inefficiently transported by the gastric mucosal pump[5,6]). The effects in isethionate solutions were completely reversible for at least 7 h.

Fig. 1 shows the changes in potential difference following serial addition of chloride to isethionate solutions on the (*a*) nutrient and (*b*) secretory sides. Fig. 2 shows similar changes during anaerobiosis and following the reintroduction of oxygen during conditions where acid secretion occurred or was inhibited.

The results support the view that there is a chloride diffusion potential across the nutrient (but not the secretory) surface, and that there are electrogenic hydrogen and chloride ion pumps at the secretory surface[4]. The evidence for this view is as follows.

The reversible change from a negative to a positive polarity when isethionate completely replaced chloride on the nutrient side shows that chloride is necessary for part of the naturally occurring potential difference. In isethionate solutions, with no external chloride, the rates of acid secretion were considerably less than those observed in the normal chloride solutions, presumably because the isethionate accompanying the hydrogen ion is at least ten times less permeable. Fig. 1 shows that for the change from 1–6 mM chloride on the nutrient side the Q_{H^+} more than doubled ($\Delta Q_{H^+} = 1{\cdot}3$) and the potential difference increased at the rate of − 15 mV for a ten-fold concentration change. However, for the change from approximately 15 to 60 mM chloride the ΔQ_{H^+} was only 0·4, but the rate of increase of the potential difference was − 54 mV for a ten-fold concentration change in chloride and thus approached the value of − 58 mV for a ten-fold change predicted by the Nernst equation for a diffusion potential resulting from an ionic concentration gradient. This suggests that there is a chloride diffusion potential

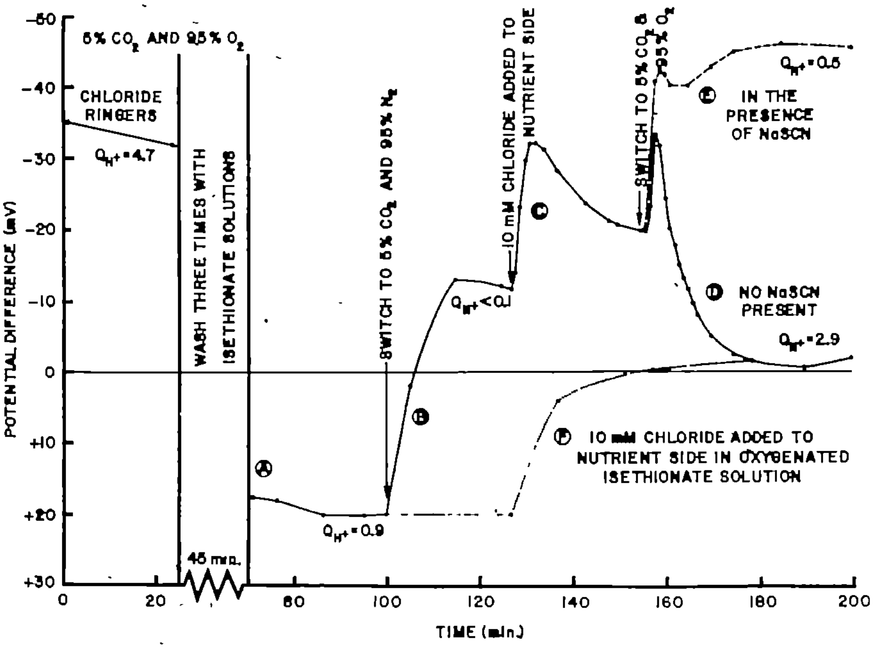

Fig. 2. Typical composite experiments showing effects of removal and reintroduction of oxygen and chloride from the solutions bathing the gastric mucosa. Conditions *b* and *c* of Fig. 3 obtain in normal chloride Ringer's, while in isethionate Ringer's condition *a* operates. *C* shows the transient chloride diffusion potential (Fig. 3*b*) which dissipates in the absence of oxidative metabolism. *E* represents the potential difference when 5 mM sodium thiocyanate has previously been added to the secretory side at time 127 min and shows that the chloride pump is re-established on the introduction of oxygen (Fig. 3*b*), while at *D* both the hydrogen pump and the hydrochloric acid pump (Fig. 3*a* and *c*) are operating. This latter state can be reached directly as shown in *F*.

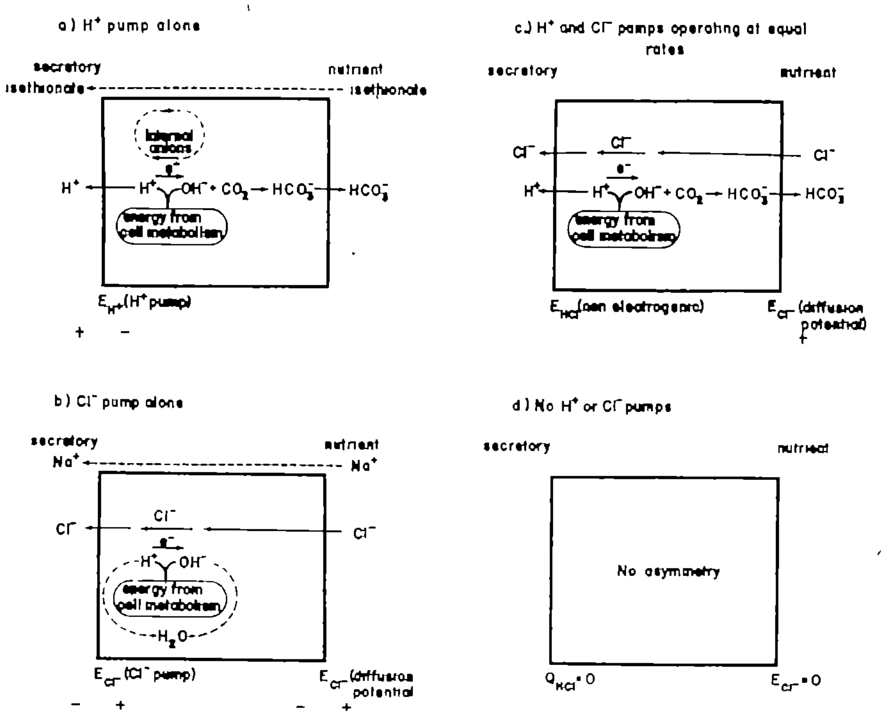

Fig. 3. Schematic representation of the mechanism of chloride and hydrogen ion transport under four operating conditions (see also Davies (ref. 14)). The secretory surface is probably relatively impermeable to hydrogen and chloride ions. The nutrient surface is permeable to chloride and bicarbonate ions. (*a*) H^+ pump alone in isethionate Ringer's. Note that traces of Cl^- remain in the mucosa even after many hours in this solution; (*b*) Cl^- pump alone, but no acid secretion, either naturally or in the presence of sodium thiocyanate; (*c*) the H^+ and Cl^- pumps are normally in operation during acid secretion; and (*d*) no H^+ or Cl^- pumps are in operation under conditions such as anoxia, 2,4-dinitrophenol, death, etc.

across the nutrient surface, but that normally it is masked by the positive potential caused by hydrogen ion secretion. Furthermore, the insensitivity of the potential difference to changes of chloride on the secretory side shows that no chloride diffusion potential is developed across this surface. The small changes in potential shown in Fig. 1*b* might be the result of slight leaks of chloride from the secretory to the nutrient solutions. Changes in the bicarbonate concentration from 5 to 30 mM in the nutrient solution were without significant effect on the potential difference, so it is unlikely that there is a bicarbonate diffusion potential across the nutrient surface (Fig. 3*c*).

Fig. 2*C* shows that a chloride diffusion potential at the nutrient surface can develop in the absence of oxygen although it dissipated as chloride entered the cells. On re-establishment of the energy supply (Fig. 2*D* and *E*) the cells were able to pump out the chloride which had accumulated in the absence of oxygen; thus they could restore the anionic concentration gradient and the resulting diffusion potential. These findings agree with the results of several authors that the chloride concentration within the oxyntic cells is normally low relative to the external concentration[7,8], and that the intracellular chloride concentration increases when oxidative phosphorylation is inhibited by 2,4-dinitrophenol[8] which also destroys the transmucosal potential difference[9].

The chloride pump causes a net flow of chloride through the secretory surface[10]. Since it can operate in the absence of acid secretion and with no exchangeable anion on the secretory surface, and since the chloride concentration within the cells is low, the chloride pump seems to be electrogenic, that is, capable of producing a net transport of electric charge.

It has been shown that the potential difference across the gastric mucosa in chloride Ringer's is unaffected by changes in *p*H from 2 to 7 on the secretory side[11]. In isethionate solutions we have similarly found that the potential difference is insensitive to the aforementioned *p*H changes. Thus, there is no significant hydrogen ion diffusion potential across the secretory surface. Since the secretory surface is relatively impermeable to hydrogen ions, the positive potential found during acid secretion in isethionate solutions (Fig. 2 *A* and *B*) is taken to be the result of an electrogenic hydrogen pump. This potential can be abolished by suitable inhibitors of acid secretion (anoxia or thiocyanate, Fig. 2 *B* and *E*). The magnitude of the measured hydrogen ion potential would depend on local short circuits through the mucosa which, in turn, are related to the permeability of the membrane. Thus in solutions containing isethionate, but no chloride, where the mucosal conductance was low, we have found that inhibition of acid secretion resulted in larger transmucosal potential changes than in chloride-containing solutions.

Fig. 3 is a schematic representation of the sources of the gastric mucosal potential difference under four different conditions. (It should be noted that states *b* and *c* are usually found together in any given mucosa.) Both the hydrogen and the chloride pumps are taken to be electrogenic in that if either of them operates alone it produces a separation of charge and hence an electromotive force. When the mucosa is secreting hydrochloric acid, the hydrogen pump and the chloride pump both operate, and, since the ions are equal and opposite in sign, there is no net charge transfer and hence no developed electromotive force associated with hydrochloric acid secretion *per se*. So long as the chloride pump is in operation, it maintains the intracellular chloride concentration at values below that of the bathing solutions, and hence a chloride diffusion potential develops across the nutrient side of the oxyntic cells. This causes a polarity across the mucosa—the secretory side being negative with respect to the nutrient side. In the absence of a permeable anion, such as chloride (or bromide, iodide, nitrate, etc.[12,13]), or in the presence of metabolic inhibitors which reduce or abolish the concentration gradient, the chloride ion diffusion potential is lowered or absent.

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PRESENCE OF IODINE-127 IODOTYROSINES IN EXTRACTS OF NORMAL HUMAN SERUM

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WERNER and Block[1] observed a discrepancy between iodine-131 and iodine-127 content of iodotyrosines in chromatograms of serum of healthy human subjects given a labelling dose of iodine-131. No iodine-131 iodotyrosines were found, whereas in the same chromatograms, iodine-127 iodotyrosines, both diiodotyrosine (DIT) and monoiodotyrosine (MIT), constituted a sizeable percentage of the total iodine. The same group of workers further demonstrated that neither radioiodide nor labelled thyroxine (T_4*) was converted to labelled iodotyrosines by the extraction and chromatographic procedures originally used[2] or by several other methods. It was also noted that stable iodotyrosines were present in chromatograms of normal rat serum, and additional evidence was furnished[2-4] that the iodotyrosine titre in human serum extracts probably varied with pathophysiological change.

The present experiments were designed to determine further the identity of the iodine-127 iodotyrosines extracted from serum with standard compounds, MIT and DIT. Two techniques were adopted: isotope dilution and derivative formation by dinitrophenylation. With the isotope dilution method, small concentrations of iodocompounds were determined when dilution occurred of the appropriate standard compound of known specific activity added to the serum before processing. By dinitrophenylation, *bis*(2,4-dinitrophenyl)-derivatives of both DIT and MIT were formed which were then identifiable chromatographically by comparison with diphenylated standard MIT or DIT of known specific activity. Addition of either of these compounds to the serum prior to dinitrophenylation permitted both autoradiography and isotope dilution studies.

Blood for the experiments was secured from healthy medical students, and the sera therefrom pooled and used immediately. Iodine-131 diiodotyrosine and monoiodotyrosine (Abbott) of known specific activity were then added to separate aliquots of pooled serum and one of the two types of experiment mentioned here conducted. Several pools of serum were used during the study.

In all experiments, two-dimensional chromatography was performed with two different solvent systems. Radioactive areas were then identified by autoradiography, the appropriate areas of the paper cut out, and the radioactivities measured. Iodine-127 concentrations in these areas were determined either directly as DIT or MIT by the ceric sulphate–arsenious acid reaction, or in the case of the dinitrophenylated derivatives, as iodine-127 following combustion in oxygen.

Serum for the isotope dilution experiments was processed by the methods usually adopted in this laboratory[5], and the final extract chromatographed on paper. For dinitrophenylation, the iodotyrosines were first separated from the other iodinated compounds in serum by column or by paper chromatography, and the derivatives then prepared.

In all experiments, saline solutions containing 0·59 per cent sodium chloride, the average of human serum, were used as controls and processed the same as the serum. Full details of the procedures as well as the recovery and other salient data not presented here are being published elsewhere[6,7].

Tables 1 and 2 show the pertinent results. The wide range of individual values shown for the isotope dilution experiments was accounted for by several factors: (*a*) the amount of isotope added to the serum; (*b*) the serum sample used which may affect the chemical reaction used to quantitate iodine-127; (*c*) to a limited degree, the solvent systems. Since greater purification is achieved with the compounds to be dinitrophenylated, this probably accounts for the greater uniformity of the values for this part of the study. Also, the quantitation of stable DIT and MIT is more reliable with the combustion procedure than with the catalytic method used for the isotope dilution study.

Table 1. IDENTIFICATION BY ISOTOPE DILUTION OF IODINE-127-MONOIODOTYROSINE AND IODINE-127-DIIODOTYROSINE IN EXTRACTS OF NORMAL HUMAN SERUM

Solvent system	Isotope dilution (ratio of initial to final specific activities) MIT	DIT
BDA : 2 BA	2·3	*
2 BA : BDA	1·9	5·3
BDA : BAc	3·0	1·7
BAc : BDA	4·1	1·4
2 BA : BAc	5·6	8·6
BAc : 2 BA	2·8	2·1

* Value not determined

BDA, *n*-butanol : dioxane : 2N ammonia (4:1:5)

2 BA, sec.butanol : 1·6 N ammonia (3:1)

BAc, *n*-butanol : glacial acetic acid : water (9:1:2·5)

Table 2. IDENTIFICATION BY DERIVATIVE FORMATION THROUGH DINITROPHENYLATION OF IODINE-127-MONOIODOTYROSINE AND IODINE-127-DIIODOTYROSINE IN NORMAL HUMAN SERUM

Exp. No.	Isotope dilution (ratio of initial to final specific activities) MIT	DIT	Estimated serum content† MIT µg/100 ml.	DIT µg/100 ml.
A	7·5	4·3	1·21	0·48
B	*	7·2	*	0·86
C	14·4	8·9	1·31	1·01
D	*	10·7	*	0·74

* Values not determined.

† Corrected for losses during extraction.

The average concentrations of diiodotyrosine in the original serum before extraction have been estimated from the dinitrophenylation procedure (Table 2). The results agree reasonably well with those originally reported by this laboratory[1] and by Beale and Whitehead[8]. These latter used a double isotope dilution technique although the completeness of the chromatographic separation of the final products has been questioned. About a third of the extractable serum iodine appears to be present as iodotyrosines.

Proof that the compounds considered to be iodotyrosines do in fact contain iodine and hence that the catalytic reaction to measure iodine was not being produced by non-iodinated materials has been furnished by neutron-activation analysis. Both Stark and Knorr[9], and Dimitriadou, Turner, Slater and Fraser[10] have extracted normal human serum and identified the formation of iodine-128 in the iodotyrosine areas of the chromatograms. Their calculations of the amounts of iodine-127 present in serum correspond reasonably well with the original estimates of Werner and Block[1].

Dimitriadou *et al.*[11] later reported that the proportion of iodine-127 iodotyrosines in normal sera and in sera from thyrotoxic patients was less if a resin column was used for the extraction procedure. A similar observation has been made in our laboratory. Dimitriadou *et al.*[11] and Wellby and Hetzel[12], using different methods, also confirmed the fact that iodotyrosines are not formed during extraction from the other iodocompounds normally present in serum or plasma. The latter workers, however, were able to identify iodine-127 iodotyrosines in normal serum only when the serum protein-bound iodine values were at the upper limits of the normal range or were increased by pre-treatment of the donor with thyrotropin.

All in all, the evidence seems almost incontrovertible that iodotyrosines are extracted from normal human serum by the usual procedures for chromatography. The present investigation indicates that both DIT and MIT are involved. The materials extracted from serum and the appropriate standard compounds behaved identically on isotope dilution and derivative formation. It is improbable that two other iodinated compounds have been behaving in so many systems like DIT and MIT and yet are different.

If iodotyrosines are present in extracts of normal serum, the question remains whether they exist as such in the original serum. It is more probable that they do not, in view of the short half-life *in vivo* of several hours for these substances[13]. Rather they may be liberated from other compounds in the course of preparation for chromatography by splitting of bonds which are unstable at *p*H 3–4. This possibility is at present under investigation.

We are indebted to (the late) Dr. Richard J. Block and also thank Dr. Seymour Lieberman for his advice.

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BINDING OF INSULIN TO A MUSCLE CELL MEMBRANE PREPARATION

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RECENT investigations[1-4] suggest that the primary action of insulin is on the permeability of cell membranes. This idea may account for the effects of the hormone in broken-cell preparations as well as in tissue preparations with intact cells if the concept of the cell membrane is broadened to include endoplasmic reticular extensions and the membranes of sub-cellular organelles.

Following on the initial demonstration that insulin was bound to muscle[7,8] and the elucidation of the structure of insulin[9,10], evidence was presented that this binding involves a sulphur–sulphur linkage between hormone and target tissue[11-13]. In the work reported here, skeletal muscle cell ghosts were prepared for use in studying the chemical nature of the attachment of insulin to a relatively pure cell-membrane preparation.

Sprague–Dawley female rats (150–200 g) were fasted for 18–20 h, killed by decapitation, and the femoral musculature removed. The intact excised muscles were then incubated in 15 ml. of phosphate–bicarbonate medium[14] containing 1–2 ml. of ^{131}I-iodoinsulin (specific activity 7·85 and 2·96 mc./mg, Abbott Lot No. *IN* 096–9, No. *IN* 099–22, respectively, and containing 0·05 mg insulin/ml.), at *p*H 7·4 and 37° C. 95 per cent oxygen and 5 per cent carbon dioxide were gently bubbled through the medium. The contralateral limb musculature was used for control studies.

After 30 min incubation, the muscles were removed from the medium, washed 3 times in normal saline, bathed in 10^{-3} M *N*-ethylmaleimide (NEM) for 3 min to bind available SH groups, washed 3 times in 0·01 N hydrochloric acid and then 6–8 times in a solution of 8 M urea in 0·015 N hydrochloric acid, after which the radioactivity in the supernate was at background levels.

The muscle was then sheared and shredded by a modification of the method of Wang[15] and membranes were prepared by a modification of the procedure of McCollester[16]. During the preparation, samples were inspected in polarized light[17] and the washings discontinued when half the muscle cell membranes appeared as hyaline tubules. The membrane suspension was divided into 4 equal parts in 4 separate tubes, and aliquots of the uniformly dispersed membrane preparations were assayed for protein by the method of Lowry[18] and for radioactivity in a well-type scintillation counter.

To free the preparation further of insulin bound in electrovalent linkage, these four tubes were shaken for 1 h at 37° C in a solution of 8 M urea and 0·1 M sodium bicarbonate (*p*H 8) under an atmosphere of nitrogen. After this and subsequent incubations, the membrane suspensions were centrifuged (1,200*g*) and the radioactivity and protein of the membrane and supernate determined. The membranes were then resuspended in 5 ml. of fresh media containing 0·1 M serine, 0·1 M methionine, 0·1 M cysteine or 0·1 M β-mercaptoethylamine in addition to 8 M urea, and 0·1 M sodium bicarbonate (*p*H 8). After replacing the air with nitrogen, the tubes were stoppered and shaken (100 oscillations per min) at 37° C. At 30-min intervals the tubes were centrifuged and an aliquot of each membrane preparation and the supernate derived from it were assayed for protein and radioactivity. The membranes in each of the 4 tubes were then redispersed in fresh aliquots of their respective suspension solutions, the tubes again gassed with nitrogen, stoppered, and the incubation continued with shaking at 37° C.

After 210 min of washing, the specific radioactivity of the membranes (defined as c.p.m./mg membrane protein) exposed to methionine or serine (the controls) reached a plateau at a level of 25 (±2 per cent) of their initial radioactivity. When these membranes were then incubated and washed with either β-mercaptoethylamine or cysteine, their specific radioactivity consistently dropped to a new plateau within 2 h; the residual labelling of the membranes now amounting to 5–8 per cent of the original (pre-incubation) level. Membranes that were washed in thiol compounds from the beginning also dropped to the 5–8 per cent plateau-level within 3 h (Figs. 1 and 2). (We observed that the thiol compounds caused a continuous dissolution of the muscle membrane which was minor but significantly greater than that noted in the control experiments. This phenomenon was, of course, taken into consideration in the calculation of the specific activity. It should be noted that the specific activity of the membrane preparation ultimately rises, indicating solution of unlabelled or relatively lightly labelled protein components.)

To determine the amount of ^{131}I labelling in each chain, an aliquot of ^{131}I–insulin was oxidized with performic acid[19,20] and the chains separated by counter-current distribution[20]. The *A* and *B* chains were found to have 56 and 44 per cent, respectively, of the total radioactivity.

Thus, when insulin was attached to muscle tissue under the conditions already described (and when the remaining free SH groups in the membrane were then reacted with NEM) it was found that, after the removal of the trapped and electrovalently bonded ^{131}I–iodoinsulin, a significant amount of radioactivity (25 per cent of the original level) remained bound to the membrane. This residual radioactivity was further reduced to 5–8 per cent of the original level when these membranes were subjected to thiol reagents, suggesting that this final reduction in membrane-bound iodine-131 was the result of cleavage of an SS bond between hormone and muscle cell membrane protein.

Since insulin possesses 2 S—S bonds linking the *A* and *B* chains, one must consider the possibility that it is these interchain S—S bonds which are cleaved by the thiol treatment. However, this possibility would allow a maximum 44 per cent release of additional radioactivity to be effected specifically by the final thiol treatment. The fact that a 67–80 per cent release of additional radioactivity occurred as a result of the thiol treatment strongly suggests that the initial hormone-tissue reaction involved the formation of an S—S bond between membrane protein and the half-cystine moiety in position 6 and/or position 11 on the *A* chain of the hormone.

Another possibility not excluded is that the *A* and *B* chains are involved in the separate attachment of these chains to the tissue. This alternative seems unlikely, however, because the individual chains are neither easily separated nor physiologically active[21].

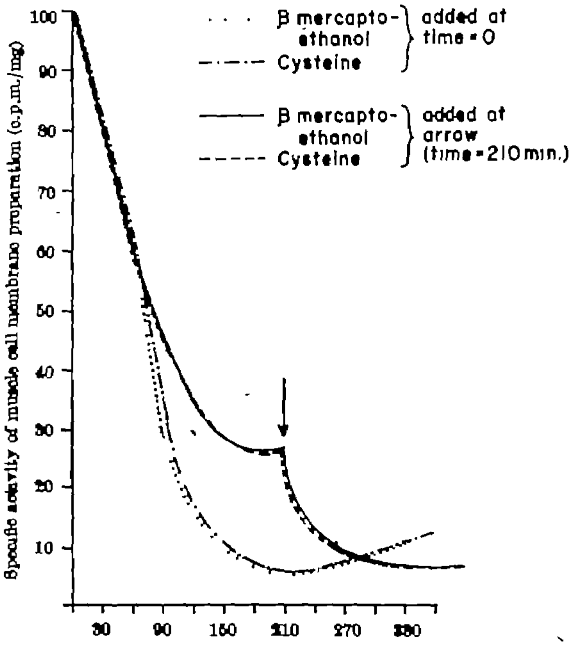

Fig. 1. The specific radioactivity of preparations of iodine-131-insulin-labelled muscle cell membranes at various times after incubation in media containing thiol reagents and in control media. The curves show data averaged from 10 experiments. Note the drop of the membrane specific activity after the addition (arrow) of the thiol reagents. The specific activity has been normalized to a zero time pre-incubation value of 100 c.p.m./mg membrane protein

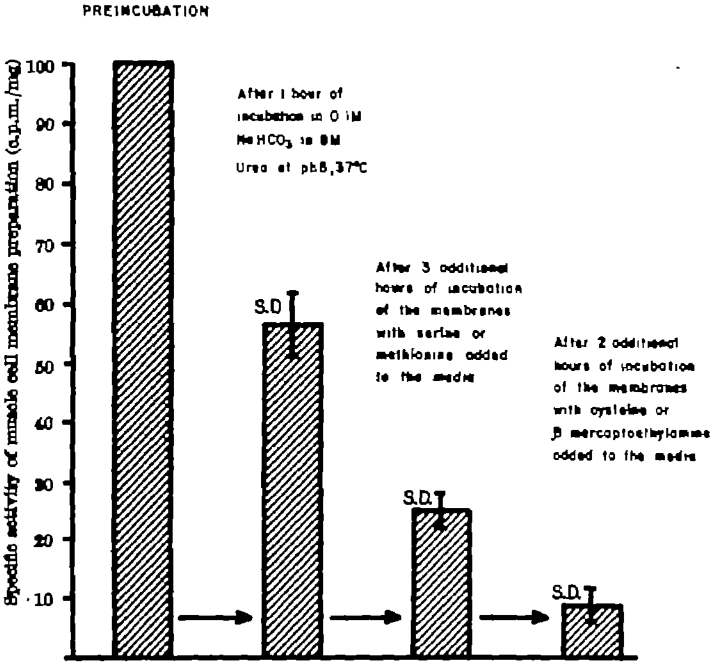

Fig. 2. Residual specific radioactivity of preparations of ^{131}I-insulin-labelled muscle cell membranes before and after incubation in various media. The specific activity has been normalized to a zero time pre-incubation value of 100 c.p.m./mg membrane protein. All incubations were performed with shaking at 37° C in media of 8 M urea in 0·1 M sodium bicarbonate to which the reagents were added

Whole muscle pretreated with 10^{-3} M NEM (to block SH groups) bound approximately 50 per cent less ^{131}I–iodoinsulin than did the control tissue which was not pretreated with NEM. This is in agreement with the findings of Cadenas *et al.*[11]. However, the amount of iodoinsulin bound in both the NEM-pretreated and the control whole muscle varied greatly; the bound iodoinsulin was apparently influenced by the amount of connective tissue present as suggested by Mirsky and Perisutti[22]. The specific activity of membranes prepared from NEM-treated muscle was 70 per cent of the specific activity of control membranes prepared from non-treated muscle. This relationship was maintained throughout the procedures designed to release electrovalently bound radioactivity. However, the thiol compounds reduced the specific activity of the control membranes and the membranes from NEM-pretreated muscle to the same level. This suggests that not all the membrane SH groups which may be serving as insulin-binding sites were chemically or physically available to NEM, but that the NEM which did reach and react with the muscle membrane indeed blocked hormone attachment at 30 per cent of the available sites. In view of the finding that the release of bound radioactivity was effected similarly from membranes prepared from NEM-pretreated and from control muscle, it appears that the mode of attachment of hormone to membrane may be qualitatively unaltered by the partial binding of NEM to the membrane as a whole.

Whole muscle boiled in saline for 3 min produced extremely variable results: the specific activity of the boiled muscle seemed to be in inverse relationship to the amount of connective tissue as determined by histological appearance. When moderate amounts of connective tissue were present, the boiled whole muscle bound approximately 75 per cent more radioactivity, a finding which corresponds to that of Mirsky and Perisutti[22] on whole muscle tissue. The muscle membranes obtained from boiled tissue had a specific radioactivity amounting to as much as 600 per cent of that of membranes prepared from the unboiled control muscle. However, the boiled membranes dissolved readily in the urea–bicarbonate media and even more so in the media containing thiol reagents. During the washing of the membranes to remove electrovalently- and disulphide-bound iodoinsulin, the specific activity of the 'boiled' membranes decreased at twice the rate (200 per cent) of the 'unboiled' control membranes. It appears, therefore, that boiling disrupts the membranes, causing a variably greater non-specific binding and trapping; the heat denatured membrane neither binds nor releases ^{131}I–iodoinsulin in a reproducible manner.

In summary, a relatively pure preparation of cell membranes was isolated from rat skeletal muscle which had been allowed to react with physiologically active iodoinsulin labelled with iodine-131. These membranes were found to be extensively labelled, the radioactivity being bound through both electrovalent and covalent (disulphide) linkages. These findings are consistent with the hypothesis that the action of insulin on membrane permeability is initiated by a thiol-disulphide interchange reaction involving the cyclic disulphide on the *A* chain and sulphydril groups on the receptor protein of the target tissue.

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SPECIFIC METABOLIC REPRESSION OF INDUCED ENZYMES IN *ESCHERICHIA COLI*

By Dr. ELIZABETH McFALL and Dr. J. MANDELSTAM

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INDUCTION and repression are two mechanisms which appear to have been evolved in bacteria to ensure that certain enzymes will be produced at a high rate only when they can function usefully in the chemical environment. The selective advantages of directed enzyme synthesis are obvious, and the fact that at least 100 enzymes may be controlled in this way[1] means that a substantial saving in protein synthesis is achieved. In general, repression is the control mechanism for biosynthetic enzymes concerned in the production of amino-acids, purines, pyrimidines and possibly co-factors. The inhibitory effect of amino-acids on the formation of enzymes involved in their own synthesis was first noted by Monod *et al.*[2], and later termed 'repression'[3]. The best-known example is that in which arginine, in *E. coli*, represses the synthesis of eight enzymes in the arginine biosynthetic pathway[4–6].

On the other hand, many degradative enzymes are controlled by induction, that is, they are not produced unless their substrates or chemically similar inducers are present. On the basis of genetic experiments with the β-galactosidase system the following explanation of induction was proposed (see Jacob and Monod[7]). The gene responsible for inducibility (i^+) causes the formation of a specific repressor (*R*). This prevents the functioning of the gene (*z*) which specifies the structure of β-galactosidase so that little or no enzyme is formed. If an inducer is added it reverses the action of *R*, and the enzyme is produced freely.

There is, however, an additional control on the production of β-galactosidase and other degradative-induced enzymes. This is commonly manifested as the 'glucose effect'; in which glucose prevents enzyme formation even in the presence of inducer. This is not a specific property of glucose; if any source of carbon and energy is made excessive (for example, by restricting nitrogen), it will exert a similar repression[8]. This is consistent with the earlier suggestion[9] that it is not glucose itself but metabolites derived from it which produce the effect.

We have examined the glucose effect to determine whether the repression is due to formation of greater amounts of *R*, or whether there is a separate metabolic repressor. If the metabolic repressor were the same as *R*, it should have the following properties: (*a*) it should not be formed at all by constitutive (i^-) strains because these have lost the ability to produce functionally active *R*; (*b*) its action should be reversed by adding sufficient inducer; (*c*) it should have no effect on the constitutive strains designated O^c. These are still i^+, that is, they still produce *R*, but have undergone a mutation which renders them insensitive to it.

An earlier investigation showed that the constitutive (i^-) strain was in fact susceptible to repression by glucose and all other carbon sources tested, and that even a moderate degree of repression was not reversed by adding large amounts of inducer[10]. Finally, we have confirmed Brown's observation[11] that the O^c mutants are also subject to carbon source repression. On all these criteria, therefore, the metabolic repressor is a separate control factor acting at some point which does not involve the three factors of the Jacob–Monod model: the *i* gene, the inducer or the O^c locus on the chromosome.

The next problem was to determine whether a common metabolic repressor exists for all enzymes showing the 'glucose effect' or whether there is a specific repressor for each. This was examined using three enzymes known to be subject to metabolic repression: β-galactosidase, D-serine deaminase and L-tryptophanase. When a culture of *E. coli* induced for the three enzymes was subjected to nitrogen- or magnesium-deprivation in a continuous-flow apparatus, it was often possible to dissociate the repression of β-galactosidase from that of the other two enzymes. The fact that the two latter enzymes behaved so similarly suggested that they had a common repressor which differed from that of β-galactosidase. This led us to test the effect of pyruvate, which is an end-product of enzyme action both for serine deaminase and for tryptophanase, and of galactose, an end product of β-galactosidase action. Pyruvate proved to be an even more effective repressor for tryptophanase than glucose while galactose was almost inactive (Fig. 1). Serine deaminase was examined in the continuous flow apparatus because a transient toxic effect of D-serine made the results of flask culture experiments difficult to interpret. Again, pyruvate repressed, while galactose did not. On the other hand, galactose was an efficient repressor for β-galactosidase (Fig. 2). In most experiments it was somewhat more effective than glucose itself, while pyruvate failed to repress at all (Fig. 2). Other carbon sources have been tested for repressor action but none was as efficient as galactose for β-galactosidase or as pyruvate for tryptophanase. These results are consistent with the assumption (see above) that glucose acts, not as such, but because it is rapidly metabolized and is thus capable of giving rise to large amounts of intermediary metabolites like galactose and pyruvate, which act as specific repressors for different enzymes. The validity of this interpretation and objections to it are considered in more detail elsewhere.

It appears, therefore, that inducible enzymes producing carbon and energy for the cell are each controlled by two specific repressors. Production of one repressor is controlled by the *i* gene, which determines the property of inducibility or constitutivity. The other repressor controls enzyme production both in constitutive and inducible strains, and seems to be the immediate end-product of the reaction or some closely related substance.

Other instances have been reported in which end-products repress the synthesis of degradative enzymes; see, for example, the effect of succinate on isocitratase production[12].

These findings suggest that end-product repression is a fairly general mechanism for controlling enzyme biosynthesis, and that it operates both in biosynthetic and catabolic pathways. However, in some of the latter induction provides an additional and independent control. The value, in terms of protein economy, of having this second type of regulation can best be appraised by considering what would happen if only one type of repressor were present. If the cells possessed the genetic, but not the metabolic, repressor, the enzyme would be produced in response to the substrate even in the presence of good carbon sources like glucose, which were already supplying sufficient carbon and energy. Conversely, if there were only the metabolic repressor, a state of carbon starvation would cause the synthesis of the previously repressed enzyme, whether its substrate were present or not. The possession of two repressor systems ensures that the enzyme will not be synthesized unless the substrate is present, and even then only if the level of intermediary metabolites has fallen to a low level.

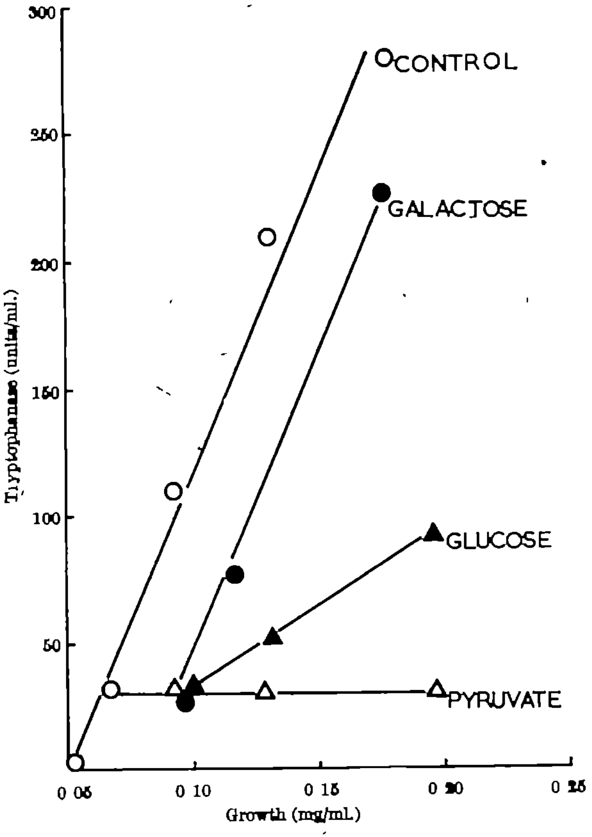

Fig. 1. Repression of L-tryptophanase by various carbon substrates. *E. coli* (K12) growing in 1 per cent glycerol–ammonium salts medium was induced with L-tryptophan (300 μg/ml.). The metabolites (5 mgm/ml. final concentration) were added to different portions of the culture. The rate of enzyme synthesis is given by the slope of the line. One unit of enzyme produces 1 mμmole indole/h from L-tryptophan

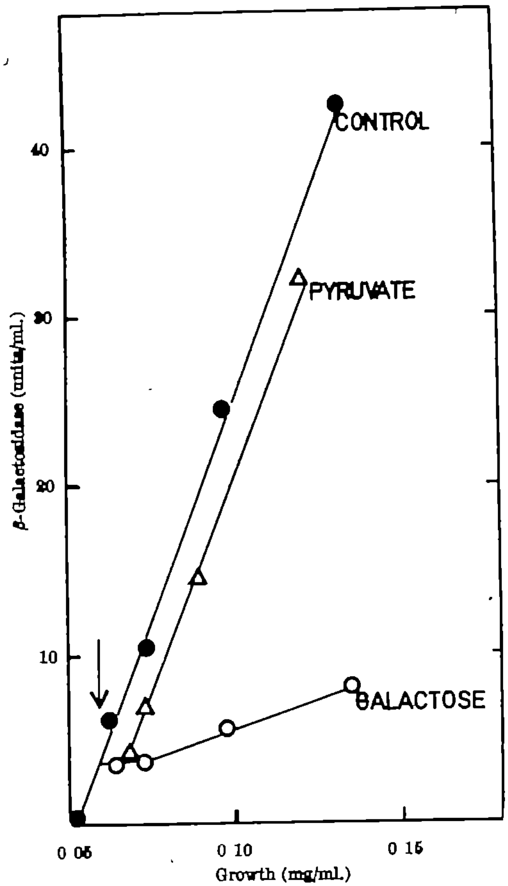

Fig. 2. Repression of β-galactosidase by carbon substrates. Medium and additions were as in Fig. 1 except that methyl-thiogalactoside was used as inducer and additions were made at the point indicated by the arrow. One unit of enzyme hydrolyses 1 μmole of *O*-nitrophenyl-β-D-galactoside/h

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ENVIRONMENTAL CONTROL OF FLOWERING IN *DACTYLIS GLOMERATA* L.

By D. M. CALDER

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DACTYLIS glomerata has been shown to be a long-day plant[1], but before responding to long days it must be fully induced and there is some confusion over the nature of induction in this species. Some workers claim an inductive requirement of cold temperatures only[2], others short photoperiods[3], while still others suggest that both low temperatures and short days are necessary[4]. In a few instances[5,6] complete failure of induction has been reported.

In recent investigations at the Welsh Plant Breeding Station on the environmental control of flowering in *D. glomerata* certain aspects of behaviour have been revealed of which the following are significant: (1) germinating seed and young seedlings do not respond to cold vernalization; (2) plants grown from germination in continuous light fail to head; (3) plants grown in natural long days without any cold or short-day induction can head; (4) overwintered plants head more rapidly in continuous light compared with 16-h photoperiods.

These apparently confusing results may be explained by suggesting that from germination individual plants must pass through a number of developmental stages, each with its own environmental requirement for most rapid completion. The following three stages are postulated for *D. glomerata*: (1) juvenile, (2) inductive, and (3) post-inductive.

Juvenile. During this stage the plant is completely insensitive to environmental conditions which later promote flowering. It is believed to be a stage when the plant is increasing in size and weight but is not making any progress towards flowering. The existence of such a stage could account for the failure of seedling induction.

Inductive. On the completion of the juvenile stage plants enter the inductive stage during which they are sensitive to inductive conditions. It is suggested that darkness is the essential environmental factor for completion of induction, since plants of *D. glomerata* fail to head when grown from germination in continuous light, but will head in long days without exposure to cold temperatures or short photoperiods. It is possible that complete induction is achieved either by exposure to a certain number of hours of darkness, or by exposure to a certain number of diurnal cycles of light and dark. Temperatures during induction may influence the efficiency of the dark exposure.

Post-inductive. This state is achieved on the completion of induction, and during it *D. glomerata* behaves as a typical long-day plant.

It should be possible to test these postulates by growing plants for various lengths of time under continuous light conditions and then transferring them to long days and recording the number of days from germination to heading. Fig. 1 is the hypothetical graph which may be expected from the foregoing postulates when plotting time from germination to heading (Y axis) against length of time in continuous light (X axis). To achieve a graph of this form there must be a number of assumptions: (1) that there is a juvenile stage which, for the purposes of the present argument lasts, say, 4 weeks: (2) that continuous light is developmentally neutral when experienced before complete induction; (3) that darkness alone, as opposed to short-days, is necessary for induction.

In this hypothetical graph the time from germination to heading in plants which have had no exposure to continuous light (vertical line A) is taken up with the

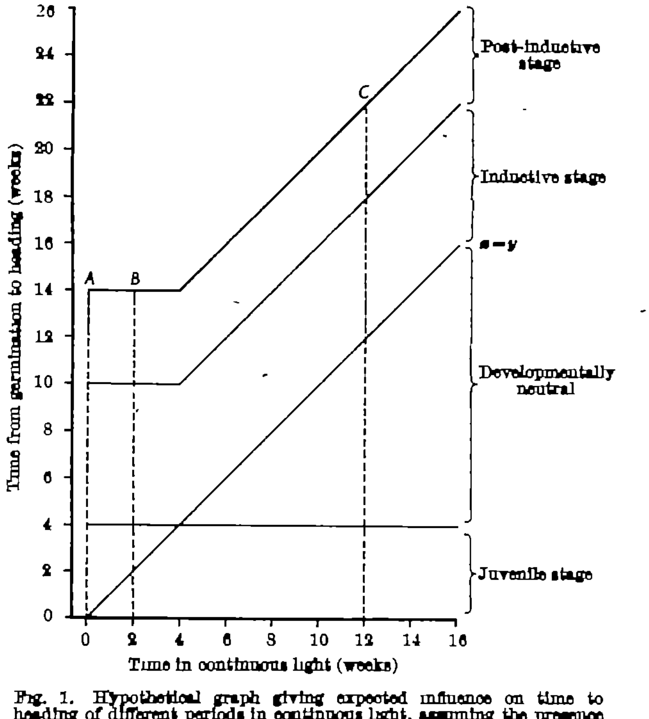

Fig. 1. Hypothetical graph giving expected influence on time to heading of different periods in continuous light, assuming the presence of a three-stage development as outlined

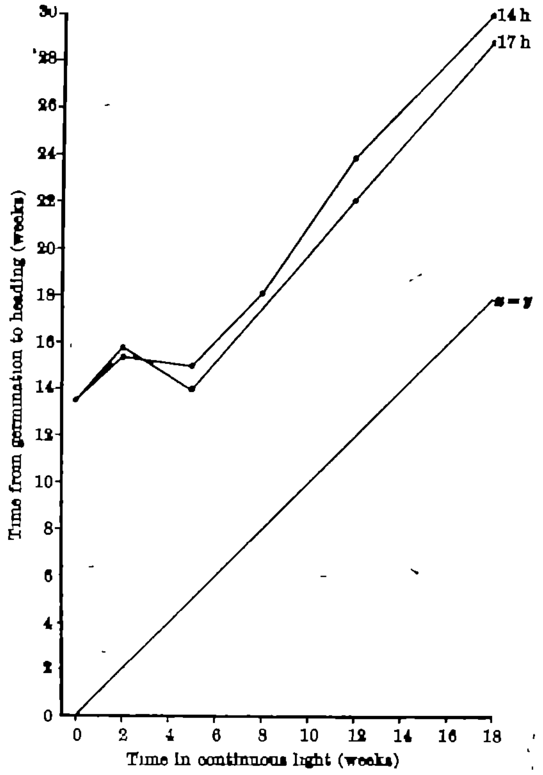

Fig. 2. The influence of increasing lengths of exposure to continuous light on time to heading in *D. glomerata*, variety *S.*143

length of the juvenile stage, plus the time taken for the plant to be fully induced (6 weeks), plus the time necessary for post-inductive development (4 weeks). After a period in continuous light shorter than the juvenile stage (vertical line *B*) the time taken to head is the same as for those plants which had no exposure to continuous light, because the juvenile stage is not completed while the plants are in continuous light and on completion of the juvenile stage these plants are in the inductive environment of long days. Thus for any period of time in continuous light shorter than the juvenile stage there should be no increase in the time taken to head. After a longer period in continuous light, say, 12 weeks, the time taken to head is longer (vertical line *C*). For the first four weeks in continuous light the plants are juvenile. On completion of the juvenile stage these plants cannot become induced since they are still in continuous light; they are increasing in size but are making no progress to flowering. On being placed in long-days they immediately respond to darkness, and head after induction and post-inductive development. Thus for every increase in exposure to continuous light after the completion of the juvenile stage there should be a similar increase in the time taken to head, resulting in a slope of 1. This proportional increase in time will hold, provided that exposure to continuous light does not inhibit later stages of development, provided also that there is no progress to flowering in continuous light, and that neither inductive nor post-inductive development is more rapid in older plants.

An experiment as outlined here was carried out using *D. glomerata* variety *S*.143. After various times in continuous light in a warm glasshouse the plants were placed in either a 17- or 14-h photoperiod. The results are seen in Fig. 2, each point representing the median days to heading of ten plants.

The correspondence between this graph and the hypothetical graph is close and it is evident that with increasing exposure to continuous light above 5 weeks there is a corresponding increase in the time to heading. Exposure to continuous light before the plants are 5 weeks old has apparently no effect. It is also evident that heading is more rapid in 17-h than in 14-h photoperiods.

On the basis of this experiment it is suggested (1) that *D. glomerata S*.143 has a juvenile stage which under the present conditions lasts about 5 weeks; (2) that there is no progress to flowering in continuous light prior to induction; (3) that continuous light before induction does not inhibit induction or post-inductive development; (4) that the time necessary for induction and post-inductive development is not altered by the age of the plant. The net effect of a 17-h photoperiod compared with one of 14 h is to decrease the time to heading, but since at present there is no known way of determining when induction is complete, apart from observing subsequent behaviour, it is not possible to say whether this effect is brought about by influencing the time needed for induction or post-inductive development.

Such a three-stage development has not been described previously in grasses and it may be general for *D. glomerata* since other varieties investigated behaved in a similar manner. It is believed that this rather complex relationship between stage of development and photoperiod could account for the varying interpretations of the nature of induction in this species. These investigations were carried out on the main tiller of each plant; whether later-formed tillers behave in the same way and, in particular, whether they have a juvenile stage has yet to be determined.

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RADIOACTIVITY IN LARGE MARINE PLANKTON AS A FUNCTION OF SURFACE AREA

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THERE are two sources of artificially produced radionuclides at present available to marine plankton in the north-east Pacific Ocean. The source of the first group is the Columbia River water used for cooling the nuclear reactors at Hanford, Washington. Trace elements in the water are activated by the high neutron flux in the reactors and ultimately returned to the river. The other source is the fall-out of fission products from nuclear weapons testing.

Two macroplankters, *Salpa* spp. and *Euphausia pacifica*, have been shown to concentrate zinc-65 (ref. 1), most of which originates at Hanford and reaches the ocean by way of the Columbia River[2]. Euphausiids are also known to concentrate fission products[3]. Because the organisms have a large total surface area, it was thought that adsorption should play a vital part in the uptake of radionuclides. Three experiments were designed to investigate this effect.

The animals used in the three experiments were collected with a 6-ft. Isaacs-Kidd midwater trawl off the Oregon coast, then preserved in formaldehyde, sorted by species, freeze-dried, pulverized, and packed into 15-c.c. spectrometer counting tubes. The samples were analysed with the low background anticoincidence spectrometer[4] at General Electric's Hanford Laboratories, Richland, Washington. Data reduction was carried out with an I.B.M. 1620 computer.

Since adsorption is probably related to surface area, the first experiment was to compare two species of pelagic crustaceans of different size, both from the same sample. *Euphausia pacifica*, which averages almost 20 mm in length, was compared for radioactivity with the copepod *Calanus cristatus*, averaging about 9–10 mm. The radioactivity per gram dry weight was found to be greater in the euphausiid (Fig. 1), despite its smaller surface area per unit weight, which would indicate that other factors are more significant than surface area.

The second experiment, a variation of the first, compared large adults with small adults and juveniles. Euphausiids from one particularly bountiful haul were separated into the two size groups. After fractioning, sub-samples were measured; the small euphausiids averaged 13·3 ± 2·3 mm in length, the large adults averaged 21·6 ± 2·6 mm. Assuming that the shape of the shrimp-like euphausiid approximates two cones (one long, one short) joined at their bases, this difference in length means that the smaller organisms have almost twice as much surface area per unit volume as the larger ones. Since weight varies directly with volume, the smaller euphausiids would have twice the surface area per unit

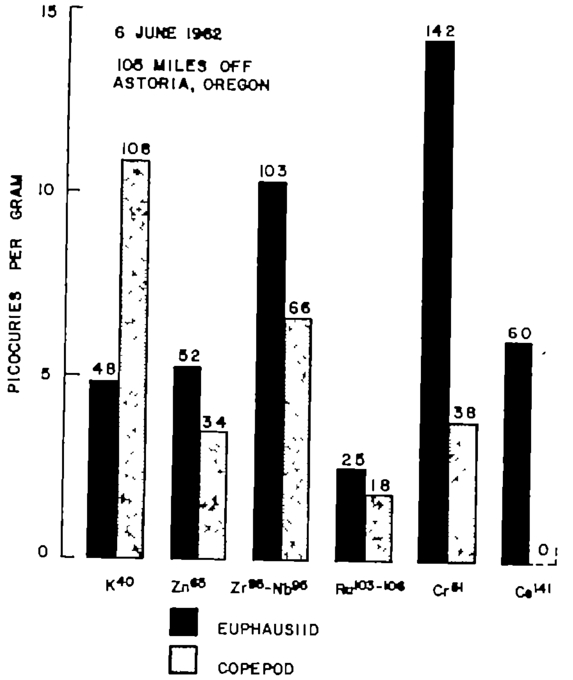

Fig. 1. *Euphausia pacifica* compared with *Calanus cristatus*
The heights of the bars are proportional to the radioactivity in picocuries ($\mu\mu c.$)/g dry weight. Zinc-65 and chromium-51 are from the Columbia River, zirconium-95–niobium-95, ruthenium-103 and cerium-141 are from fall-out, while potassium-40 is the natural radioisotope of potassium. Counting time was 30 min

weight. In general, our results (Fig. 2) indicated that the radioactivity per unit of weight was not greater in small euphausiids. The major exception, chromium-51, may be significant or it may merely be due to the particle size of chromium. However, not only do our techniques yield relatively poor data for chromium-51 (because of the nature of its nuclear decay scheme), but also its form and behaviour in the marine environment are uncertain. Zinc-65 and zirconium-95–niobium-95, on the other hand, are most amenable to the techniques of γ-ray spectrometry.

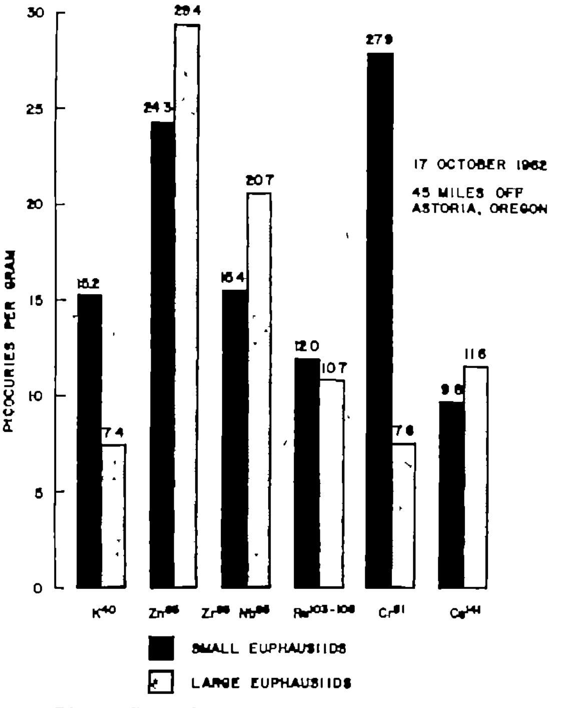

Fig. 2. Comparison of large and small euphausiids (see also legend to Fig. 1)

Pelagic tunicates, *Salpa* spp., were used in the third experiment, which compared the radioactivity of the internal organs with the external parts of the body. Salps are transparent, barrel-shaped animals with a central dark 'nucleus' which contains the digestive tract. In feeding, water is passed through the cylindrical transparent body and through a mucous filter on which particulate material is trapped and ultimately carried to the stomach in the 'nucleus'. Surface adsorption would occur on both the inner and outer walls of the transparent portion, since sea water is in contact with both. In our experiment, the 'nuclei' were removed from the transparent bodies of salps with a dissecting needle, and the radioactivities of the two fractions were compared (Fig. 3*a*). Fig. 3*b* is a similar comparison made of salps taken at a later date.

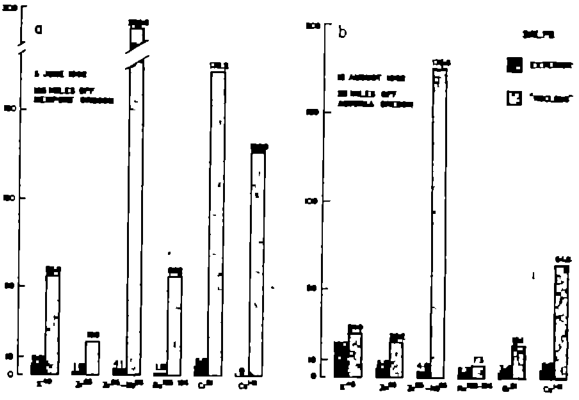

Fig. 3. *a*, Comparison of 'nucleus' and exterior of salp; *b*, similar comparison made at a different time and place (see also legend to Fig. 1)

A comparison of the radioactivity in the 'nucleus' to radioactivity in the transparent outer body (on a gram dry-weight basis) always revealed a higher concentration in the 'nucleus' (Fig. 3*a* and *b*). This is undoubtedly a direct result of feeding. The ratio of radioactivity in the 'nucleus' to radioactivity in the outer body varied, however, depending on the chemical species. Zirconium-95–niobium-95 and cerium-141, which are particulate in sea water[4], were most strongly concentrated in the 'nucleus'. Zinc-65 and potassium-40, which are in ionic form[5], show much smaller concentration ratios. After entering the digestive tract, zinc-65 and potassium-40 are probably somewhat mobile, while zirconium-95–niobium-95 and cerium-141 seem to be restricted to the gut. Preferential washing away of the particulate radionuclides from the surface might also be suspect here. However, Martin[7] has shown that washing with uncontaminated sea water did not remove radioelements (including zinc-65, zirconium-95–niobium-95 and ruthenium-106) from Foraminifera.

All three experiments lead us to the conclusion that, in general, surface adsorption plays a relatively insignificant part in the accumulation by macroplankton of these six radionuclides.

We thank R. W. Perkins of the General Electric Co., Richland, Washington, for the use of equipment and technical assistance.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Occultation of the Crab Nebula by the Solar Corona in June 1962

It has been established that the radiation from Taurus (*IAU 05N2A*) is profoundly modified every year around June 15 when this radiation passes through the solar corona. Extensive observations have been made in the metre wave-length range by the British, Russian, French, and Australian groups[1-4]. It has been found that there is a progressive increase of the angular diameter and a reduction of its intensity as the angular distance of the source from the Sun decreases. This has been attributed to the scattering by a non-uniform corona with randomly distributed irregularities of electron density.

The work reported here was undertaken to examine this effect at much shorter wave-lengths, namely, 25 cm and 10 cm. Preliminary observations were first made in June 1961 with the 84-ft. paraboloid at Sagamore Hill Radio Observatory, Hamilton, Massachusetts. The beam-width of the antenna is 35 min of arc in right ascension and 41 min in declination at 25 cm and 17·5 min of arc (approximately circular) at 10 cm. The observations in 1961 suggested a decrease in intensity of the source with decrease of its angular distance from the Sun. As such, these were repeated in June 1962 with an improvement in the technique of observation and reduction.

The object of these observations was to measure the temperature of the source and its angular diameter as a function of its radial distance from the Sun. This was carried out by taking successive drifts of Taurus in right ascension through the antenna beam. It was realized that, with the beam-width available, the solar contribution has to be carefully computed at least for the days when the source is close to the Sun. As such, at the end of the occulting period, the antenna was successively placed at the same relative right ascension and declination with respect to the Sun for each of the occulting days and a drift pattern was obtained. Each of these records was then normalized with respect to the corresponding solar temperature on the occulting day and the solar contribution was subtracted to give the antenna response due to the source alone. Records taken on June 14 and June 15 at 25 cm wave-length were rejected as the source contribution was less than 10 per cent of the total response.

The relative position of the source and the Sun is shown in Fig. 1 for each of the occulting days at 1700 G.M.T., which corresponded to the observation period. The plot of the intensity and the angular diameter of the source in the right ascension as a function of its radial distance from the centre of the Sun is shown in Figs. 2 and 3. This shows that there is a decrease of the intensity and an increase of the angular diameter of the source as the angular distance of the source from the centre of the Sun decreases. The difference in scattering between June 14 and June 15 at 10 cm, where the angular distance of the source is approximately $5\,R_{\odot}$, can be explained as we note that on June 14 the source was situated on the prolonged solar axis. If we assumed that there are field aligned irregularities then the geometry suggests that there will be more scattering in right ascension.

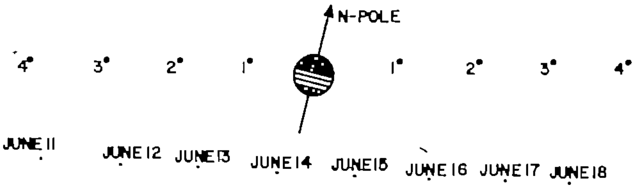

Fig. 1. Position of Taurus relative to the Sun at 1200 D.S.T.

Fejer[5] has made theoretical investigations on scattering of radio waves in a region of mean dielectric constant ε associated with a mean square deviation $\overline{(\Delta\varepsilon)^2}$, the scale of the deviations in any direction r being defined by the auto-correlation function $\rho(r) = e^{-r^2/l^2}$. It was shown that when a radiation of wave-length λ traverses a distance z through such a medium, the energy of the unscattered wave is:

$$Q = Q_0 e^{-B}$$

where

$$B = \frac{\overline{(\Delta\varepsilon)^2}}{\varepsilon^2} \frac{\pi^{5/2}}{\lambda^2} lz.$$

In the case of single scattering $B < 1$, and the angular spectrum of the scattered energy is given by:

$$P(\varphi) \propto \exp\left(-\frac{\pi^2 l^2 \varphi^2}{\lambda^2}\right)$$

However, in the case of multiple scattering $B > 1$, and the angular spectrum is expressed by the following relation:

$$P(\varphi) \propto \exp\left(-\frac{\pi^2 l^2 \varphi^2}{B\lambda^2}\right)$$

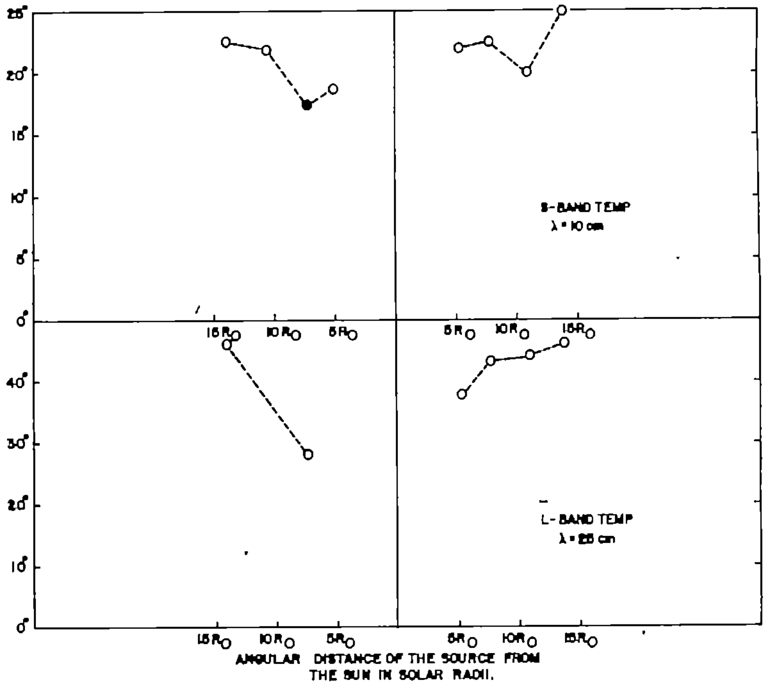

Fig. 2. Variation of the temperature of the source with angular distance from the Sun at $\lambda = 10$ cm and $\lambda = 25$ cm

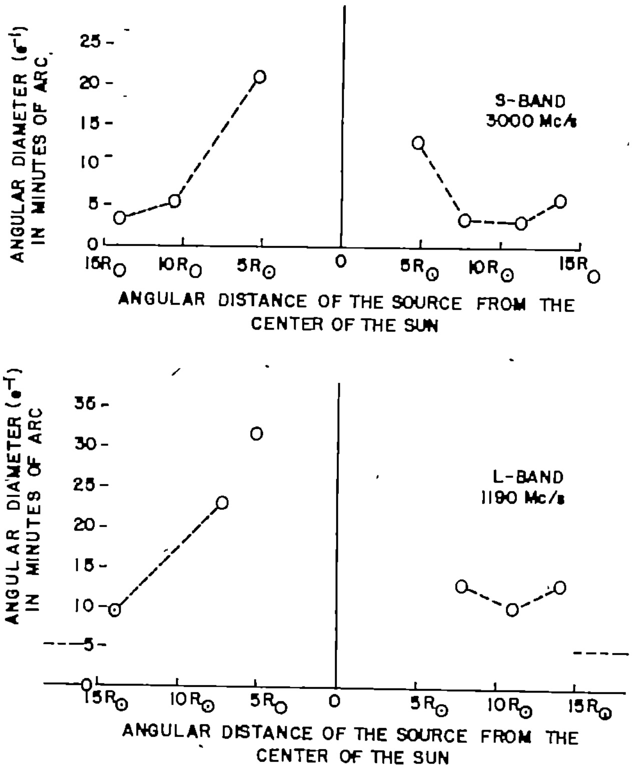

Fig. 3. Variation of angular diameter of the source with angular distance from the Sun at $\lambda = 10$ cm and $\lambda = 25$ cm

Hence the width (e^{-1}) of the angular spectrum is given by:

$$\varphi = \left[\pi^{1/2}\,\frac{z}{l}\,\frac{\overline{(\Delta\epsilon)^2}}{\epsilon^2}\right]^{\frac{1}{2}} \tag{1}$$

Considering the limiting case of multiple scattering when $B = 1$, we get:

$$\frac{\overline{(\Delta\epsilon)^2}}{\epsilon^2}\,\pi^{5/2}\,\frac{lz}{\lambda^2} = 1 \tag{2}$$

Combining (1) and (2), we get:

$$\varphi = \frac{\lambda}{\pi l}$$

or,

$$l = \frac{\lambda}{\pi\varphi} \tag{3}$$

It should be noted that this value of l is the minimum scale of irregularity consistent with $B = 1$. Further, it can be shown that:

$$\frac{\overline{(\Delta\epsilon)^2}}{\epsilon^2} = \frac{\overline{e^4(\Delta N)^2}}{\pi^2 m^2 f^4} \tag{4}$$

where,

$\overline{(\Delta N)^2}$ is the mean square deviation of electron density and e, m, the charge and mass of an electron in E.S.U.

Substituting (4) in (2), we obtain:

$$\overline{(\Delta N)^2} = \frac{m^2 f^4 \lambda^2}{\pi^{1/2} e^4 l z} \tag{5}$$

Hewish[1], assuming that z is equal to the minimum distance between the ray and the centre of the Sun, used equations (3) and (5) to arrive at the value of the minimum scale of irregularity $l = 0{\cdot}27$ km and $(\Delta N) = 70$ electrons/c.c. Considering June 14 data for $\lambda = 10$ cm, when we obtained the maximum scattering with $\lambda = 21$, calculations yield $l = 0{\cdot}52$ km and $\Delta N = 1{\cdot}7 \times 10^2$ electrons/c.c.

We thank Dr. Jules Aarons, chief of the Radio Astronomy Branch of Air Force Cambridge Research Laboratories, for his advice. We also thank Mr. C. Ferioli for help during observations.

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RADIOPHYSICS

Focusing Effects in Spherical Earth Radio Propagation

The flat earth scattering theory[1] which explained many of the observed effects in relatively long-distance propagation of short radio waves has been extended to cover propagation around a spherical earth. The resulting formulæ may be expressed as here, using a co-ordinate system in which the transmitter is located at a pole; Λ is the co-latitude of the receiver, θ is the longitude of the receiver measured from the longitude of the direction of the maximum of the transmitted beam. The power polar diagram of the transmitter is assumed to be of the form $\exp(-B\theta^2)$. The distribution of received power in the direction ξ measured from the great circle direction between the receiver and the transmitter is then of the form:

$$P(\Lambda, \theta) \propto \exp - (L\theta^2 - M\theta\xi + N\xi^2)$$

where-

$$L = AB/D$$

$$D = A + \frac{\overline{n-1}^2 S_1^2 - S_{n-1}^2}{2S_1[nS_1 - S_n C_{n-1}]}\,B$$

$$M = \frac{2AB}{D}\cdot\left\{\frac{S_n C - nSC_n}{nS_1 - S_n C_{n-1}}\right\}$$

$$N = \frac{A}{D}\cdot\left\{B + \frac{2S_1 S_n^2 A}{nS_1 - S_n C_{n-1}}\right\}$$

n = number of equal steps (half-hops) between the transmitter and the receiver, each of angular length λ,

$$S_n = \sin n\lambda$$
$$C_n = \cos n\lambda$$

A = average ionospheric scattering factor obtained from a scattering function of the form $\exp(-A\xi^2)$.

The standard deviation of the spread of the incoming fan of waves may be interpreted as a measure of either the average power distribution or of the probability distribution of obtaining a particular bearing at any time. It is given by:

$$\sigma = \frac{1}{\sqrt{2N}}$$

The extent of the antipodal area[2] can be defined for an omnidirectional transmitter ($B = 0$) as the area within which $\sigma > \frac{1}{2}$ (that is, the standard deviation of the spread is greater than 30°, interpreting σ as sin(spread)). Then, approximately:

$$S_n = \sqrt{\frac{2n}{A}} = \sin^{-1} 10{\cdot}3° \text{ for } n = 16,\ A = 1.000$$

This is the angular size of the radius of the antipodal area which is centred on the point antipodal to the transmitter.

There is another position of interest. The spread becomes independent of B at a distance given by:

$$\tan \Lambda = n \tan \lambda$$

that is, at about 260° angular distance from the transmitter. There the spread is given by:

$$\sigma = \tfrac{1}{2}\sqrt{\frac{n-1}{A}}$$

Along this particular latitude line (measured from the transmitter), the deviation of the main incoming direction from the great circle is zero, irrespective of the beaming direction of the transmitter, or its beam-width or the ionospheric scattering coefficient. This arises since the deviation is given by:

$\xi \propto \frac{M}{2N}$ and M becomes zero at this distance. This is a long path but offers significant possibilities in separating ionospheric scattering effects from effects arising from the fact that the average ionosphere is eccentrically situated with respect to the centre of the Earth. It is proposed that the line at about 260° be called the reciprocal line to the transmitter.

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Effect of the U.S. High-altitude Nuclear Explosion on Radio Wave Propagation

The U.S. high-altitude nuclear test at Johnston Island on July 9, 1962, when explosion occurred at a height of about 200 miles, offered a rare opportunity to observe the effect of the ionization caused by a nuclear explosion on the propagation of radio waves.

The 18·6 kc/s signals of the American very-low-frequency transmitter *NPG*, located near Seattle, Washington (38° 05′ N., 121° 35′ W.), the great circle connexion of which with Salisbury, South Australia (34° 43·9′ S., 138° 38·7′ E.), runs close to the site of the tests, have been monitored together with the 18 kc/s signals of *NBA* in the Panama Canal Zone (08° 22′ N., 79° 21′ W.), the 16 kc/s signals of *GBR* (52° 22′ N., 01° 11′ W.) and the 10 Mc/s signals of stations *WWV* and *WWVH* (39° 00′ N., 76° 51′ W. and 20° 46′ N., 156° 28′ W. respectively).

The beat frequency method, by which the beat between the received frequency and a suitable frequency derived from a stable local source is recorded in order to determine phase variations, has been used for the very-low-frequency signals, while only the signal strength was monitored on 10 Mc/s.

Fig. 1 shows the 10-Mc/s signal strength records of July 8, 9 and 10. The instant of detonation, which according to our timing occurred at 090009 u.t.2 (183009 local time) on July 9, is marked by *H* on the appropriate record. The signal strength dropped suddenly but recovered after about 15 min, to drop again at about 1900 h. After that it rose to a more or less steady value which was lower than that on the previous or following day and which would be expected here at this time of the year and day.

Fig. 2 shows the superimposed phase records of *NPG* during the three days in question. It is unfortunate that

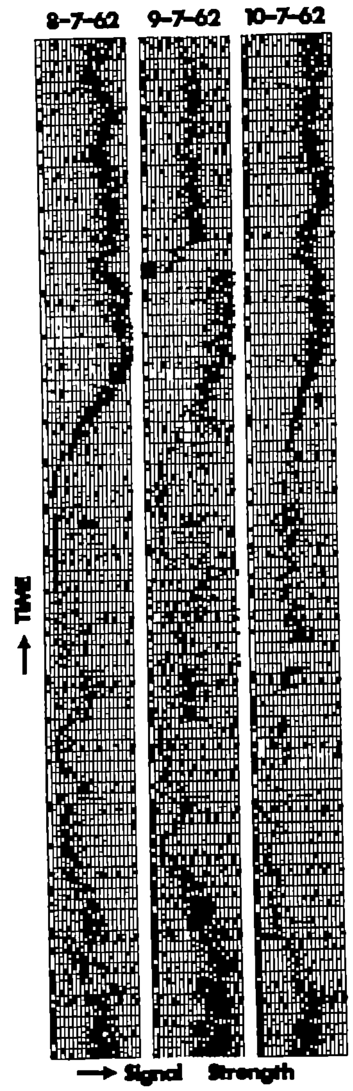

Fig. 1. Signal strength of *WWV* and *WWVH* on 10 Mc/s. Time-scale is Central Australian Standard Time, which is 9½ h ahead of u.t.2

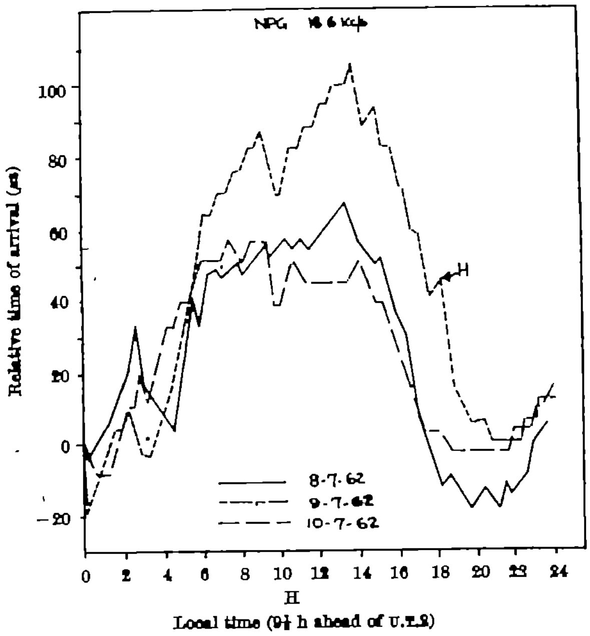

Fig. 2. Relative time of arrival of *NPG* signals

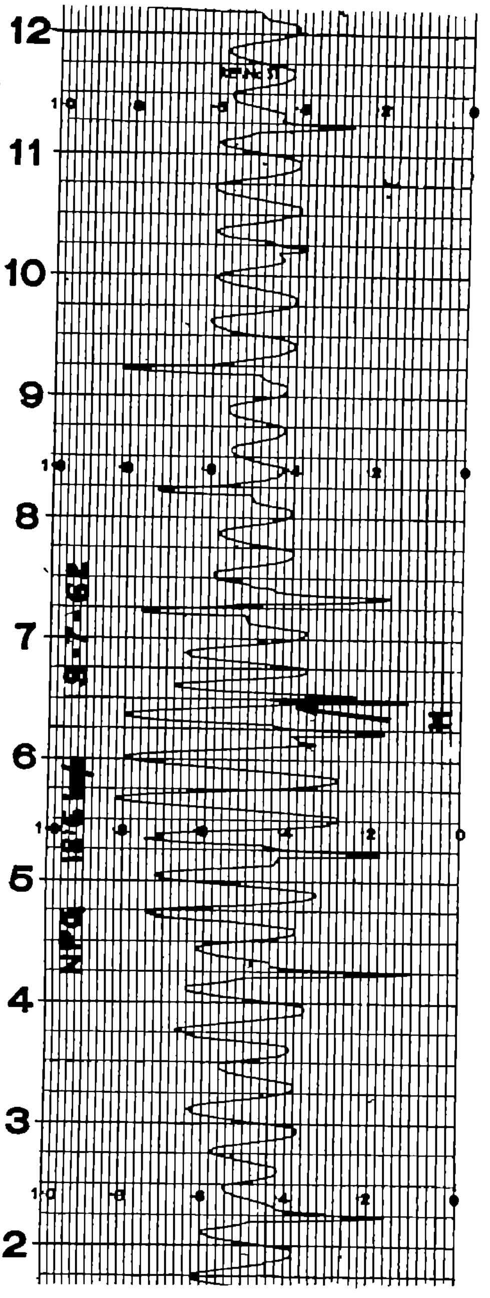

Fig. 3. Beat signal record of *NPG* between 1400 h and 2400 h C.A.S.T. on July 9, 1962

the explosion occurred at a time when the phase shift of the *NPG* signal is normally decreasing rather rapidly so that its effect is not readily discernible.

Fig. 3 is part of the beat signal record of *NPG* for July 9. A marked effect of the explosion on the phase is noticeable. This method of phase monitoring does not follow rapid changes of complete 360° and it is not known, therefore, whether a phase shift of such a magnitude has occurred. The receivers used for the reception of the very-low-frequency signals have a band width of 0·01 c/s approximately.

No effect was observed on the signals of *GBR*.

Fig. 4*a* and *b* show the variation of the relative signal strength of the signals of *NPG* and *NBA* for the three days of observation. It is interesting to note that the strength of the *NBA* signals has been also affected by the explosion although the great circle connecting the site of *NBA* with Salisbury is very far removed from the site of the tests.

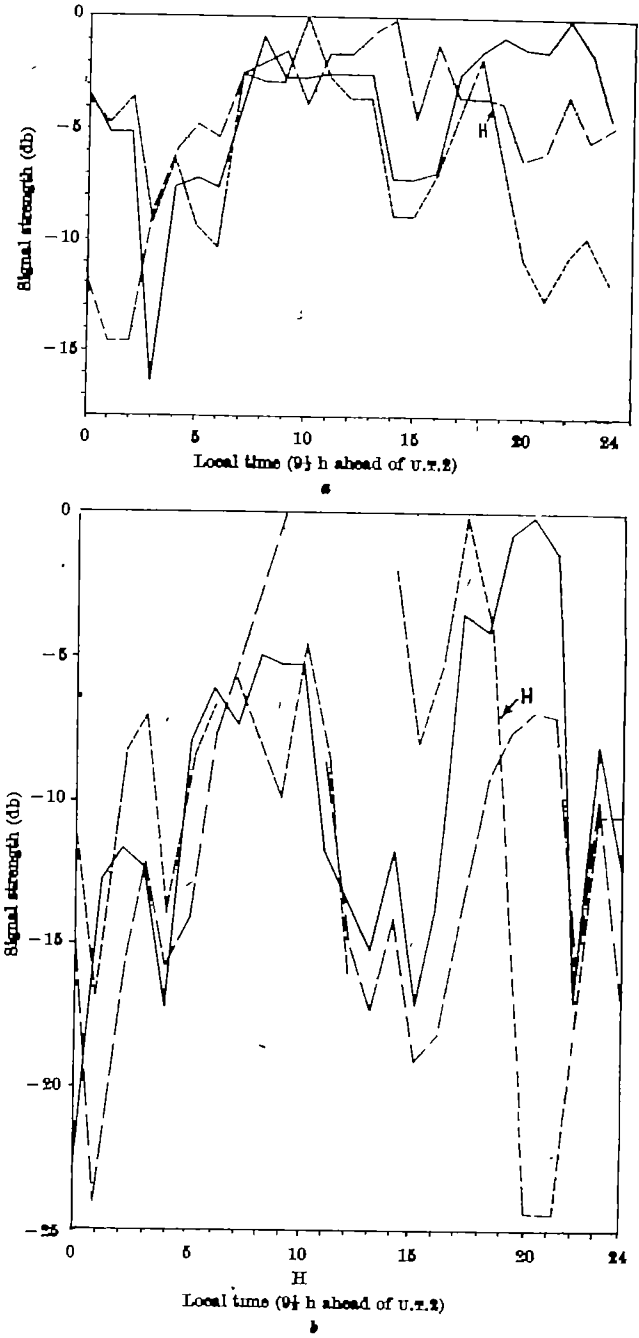

Fig. 4. *a*, Relative signal strength of *NPG*.; *b*, Relative signal strength of *NBA*. ——, July 8, – – –, July 9; —·—, July 10

We thank the Chief Scientist, Department of Supply, for his permission to publish this communication.

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GEOPHYSICS

Magnetic Anomalies over the Mid-Atlantic Ridge

The intensity of the Earth's magnetic field over the Atlantic Ocean has been measured on a number of flights organized by the Department of Mines and Technical Surveys of Canada and also under Project Magnet (U.S.

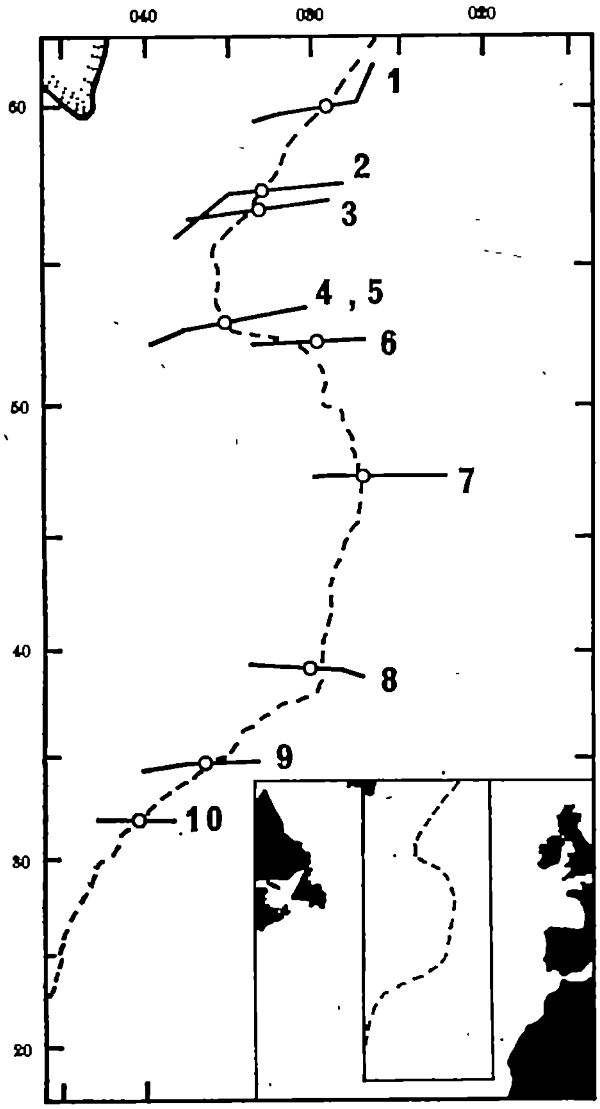

Fig. 1. Tracks of the flights. The heavy broken line is the approximate position of the crest of the Mid-Atlantic Ridge (see text). The circles correspond to the points on the centre line of Fig. 2

Hydrographic Office). Some of these flights passed over the Mid-Atlantic Ridge and the magnetic observations show that there are large anomalies associated with the crest of the Ridge in some crossings, but not in all.

Ewing, Heezen and Hirshman[1] have pointed out that work at sea has shown that the crest of the Ridge has a characteristic anomaly pattern associated with it, and Heezen, Tharp and Ewing[2] have published a profile of the total magnetic intensity across the Ridge, which indicates a positive magnetic anomaly flanked by two negative anomalies. M. N. Hill[3] has shown that the anomalies measured at sea-level in the area around 46° 50′ N., 027° 20′ W. range from about (+300) to (−500) gammas (one gamma is 10^{-5} oersted). Further work by his group in 1960 showed that the anomalies in the area of the median valley of the Ridge at 45° 50′ N., 027° 45′ W. range from about (−500) to (+500) gammas. The magnetic measurements made on the flights of the Department of Mines and Technical Surveys and under Project Magnet were therefore examined to see if anomalies occurred over the Ridge.

The flights were made at heights which varied from 8,000–15,100 ft. above sea-level. Measurements of all the magnetic elements were made but only the total intensity of the field is considered in this communication. Details of the parts of the flights which passed over the Ridge are given in Table 1. The values of the intensity found on the Department of Mines and Technical Surveys flights are values averaged over five minutes of flying time, which represents about forty kilometres in distance. The probable error of a single observation is about ± 50 gammas. The values found on the Project Magnet flights have been reduced from the original continuous strip chart records; the relative error between observations is not likely to be significant. The geographical positions at which the observations were made are likely to be uncertain by several kilometres.

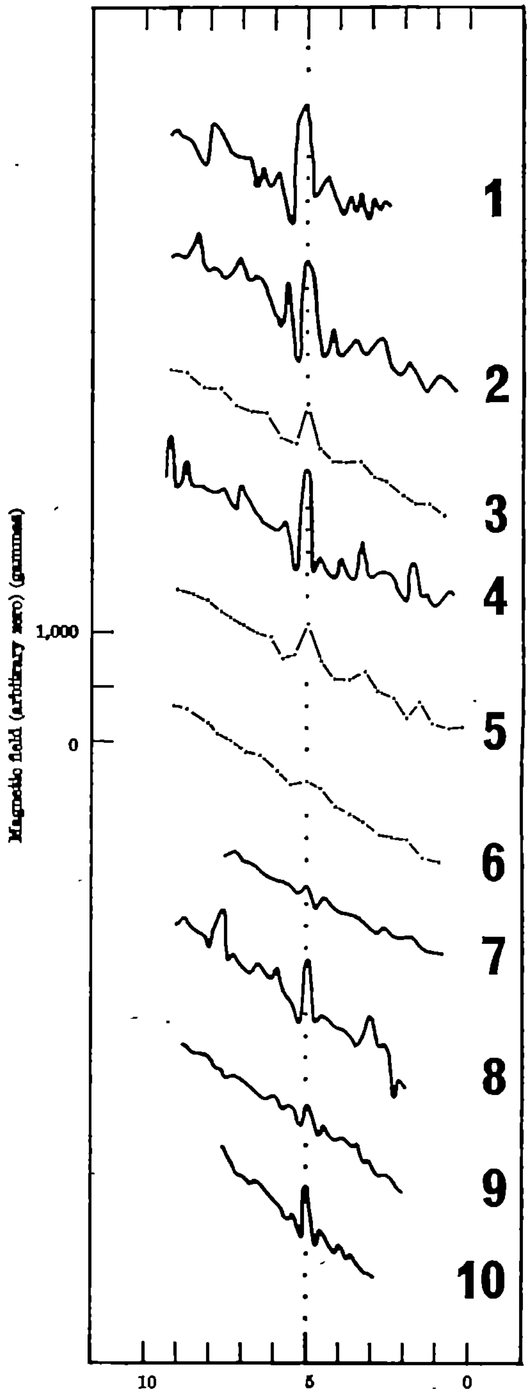

Fig. 2. Total intensity of the magnetic field. The profiles show the intensity of the field plotted against a scale of longitude. The zero values of the field are arbitrary; a scale in gammas is shown on the left-hand side. Numbers correspond to the flight tracks of Fig. 1

Table 1

Track No. on figs.	Dept of Mines and Tech. Surv. or Project Magnet No.	Height (ft.)	Position of points marked by circles
1	*PM* 008	11,000	60° 01′ N. 029° 00′ W.
2	*PM* 89	10,000	57° 35′ 033° 00′
3	*DS* 20 (*B*)	9,000–11,000	57° 02′ 033° 08′
4	*PM* 73	7,000	53° 02′ 035° 40′
5	*DS* 23 (*B*)	8,000–10,000	53° 10′ 035° 12′
6	*DS* 13 (*A*)	9,000	52° 25′ 029° 25′
7	*PM* 106	15,100	47° 24′ 027° 20′
8	*PM* 109	7,000–9,100	39° 08′ 030° 03′
9	*PM* 76	9,000	34° 38′ 036° 20′
10	*PM* 114	8,000–10,000	31° 56′ 040° 24′

Note.—Project Magnet lines marked *PM*, Department of Mines and Technical Survey lines marked *DS*.

The tracks of the flights are shown in Fig. 1, in relation to the approximate position of the central part of the Mid-Atlantic Ridge. This has been plotted from the map prepared by Heezen *et al.*[2] and from the British Admiralty Chart 1904. Track 8 is to the west of the Azores and track 7 passes over the Ridge at about 47° N. Two flights, 4 and 5, are close together and were made independently by Project Magnet and by the Department of Mines and Technical Surveys respectively.

Profiles of the total field along these tracks are shown in Fig. 2. There is a regional trend which has not been removed. The observations have been plotted against longitude and the points which lie on the dotted centre line of Fig. 2 correspond to the circles on the tracks of Fig. 1. There are peaks on the profiles of tracks 1–5 between 60° N. and 53° N., on track 8 at 39° N. and on track 10 at 32° N., and these vary in size from about 200 gammas to 800 gammas. The peaks on the profiles of tracks 1 to 5 and track 10, and one of the peaks of track 8 lie on or close to the central part of the Ridge. A flight adjacent to track 8, which is not shown, also has more than one peak on its profile in the area to the west of the Azores. There are no peaks on tracks 6 and 7 at 52° 25′ N. and 47° 24′ N. respectively. The peaks of tracks 4 and 5 are separated by several kilometres.

These results suggest that a magnetic anomaly is associated with some parts, but not all, of the central portion of the Mid-Atlantic Ridge, which agrees with the observations made by Ewing and others[1] and by Hill[3]. The apparent lack of coincidence of the peaks of the profiles with the exact centre of the Ridge is unlikely to be significant in view of the uncertainties in navigation.

I thank the Department of Mines and Technical Surveys of Canada and the U.S. Hydrographic Office for making their results available to me. This work was supported by the National Research Council of Canada and by the Defence Research Board of Canada.

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OCEANOGRAPHY

Origin of Drift-logs on the Beaches of Hawaii

PACIFIC ocean currents can carry objects to the Hawaiian Islands not only from North America, but also from Western Pacific islands and perhaps from the mainland of Asia. This is our conclusion from a recent examination of logs that have drifted to beaches of all the main Hawaiian Islands.

We were stimulated to pursue this investigation by the forester's interest in the species and forest areas represented in these logs, but we have learned that our findings may interest oceanographers, botanists, and others concerned with the part that ocean currents have played in the development of the Islands.

Most of the logs were from the west coast of North America: Douglas fir, western red cedar, and redwood were most frequent (Table 1). The origin of some of these could be pinpointed by checking brands placed on the logs by west coast timber companies. But some logs were of species native to such far-away places as the Philippines, Japan, and Malaya.

Table 1. SPECIES, NUMBER, AND ORIGIN OF LOGS FOUND ON THE BEACHES OF HAWAII

Species	Common name	No. examined	Probable origin
Pseudotsuga menziesii	Douglas fir	75	W. North America *
Thuja plicata	Western red cedar	27	W. North America
Sequoia sempervirens	Redwood	25	California
Abies spp.	True fir	7	W. North America
Tsuga heterophylla	Western hemlock	4	W. North America †
Chamaecyparis lawsoniana	Port Orford cedar	4	W. North America
Pinus monticola	Western white pine	2	W. North America
Picea sitchensis	Sitka spruce	1	W. North America
Quercus sp.	Oak (red)	1	W. North America
Populus sp.	Cottonwood	1	W. North America
Shorea spp.	Several 'Philippine mahoganies'	6	Philippines
Parashorea bagtikan	'White Lauan'	1	Philippines
Cercidiphyllum japonicum	Kadsura	1	Japan
Dryobalanops sp.	Kapur	1	Malaya, Borneo or Sumatra
Melia sp.	—	1	Philippines or S.-W. Pacific ‡
Agathis sp.	—	1	Philippines or S.-W. Pacific
Albizzia sp.	—	1	Philippines or S.-W. Pacific ‡
Podocarpus sp.	—	1	S.-W. Pacific or Chile
Cybistax donnell-smithii	Primavera	1	Central America ‡

* Five logs carried brand from Gold River, Vancouver Island, British Columbia.

† One log carried brand from Coos Bay, Oregon.

‡ Possibly of Hawaiian origin.

It is quite certain that none of the logs listed in Table 1 arrived other than by drifting. The only logs Hawaii imports are monkey-pod (*Samanea saman*) from Fiji, and her forests grow only a few of the species listed. In all but three cases, Hawaiian forest trees could not produce logs as large as those found. Wood identification of the lesser known species was provided by the U.S. Forest Products Laboratory, at Madison, Wisconsin.

Since logs float very low in the water, they should be little influenced by wind action. The movement of drift logs is therefore probably due almost entirely to ocean currents.

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PHYSICS

Multiple-beam 'Transmission-like' Fizeau Fringes in the Reflexion Interference System

MULTIPLE reflexions between close, nearly parallel surfaces of high reflectivity give rise to the well-known sharpened Fizeau fringes of equal thickness[1]. For two surfaces of equal reflectivity R and equal transmittivity T, neglecting absorption, the successive interfering beams in the transmitted system have intensities $T^2, R^2T^2, R^4T^2 \ldots = T^2 (1, R^2, R^4, \ldots)$. For the reflected system the intensities are $R, RT^2, R^2T^2 \ldots = R, RT^2(1, R^2, R^4, \ldots)$. As shown by Tolansky, the Airy summation of the corresponding amplitudes is valid for non-parallel plates provided certain conditions are met, and we thus obtain the intensity distribution of the transmitted and reflected fringe systems. It is found that, except for the term R

(and neglecting the *factor* R, close to unity for high-reflectivities), the reflected series is identical to the transmitted series. The term R represents the beams directly reflected from the upper surface. If these beams can be eliminated from the reflected system we are left with exactly the transmitted fringe system (reduced slightly in intensity by the factor R). That the usual reflexion fringes (minima on a light field) then become 'transmission-like' (maxima on a dark field) was shown by Lummer[1] for fringes of equal inclination at infinity. Tolansky[2] refers to the effect as "a property that can be exploited in special cases".

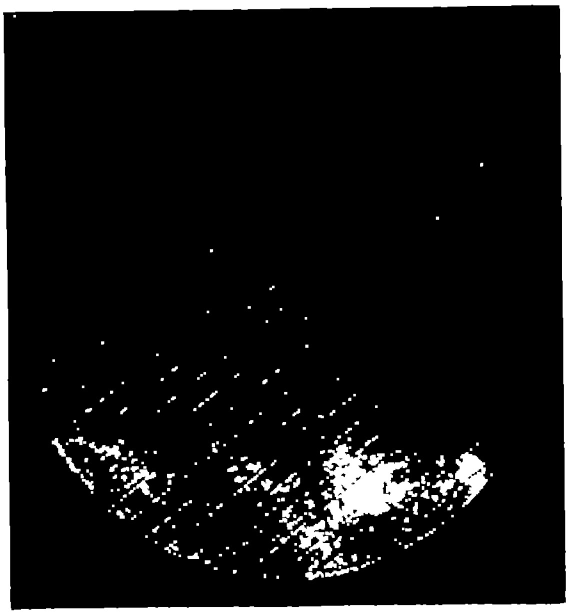

Fig. 1. Multiple-beam Fizeau fringes in *reflexion* for a flat ($R \approx 90$ per cent) against a surface of reflectivity close to 100 per cent. The fringes are 'transmission-like', that is, bright fringes on a dark field, having been reversed by intercepting the directly-reflected beams with a stop In the lower part of the field the directly-reflected beams are not intercepted and normal reflexion fringes result (minima on a bright ground). Ghosts are present due to interception of some of the higher-order reflexions. $\lambda = 5461$ Å

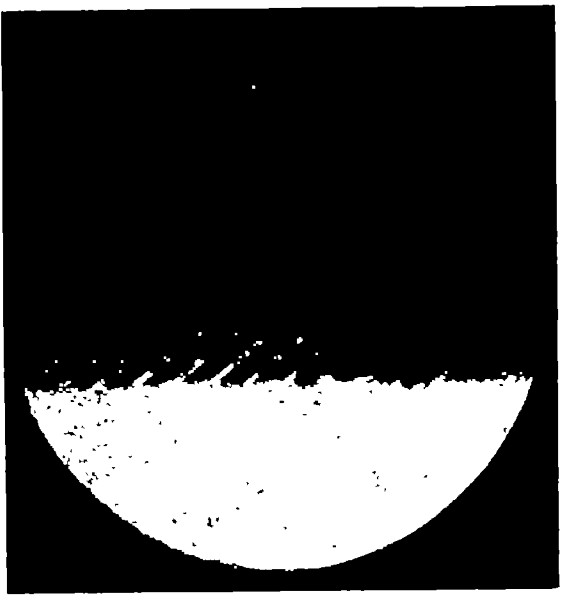

Fig. 2. Multiple-beam *reflexion* fringes produced by a linear wedge, using several wave-lengths (5461 Å, 5770 Å and 5791 Å). The contrast of the normal reflexion fringes is drastically reduced; that of the reversed reflexion fringes is scarcely affected. The bright fringe pairs come from the yellow doublet, the fainter fringes from the green line 5461 Å

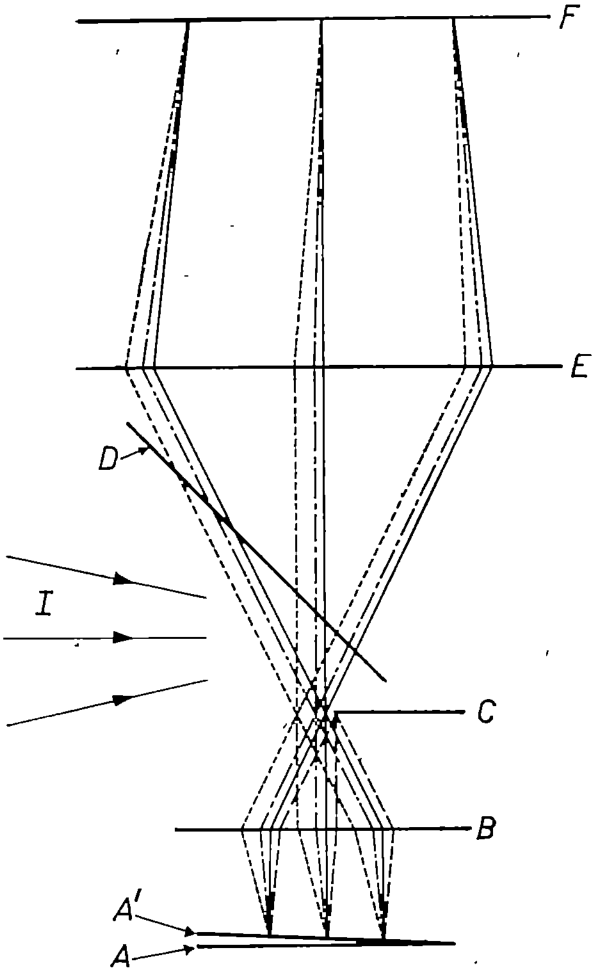

Fig. 3. Production of the reversed reflexion fringes. The diagram shows one of the possible configurations for intercepting the first reflected beams only. AA', interferometer wedge; B, objective lens; C, stop, intercepting the first reflected beams (— — —); D, half-silvered mirror; I, incoming convergent light, brought to focus in the back focal plane of B and producing collimated incident light on AA'; E, projection lens; F, plane on which the fringes are formed. The full lines represent the second set of reflected beams, the lines (- - -) the third set and the lines (---) the n^{th} set of reflected beams

It is possible to obtain this effect with Fizeau fringes of equal thickness. It seems remarkable that this fact has lain dormant until now, for 'reversed' Fizeau reflexion fringes can be useful in the examination of opaque surfaces. The accompanying photographs (Figs. 1 and 2) show topographic Fizeau fringes *in reflexion* for an upper flat of about 90 per cent reflectivity against an opaque highly-reflecting flat surface. Part of the field has been allowed to remain as normal reflexion fringes (minima on light field). Fig. 1 was made with the mercury green line 5461 Å; Fig. 2 with the three wave-lengths 5461, 5770 and 5791 Å.

The conditions under which reversal of the reflexion fringes was observed are outlined in Fig. 3. Collimated light falls nearly normally on the interferometer surfaces via a microscope objective. The light reflected directly from all points on the upper surface all comes to a single focus in or near the back focal plane of the objective. (In the microscope used the objective is used in conjunction with an auxiliary lens, E in Fig. 3, but this lens is not an essential feature in the present context.) The second and multiply-reflected beams all have slightly different directions, depending on the wedge angle, and are imaged at other locations in the back focal plane.

The directly-reflected beams are interrupted by a stop introduced in the back focal plane. Provided the upper surface is suitably oriented, the stop can be adjusted so as to intercept neither the incident light nor any of the multiply-reflected beams. If beams other than the first are intercepted the fringes are broadened, ghosts may develop and contrast may be affected. To obtain sharp reversed fringes over the whole field, critical adjustment of the stop and of the reflecting surfaces is required; it is not always possible to entirely avoid ghost formation or fringe broadening. However, these unwelcome effects may be suppressed or minimized if one is content with fringe reversal over only part of the field. In the interferograms shown there is nevertheless some ghost formation and fringe broadening. (The latter was found to be partly due to pressure broadening of the spectral lines of the source.)

Reversal of reflexion fringes also occurs when the upper flat has a metallic film of low reflectivity. This was first observed by Hamy[4] and further investigated by Holden[5]. This effect is due to the phase defect of the first reflected beams; but the fringes are necessarily broad and for this reason the effect has found little application.

The effect described here can be obtained with extremely sharp, narrow fringes and opens up the possibility of applying to opaque surfaces various techniques hitherto limited to transparent substances. Among these techniques are the use of two or more wave-lengths (for example, for order determination) and the use of low-dispersion crossed-fringes[6]. The very sensitive interference-contrast effect obtained with high dispersion, also described by Tolansky, seems to be impracticable here because the focus of the directly-reflected light is not sufficiently separated from the foci of the multiply-reflected beams when the wedge angle is small (as it must be for high dispersion). However, even with low dispersion, the reversed reflexion fringes appear to be more sensitive to certain surface features than either normal reflexion fringes or normal transmission fringes. For this reason some caution is necessary in the interpretation of details of reversed reflexion-fringe patterns.

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GEOCHEMISTRY

Discrepancy in Age-determination Involving Radiogenic Isotopes of Lead

As is well known, the ages determined by the ratios $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$, $^{207}Pb/^{206}Pb$, and $^{208}Pb/^{232}Th$ are not always identical even for one and the same radioactive mineral. Wetherill[1] explained such a discrepancy as a result of chemical removal of radiogenic isotopes of lead from the mineral.

Where ages calculated from the aforementioned ratios do not agree with each other, it occurs most frequently that the age calculated from the ratio $^{208}Pb/^{232}Th$ is the lowest of all. This is believed to be due to the greater degree of deficiency or loss of lead-208 than those of other radiogenic lead isotopes lead-206 and -207. On the other hand, the acid-wash experiment conducted by Tilton[2] showed that there is a correlation between the isotopic composition of the lead removed by dilute acid and the mode of age discrepancy, and suggested that part of lead contained in the mineral exists in the loosely bound state. Kulp and Eckelmann[3] remarked that, for a given mineral, the isotopes of lead derived from decay of uranium must be in a different environment of lattice from the lead-208 derived from decay of thorium. However, any probable interpretation in terms of a basic parameter has not been successfully presented with respect to the genesis of loosely bound material and the different behaviours of radiogenic isotopes of lead on removal from the mineral.

Process of stepwise disintegrations complicates matters as compared with a process of single disintegration. Thorium-232 and uranium-235, the decays of which terminate in lead-208 and -207, respectively, are roughly similar to each other in the mode of disintegrations, while uranium-238 terminating in lead-206 differs somewhat from them. However, observed facts show that the behaviours of lead-206 and -207 on removal from a radioactive mineral are approximately similar mutually. This fact means that the difference in the scheme of disintegrations is not a leading factor in bringing about the difference in the degree of removal or loss of lead isotope. Therefore attention may be focused on the final stage of disintegrations and the stable lead isotopes which are the ultimate products of the concerned disintegrations.

The process of radioactive decay brings atoms involved in a limited range into the state of excited disorder and causes the ruptures of chemical bonds. But as the excitation of atoms abates with lapse of time, they will tend to compete for re-establishment of chemical bonds. However, the complete reversion to the initial orderly lattice is more or less impossible. That is, the disorder or defects in lattice and the half-satisfied bonds of atoms are thought to remain. It is reasonable to infer that at the stage of re-establishment of chemical bonds an important part is played by the difference in bond-strength of the oxide, because the easiness of re-establishment of bond can be inferred to depend on the bond-strength.

Table 1

	PbO	UO_3	U_3O_8	UO_2	ThO_2
Heat of formation at 25° C (kcal/mole)	52·4	302	898	270	292
Bond-strength (kcal/equivalent)	26·2	50·3	56·1	67·5	73·0

In this connexion the value obtained by dividing the heat of formation by twice the number of atoms of oxygen involved in molecular formula is used as a measure of bond-strength (Table 1). As seen in the bottom row of Table 1, the difference in bond-strength between Th-O and Pb-O is larger than between U-O and Pb-O. Accordingly, lead-208 in the radiation-affected zone where thorium co-exists can be expected to be prevented to greater extent from re-establishing a normally stable bond with oxygen than lead-206 and -207 in the environment where uranium co-exists. In other words, to formation of stable bond of lead with oxygen, the obstruction by thorium is expected to be greater than by uranium. This forms a greater potentiality for lead-208 to be segregated from phase of thorium oxide than for lead-206 and -207 to be segregated from phase of uranium oxide. Thus the structural damage caused by ejection of α-, β-, and γ-rays and the unstable existence of lead make ready the mobilization or removal of this element from host mineral. For a similar reason, on the other hand, it is conceivable that the distributional heterogeneity between thorium and its ultimate product which are contained in a microphase of a mineral is larger than that between uranium and its ultimate product. This can also be the condition parallel with the observed discrepancy.

According to the foregoing interpretation, the oxidation state of uranium bears on the instability of radiogenic lead, for bond-strength of oxide depends on the oxidation state. Very roughly, assuming that the relative instability of radiogenic isotopes of lead stands in the same ratio as the difference in bond-strength, the instability of lead-208 in thorium oxide might be 1·94 times those of lead-206 and -207 in UO_3, 1·53 times in U_3O_8, and 1·13 times in UO_2. These relative instabilities were calculated

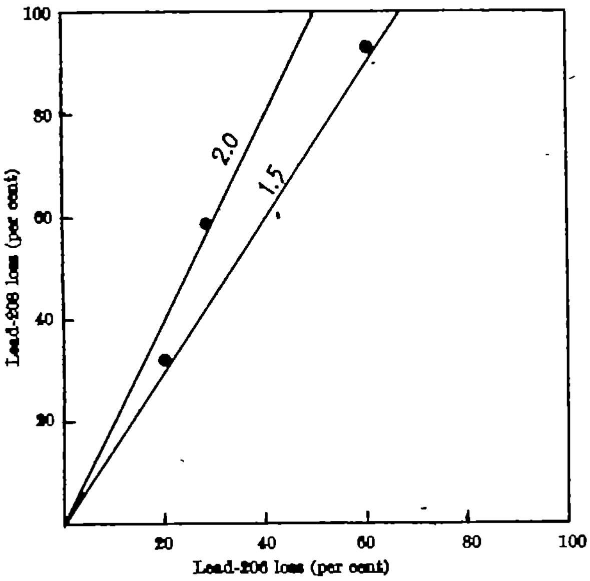

Fig. 1

as follows: for example, 1·94 was derived from (73·0–26·2)/(50·3–26·2).

Also the existence of other elements in the radiation-affected zone may have bearing on the problem under consideration. However, attention might be attracted to the fact that the ratios of lost percentage of lead-208 to lead-206 in specimens participating in Rhodesia pattern scrutinized by Ahrens[4,5] are about 1·5 and 2·0 (Fig. 1).

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Age Determination of some Yugoslav Granites and Granodiorites by the Rubidium/Strontium Method

THE age of four samples of granitic rocks selected by the Establishment for Nuclear Materials (Beograd, Yugoslavia) has been measured by the rubidium/strontium method. All analyses were made on biotite fractions; the usual techniques of isotopic dilution were used for the determinations of rubidium and strontium, utilizing an *Atlas CH*4 mass-spectrometer equipped with a normal collector.

The following results have been obtained:

39025—*Stara Planina*. Granitic rock, coarse grained, with ipidiomorphic structure with a reddish coloration due to abundant large potassium feldspar crystals. The biotite frequently appears with idiomorphic crystals and seems rather fresh.

38772—*Biotite from a granitic rock of the same locality.* Stara Planina granite is considered to be one of the oldest Yugoslav intrusive rocks, and it seems very probable that this granite was formed during the Hercynian orogeny.

49438—*Dunye* (*Pelagonia—Macedonia*). Biotite from a rock intermediate between granite and granodiorite.

The sample comes from a geologically not well-known locality in which the rocks are characterized by a heavy metasomatic action and seem to form a para-series including gneiss, granitic gneisses, migmatites, etc. A pegmatite sample was analysed in a Russian laboratory. The age obtained on the muscovite by the potassium/argon method is 140 million years.

Table 1

Sample No.	Total Rb (p.p.m.)	Total Sr (p.p.m.)	Radiogenic ^{87}Sr (p.p.m.)	$\frac{\text{Radiogenic } ^{87}\text{Sr}}{\text{Total } ^{87}\text{Sr}}$	Age (m.y.)
39025	516 ± 9	9·17 ± 0·13	0·6474 ± 0·0160	0·50	300 ± 11
38772	642 ± 11	5·65 ± 0·08	0·7565 ± 0·0190	0·65	282 ± 10
59052	413 ± 7	8·40 ± 0·12	0·0542 ± 0·0077	0·10	37·3 ± 5
49438	708 ± 13	2·66 ± 0·04	0·3375 ± 0·0096	0·67	131 ± 5

59052—*Iastreboc* (*Central Serbia*). Biotite from a granodiorite with ipidiomorphic structure without any mechanical deformation. Biotite crystals, sometimes lightly chloritized, are very large.

The granodiorite intruded a Palæozoic series causing a strong contact metamorphism.

The results obtained for the samples 39025 and 38772 confirm the Hercynian age postulated for the Stara Planina granite.

The age of 131 m.y. for the sample 49438 agrees with that determined by the potassium/argon method, and is probably related to an ultrametamorphic phase concluded at the end of the Jurassic period.

The sample 59052 gives an Alpine age which agrees with geological information.

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GEOLOGY

The Precambrian Atmosphere

IN his recent book on *The Geological Aspects of the Origin of Life on Earth*, Prof. M. G. Rutten has adopted as his central thesis the postulate that the Earth retained its primæval reducing atmosphere up to 1,500–2,000 million years ago. This conclusion is based on the premise that the gold–uranium ores of the Witwatersrand (South Africa), Blind River (Canada), and Serra de Jacobina (Brazil) are fossil alluvial deposits, in which the notionally detrital uraninite and pyrite are supposed to have survived weathering because the deposits reputedly accumulated under anoxygenic conditions. Nowhere in his work does Prof. Rutten mention that scores of papers have been written by geologists of international reputation to show that these ore-bodies ('bankets') have been formed within the Earth's crust long after the mother-rock sediments were laid down, by the infiltration of hydrothermal metal-bearing solutions. A lengthy review by Prof. J. D. Bernal[1] commends this book to *Nature*'s wide circle of readers as "a welcome fresh light on one of the most exciting problems of the day", the reviewer apparently being unaware that the origin of the Witwatersrand gold deposits has remained one of the most controversial issues of economic geology for more than half a century, and that since the discovery of commercial uranium in these ores the arguments have increased in number, vigour and acerbity. Most geologists will know that both sides of the question are recounted at length in the leading American, French and Russian text-books on uranium geology, as well as in the official publications of the South African, Canadian, and United States Geological Surveys. Other readers, however, should be warned that the validity of Prof. Rutten's conclusions is no greater than that of his fundamental premise, and that this is very debatable indeed.

The arguments against the hypothetical alluvial origin of these ore-bodies are much too numerous to be summarized here, and it will suffice to say that the placerist conceptions can only be sustained by a long series of perversions of chemical and physical laws[2]. It is relevant that in the Mozaan rocks of Swaziland deposited concurrently with the Witwatersrand strata, and in the Lorrain sandstones of Ontario almost contemporary with the Blind River conglomerates, heavy mineral assemblages completely of modern aspect are widely developed, indicating that the mineralogy of the banket ores cannot be explained in terms of abnormal atmospheric conditions. The latest work on the Serra de Jacobina ore-body shows clearly that this deposit is not alluvial, and that the mineralization was probably emplaced hydrothermally[3]. The recently discovered Russian deposits of banket-type gold–uranium ores[4], the locality of which has not yet been revealed, exhibit both sulphidic (pyrite–uraninite) and oxidic (magnetite–brannerite) phases in the same conglomeratic ore-body, demonstrating beyond a shadow of doubt that the mineral assemblages are of hydrothermal origin.

The orthodox geological conceptions on the history of the atmosphere have recently been reviewed by A. P. Vinogradov[5] in his Vernadsky Memorial Lecture, in which he concludes that the primæval atmosphere of methane, hydrogen and carbon dioxide (akin to the gases of meteorites and of terrestrial rocks of mantle origin such as kimberlites, carbonatites and dunites) lasted only a relatively short time. Probably it was lost in the 1,000 million years of the Earth's history before the earliest known rocks came into being. A. A. Polkanov[6] has lately observed that even for these earliest Katarchaean rocks of 3,500 million years ago, Lyell's principle of actualism may not, and should not, be rejected.

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The origin of early Precambrian gold–uranium ore deposits has indeed been a controversial issue. But, as I stated explicitly in my book, I have become convinced that the features acquired by hydrothermal processes are due to younger alterations. These deposits are placers originally. The hydrothermal alterations have been important in enriching the original placers. They may even be at the origin of the money value of a given deposit, and consequently seen as the most important process by an economic geologist.

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CRYSTALLOGRAPHY

Commination of Crystal Nucleation by a Precipitation Method

There are two ways to precipitate sediments of slightly soluble salts, namely, by simple mixing of the reagents or by the homogeneous method, that is, by developing the reagent *in situ* and thus precipitating the insoluble salt homogeneously in the whole bulk of the solution. In both cases no special care is taken with regard to nucleation and the parameter of time.

The formation of a sediment of an insoluble salt comprises two basic processes, the character of which is quite different: a chemical process involving the formation of a new compound of the ions existing in or introduced into the solution, and a physical process of solid phase formation. Different kinds of this general scheme, comprising extreme and intermediate cases, are being examined. The ionic process of chemical compound formation proceeds very fast. The physical process is rather slow and thus—depending on existing conditions—the solid phase is obtained as an amorphous body, that is, of poorly ordered crystal lattice or in the crystalline, well-ordered state.

The principle of the new technique which I have developed is to perform the chemical reaction as slowly as is necessary to obtain a well-ordered solid phase, that is, a crystalline form of the sediment. Under such conditions many new features, unknown until now, have been observed[1–3].

Now further work has revealed that:

(1) Crystallization proceeds on nuclei of symmetric shape and a mass of only 10^{-18} g or smaller.

(2) A great, but limited, number of crystallite shapes are obtained. The shape depends on the nucleus acting as a centre of crystallization.

(3) The different shapes are obtained simultaneously, which shows that just the specific properties of the nuclei are responsible for the shape and not the properties of the medium, for example, the concentration, surface tension, pH, etc. (Fig. 1).

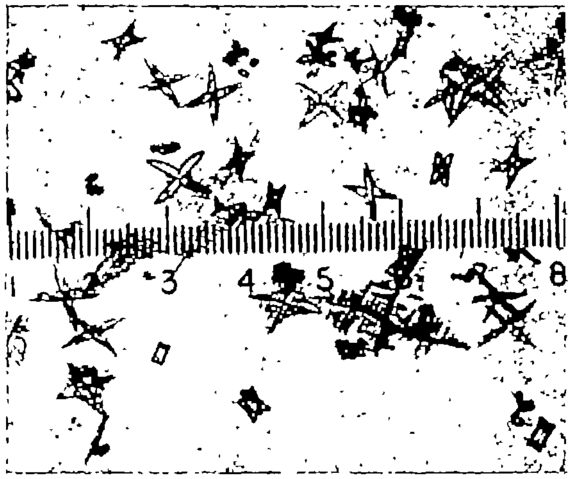

Fig. 1. × 400

(4) While introducing small quantities of foreign ions (the basic precipitate being barium chromate) crystallites of quite different shape are obtained. Because of the lack of separating methods it could not be established until now whether they were other compounds or other crystalline forms.

(5) A fractional precipitation performed with the aid of the new method removes the existing nuclei. On further precipitation the number of crystallite forms diminishes.

(6) In spite of their very delicate structure the crystallites are very stable. It has been stated that sediments do not change while in contact with the mother liquor for a period of even four years, showing that the solid phase has been formed under conditions enabling the building of an ideal crystal lattice, that is, a lattice of lowest energy.

(7) Observations have shown that nuclei formation proceeds possibly on amorphous matter of indefinite composition (hydroxides ?, silica ?).

I thank Mr. L. Trojanowski for assistance in performing the experimental work.

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ENGINEERING

Role of Extreme Pressure Lubricants in Boundary Lubrication and in Metal Cutting

Rebinder *et al.*[1] have examined the effects of surface-active agents such as mineral oil solutions of oleic acid to alter the creep and fatigue character of metals. Observed changes are attributed to the adsorption of oleic acid by the metal, thereby hastening the strain-hardening of the metal's surface.

In metal cutting, the reduction of tool forces is attributed by Rebinder *et al.*[2] to the intensification of strain-hardening in the cutting zone by these agents, while the contributions made to tool force reductions as a result of the lubricant's action to reduce friction is regarded as unimportant[3].

Little attention has been paid by these investigators to liquids of other types, containing labile atoms of sulphur or chlorine, which are commonly regarded as effective metal-cutting fluids and extreme pressure or boundary lubricants. We have examined the effect of such liquids to modify the shear-strength of metals and a clearer picture of the part played by these liquids in boundary lubrication and metal-cutting has emerged from these studies.

The instrument used in these studies is shown schematically in Fig. 1*A*. A metal specimen ¼ in. × ⅜ in. × 2¼ in. made of steel, aluminium, etc., protrudes approximately ¼ in. above the surface of a split block, which clamps it tightly. The specimen yields plastically, as in a hardness test measurement, to form a V-shaped notch when the hardened tool-steel indenter, having $0{\cdot}25$-in.^{2} cross-section, is loaded hydraulically into the centred specimen at 2 in./min, Fig. 1*B*. The indenter's knife-edge overhangs the front and back of the specimen.

Shear-failure of cold-rolled steel takes place, with the production of a tiny chip (see Fig. 1*C*) when the applied load, which is increased continuously, reaches 4,400 lb. ± 2 per cent, provided that carbon tetrachloride saturates the specimen-indenter zone. No such shear-failure occurs in air, however, but only further deformation, as shown in Fig. 2, even at loads as high as 7,000 lb., provided that proper attention is paid to the cleanliness of the tool.

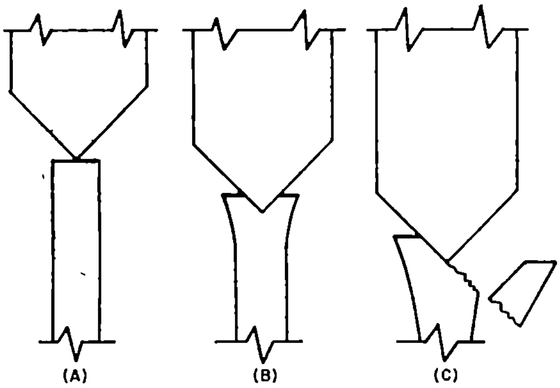

Fig. 1. *A*, Schematic diagram of shear-failure tester; *B*, plastic deformation of a specimen under load; *C*, shear-failure resulting from deformation in the presence of carbon tetrachloride

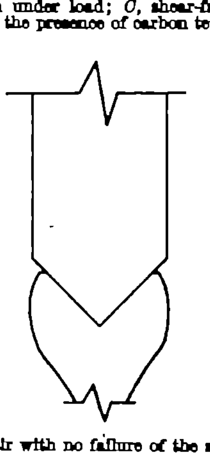

Fig. 2. Deformation in air with no failure of the specimen at 7,000 lb.

It is significant that the deformation of all specimens is the same, whether the deformation is conducted in air or in carbon tetrachloride, for loads up to at least 200 lb. short of the carbon tetrachloride shear-failure load. The deformation is judged both in terms of build-up of load compared with tool advance into the specimen, and from microphotographs of etched specimens that have been deformed.

For this and other reasons it has been concluded that the loading forces required to shear metal which has been strained is determined largely by the interaction between the environment and metal at points of maximum shear-stress. However, the process is not due to an acceleration of strain-hardening, as proposed by Rebinder, and is not due to the formation of a low shear-strength film along the tool-chip interface, as is commonly accepted in explaining reduced forces in problems of this nature which deal with boundary lubrication[3]. Rather, the source of activity arises from the generation of products, such as ferric chloride, formed during creation of fresh metal surfaces in the presence of lubricant, which stabilize micro-cracks generated prior to shear-failure in much the way liquid metals function in the embrittlement of aluminium[4]. This leads to shear at reduced stress-levels. The same mechanism applies in the case of surface-active agents.

Extreme pressure lubricants, therefore, because of their low-stress micro-crack stabilization factor, will reduce the friction, wear, and metal transfer of sliding surfaces by promoting the rapid shear-failure of welded asperities, of the type envisaged by Greenwood and Tabor[5], at reduced stress-levels, before build-up of the junction becomes extensive. Hence, cutting fluids reduce tool forces by promoting the shear-failure of the work-piece ahead of the tool-tip and the shear-failure of numerous welded asperities arising during chip-flow over the tool face, all at reduced stress-levels, with the former effect believed to be the more important.

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RADIATION CHEMISTRY

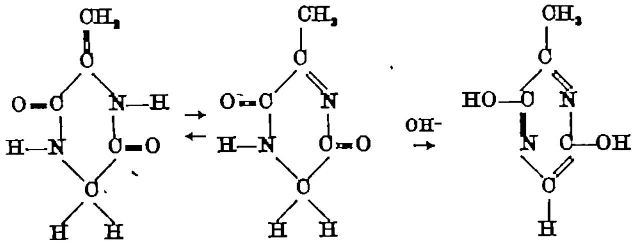
Dehydropeptide Production in the Radiolysis of Solid Peptides: a Radiolytic Synthesis of Pyrazine Derivatives from Cyclic Dipeptides

Among the chemical changes observed on radiolysis of compounds containing the peptide bond are those attributed[1-3] to the production of unsaturated linkages which may be represented in terms of the tautomeric structures, $\sim \mathrm{CON{=}C(CH_2}R) \sim \rightleftarrows \sim \mathrm{CONH{-}C({=}CH}R) \sim$. Compounds containing this type of chemical function have been designated dehydropeptides; such compounds are stable in water yet are readily hydrolysed to yield ammonia and a carbonyl product on heating in dilute mineral acid, $\sim \mathrm{CON{=}C(CH_2}R) \sim + 2\mathrm{H_2O} \rightarrow \sim \mathrm{COOH} + \mathrm{NH_3} + R\mathrm{CH_2CO} \sim$ (ref. 4). The experimental evidence for production of dehydropeptides in the radiolysis of proteins and simpler peptide systems has recently been reviewed[5]. Identification has been based primarily on studies of the formation of ammonia and carbonyl function during hydrolysis of the irradiated peptide system. For example, the hydrolytic production of ammonia and pyruvic acid from γ-irradiated acetylalanine (solid, evacuated) has been found to parallel very closely the hydrolytic degradation of authentic acetyldehydroalanine.

The cyclic dipeptides, such as glycine anhydride and alanine anhydride, also show an analogous post-irradiation production of ammonia and α-keto acid during acid hydrolysis[6]. We note, too, that the dehydropeptide derivatives of these cyclic compounds represent isomeric forms of the 2,5-dihydroxypyrazines. Quite recently, Sorkin[7] has given evidence that the dehydro derivative of glycylalanine anhydride, that is, 3-methylene-2,5-diketopiperazine (prepared by the classical chemical method of Bergmann and Miekely[8]), undergoes isomerization in alkaline solution to give 3-methyl-2,5-dihydroxypyrazine. We report here analogous evidence for the formation of pyrazine derivatives through γ-radiolysis of the amino-acid anhydrides followed by dissolution of the irradiated solids in alkaline solution.

Glycylalanine anhydride was synthesized after the method of Fischer and Scheibler[9]. Glycine anhydride and alanine anhydride were obtained from the Nutritional Biochemicals Corporation. The anhydrides were recrystallized three times from water, dried, and ground to a microcrystalline powder. Samples were evacuated on the vacuum line in 'Pyrex' ampoules for a minimum of 48 h. After irradiation (cobalt-60 γ-rays, $1\cdot7 \times 10^{18}$ eV/g/min), the ampoules were opened to the atmosphere and the contents were dissolved to a concentration of 0·1 M in 0·02 N sodium hydroxide under nitrogen aeration. Complete dissolution required 2–3 min. Changes in the absorption spectra of the oxygen-free solutions were followed differentially (against a 0·1 M solution of unirradiated anhydride) over the range 300–400 mμ.

Solutions of irradiated glycylalanine anhydride (3-methyl-2,5-diketopiperazine) show a rapid increase in optical density with time over the 300–400 mμ range with λ_{max} = 370 mμ. The spectrum is completely developed after about 2 h. Authentic 3-methylene-2,5-diketopiperazine[8] was found to exhibit almost identical spectrophotometric properties. The fully developed spectra obtained with irradiated glycylalanine anhydride and authentic 3-methylene-2,5-diketopiperazine in oxygen-free 0·02 N sodium hydroxide solution are shown in Fig. 1. Appearance of the absorption maximum at 370 mμ is attributed to the formation of the dihydroxypyrazine nucleus (II) via the isomerization reaction:



Ia Ib II

The absorption spectrum of an authentic dihydroxypyrazine (3,6-dimethyl) prepared after Karmas and Spoerri[10] is included in Fig. 1 for purposes of comparison. It is noted that the dihydroxypyrazines in alkaline solution are rapidly destroyed on exposure to air.

Isolation of 3-methylene-2,5-diketopiperazine (*Ia*, *Ib*) from irradiated glycylalanine anhydride has been accomplished. A methanolic extract of the irradiated solid was transferred to filter paper and chromatographed in parallel with authentic 3-methylene-2,5-diketopiperazine; butanol saturated with water was used as the developing liquid. The authentic methylene derivative is readily detected at $RF \simeq 0\cdot4$ under ultra-violet illumination; the irradiated glycylalanine anhydride also gives a strongly absorbing component at this RF value. The two areas were separately eluted and examined spectrophotometrically in oxygen-free 0·02 N sodium hydroxide. The characteristic growth in the 300–400 mμ region was observed in both cases and the final curves were essentially identical to those given in Fig. 1. The yield of 3-methylene-2,5-diketopiperazine from glycylalanine anhydride at the lowest dosage studied (5×10^{20} eV/g) corresponds to a G value of ~ 0·55.

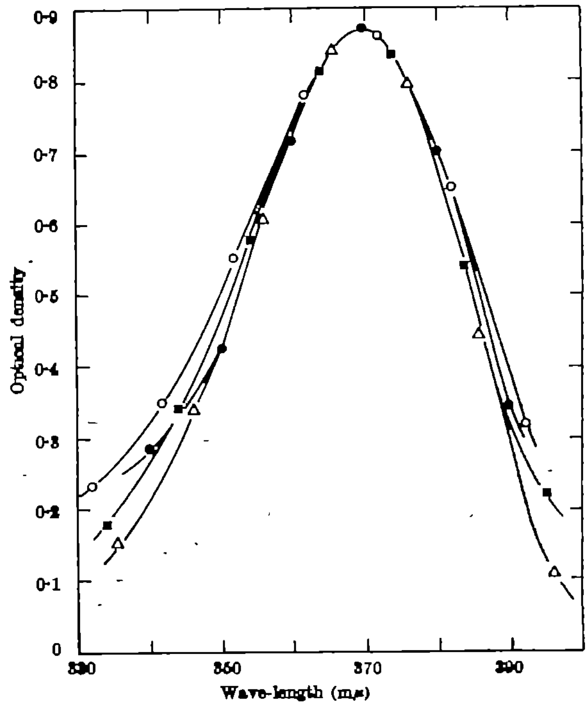


Fig. 1. Absorption spectra of irradiated cyclic dipeptides and authentic dehydropeptides in 0·02 N sodium hydroxide. ○, Irradiated glycylalanine anhydride; △, irradiated glycine anhydride; ■, authentic 3-methylene-2,5-diketopiperazine; ●, authentic 2,5-dihydroxy-3,6-dimethylpyrazine

The results obtained with alanine anhydride appear to be somewhat more complicated. In this case the development of the absorption curve is accompanied by a gradual shift in λ_{max} from 370 to 360 mμ. However, the spectra obtained within 15 min of the time of dissolution coincide with that given by authentic 2,5-dihydroxy-3,6-dimethylpyrazine.

Solutions prepared from irradiated glycine anhydride show the characteristic dihydroxypyrazine spectrum and on standing there is no detectable shift in λ_{max} from 370 mμ to shorter wave-lengths. However, glycine anhydride differs from both glycylalanine and alanine anhydrides in that the absorption spectrum shown in Fig. 1 is fully developed within the 2- to 3-min period required for dissolution. This behaviour may be interpreted as evidence for rapid isomerization of dehydroglycine anhydride, which must necessarily have only the endocyclic form as given by structure *Ib*.

Finally, we should like to comment on the fact that all three solid anhydrides develop a yellow coloration which is visually detectable at radiation dosages as low as ~ 5×10^{18} eV/g. The effect is most pronounced in the case of glycine anhydride. Coloration is not appreciably diminished by exposure to oxygen and remains visible in water extracts of the irradiated solids. This coloration, then, cannot be ascribed to an optical absorption by free radicals or ionic transients. It is noted that the authentic dihydroxypyrazines are yellow in the solid state and, although we have made only preliminary spectrophotometric studies of the irradiated solid anhydrides, the available evidence suggests that isomerization of dehydropeptides occurs to some extent in the solid state. A mechanism for the radiation-induced isomerization of the ~CO—NH~ linkage in solid peptide systems has recently been described[6]. A detailed report of the various aspects of the radiation chemistry of the cyclic dipeptides will be forthcoming.

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CHEMISTRY

Binding of Xenon to Halogens, to Alkali Metals, and to Itself

THE existence of stable XeF_4 and XeF_2 molecules has recently been demonstrated[1]. A description of the binding and molecular geometry using the conventional electron pair model has been proposed[2]. Twelve of the XeF_4 electrons are assumed to be distributed in four electron pair bonds and two sets of lone pairs. This model is based on analogies with the chemistry and geometry of a large number of inorganic compounds. Simple chemical analogy must likewise predict that molecules such as $Xe(Cs)_4$ or $Xe(Rb)_4$ will not exist. Be_2 offers a partial analogy to Xe_2 and implies that the latter molecule will not be bound.

It is also important to understand XeF_4 in terms of approximate solutions to Schrödinger's equation, and an explanation based primarily on our knowledge of the electronic structure of isolated atoms and ions has been given[3]. The xenon fluoride bond is attributed to the atomic distortion[4] produced by electron correlation and the resultant opportunity for a xenon electron of one spin to partially fill the outer shell of a fluorine atom to a slightly greater extent than its partner of opposite spin.

However, in many quantum mechanical problems we find an inherent symmetry between systems possessing closed shells plus one electron and those possessing closed shells minus one electron, and thus we must examine the binding of $Xe(Cs)_4$ and $Xe(Rb)_4$ relative to that in XeF_4. In addition to numerous atomic calculations there exists one *ab initio* molecular calculation which can aid our discussion. This is a wave function for HeH determined by Harris[5], and we may use it as a model for the binding of xenon to an alkali metal atom. His wave function is particularly important because it was carried out by the full permutation group method, thus allowing different orbitals for different spins and building in the correlation induced orbital distortions that have been used to explain binding in XeF_4 (ref. 3).

Harris's HeH calculation shows no binding and we can interpret this through the properties of the constituent atoms. First, the noble gas atoms will have absolutely no electron affinity—even though there is a slight correlation induced separation of α and β spin there can be no stable orbit for an additional electron—so that the ease with which an alkali metal atom loses an electron has no influence on potential binding. Second, the electron affinity of both hydrogen and the alkali metal atoms is considerably less than fluorine. On the other hand, it might be argued that the unfilled *s* shell of hydrogen could provide as favourable a potential energy environment for a rare gas electron as does fluorine. This reasoning is incorrect, however, since not only is the electron affinity less but also the hole in the fluorine has a *p*-like angular dependence and the partially unpaired xenon electron which is most available for bonding also has a *p*-like angular distribution. Similarly, Xe_2 cannot exist because xenon shows no tendency to accept an electron.

One of the principal purposes in presenting this article is to point out the paucity of detailed theoretical knowledge available on the electronic structure of even small molecules, and short of very elaborate and extensive *ab initio* calculations (which are just now on the outer edge of feasibility for XeF_4 and XeF_2) it is not possible at present to give a thoroughly convincing theoretical prediction of inert gas compounds. In hindsight it appears that the geometry of XeF_4 is the same as ICl_4^- and thus empirical or semi-empirical model calculations bring out and tend to support the similarity in electronic structure of these compounds at their equilibrium separation[6]. However, there does not now exist any really adequate connexion between the effective Hamiltonians of the simple model schemes (such as the simple LCAO MO method) and direct solutions of Schrödinger's equation. Particularly at larger atomic separations, the xenon–fluoride bond involves subtle correlation effects and the partial breakdown of the simple idea of closed shells which are not directly represented in calculations with the simple models. Certainly a major reason for the lack of extensive investigations into inert gas compounds has been the overwhelming success of the Periodic Table based on free atoms along with the concept of closed shell configurations in organizing chemical experience.

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Breeding of Nuclei

IN 1911 Miers[1] wrote: "I have many times noticed that when the appropriate crystal is introduced into a supersaturated solution, which is not strong enough to crystallize spontaneously, it may cause crystals to grow not only in actual contact with itself, but at some little distance in its neighbourhood". McCabe[2] has also discussed this subject: other references are rare. It is proposed to refer to this phenomenon as 'breeding' of nuclei. We have recently studied this question experimentally, using aqueous $MgSO_4 7H_2O$, and have concluded that various categories of breeding may be observed.

(1) If a crystal from a stock bottle is dipped into an under-cooled solution, a considerable number of small crystals appear after only a few seconds, usually on the bottom or surface of the solution. The breeding in this case is believed to be due to detached crystallites present on the surface of the crystal, which were produced either by rapid evaporation of solution when the crystal was dried, or by mechanical impact. This form of breeding will be called 'initial' breeding.

(2) If however the crystal, after removal from the bottle, is pretreated by growing at a low under-cooling (say 1° C)

and is then transferred directly to another solution, the phenomenon observed in (1) no longer occurs. But if the latter solution is strongly under-cooled (> 7° C) and if it is stirred, needles tend to grow out of the ends of the crystal, and these break off from time to time, and grow independently. This effect we call 'true' breeding.

(3) At the moment the aforementioned needles break off there is often also produced a shower of very small crystallites. This we call 'splinter' breeding.

(4) If the crystals growing in a stirred solution are free to collide with each other or with the walls fresh nuclei are very easily produced, even at low supersaturation, and with relatively gentle collisions. This we call 'attrition' breeding.

Results obtained with aqueous potassium bromide were generally similar to the above, but differed in some details. Fuller results will be published elsewhere.

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Conduction in Nucleic Acid Components

In a recent paper[1] DNA was shown to possess a small semiconductivity in the dry state, the specific conductivity following the equation:

$$\varkappa = \varkappa_0 \exp(-\Delta\varepsilon/2kT),\ \Omega^{-1}\text{cm}^{-1}$$

with $\Delta\varepsilon = 2{\cdot}42$ eV, $\log_{10}(\varkappa_0\ \Omega^{-1}\text{cm}^{-1}) = 3{\cdot}4$. It was suggested that the conductivity was associated with the π-electron overlap of the paired bases, adenine–thymine, and guanine–cytosine. In the Crick and Watson double-helix for DNA structure these base pairs are arranged one on top of the other, with an interplane spacing of 3·4 Å, similar to that found in graphite[2]. A similar result was found for the electrical conductivity of RNA, which may now be related to its high content of double-helix structure[3]. We have examined the solid-state electrical conductivity of some of the component structures of the nucleic acid, using d.c. with the polycrystalline specimen compressed between nickel electrodes at 80 kg cm^{-2}, in a vacuum of 10^{-5} mm mercury. The bases adenine, thymine, guanine, cytosine and uracil were first examined. These gave very low conductivities, around $10^{-15}\ \Omega^{-1}\text{cm}^{-1}$ at 400° K, but sublimation (even in a nitrogen atmosphere) and thermal decomposition precluded the establishment of accurate Δε values. The nucleosides had conductivities of 10^{-11}–$10^{-13}\ \Omega^{-1}\text{cm}^{-1}$ and the nucleotides 10^{-7}–$10^{-13}\ \Omega^{-1}\text{cm}^{-1}$ at 400° K. The results are listed in Table 1.

Table 1. Nucleosides and Nucleotides

Substance	Δε, eV	$\log_{10}(\varkappa_0, \Omega^{-1}\text{cm}^{-1})$
Adenosine*	4·5	15
Yeast adenylic acid†	1·8	2·7
Muscle adenylic acid†	2·0	2·0
Adenosine triphosphate (DiNa, DiH salt)	2·0	2·5
Adenine phosphate	2·0	3·3
Uridine*	5·2	22
Uridylic acid†	1·3	0·6
Guanosine*	2·1	2·6
Guanylic acid†	1·5	1·8
Thymidine*	4·7	17·4
Cytidine*	4·9	19·4
Cytidilic acid†	2·2	1·6
Riboflavin	2·4	1·6
Riboflavin-5-phosphate (sodium salt)	2·2–2·3	5·0

*Denotes nucleoside †Denotes nucleotide.

We have to compare the following Δε values: (*a*) base + ribose (nucleoside) 4·5–5·2 eV; (*b*) base + ribose + phosphate (nucleotide) 2·0–2·2 eV; (*c*) RNA, DNA 2·4 eV.

It has been suggested that Δε for DNA corresponds to excitation of π electrons to the first excited state π→π* (or n→π*) followed by tunnelling of electrons and holes at right angles to the planes of the bases down the axis of the double helix[1]. This mechanism is made possible by the overlap of π-orbitals between the parallel-stacked bases[4], which stacking does not occur apparently in the nucleosides or nucleotides. It is suggested that to obtain electron tunnelling between the widely spaced bases in the nucleosides excitation of π electrons to the second excited state is necessary, π→π**. In nucleotides, in the specific case of cytidilic acid, the bases are connected by hydrogen bridges via phosphate groups[5]. If generally true for nucleotides, this may explain the Δε of 2 eV, since again the π→π* excitation would be adequate for electron tunnelling. Clearly, there may be other possible mechanisms for bridging of charge-carriers since guanosine has Δε = 2·1 eV, and riboflavin 2·4 eV, and an explanation of these low values, similar to the nucleotides, must await information on their crystal structure.

On band theory $\varkappa_0 \sim 4\mu$, where μ is the average mobility of electrons and holes. On this basis the observed values of μ are comparable for DNA and nucleotides, but the values for nucleosides, as for small aromatic molecules[6], are much too high to be interpreted in this fashion, and an alternative to band theory must be sought in such cases.

These substances obeyed Ohm's law accurately up to 3,000 V/cm, deviations at this voltage being adenine 5 per cent, uridine 7 per cent, thymidine 2 per cent, riboflavin-5-phosphate 5 per cent. This is a clear indication that we are not dealing with space-charge limited currents[7,8]. This interesting fact, which also holds for the proteins, also of very high resistivity[9], is not explicable on band theory.

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Helix-coil Transmission and Electronic Conductivity of the Amylose–Iodine Complex

The first linear polymer of biological origin which was shown to exist in a helical configuration was amylose, the linear polymer of glucose. This polymer has long been known to exist in a helical form in its familiar blue complex with iodine[1–3]. Subsequent discoveries of the helical structure of other biological polymers such as polypeptides[4] and polynucleotides[5] have created great excitement and stimulated many detailed studies into the properties of these polymers. However, possibly because amylose fulfils a rather mundane biological function and is derived solely from plant origin, equivalent studies on this material have not been carried out.

One of the most interesting properties which has been intensively studied in the aforementioned polymers is their ability to undergo rather sharp transitions from a highly ordered helical rod to a disordered randomly kinked structure[6,7]. Furthermore, electronic conductivity has recently been reported for polypeptides and proteins[8,9]. In this communication I present results of experiments which show the iodine–amylose helical complex exhibits similar properties.

Fig. 1 shows the dependence on temperature of the adsorption maximum of the helical amylose–iodine complex in a mixed solvent of five parts water and one part dimethyl sulphoxide. No added ions are present. The molecular weight of the fractionated amylose sample was

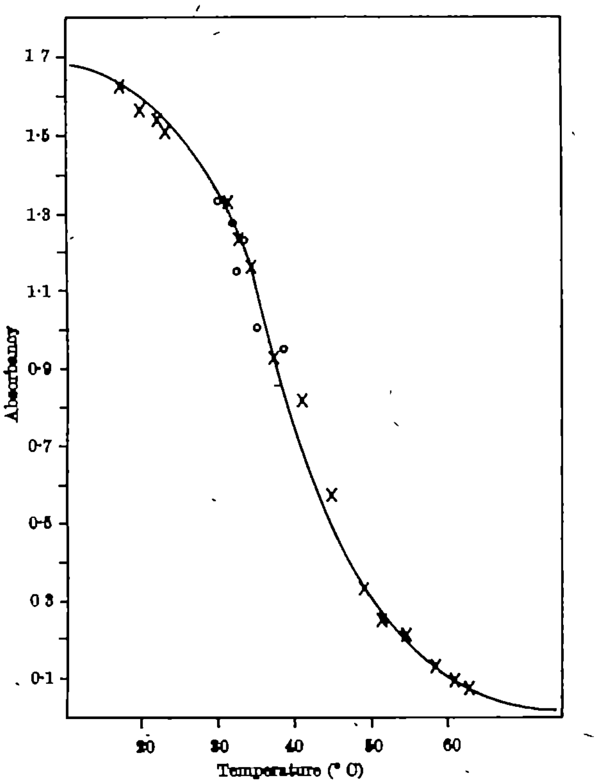

Fig. 1. The 6000 Å absorption band of the amylose–iodine complex as a function of temperature. The crosses were measured during the heating and the circles during the cooling-off cycle

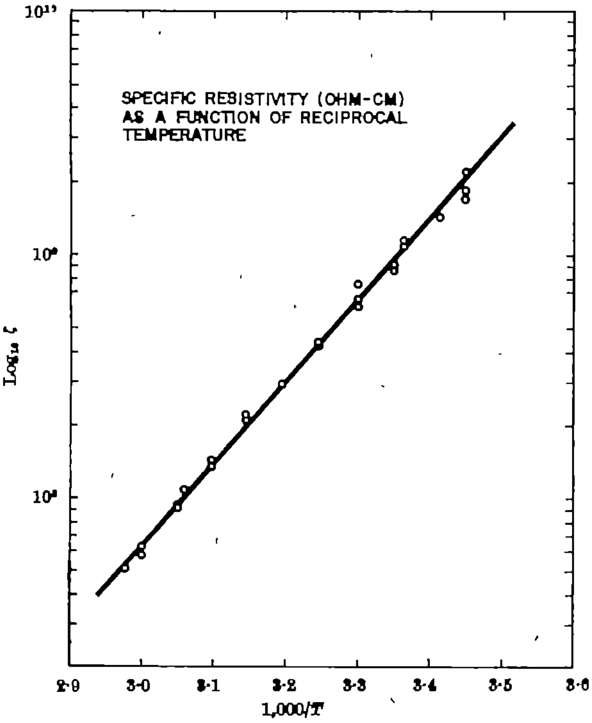

Fig. 2. Electric resistivity of a pressed sample of the amylose–iodine complex as a function of temperature

$1·6 \times 10^5$ and the concentration of iodine and amylose was $3·5 \times 10^{-4}$ g/ml. for each. This figure is in qualitative agreement with the statistical mechanical theory of such transitions[10–12] and shows the characteristic sigmoidal shape of such curves[6,7]. (A more quantitative evaluation is under way.)

Fig. 2 shows the resistivity, ζ, of a powder sample of amylose–iodine complex prepared in the following manner. Purified amylose and iodine were dissolved in dimethyl sulphoxide and then dialysed exhaustively against deionized water. The blue amylose–iodine complex will not form in dimethyl sulphoxide but forms sharply when the concentration of water reaches a critical value. The complex was dried by lyophilization and compressed into pellets for d.c. conductivity measurements by standard 4-pole methods. That electronic conductivity is involved seems assured by my experiments which show that the conductivity does not change with current flow. For any ionic mechanism one would expect an increase in resistivity due to polarization effects at the electrode.

My original observation led me to believe that this conductivity was evidence of the metallic behaviour of the iodine in this complex, which has been suggested theoretically by a number of investigators[13–15]. However, I afterwards found that this crystalline powder possesses a conductivity strongly dependent on the adsorbed water. In this respect it strongly resembles conductivity measurements of Rosenberg on hæmoglobin[9], who has shown that electronic conductivity increases in hæmoglobin with increasing moisture content.

Consequently, it is apparent that amylose–iodine helical complex possesses a number of the thermodynamic and electronic properties equivalent to those of other polymers of biological origin.

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BIOCHEMISTRY

Capsular Polysaccharide of *Klebsiella pneumoniae* Type *A* (Strain 1265)

PREVIOUS investigations[1–3] have shown that the capsular polysaccharides of *Klebsiella pneumoniae* types *A* and *B* are glucose-containing polyuronides. Eriksen and Henriksen[3] have tentatively identified glucose, fucose and a uronic acid as components of type *A* capsular polysaccharide. Hitherto, little work has been directed towards elucidation of the structure of these polysaccharides and we now report briefly on the structure of the acidic polysaccharide of *Klebsiella pneumoniae* type *A* (strain 1265).

The bacterium was grown on a 2 per cent lactose–agar medium at 37° for 24 h, and the crude polysaccharide fraction precipitated from aqueous solution containing sodium acetate by 3 volumes of ethanol. After deproteinization, the polysaccharide fraction had $[\alpha]_D^{22}$ = −89°, N, 0·3 per cent, and was shown by hydrolysis to contain glucose, fucose, galactose, and a uronic acid. Earlier work[4] indicated that the galactose may result from contamination of the acidic polysaccharide with an intracellular galactan. The acidic polysaccharide was further purified by precipitation with cetyl pyridinium chloride[5] to yield a fraction, $[\alpha]_D^{20}$ − 102·5° (as sodium salt), containing uronic acid, glucose and fucose. A similar fraction, $[\alpha]_D^{20}$ − 107·8°, could be obtained by fractional precipitation with ethanol.

Hydrolysis of the acidic polysaccharide (700 mg) with 2 N sulphuric acid at 100° for 3 h yielded a uronic acid, glucose, fucose, and an oligosaccharide fraction containing uronic acid. The acidic sugars were absorbed on 'Deacidite *FF*' (CO_3^{2-}) and the neutral sugars (330 mg) chromatographed on a cellulose column to give D-glucose (180 mg), characterized as the *p*-nitroanilide dihydrate, m.p. and mixed m.p. 182–184° (ref. 6) and L-fucose (132 mg), $[\alpha]_D^{20}$ − 62·5°, characterized as the 2,5-dichlorophenylhydrazone, m.p. and mixed m.p. 206° (ref. 7). The acidic sugars (412 mg), after elution from the resin with N ammonium carbonate and conversion to the free acid forms, were chromatographed on cellulose to give a uronic acid fraction (254 mg), $[\alpha]_D^{20}$ + 12°, and a uronic acid-containing oligosaccharide(s) fraction (43 mg). The uronic acid was characterized as glucuronic acid by glycosidation and esterification with methanolic hydrogen chloride, reduction with lithium aluminium hydride, and hydrolysis. Chromatograms of the neutralized hydrolysate revealed a single component with mobility indistinguishable from that of D-glucose, and which was not detected after pretreatment of the hydrolysate with glucose oxidase. On hydrolysis the uronic acid-containing oligosaccharide(s) gave glucuronic acid, glucose and a sugar with the paper chromatographic and ionophoretic properties of lyxose. The reduced methyl ester methylglycoside of the oligosaccharide(s) fraction gave only D-glucose and lyxose on hydrolysis. The origin of the lyxose has still to be determined.

The polysaccharide rapidly lost the capacity to precipitate with antiserum after treatment with dilute alkali at room temperature. Approximately 45 per cent degradation resulted after exposure to 0·04 N calcium hydroxide solution under nitrogen for 96 h. Degradation was accompanied by the formation of saccharinic acids, including saccharinic acids derived from glucuronic acid and fucose. It has been established[8] that alkaline degradation of 1,3- and 1,4- linked glucans, and partially substituted reducing sugars, results in the formation of saccharinic acids. However, the formation of saccharinic acids is most rapid with 3-*O*- substituted derivatives[9]. The relatively rapid formation of saccharinic acids in this case suggests the presence of 1,3-linkages in the polysaccharide. This contention is supported by the fact that no uptake of oxidant was observed after prolonged treatment with periodate. The polysaccharide reisolated after treatment with periodate still precipitated with its antiserum using the gel precipitation technique.

The results of methylation studies also implied that 1,3-linkages predominated in the acidic polysaccharide. Reduction of the reducing end group with buffered sodium borohydride was necessary prior to methylation to avoid degradation of the polysaccharide under the alkaline conditions of methylation[10]. Hydrolysis of an incompletely methylated polysaccharide fraction ($[\alpha]_D^{20}$ − 92·5° in water) afforded components chromatographically indistinguishable from 2,4-di-*O*-methyl-L-fucose; 2,3,4,6-tetra-*O*-methyl-D-glucose (trace); 2,4,6-tri-*O*-methyl-D-glucose; and 4,6-di-*O*-methyl-D-glucose. The latter component probably arises from incomplete methylation of the polysaccharide as did two components resembling monomethylated glucose and 2-*O*-methylfucose in their chromatographic properties. However, the possibility of some branching in the polysaccharide cannot be excluded. A component showing similar mobility to glucuronic acid in acetate buffer at *p*H 5 indicated that it was a methylated glucuronic acid. The presence of 2,3,4,6-tetra-*O*-methyl-D-glucose in the hydrolysate implies that glucose comprises the non-reducing end unit of the polysaccharide. It is noteworthy that glucose is preferentially released, together with some glucuronic acid, on treatment of the polysaccharide with 0·01 N sulphuric acid at 80° for 30 min.

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Sulphydryl Groups Essential for the Penicillinase Activity of *Aerobacter cloacae*

ALTHOUGH many enzymes possess sulphydryl groups essential for enzymatic activity there have been, so far as is known, no reports of these or indeed of any other types of essential group necessary for the enzymatic activity of a penicillinase. Evidence is given here to show that sulphydryl groups are essential for the penicillinase activity of a strain of *Aerobacter cloacae*.

Cells from an overnight broth culture of *Aerobacter cloacae* (laboratory reference No. 53) were collected by centrifugation, resuspended in 1/30 of their original volume with 0·025 M Sørensen's sodium phosphate buffer (*p*H 7·4) and disrupted, as described by Smith[1], in a liquid extrusion press. This suspension was centrifuged at 33,000*g* for 90 min to remove cell wall and other débris and the clear supernatant fluid stored at 4° C. This crude penicillinase preparation was diluted 1/100 with 0·025 M phosphate buffer and assayed against benzylpenicillin (2 mg/ml.) as described by Knox and Smith[2]. Penicillinase activity at different *p*H values was determined using 0·025 M phosphate buffer and ½ strength McIlvaine's[3] buffer. The *p*H optimum was found to lie between 5·2 and 6·2. Subsequent studies of penicillinase activity were made, using 0·025 M phosphate buffer, at *p*H 6·0, and as some penicillinases from other Gram-negative bacteria have been studied at *p*H 7·4 (ref. 4) the latter *p*H value was also used in order to make results obtained with this enzyme preparation comparable.

To investigate the effect of various enzyme inhibitors a penicillinase preparation was used which, in the absence of inhibitor, would hydrolyse about 20 µg/ml./min of benzylpenicillin. This was incubated with each inhibitor (2 mM) for 10 min at 37° C; an equal volume of benzylpenicillin solution was added and the enzyme assayed. The results (Table 1) show that, under these conditions, *p*-chlormercuribenzoate, which forms mercaptides with sulphydryl groups[5], was the only reagent which completely inhibited the penicillinase activity. Therefore the effect of *p*-chlormercuribenzoate was investigated more closely; and in addition *N*-ethylmaleimide, which forms, by addition, a thio-ether with sulphydryl groups[6–8], and iodoacetamide[9,10], which alkylates sulphydryl groups, were tested. *N*-ethylmaleimide and iodoacetamide reacted much more slowly than *p*-chlormercuribenzoate at concentrations

Table 1. EFFECT OF VARIOUS ENZYME INHIBITORS (2 mM) ON PENICILLINASE ACTIVITY

Inhibitor	Inhibition (per cent) At *p*H 7·4	At *p*H 6·0
2,4-Dinitrophenol	0	0
2,4-Dinitrofluorobenzene	17	13
Hydroxylamine	0	0
Sodium azide	0	0
Potassium cyanide	0	0
Ethylenediaminetetraacetate	0	0
Potassium ferricyanide	14	12
Ethyl urethane	0	0
Formaldehyde	0	0
Sodium fluoride	0	0
o-Iodosobenzoate	29	0
Phenylhydrazine hydrochloride	15	0
p-Chlormercuribenzoate	100	100

Table 2. EFFECT OF SULPHYDRYL GROUP REAGENTS ON PENICILLINASE ACTIVITY

Inhibitor	At *p*H	Concentration of inhibitor required to inhibit penicillinase activity by 50%	100%
p-Chlormercuribenzoate	7·4	2 μM	10 μM
	6·0	14 μM	60 μM
N-Ethylmaleimide	7·4	16 μM	200 μM
	6·0	2 mM	20 mM
Iodoacetamide	7·4	2 mM	20 mM
	6·0	> 20 mM*	—

* 20 mM iodoacetamide caused 10 per cent inhibition at *p*H 6·0; higher concentrations could not be tested under the conditions used.

producing equivalent inhibition and these inhibitors were therefore incubated with the penicillinase preparation for 90 min. The results (Table 2) show that *p*-chlormercuribenzoate was the most efficient inhibitor, *N*-ethylmaleimide had less effect and iodoacetamide had the least effect. Similar results showing that *p*-chlormercuribenzoate was more effective than iodoacetamide in inhibiting other enzymes containing sulphydryl groups have been reported[11]. All inhibitors were more effective at *p*H 7·4 than at *p*H 6·0; similar findings have been made[12,13] in the titration of the sulphydryl groups of egg albumin.

As the inhibition described here was caused by reagents which react with sulphydryl groups, various thiol compounds were tested to see if they caused reactivation. The results are shown in Table 3. With *p*-chlormercuribenzoate, when the enzyme preparation was incubated at 37° C with this inhibitor for 10 min then for 10 min with the thiol compound before benzylpenicillin was added, some reactivation occurred with all the thiol compounds tested. With *N*-ethylmaleimide or iodoacetamide, on the other hand, after the long period of incubation (90 min) necessary to complete the inhibitory process no reactivation occurred when any of the thiol compounds was added afterwards; though some reactivation did occur when the enzyme was incubated with these inhibitors for only 10 min at 37° C then for 90 min with the thiol compound before the addition of benzylpenicillin. Thus the inhibition caused by *p*-chlormercuribenzoate was more easily reversed than the inhibition caused by the other reagents. It is known that in general alkylating agents, in contrast to mercaptide-forming agents, combine irreversibly with the sulphydryl groups of enzymes[14].

Table 3. REVERSAL, BY CERTAIN THIOL COMPOUNDS, OF INHIBITION OF PENICILLINASE ACTIVITY

Concentration of inhibitor	Percentage of penicillinase activity in presence of inhibitor	*p*H value	Thiol compound	Percentage of penicillinase activity in presence of inhibitor + 5 times its molar concentration of thiol compound
4 μM *p*-Chlormercuribenzoate	28	7·4	Thioglycollate	96
			β-Mercaptoethylamine	44
			L-Cysteine	89
20 μM *p*-Chlormercuribenzoate	32	6·0	Thioglycollate	96
			β-Mercaptoethylamine	96
			L-Cysteine	96
20 μM *N*-Ethylmaleimide	35	7·4	Thioglycollate	78
			β-Mercaptoethylamine	35
			L-Cysteine	80
2 mM *N*-Ethylmaleimide	61	6·0	Thioglycollate	61
			β-Mercaptoethylamine	93
			L-Cysteine	91
2 mM Iodoacetamide	41	7·4	Thioglycollate	76
			β-Mercaptoethylamine	*
			L-Cysteine	†

* Under these conditions β-mercaptoethylamine caused further inhibition of penicillinase activity.

† Under these conditions L-cysteine destroyed benzylpenicillin.

Penicillinase preparation was incubated with *p*-chlormercuribenzoate for 10 min, thiol compound added and incubation continued for a further 10 min before penicillinase activity was estimated. Penicillinase preparation was incubated with the other inhibitors for 10 min, thiol compound added and incubation continued for a further 90 min before penicillinase activity was estimated.

The activity of this penicillinase preparation was found to be little affected by the oxidizing agents potassium ferricyanide and *o*-iodosobenzoate. It has been suggested[11,15] that sulphydryl groups in proteins can be readily oxidized only when two of them are sufficiently close for disulphide linkages to be formed. The resistance to oxidation shown by the penicillinase preparation may indicate that its sulphydryl groups occur singly or are not sufficiently close to allow oxidation. In any event, the finding that the penicillinase activity of this strain of *Aerobacter cloacae* can be inhibited by reagents which inactivate sulphydryl groups and that this inhibition can be reversed by thiol compounds suggests that sulphydryl groups are essential for activity. In this respect it seems to be unique among penicillinases so far described. It would be interesting to know if any other strains of bacteria, particularly of *Aerobacter cloacae*, possess a penicillinase with similar properties.

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Occurrence of γ-Hydroxyarginine in Plants

AN examination of free amino-acids and related compounds in the genus *Vicia* revealed the presence of an unidentified ninhydrin-reacting guanidino compound in the seeds of 17 species (*V. alba*, *V. calcarata*, *V. cornigera*, *V. cordata*, *V. dumetorum*, *V. ferruginea*, *V. grandiflora*, *V. ludoviciana*, *V. monantha*, *V. nichouxii*, *V. onobrychioides*, *V. peregrina*, *V. pyrenaica*, *V. sativa*, *V. sepium*, *V. unijuga*, and *V. varia*).

This compound could be separated from arginine and homoarginine on paper in 17 h using methylethylketone/propionic acid/water (2 : 1 : 2 by vol.)[1] as solvent or in 72 h using butan-1-ol/acetic acid/water (12 : 3 : 5 by vol.)[2] as solvent. It gave a scarlet reaction with Sakaguchi's reagent[3], unlike the orange-red given by arginine and homoarginine, and behaved as an α-monoamino carboxylic acid when treated successively on paper with cupric nitrate and ninhydrin[4].

After extracting with water from seeds of *V. sativa*, and partially purifying by ion exchange chromatography, an attempt was made to isolate the compound as the monohydrochloride by acidifying the crude material (concentrated to a syrup under reduced pressure) with concentrated hydrochloric acid, removing excess acid with aniline and recrystallizing repeatedly from hot aqueous ethanol. During recrystallization the compound underwent transformation and the salt of a more basic compound (as indicated by its ionic mobility) was isolated in long colourless needles, m.p. 158° (uncorrected). This artefact resembled the original compound in giving a scarlet reaction with Sakaguchi's reagent but differed from it in giving a yellow and not a purple with ninhydrin. On standing in 0·1 N sodium hydroxide at room temperature the artefact reverted to the original compound in less than 17 h. These changes suggested the formation and subsequent hydrolysis of a lactone ring. The elementary analysis of the isolated salt was in agreement with it being the monohydrated dihydrochloride of a hydroxyarginine lactone (found: C, 27·72; H, 6·17; N, 20·40; Cl, 26·40; calc. for $C_6H_{12}N_4O_2.2HCl.H_2O$: C, 27·38: H, 6·08; N, 21·29; Cl, 27·00 per cent).

Although hydroxy derivatives of arginine have not been found previously in plant material, γ-hydroxyarginine, the derivative most likely to form a lactone, has been isolated from the sea-cucumber *Polycheira rufescens*[5] and the sea-anemone *Anthopholeura japonica* Verrill[6].

Using authentic γ-hydroxyarginine and γ-hydroxyornithine (kindly supplied by Dr. Y. Fujita) as markers, the original compound from *V. sativa* and the product obtained by refluxing it in 0·1 sodium hydroxide for 3 h were chromatographed on paper in six solvent systems and subjected to high-voltage ionophoresis on paper at pH 1·9 and pH 3·6. The R_F values and mobilities of the plant amino-acid and the product of its alkaline hydrolysis were, in each solvent system and at each value of pH, identical with those of γ-hydroxyarginine and γ-hydroxyornithine respectively. The plant amino-acid and γ-hydroxyarginine gave identical reactions with Sakaguchi's reagent as did the hydrolysis product and γ hydroxyornithine with periodic acid, and acetylacetone[7].

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Enzyme Reactions with Phenolic Compounds[1]: Effect of O-Methyltransferase on a Natural Substrate of Fruit Polyphenol Oxidase[2]

PHENOLIC compounds constitute the natural substrates of the enzyme polyphenol oxidase (PPO). Of these, the major substrates of the enzyme in fruits are reported to be *o*-diphenols (catechols), for example, chlorogenic acid[3] (CGA), *l*-epicatechin[4], and dopamine[5]. If it were possible to modify irreversibly one of the catechol hydroxyl groups in any manner, this would have the following effects of preventing participation in PPO reactions by constituent phenolic compounds: (1) The modified catechols would themselves no longer be substrates of PPO[6], (2) the absence of catechols as primers of monophenolase oxidations could, in turn, eliminate the oxidation of constituent monophenols[7]. Even the first effect alone would eliminate the major proportion of PPO substrates in many plant tissues, as stated here[8].

The enzyme *O*-methyltransferase is capable of methylating the 3-position of 3,4-dihydroxy aromatic compounds[8,9] and *O*-methylation would be an irreversible modification of the type described. The substrates reported for rat-liver *O*-methyltransferase are such as to suggest a broad specificity, possibly including many of the plant *o*-dihydroxy aromatic acids. What would be the effect on PPO reactions after interaction of *O*-methyltransferase with PPO substrates? Methyl transfer was tested on two plant constituents, caffeic acid and CGA, after which their participation in PPO reactions was evaluated.

Synthetic caffeic acid[10] (3,4-dihydroxycinnamic acid) was used as a model compound because it was known to be methylated by the animal transferase[8] and the methylated product could be easily identified. The methylating enzyme was prepared according to Axelrod[8], taken through the ammonium sulphate precipitation step, dialysed against 0·001 M pH 7·0 potassium phosphate, and frozen.

Table 1. OPTICAL DENSITY CHANGES IN CGA AND ITS PRODUCT

Reaction time (h)	Optical density R_F 0·2 spot (CGA)	 R_F 0·6 spot (product)	O.D.—O.D. of control R_F 0·2 spot (CGA)	 R_F 0·6 spot (product)
(Boiled control)	0·228	0·057	0·00	0·00
1	0·170	0·112	−0·058	+0·055
2	0·154	0·147	−0·074	+0·090
5	0·071	0·263	−0·157	+0·206

O-Methyltransferase preparation (ref. 8) containing 8 mg protein was added to the following quantities of reagents (μmol/ml.): pH 9·5 potassium phosphate, 375; magnesium chloride, 10; sodium chlorogenate, 2·3; and *S*-adenosylmethionine, 15. Resultant pH, 7·7. The mixture was incubated at 38° C. 20 λ aliquots were spotted on paper at the designated times and the paper was chromatographed in 7 : 3 solvent mixture (see text). Excised spots were eluted by shaking in 5 ml. 70 per cent ethanol, and optical density was determined spectrophotometrically at 325 mμ. A control sample containing boiled enzyme was treated in the same manner.

Aliquots from a reaction mixture comparable with that shown in Table 1, except that sodium caffeate was used as substrate, were withdrawn periodically, spotted on paper, and chromatographed in chloroform/acetic acid/water, 2 : 1 : 1, lower layer[11]. During the incubation, caffeic acid decreased (R_F 0·1, detected by its ultra-violet fluorescence). A new fluorescent product, which increased in quantity with time, appeared at R_F 0·9. Its tested properties (R_F and absorption spectrum) coincided with those of ferulic acid (4-hydroxy-3-methoxycinnamic acid) as reported[8].

Chlorogenic acid (3-caffeoylquinic acid) isolated from green coffee beans[12] was subjected to the methylating system described in Table 1, and aliquots were chromatographed in several solvents, including a mixture of 7 parts chloroform–acetic acid–water (see above) and 3 parts methylcellosolve/acetic acid/water, 112 : 1 : 12 (ref. 13). This mixture was the only solvent system of many tried that distinctly separates CGA from its product substance. A fluorescent product appeared on the chromatograms; it increased in amount with time in proportion to the chlorogenic acid decrease (Table 1), suggesting their precursor–product relationship. The following properties were used to tentatively identify the product compound as 3-feruloylquinic acid, recently isolated from coffee bean and obtained through the kindness of Dr. J. Corse[14]: its R_F's in several solvents (for example, R_F 0·6 in the 7 : 3 mixture described here and R_F 0·7 in *n*-butanol/acetic acid/water, 4 : 1 : 2·2; as against R_F's 0·2 and 0·6, respectively, for chlorogenic acid), the bright blue-fluorescent colour of its spot on paper, and its 290–350 mμ absorption spectrum after elution from a paper chromatogram (procedure, Table 1).

In order to test the effect of PPO on the reaction products, caffeic acid and CGA reaction mixtures were chromatographed, and the chromatograms were sprayed with crude PPO prepared from peach or apple fruit according to the procedure of Siegelman[4]. When the sprayed papers were incubated at room temperature in a moist chamber, brown coloration soon appeared where

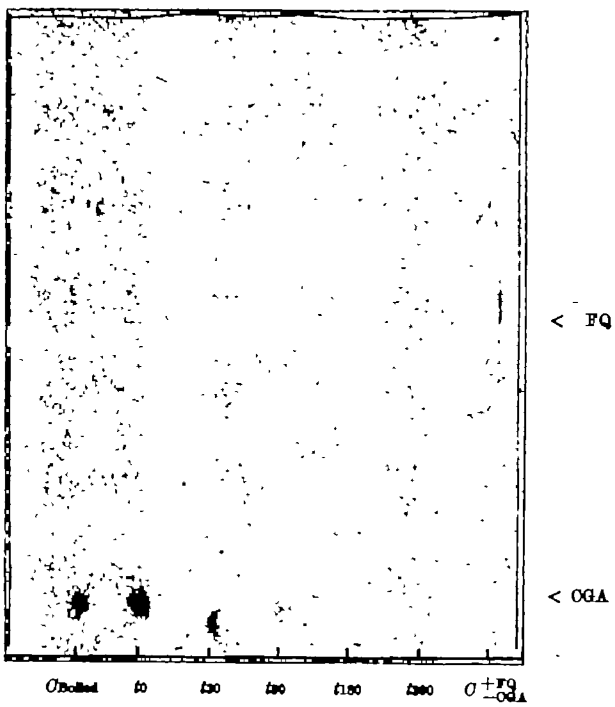

Fig. 1. Paper chromatogram of OGA reaction mixture, paper uniformly sprayed with PPO. Conditions of incubation and chromatography similar to those in Table 1 were used, except for an increase in enzyme concentration (40 mg/ml.). Aliquots were withdrawn at the designated times ($t_{\min}$). Two control mixtures are also shown: in C_{Boiled}, boiled enzyme was used; in C^{+FQ}_{-OGA}, feruloylquinic acid was substituted for OGA. R_F positions of the compounds are indicated at the right margin and by circling of the feruloylquinic acid control spot, visible when under ultra-violet light. (Note.—The migration of OGA is retarded somewhat by components of the incubation mixtures)

oxidizable substrates were present. Only caffeic acid, not methylated product, reacted to give a dark colour. Parallel effects, illustrated in Fig. 1, were obtained with OGA (R_F 0·2) and its methylation product (R_F 0·6). In each case, the methylated compound was no longer a PPO substrate. Similar results might be expected[6] with the enzymatically 3-methylated products of dopamine[8] and other *o*-diphenolic plant constituents.

Since enzymatic methylation of substrates of PPO prevents their oxidative reaction, the methylation would be of obvious interest with regard to the oxidative darkening of plant tissues. Interaction of *O*-methyltransferase with fruit tissues has given the expected effect on their PPO reaction, which will be reported elsewhere.

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Tryptophan–Nicotinic Acid Metabolism in Subjects recently or long since recovered from Viral Hepatitis

In previous work we have shown that metabolic alterations in the chain of reactions leading from tryptophan to nicotinic acid are present in both experimental hepatitis by mouse hepatitis virus (*MHV*-3 virus)[1] and in human viral hepatitis in the acute stage[2].

We have now investigated the persistence of the aforementioned alterations in subjects clinically recovered from the latter disease.

The pattern of urinary metabolites involved in the tryptophan–nicotinic acid interrelation was examined in urine samples collected over a 24 h period from normal subjects and from 34 subjects who had recovered from viral hepatitis: 10 recovered for 2 months–1 year (group I); 12 for 1–5 years (group II); 12 for 5–15 years (group III).

For the chromatographic analysis of the aforementioned metabolites Dalgliesh's technique[3] was used. Identification of the compounds was carried out by examination of the fluorescence by ultra-violet light (3660 Å) and by the characteristic colour reactions. Several chromatographic analyses were carried out on different days for each subject.

Furthermore, in the same subjects the electrophoretic protein pattern, the flocculation or turbidity tests (Takata-Ara test and thymol–turbidity test), the activity of serum transaminases and the determination of serum bilirubin were carried out in order to discover whether the liver function of these subjects was altered or not.

Of the 10 cases of group I, we found kynurenine in 4, kynurenine and 3-hydroxykynurenine in 2 (only one of these presented a slight increase of γ-globulin and a partially positive Takata-Ara and thymol–turbidity tests).

Of the 12 cases of group II, we found kynurenine in 4, kynurenine and 3-hydroxykynurenine in 3 (the other tests were normal in all 12).

Of the 12 cases of group III, we found kynurenine in 5, and kynurenine and 3-hydroxykynurenine in 3 (only one of these presented a slight increase of γ-globulin and a partially positive Takata-Ara and thymol–turbidity tests).

The results obtained show that in a high percentage (about 60 per cent) of the completely recovered subjects examined (on the basis of clinical examination and of many tests for liver functions) a certain alteration of the tryptophan–nicotinic acid metabolism was still present. It seems that this lesion is connected chiefly with kynurenine oxidase activity.

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Bacterial Transformation: an Antigen Specific for 'Competent' Pneumococci

The ability of a strain of pneumococcus to be transformed by deoxyribonucleic acid (DNA) isolated from another strain is termed 'competence'. The basis for 'competence' is not yet fully understood, but evidence has been obtained[1] that it may depend on the capacity of a cell to incorporate DNA. The finding by Hotchkiss[2] that non-transforming DNA inhibits transformation indicated that specific receptors for DNA are present in 'competent' cells. Schaeffer[3], after extensive investigation of this phenomenon, concluded that the receptor is on the surface of 'competent' bacteria.

The probability that the receptor on 'competent' pneumococci may be a protein[1,2] suggested that it might be antigenic. Immunization with 'competent' pneumococci accordingly should stimulate the production of antibodies which inhibit transformation, whereas antibodies to 'non-competent' cells should not inhibit transformation. The results of experiments designed to test this possibility are reported here.

All experiments were performed with DNA prepared from a strain of pneumococcus resistant to streptomycin, micrococcin, and erythromycin. The DNA was isolated from cells lysed with deoxycholate, deproteinized by shaking with chloroform and isoamyl alcohol, and precipitated with ethanol[3]. The procedures for transformation and the method of quantitation using anti-R serum were those described by Hotchkiss[2,3].

To prepare 'competent' cells for immunization, pneumococci were grown under conditions identical to those used for preparing a culture for transformation[3]. When 'competent' cells were presumably present, an aliquot was removed to test for transformability. Formaldehyde in a final concentration of 0·2 per cent was added to the remainder of the culture, and the pneumococci were kept at room temperature overnight. The cells were then separated by centrifugation and concentrated fifty-fold for immunization.

'Non-competent' cells were prepared in an identical fashion, except that bovine serum albumin was eliminated from the growth medium. It was usually necessary to transfer the culture several times in a medium lacking bovine serum albumin before a culture which gave no evidence of transformability could be obtained.

It had been found, in connexion with other investigations, that sera from presumably normal rabbits occasionally inhibit transformation. Consequently globulin fractions prepared by sodium sulphate precipitation[7] were used in these investigations. Such fractions do not inhibit transformation non-specifically.

The results of experiments in which three globulin fractions from the sera of rabbits immunized with 'competent' cells were compared with three corresponding fractions from rabbits immunized with 'non-competent' pneumococci are recorded in Table 1. All the globulin fractions obtained from the rabbits immunized with transformable pneumococci inhibited transformation for all three markers studied, whereas no inhibition was noted with the globulin fractions obtained from the animals immunized with 'non-competent' cells. In fact, the latter, for reasons not apparent, actually increased the yield of transformants as compared with the control tubes to which only the transforming DNA was added. The difference between the activities of the two sets of globulin preparations was not due to a difference in titre, because all six fractions had identical titres as determined by agglutination of either formalinized 'competent' or 'non-competent' pneumococci.

To eliminate the possibility that the results might be due to fortuitous variations among rabbit sera, globulin fractions prepared from pre- and post-immunization sera were compared (Table 2). Here, again, the inhibitory effect of fractions obtained from immunized rabbits is apparent, in contrast to the usual enhancement observed with globulin fractions obtained from the same rabbits before immunization.

Table 1. EFFECT OF GLOBULIN FRACTIONS FROM SERA OF RABBITS IMMUNIZED WITH 'COMPETENT' (C) AND 'NON-COMPETENT' (NC) PNEUMOCOCCI ON TRANSFORMATION

Globulin fraction	No. of cells transformed/ml. Streptomycin	Erythromycin	Micrococcin
$E6$–1 (C)	680	390	$1·0 \times 10^3$
$E6$–26 (NC)	$1·9 \times 10^5$	$1·4 \times 10^5$	$2·2 \times 10^5$
Control	$5·5 \times 10^4$	$1·0 \times 10^5$	$1·7 \times 10^5$
$E6$–2 (C)	$8·4 \times 10^2$	$4·2 \times 10^4$	$1·6 \times 10^4$
$E6$–27 (NC)	$1·3 \times 10^5$	$3·5 \times 10^5$	$1·8 \times 10^5$
Control	$8·6 \times 10^4$	$3·7 \times 10^5$	$1·8 \times 10^5$
$E6$–3 (C)	$1·4 \times 10^4$	$6·5 \times 10^4$	$2·7 \times 10^4$
$E6$–22 (NC)	$1·2 \times 10^5$	$4·5 \times 10^5$	$1·9 \times 10^5$
Control	$6·2 \times 10^4$	$3·2 \times 10^5$	$2·0 \times 10^5$

Table 2. COMPARISON OF GLOBULIN FRACTIONS FROM PRE- AND POST-IMMUNIZATION SERA OF RABBITS IMMUNIZED WITH 'COMPETENT' PNEUMOCOCCI

Globulin fraction	No. of cells transformed/ml. Streptomycin	Micrococcin
$E6$–35	0	0
$E6$–35s	$8·8 \times 10^5$	$2·7 \times 10^5$
$E6$–38	$1·5 \times 10^4$	$1·5 \times 10^4$
$E6$–38s	4×10^5	$3·3 \times 10^5$
$E6$–40	30	130
$E6$–40s	$2·2 \times 10^5$	$2·2 \times 10^5$
Control	$2·4 \times 10^5$	$2·2 \times 10^5$

s denotes pre-immunization sera.

The results provide direct evidence for the presence of an antigen associated with 'competence' in pneumococci. It is reasonable to assume that this antigen is the receptor that has been postulated to occur on the surface of transformable cells. This antigen must be lost or altered in the 'non-competent' cell, for if it were merely 'masked', that is, covered so that it was no longer on the surface, it should still be capable of acting as an antigen to stimulate the formation of inhibiting antibodies. Whether or not this antigen is responsible for the cyclical appearance of transformability in synchronously cultured pneumococci[4] remains to be determined. Obviously such variations in transformability could also result from changes in the ability of the cells to integrate DNA into their genomes, and bear no relation to a surface receptor or antigen.

The 'competent' pneumococci were grown in the presence of bovine serum albumin. As an additional control, therefore, globulin fractions prepared from the sera of rabbits immunized with bovine serum albumin were tested in the transforming system and were found to be without effect.

The sera, whether obtained from rabbits immunized with 'competent' or 'non-competent' cells, exhibited identical agglutinin titres with both types of cells. They undoubtedly contain a number of different antibodies, most of which are present in all the sera. It is not possible, therefore, to measure the specific antibody responsible for inhibiting transformation, but it has been found that only globulin fractions with relatively high titres (> 10,000) were active in this regard. Dilution by as little as ten-fold eliminated their capacity to inhibit transformation, indicating that the specific inhibitory antibody probably is present in small concentrations in comparison with antibodies directed against other cell components. Despite this complication, it is hoped to use such antisera as a guide to the isolation of the 'competent' antigen by fractionating transformable pneumococci and determining which fractions absorb the inhibiting activity.

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PHYSIOLOGY

Rate of Restitution and Self-regulation of Contractility in Mammalian Heart Muscle

In a suitable environment a heart muscle preparation stimulated at a fixed rate maintains a steady-state strength of contractions for long periods. The muscle thus completely recovers between two successive beats. However, the recovery does not occur immediately with repolarization of the membrane. The recovery or restitution of contractility lags behind that of excitability[1] and it is not instantaneous but progressive, as revealed by the premature and delayed beats (Fig. 1): if the interval is shorter, the amplitude of contraction is smaller, that is, the restitution of contractility is not complete. If, on the other hand, the interval is longer than the regular one, the contraction is higher, the restitution of contractility being even more complete.

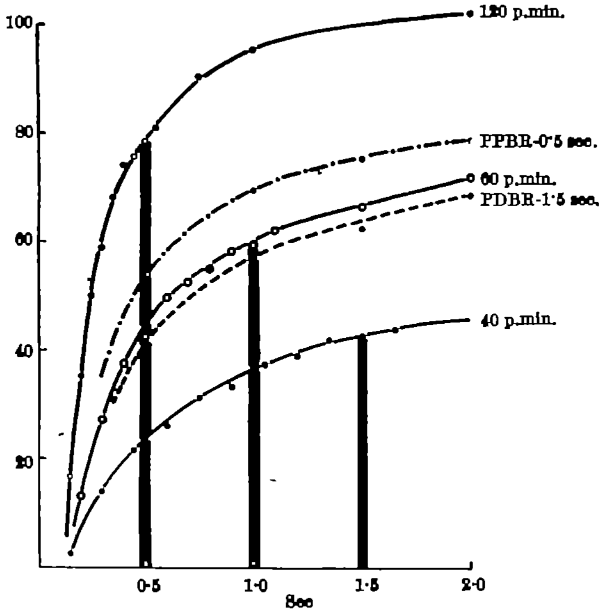

Fig. 1. Restitution of contractility in guinea pig atrium at 32·2° C and at basal rates of 40, 60 and 120 per min, as revealed by the strength of premature and delayed contractions (ordinate). Abscissa: interval preceding the measured contraction. The steady state amplitude for each of the 3 frequencies is marked by a black column. *PPBR*, post-premature beat restitution—accelerated (potentiated) restitution following a premature beat (interval 0·5 sec); *PDBR*, post-delayed beat restitution—slowed restitution following after a single delayed beat (interval 1·5 sec). Ordinate, amplitude; abscissa, interval. Differences between the two upper (120 per min and *PPBR*) and two lower (*PDBR* and 40 per min) curves indicate the effects due to cumulation (single and repeated intervals of 0·5 and 1·5 respectively)

Moreover, besides this change in the amplitude of the irregular contraction, the change of a single interval causes an augmentation of the next beat, even when it follows after a regular interval, that is, without a compensatory pause (post-extrasystolic potentiation)[2]. In our experiments it was possible to show that within certain limits—at medium rates—the velocity of contractility restitution, which follows the premature beat, is increased (Fig. 1, *PPBR*). On the other hand, a longer interval causes a decrease in the rate of restitution. It looks as if the speed of contractility recovery were set according to the length of the preceding interval. A shorter interval between contractions accelerates the restitution of contractility so that the amplitude of the following beat reaches a higher level than previously during the same time period (Fig. 1, *PPBR*); a longer interval causes just the contrary (Fig. 1, *PDBR*).

If not only one but all intervals are shortened, that is if frequency is increased, the speed of the restitution process gradually increases and this change results in gradual augmentation of the contractile force or shortening (staircase phenomenon). The speed of restitution of contractility is thus set progressively to a higher level corresponding to the final steady-state of the new frequency. The change of frequency in the opposite sense, its slowing within the limits of medium rates, results in a progressive decrease of restitution velocity to a new lower steady-state level (negative staircase).

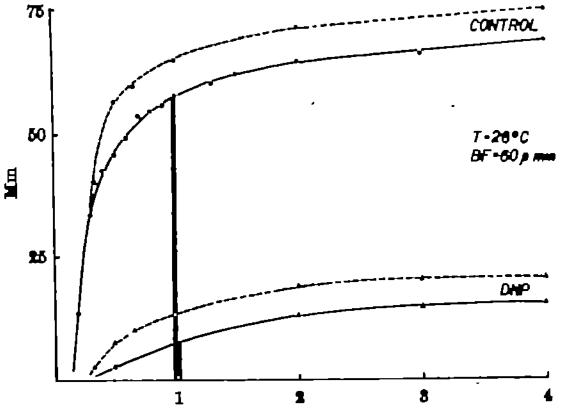

Fig. 2. Restitution of contractility in guinea pig atrium at 28° C and basal frequency 60 per min before and after 1 h exposure to 2,4-DNP ($7\cdot5 \times 10^{-5}$ M)

Solid line, normal restitution curve; broken line, post-premature beat restitution. Steady-state amplitudes indicated by dark columns. Elevation of the curve due to single inotropic effect of premature beat is unchanged, but its maintenance on the higher level is abolished. Note the two phases of restitution development: an initial rapid, followed by a slow phase. The change in rate of restitution occurs almost entirely in the initial phase (cf. also Fig. 1)

This cumulation of the individual inotropic effects of premature or delayed beats seems due to a certain inertia in the return of the restitution, deviated from its steady rate. It may be observed on several beats after a premature or delayed contraction: the initial change is abrupt, but the return only gradual.

Potentiation thus seems to be essentially a change which accelerates or facilitates the process of restitution of contractility. The immediate effect due to a premature contraction, and the cumulative effect brought about by an increase of frequency, are closely related; but they are not identical. The difference between them is well revealed by anoxia or some metabolic inhibitors (fluoroacetate, dinitrophenol), which first suppress the cumulation, leaving the immediate effect almost unchanged (Fig. 2). It is affected only later, when, owing to the progressing metabolic lesion, the mechanical response of the heart muscle is greatly altered. Thus the immediate effect is largely independent of energy-yielding metabolic reactions, while cumulation requires integrity of the whole system of oxidative reactions.

It is to be noted that the change which results in potentiation may be elicited by very premature stimuli which are not followed by any mechanical response and is therefore connected rather with the membrane phenomena and with the process of activation than with some mechanical event of contraction[3], and may therefore be called inotropic effect of activation[4].

Some special problems arise from the behaviour of the heart muscle at very low and high frequencies, at various temperatures, and there are also some species differences in the form of the frequency-strength relationship, most important in the rat myocardium. Their implications will be discussed elsewhere.

The nature of the restitution of contractility and of the mechanism of its 'setting' is so far not clear. We know, however, that these phenomena are closely connected with the membrane depolarization and with the process of excitation–contraction coupling on one hand, and on the other with the processes of transformation and conversion of the stored energy into an available form and its utilization for the mechanical process of contraction. Recent evidence shows that many variations of contractility are related rather to changes in the efficiency of the utilization of chemical energy and its conversion to mechanical energy than to changes in the actual amount of the chemical energy used per beat[5]. This conception does not seem inconsistent with our interpretation of present results. Further research is needed, however, to elucidate the underlying processes.

The fact that the activation, besides triggering the contractile mechanism, modifies at the same time the rate of contractility restitution, and with it the strength of contraction according to the length of the preceding interval, may be of definite physiological significance in a muscle adapted to rhythmical activity. It leads to the idea of self-regulation in the heart muscle, of an efficacious mechanism—and perhaps the only possible one—allowing for a gradation of mechanical response in rhythmical activity.

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Effect of Œstradiol on Spinal Cord Convulsions in Developing Rats

Spinal cord convulsions can be elicited by direct spinal cord stimulation in 1-day-old rats[1]. In contrast, the tonic–clonic seizure pattern of adult rats produced by electroshock stimulation of the brain[2] usually does not appear until the 21st day of age. Thus, the spinal cord matures more rapidly, functionally, than do higher centres of the nervous system.

Previous investigations in this laboratory have demonstrated that œstradiol in adult rats increases brain excitability, as measured by a decrease in electroshock seizure threshold and by facilitation of the tonic phase of the maximal electroshock seizure[3]. In view of the part that spinal reflex systems play in determining the seizure pattern[4], the question arises whether or not œstradiol exerts a significant action on these systems.

In a preliminary investigation, Heim and Timiras[5] have found that in rats treated with œstradiol from the 8th to the 11th days after birth, the time of appearance of the ability to exhibit a tonic–clonic seizure was hastened by two days over that of the controls. These authors have suggested that œstradiol induces precocious maturation in the brain. However, the role of spinal reflex systems should be considered.

The following experiments were performed to examine the effect of œstradiol on spinal cord convulsion patterns in rats during maturation.

Œstradiol dipropionate in a dose of 1 mg/kg (in 0·01 ml. 'Mazola' solution/g body-weight) was administered to rats daily from the 8th to the 11th days after birth. Control animals received 0·01 ml./g body-weight of 'Mazola' for the same period. Spinal cord convulsions were elicited in both control and œstradiol-treated rats on the 12th and 21st days of age. The technique for direct spinal cord stimulation was that described by Esplin and Freston[4] and modified by Vernadakis[1]. The animals were decapitated at the cervical region and the stimulating electrode (cathode) was inserted 4–5 mm into the cord. The anode was attached to exposed tissue of the neck. Square-wave stimuli 1 msec in duration and of 100 pulses/sec frequency were delivered by a Grass stimulator at a stimulus intensity of 30 V. Spinal cord stimulation of 10-sec duration was applied 15 sec after decapitation. The end points of hind-limb flexion and extension were measured by an electronic timer. The duration of flexion is utilized as a standard index of seizure intensity; the shorter the duration of flexion the more severe is the convulsion[6].

The duration of flexion in control and œstradiol-treated rats 12 and 21 days of age are presented in Table 1. In both ages the duration of flexion in the œstradiol-treated animals is significantly decreased; the decrease in 12-day-old œstradiol-treated rats is more marked than that in the 21-day-old. These results illustrate that œstradiol administration increases the reflex excitability of the spinal cord and that this increase in excitability persisted during the experimental period; they are in agreement with previous studies[2,3] showing that œstradiol has long-lasting effects on developing rats.

Table 1. Effect of Œstradiol* on Spinal Cord Convulsions in Rats during Development

Age: days after birth	Duration of hind-limb flexion (sec)	
	Control	Œstradiol
12	1·58 ± 0·18†	0·99 ± 0·04 (< 0·001)‡
21	1·32 ± 0·13	0·95 ± 0·004 (0·05–0·02)

* Œstradiol was injected subcutaneously from the 8th to the 11th days after birth. 8–16 rats were used in each age period.

† ± *S.E.*

‡ Number in parentheses is *P* value for comparison to control.

It has been suggested in previous investigations[5] that œstradiol, given at a critical period of development, enhances the appearance of the full tonic hind-limb extensor seizure by inducing precocious maturation of the higher centres of the nervous system. In the present investigation œstradiol given at a critical period of the development of higher centres increases the level of activity of spinal reflex system. Thus, it may be suggested that the enhanced appearance of a full tonic–clonic seizure in œstradiol-treated developing rats can be attributed in part to an increased level of spinal reflex excitability.

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Permeability of Dermal Connective Tissue in Normal and Scorbutic Guinea Pigs

Recent work has shown that the permeability of capillary blood vessel walls does not seem to be affected by scurvy[1] despite the presence of hæmorrhages. However, capillary resistance was shown to decrease[2–6] and the corresponding capillary fragility increased[6,7]. No investigations of the influence of scurvy on the permeability of dermal and connective tissue have been made.

To examine the effect of scurvy on dermal and subcutaneous tissue, we used young guinea pigs weighing 250–400 g, fed *ad lib.* on Wayne guinea pig diet containing 420 mg of vitamin C (added) per 100 g of diet. To induce scurvy, the guinea pigs were placed for 15–17 days on Wayne 'Lab Blox' (mice and rats), which contained practi-

Table 1. Effect of L-Ascorbic Acid (1 mg/ml. in Saline) on the Rate of Diffusion of Evans Blue Dye in Normal and Scorbutic Guinea Pigs

	No. of animals	Weight (g)	Spread area (mm²) with: Dye alone	Spread area (mm²) with: Ascorbic acid	Increase (per cent)	Average values (males and females) Spread area (mm²) with. Dye alone	Average values (males and females) Spread area (mm²) with. Ascorbic acid	Average values (males and females) Increase (per cent)
Normal animals	3 ♂	350 ± 21	121 ± 14	144 ± 21	18	141 ± 20	162 ± 30	15
	7 ♀	430 ± 50	147 ± 11	168 ± 22	14			
Scorbutic animals	6 ♂	285 ± 42	123 ± 9	148 ± 24	20	137 ± 33	164 ± 23	20
	6 ♀	203 ± 15	151 ± 17	179 ± 22	19			

cally no ascorbic acid. On this diet, they lost an average of 22 per cent of their body-weight and at autopsy showed severe hæmorrhages of limb joints, chondrocostal junctions and of the intestines. The adrenals had increased in weight.

Modifications of Duran-Reynals's spreading test[8] and of the constant pressure infusion test of Hechter *et al.*[9-11] were used for measuring permeability. In the spreading test, 0·05 ml. of a 4 per cent Evans blue solution in saline (*p*H 7·3) with or without ascorbic acid (1 mg/ml.) was injected intradermally into the abdominal regions of animals. The areas of the spots thus produced were recorded by tracing on semi-transparent paper after 30 min.

In the constant pressure infusion method a 27-gauge needle attached to a constant pressure burette with rubber tubing was introduced into the dorsal calf region of guinea pigs. Saline (0·154 M) adjusted to *p*H 7·3 was allowed to flow into the animal for 15 min at an infusion rate of 0·4–0·6 ml./min. After the first 5 min, 0·5 ml. of ascorbic acid (1 mg/ml.) in saline was injected into the bleb. The flow-rates of the saline during the first and third 5-min periods were measured. The permeability was measured as the volume (× 100) of saline infused during the third 5-min period divided by the volume infused during the first 5-min period. Generally, two tests (on left and right calves) were made on each animal. The control ratios were obtained by injecting 0·5 ml. of saline into the bleb. (Details of these techniques will be described elsewhere.) Both methods, spreading and infusion tests, were used on normal and scorbutic guinea pigs. Before the tests, the animals were anæsthetized by use of aqueous solution of sodium nembutal ('Pentobarbital sodium', Abbott) (60 mg/ml.), diluted 1 : 4 with saline; 0·4 ml. of this dilution was injected intradermally per 100 g of body-weight.

The results of the application of the dermal diffusion method using Evans blue with or without ascorbic acid is shown in Table 1 for male and female guinea pigs. Ascorbic acid caused an increase in the spreading of the dye. However, both normal and scorbutic animals showed about the same increase. The size of the spots for dye alone was also similar for the normal and scorbutic animals. Female animals had somewhat larger spots than males, an observation found in our earlier work for both rats and guinea pigs. A similar sex difference for rabbits using indian ink and hæmoglobin as the test substances has been reported by Lurie and Zappasodi[12].

Table 2. Effect of L-Ascorbic Acid (1 mg/ml.) in Saline on the Rate of Infusion for Normal and Scorbutic Guinea Pigs

	No. of animals	Body-weight (g)	Ratio: ml. in third 5 min × 100 / ml. in first 5 min		Permeability increase (%)	
Normal animals						
Controls (with dye alone)	9 ♂	*	95 ± 13	93 ± 13†	—	
	8 ♀	*	90 ± 12		—	
With ascorbic acid	8 ♂	300 ± 20	110 ± 13	111 ± 12	15	20‡
	7 ♀	280 ± 48	114 ± 12		27	
Scorbutic animals	9 ♂	292 ± 33	101 ± 11	105 ± 12	6	18
	5 ♀	254 ± 51	113 ± 16		25	

* Animals of 250–350 g.

† The same values were also used as controls for scorbutic animals.

‡ Average.

As shown in Table 2, the increase in rate of infusion of saline was enhanced by ascorbic acid to about the same extent for normal and scorbutic animals.

These results suggest that the permeability of dermal and subcutaneous tissues is not altered in scurvy when measured by these methods.

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Diet and Histamine in the Ruminant

The presence of histamine in normal rumen liquor has not been proved decisively, and the part played by this substance in acute indigestion in ruminants has not been well established.

Dain *et al.*[1] were unable to detect histamine in normal rumen contents, whereas Shinozaki[2] found histamine base values between 2·9 and 5·6 μg/ml. rumen liquor. In both investigations the sheep were fed on hay. Dain *et al.*[1] detected histamine in ingesta of experimentally overfed sheep, levels higher than 70 μg histamine base per ml. being obtained. They also reported good correlation between increase in histamine-level, lowering of the *p*H, due to fermentation of great amounts of carbohydrates, and severity of indigestion. Tyramine was also present.

It has been established that intravenous injection of histamine in sheep will cause rumen paralysis[1,3-5] and cessation of eructation[1]. On the other hand, orally administered histamine does not influence the physiological condition of the rumen[2]. This has been attributed to poor absorption of histamine through the rumen epithelium. The consensus of opinion has been that the histamine in the rumen is of endogenous origin.

In our experiments histamine was extracted from the rumen liquor of sheep by passage through an 'Amberlite *IRC*-50' column according to Bergstrøm and Hansson[6]. After elution with hydrochloric acid, histamine was assayed conventionally on isolated atropinized guinea pig ileum. The acetyl-conjugated histamine was determined as the increase in free histamine which occurred on acid hydrolysis. All histamine values refer to the diphosphate.

Fig. 1 shows the diurnal variation of histamine concentration in the rumen liquor when the morning ration consisted of hay and concentrates and the afternoon ration

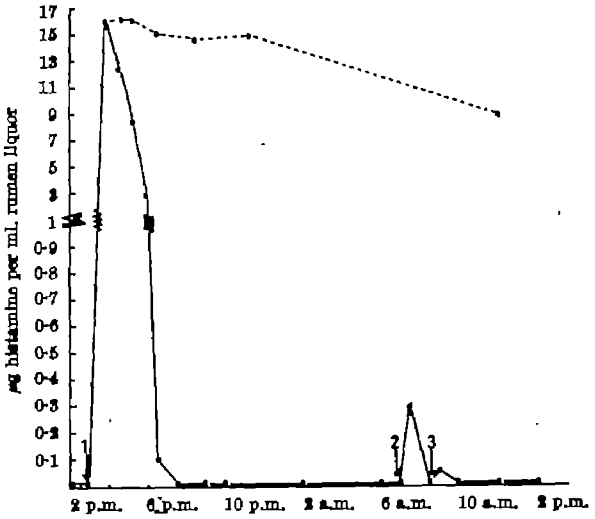

Fig. 1. Variation in the concentration of histamine in rumen liquor. ——, *In vivo*; – – –, *in vitro*; the *in vitro* sample of rumen contents was provided after ingestion of silage. Fed at hours indicated by arrows: 1, silage; 2, concentrates; 3, hay

of formic acid grass silage (460, 500, and 270 g, respectively). A large increase occurred after ingestion of silage. When the feeding schedule was altered, similar increments always occurred subsequent to silage feeding, independent of the hour of the day. The increase was of short duration, as in 3–5 h the histamine concentration in rumen reached pre-feeding levels. A moderate but distinct increase also occurred after feeding with hay or concentrates.

The quantities of preformed histamine were determined in silage, hay, fresh grass, and concentrates using the ether extraction method of Block[7]. The histamine was afterwards extracted twice from the ether with equal volumes of distilled water. By this procedure approximately 99 per cent was transferred to the water phase. When large amounts of histamine were expected, the assay could be performed directly, since small amounts of ether did not interfere. When small amounts were expected, however, the ion-exchange method was used, serving both the purpose of removing the ether and concentrating the solution.

Table 1. Content of Histamine in the Feeds used

Grass silage				
Unfractionated (μg/g dry weight)	Press juice (μg/ml.)	Hay (μg/g dry weight)	Concentrates (μg/g dry weight)	Fresh grass (μg/g dry weight)
1,110–3,000	620–1,230	0·2–0·3	0·4–0·6	0·5–0·9

Silage was found to contain large amounts of histamine, while the quantities present in the other feeds were minimal (Table 1). In samples of grass silage from 7 other farms (preserved with either HCOOH or H_2SO_4–HCl) the histamine concentration varied between 26 and 900 μg per ml. press juice.

To identify the tested substance we first showed that its effect on guinea pig ileum was completely counteracted by small amounts of antihistamine. Secondly, the ether extracts were subjected to paper chromatography (Whatman No. 1, diethyl-ether/ethanol/water/conc. ammonium = 5 : 4 : 1 : 0·1). The active compound had the same R_F value as histamine standards, and reacted positively on the Pauly reaction[8]. The chromatograms also revealed that tyramine was a component of these feeds (unpublished results).

Fig. 1 demonstrates a pronounced discrepancy between the *in vivo* and the *in vitro* disappearance of histamine in rumen contents after ingestion of silage. This indicates that micro-organisms of the rumen are not responsible for any substantial part of the metabolization. The rate of fluid transport from the rumen to more distal parts of the alimentary tract[9] does not seem to be sufficiently rapid to account for the fast disappearance from the rumen. This suggests that a considerable proportion of the histamine is absorbed. Furthermore, analysis of faeces revealed that neither free nor acetyl-conjugated histamine ever reached high levels after ingestion of silage. In urine, on the other hand, a large increase in the excretion of acetylhistamine occurred, whereas the increment of free histamine was minimal.

Table 2. Metabolism of Histamine (μg Histamine/g Wet Weight/h) *in vitro* by Liver Slices, Rumen Epithelium and Mixed Rumen Muscle

	Liver tissue	Rumen epithelium	Rumen muscle
No. of observations	28	21	8
Mean and *S.D.*	115 ± 23·4	3·1 ± 0·7	5·2 ± 1·1
Range	64–153	1·9–4·5	3·4–6·7

It is evident from Table 2 that moderate quantities of histamine can be metabolized in the rumen wall. However, after ingestion of silage its metabolic capacity will be far exceeded. Table 2 demonstrates that liver tissue possesses an activity sufficiently high to metabolize even these excessive amounts of histamine. Thus it seems likely that the absence of symptoms of intoxication and the minimal increments in free histamine in urine can to a great extent be attributed to detoxication in the liver. Further studies of these problems are in progress.

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Effect of Tetraethyl Ammonium Ion on the Electrical Activity of Smooth Muscle Cell

Tetraethyl ammonium (TEA) ion is known to be one of the most effective ions for maintaining the electrical activity of excitable tissues in sodium-free media[1]. It is also known that TEA is capable of lengthening the action potentials in crustacean muscle[2,3], crustacean nerve[4], vertebrate striated muscle[5] and in certain nerve fibres and cells[6,7]. The work reported here was planned to elucidate the effect of TEA on the smooth muscle cell, which is assumed to be different in membrane properties from many other excitable tissues.

Action potentials were recorded from the taenia coli of the guinea pig by means of intracellular microelectrode. TEA–Krebs solution, similar to the normal Krebs solution except that various amounts of sodium chloride in solution were replaced by equimolar amounts of TEA, was used.

At small concentrations of TEA (6 mM), the rate of spike discharge increased slightly but the shape of spike potential was not different from that obtained in normal Krebs solution. At relatively high concentrations of TEA (15 mM or more), its action is diphasic, causing an initial increase in spike frequency followed by the decrease in its frequency, and in the latter phase the marked prolongation of action potential was observed (Fig. 1). That is, the action potentials had shapes similar to those characteristically obtained from the cardiac muscle[8]. However, when the preparation was immersed in TEA–Krebs solution for a longer period, the resting potential tended to fall and the action potential magnitude

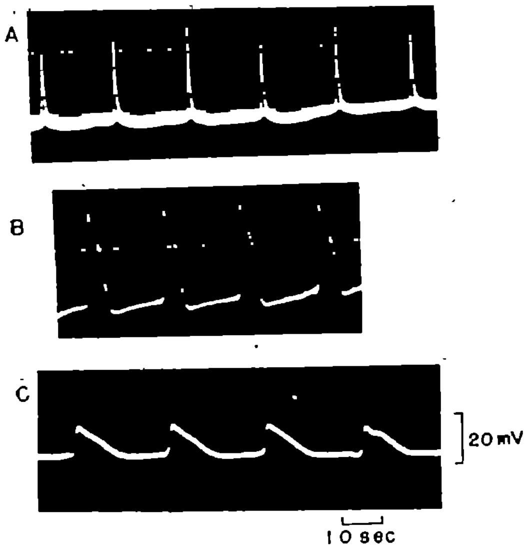

Fig. 1. The effect of TEA (38 mM) substituted for equimolar sodium in Krebs solution

A, action potential in normal Krebs solution; *B* and *C* obtained after 3 min and 30 min exposure in TEA–Krebs solution respectively. In *B* and *C*, the duration of action potential is prolonged. Note: marked depolarization in *C*

decreased. In such a depolarized condition, action potential still showed delayed repolarization. Those effects of TEA became more prominent when the amount substituted for sodium increased, but there was a variation in the degree of lengthening among individual cells of each preparation. The effect of TEA was usually reversed by washing in normal Krebs solution.

Recently, Burnstock and Prosser[9] and Barr[10] reported that barium chloride prolongs the action potential of intestinal smooth muscle. But there is not sufficient evidence to decide whether those two prolongations of action potential caused by TEA and barium chloride are similar in mechanism or not.

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Effect of Dilution of the Diet with an Indigestible Filler on Feed Intake in the Mouse

Adolph[1] and Warkentin *et al.*[2] showed that dilution of rat diets with varying proportions of indigestible filler, including cellulose, brought about an adjustment of feed intake by the animal, so that intake of digestible feed energy per day was the same on concentrated and bulky diets.

In experiments with mice at Leeds, different levels of food intake have been achieved by diluting a standard mouse food with varying amounts of cellulose meal. All diets were pelleted and fed to appetite.

Table 1. MEAN LITTER WEIGHT (g) AT WEANING (25 DAYS OLD)

Generation	Normal diet	15% Diluted	30% Diluted
0	80·46 ± 3·64 (34)	82·65 ± 3·64 (26)	60·87 ± 3·29 (46)
1	66·85 ± 4·41 (26)	68·44 ± 3·95 (30)	49·73 ± 3·25 (30)
2	84·68 ± 2·91 (40)	83·96 ± 3·97 (38)	55·14 ± 3·03 (34)

Figures in parentheses denote the number of observations for each mean.

The performance of the stock (Table 1) maintained on a normal undiluted diet and on diets diluted with 15 and 30 per cent of cellulose for 3 generations showed that, at low levels of dilution (15 per cent), performance measured by litter weight at weaning had not been impaired.

None of the differences between the means was statistically significant at $P = 0·05$. It appeared that 15 per cent dilution of the diet with cellulose was equally as effective as the normal diet.

Digestibility studies carried out showed that digestibility did not differ in the main dietary constituents between the diets, and there were limited results to show that mice on the diluted diets consumed more feed.

Recently this aspect has been pursued further in feed intake trials over a wider range of dietary dilutions using cellulose meal (Table 2).

Table 2. ANALYSIS OF DIETS USED IN FOOD INTAKE TRIALS

Diet	Dry matter (%)	Crude protein (%)	Crude fibre (%)
Normal (undiluted)	91·1	16·2	5·3
15% Diluted	91·4	14·2	14·1
30% Diluted	92·6	11·5	24·2
50% Diluted	93·5	7·7	33·4

In these trials, mean feed and water consumption per head per day were measured using 3 mice per cage. Feed wastage was recorded by sieving of waste feed and fæces. No bedding was provided in the cages. Results from male and female mice were recorded separately, but as the differences in feed consumption between sexes were small and not consistent, the values quoted refer to means of both sexes.

Trial 1. Mice were fed, some on a normal diet and others on the same diet diluted with 30 per cent cellulose meal by weight, for three continuous periods each of 7 days, commencing at 8–9 weeks of age (3 mice per cage). Feed and water consumption was expressed per gram of body-weight per day. The mice on each diet were from stocks that had been maintained on each diet throughout a long-term experiment for 20 generations. The results are expressed as the percentage extra food and water consumed by the mice on the diluted diet compared with the normal undiluted diet (Table 3).

Table 3. PERCENTAGE EXTRA FOOD AND WATER CONSUMED BY MICE ON A 30% DILUTED DIET COMPARED WITH A NORMAL DIET (PER G BODY-WEIGHT PER DAY)

Period (7 days)	No. mice per diet	$\frac{\text{30\% Diet–Normal}}{\text{Normal}} \times 100$ Food (g)	$\frac{\text{30\% Diet–Normal}}{\text{Normal}} \times 100$ Water (c.c.)
1	18	+27·6	+22·0
2	18	+17·8	+17·9
3	18	+23·1	+13·4
Mean	18	+22·8	+17·8

The animals attempted to compensate against dilution of the diet and ate 22·8 per cent more food than those on the normal diet. This compensation, however, resulted in an intake of only 86·0 per cent of the undiluted ration as eaten by the mice fed on the normal diet.

Trial 2. In this trial, mice of 8–9 weeks of age (3 per cage) were fed on diets diluted with 15, 30 and 50 per cent by weight of cellulose, and their intakes were compared with mice fed on a normal diet (Table 4).

The mice were all from stocks previously maintained on a normal diet for 20 generations and the trial extended over 35 days divided into 5 periods each of 7 days.

The intake of the animals on the 30 per cent diluted diet in Trial 1 was greater than that in Trial 2. This could be due to the different source of the animals for each trial. Adaptation of the stock to the diet for 20 generations prior to Trial 1 could have brought about a change in

Table 4. Percentage Extra Food and Water consumed by Mice on a 15, 30 and 50 per cent Diluted Diet compared with a Normal Diet (per g Body-weight per Day)

		$\frac{\text{15\% Diet–Normal}}{\text{Normal}} \times 100$		$\frac{\text{30\% Diet–Normal}}{\text{Normal}} \times 100$		$\frac{\text{50\% Diet–Normal}}{\text{Normal}} \times 100$	
Period	No.	Food (g)	Water (c.c.)	Food (g)	Water (c.c.)	Food (g)	Water (c.c.)
1	12	+15·1	+27·2	+5·6	+2·9	+14·6	+28·7
2	12	−2·4	+29·8	+16·6	+24·8	+27·3	+39·3
3	12	−4·8	−2·7	+8·5	+16·5	+26·3	+41·0
4	12	+4·6	+2·8	+12·9	+7·3	+32·1	+35·6
5	12	+4·8	+17·5	+16·4	+23·1	+34·4	+49·9
Mean	12	+3·5	+14·9	+12·0	+14·9	+26·9	+39·1

intake response, compared with the animals on the same diet in Trial 2 which had not had a similar adaptation period on the diet. Further work is required on this aspect.

The extent to which the animals on the diluted diets were successful in their attempts to compensate against lowered nutrient content of the feed is presented in Table 5. This shows the percentage intake of undiluted feed as fed on the normal diet, after the mouse had attempted to compensate.

Table 5. Percentage of Undiluted Feed consumed by Mice in Trial 2 after Attempts at Compensation

	Normal diet	15% Diluted	30% Diluted	50% Diluted
Effective nutrient intake	100	88·4	78·4	63·5
Deficiency	—	11·6	21·6	36·5

No definite conclusions could be drawn from water consumption except that it was directly related to food intake.

Dilution of the diet of the mouse with an indigestible filler, for example cellulose, is an effective means of lowering nutrient intake despite attempts by the animal to compensate for the lowered nutrient content of the diet by increased food consumption.

I thank Prof. T. L. Bywater for his interest in this work.

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HÆMATOLOGY

Hæmoglobin and Transferrin Types in the American Buffalo

When O. Smithies[1] published his first results obtained by starch-gel electrophoresis of human sera, an additional field was opened for investigations of genetic polymorphism. Consequently, a large number of investigations dealing with genetic variation in the serum proteins of several animal species have since then been reported. Smithies's technique can also be used with advantage for the purpose of examining electrophoretic patterns of hæmoglobins.

In this communication we describe results obtained by starch-gel electrophoresis of blood samples from 113 American buffalo (*Bison bison*). Sixty-five of these came from Wichita Mountains Wildlife Refuge, Cache, Oklahoma. The remaining 48 came from the U.S. Fish and Wildlife Service, National Bison Range, Moise, Montana. Blood samples were collected into tubes containing sodium citrate solution. On arrival at the laboratory, plasma samples were taken off after centrifugation and afterwards frozen. The red blood cells were washed three times in saline and then lysed by adding distilled water. The ratio of red cells to distilled water was 1 to 40.

The samples were subjected to electrophoresis in gels made from Connaught's potato starch. In the electrode vessels the *tris* buffer described by Gahne *et al.*[2] was used (20·2 g Sigma 7–9, 2 g EDTA and 1·5 g boric acid per litre). For preparing the gels a *tris* buffer similar to that described by Poulik[3] was used (Sigma 7–9, 9·196 g, citric acid 1·050 per litre). (The results showing the various types of hæmoglobins in Fig. 1 were, however, obtained by using the technique described by Gahne *et al.*[2].) The samples were inserted on filter paper and run horizontally for approximately 3·5 h at a voltage gradient of 7–8 V/cm.

In Fig. 1 is shown a photograph of the results obtained when running hæmoglobins from two bison. For comparison, the three types of cattle hæmoglobins first reported by Cabannes and Serain[4] in 1955 for Algerian hill cattle are also shown and by them called Hbα, Hbβ, and Hbα + Hbβ. These three samples here shown were from Jerseys. All the other 111 bison showed the same type of hæmoglobin as the two shown in Fig. 1; namely, two components which migrate at slightly different speeds. The slower-moving component corresponds to the hæmoglobin type of cattle now usually called *AA*. The faster-moving component moves with approximately two-thirds of the speed of hæmoglobin *BB* of cattle.

Fig. 2 is a photograph of results obtained on starch-gel electrophoresis of six bison plasma samples and three cattle serum samples. As may be seen, all six bison samples produced the same pattern. The remaining 107 samples gave this same pattern. One of the three cattle samples was from a *DE* animal; the other two were from an *AA* animal (nomenclature of Smithies and Hickman[5] and Ashton[6]). It can be seen that the cattle albumin and β-globulin bands are somewhat thicker than the bison bands. The reason for this is that the samples from cattle were serum samples and as such were not diluted, in contrast with the plasma samples which were diluted with the sodium citrate solution.

As indicated in Fig. 2 there was no difference between the β-globulin pattern of bison and the *AA* transferrin pattern of cattle. Therefore, it is concluded that these protein bands of bison are transferrins and that they are indistinguishable from the *AA* transferrin bands of cattle.

That these 113 samples of bison blood showed no polymorphism of β-globulin is in contrast with results obtained in studies of domestic animals where genetic variation in serum proteins has been the rule. Another wild species in which no genetic variation in β-globulins has so far been detected is *Alces alces*[7], but only 27 individuals were examined.

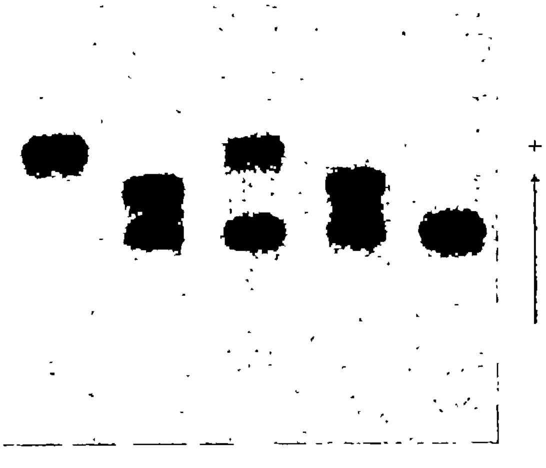

Fig. 1. Comparison between electrophoretic mobilities of bison and cattle hæmoglobins

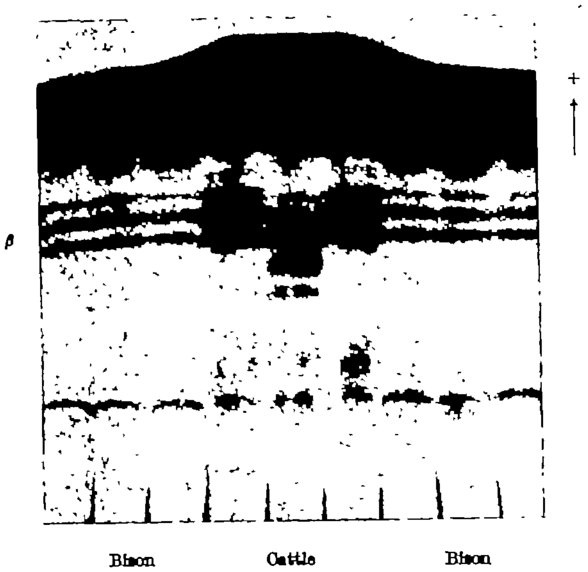

Fig. 2. Comparison between transferrin patterns in bison and in cattle

There are several explanations why we failed to detect polymorphism in bison transferrins and hæmoglobins. One is that the sample was too small and was not representative of all American buffalo. Another is that genetic variation in these traits was lost during the drastic reduction of the bison populations in the nineteenth century. For example, Owen *et al.*[8] proposed such an explanation in their discussion of the reasons for the small amount of variation in bison blood groups as detected in tests with cattle blood-typing reagents. But the possibility that bison evolved with little or no genetic variation in such traits cannot be excluded. Perhaps under natural selection mutants at the loci controlling blood groups, transferrins and hæmoglobins are not permitted to accumulate as rapidly as they do under artificial selection.

We thank Mr. Julian A. Howard, refuge manager of the Wichita Mountains Wildlife Refuge, Cache, Oklahoma, and Mr. C. J. Henry, refuge manager of the National Bison Range, Moise, Montana, for their co-operation in securing the blood samples.

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Qualitative Differences in the Mechanism of Complement Fixation

THE sensitization of human red cells by complement-fixing antibodies can be demonstrated with specific anti-human complement serum. By immunizing rabbits with egg albumen/anti-egg albumen precipitate with bound human C', an anti-complement serum was obtained, which contains at least two antibodies against human complement components, that is, C'_{3a} and C'_4 (ref. 1).

When tested in immuno-electrophoresis this antiserum forms two precipitation lines with human serum, called β_1-C(C'_{3a}) and β_1-E (C'_4) (ref. 2).

Anti-complement serum reacts, for example, with sensitized Lewis cells, red cells sensitized with naturally occurring incomplete cold antibodies, and red cells obtained from patients with cold type hæmolytic anæmia. We performed cross-absorptions of the anti-complement serum with these different types of sensitized cells.

Table 1

	Anti-C'	Anti-C' × HA–c.a.*	Anti-C' × E–c.a.†	Anti-C' × E–L.a.‡
HA–c.a.*	+	–	–	–
E–c.a.†	+	+	–	–
E–L.a.‡	+	+	+	–

* *In vivo* sensitized red cells obtained from patients with cold-type hæmolytic anæmia.

† Erythrocytes sensitized with naturally occurring incomplete cold antibodies.

‡ Sensitized Lewis red cells, which behave as sensitized sheep cells (EA) with bound $C'_{1,4,2,3a}$.

The results of the cross-absorption procedures are given in Table 1.

Fig. 1 shows the immuno-electrophoretic pattern obtained by the reaction between human serum and the non-absorbed and absorbed anti-complement serum. Sensitized Lewis cells remove both anti-C'_{3a} and anti-C'_4 antibodies; after absorption with red cells sensitized with naturally occurring incomplete cold antibodies anti-C'_4 is exhausted only, whereas the sensitized HA-c.a. cells absorb neither anti-C'_4 nor anti-C'_{3a}. The results of the immuno-electrophoretic investigation perfectly correlate with those obtained in the serological cross-absorption experiments. One outstanding feature is that C'_{3a} appears not to be bound to those systems which usually do not show hæmolysis.

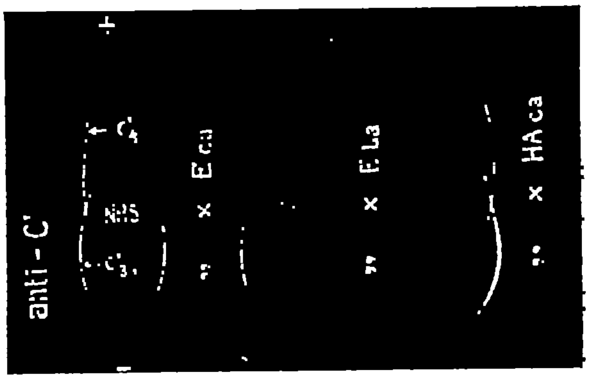

Fig. 1. Immuno-electrophoretic pattern obtained with non-absorbed and absorbed anti-C' serum

We have excluded the possibility that the agglutination of the HA-c.a. cells by the anti-C' serum was due to anti-β_2-M (19S γ-globulin) antibodies. However, several rabbit sera, even those of some non-immunized animals, possess the ability to agglutinate these HA-c.a. cells, sometimes in titres up to 32. This type of reaction, which is not caused by heterophile red cell antibodies, is under investigation.

Further experiments are needed to establish whether the reaction of anti-C' serum with HA-c.a. cells described here occurs on account of possibly present anti-C'_1, or is due to the as yet unexplained agglutinating-ability of certain rabbit sera.

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Hæmagglutination Induced by Nucleic Acids

Precipitation of some protein particles, induced by nucleic acids due to the interaction phenomenon between these two components in certain conditions, has attracted the attention of numerous workers, especially of cellular biochemists. However, very little is known concerning the interaction between nucleic acids and biological corpuscles, such as blood cells and bacteria, except for the serological reports[1] of hæmagglutination of tanned erythrocytes coated with DNA by reacting with some antibody in sera of systemic lupus erythematodes. Experiments were therefore carried out to elucidate the general effects of nucleic acids—yeast RNA and sperm DNA, regardless of their species specificity—on erythrocytes.

The buffered salines (0·147 M sodium chloride containing 0·02 M citric acid–sodium citrate buffer solution in the range of 6·0–4·0) are used as the diluents of this experiment. The *p*H of each nucleic acid solution (0·1 per cent) is adjusted with 0·2 N sodium hydroxide and diluted with stock solution of buffered sodium chloride, so as to bring its final ionic concentrations the same as the diluents. To nucleic acid solutions with two-fold serial dilution, the same volume of 2·5 per cent human erythrocytes solution, washed and resuspended in diluent, is added in the test-tube and incubated for 15 min at 25° C. The results, that is, the degree of hæmagglutination reactions, are read macro- and micro-scopically, which are shown in Table 1. Erythrocytes, which are pretreated with trypsin, exhibit the conspicuous rise of nucleic acid-induced agglutination avidity, but not any change of agglutination titres of nucleic acids. The remarkable changes of agglutination titres of nucleic acid by applying different individual human erythrocytes are not found. Agglutination activity of nucleic acid solution can be absorbed with the erythrocytes. For example, 1 ml. of diluted RNA (dilution 1 : 8, that is, 25 γ/ml.) is completely absorbed with the same volume of 2·5 per cent erythrocytes solution (5×10^8/ml.), showing that RNA molecules over $1·8 \times 10^6$ can be fixed on the surface of one erythrocyte, assuming that the molecular weight of yeast RNA is 17×10^3 and Avogadro's number 6×10^{23}.

Table 1. Hæmagglutination Induced by Nucleic Acids

*p*H*		Over 5	4·8	4·6	4·4	4·2	4·0
Agglutination titre of nucleic acid solution	RNA†	> 1 : 1	1 : 4	1 : 512	1 : 512	1 : 512	1 : 512
	DNA†	> 1 : 1	1 : 4	1 : 128	1 : 128	1 : 128	1 : 128

* Microscopic examination reveals that this aggregation of erythrocytes is true agglutination, and not the rouleaux formation, which may be encountered in acid medium. There is no remarkable acid-induced hæmagglutination in *p*H 5 to 4 in these experimental conditions. Considerable degree of hæmolysis occurs at *p*H below 4.

† The original nucleic acid concentration (1 : 1) is 20 mg per cent. RNA and DNA are preparations from Nutritional Biochemical Corporation, U.S.A.

Addition of sera (*p*H 4·5) of human, rabbit, sheep and guinea pig inhibits this nucleic acid-induced hæmagglutination. As for the proteins, so far tested, human albumin shows this inhibitory action, while α-globulin, which is precipitated at *p*H 4·5, has no effect on this hæmagglutination. Erythrocytes of sheep, guinea pig and rabbit are agglutinated by nucleic acids also. (Both proteins were fractionated by Cohn's fractionation technique of human plasma proteins.)

From these results it is not so difficult to suppose that there are some receptors on the surface of erythrocytes, more generally on the protoplasmic membrane of erythrocytes, which can combine with nucleic acids to agglutinate them. The phenomenon that some proteins, such as ovo-[2,3] and human-albumin[4], can interact with nucleic acids in *p*H below isoelectric point of the former ones is attractive in the analysis of this reaction mode. It is reasonable to expect that certain proteins, which possess the isoelectric point at about *p*H 4·6, are determining receptors in this agglutination. In the same way, inhibition of this agglutination due to the presence of human blood albumin can be elucidated by the affinity of albumin for nucleic acids, so that it may deprive nucleic acid–erythrocytes complex of nucleic acid as a competitor. It is also possible that hæmagglutination induced by some viruses may be attributed to their main components, that is, their nucleic acids or nucleoproteins. Further studies on the nature of receptors of erythrocytes are now in progress.

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PATHOLOGY

Pathology of the Effect of the Laser Beam on the Skin

The effect of the laser on the eye has been known since the work of Zaret[1,2]. The effect on the skin is not well known[3,4]. Previously, some physicists have reported subjectively no effects at all or only a slight pricking sensation of the skin[3].

The laser used for these preliminary experiments was a solid type with ruby crystal. The crystal has a diameter of ¼ in. and a length of 2 in. with a crystal optic axis of 90°. The emission is 6943 Å with a minimum energy input of 100 joules (2·0 kV). Reflectivity is 99+ to 90 per cent. Output at 400 joules input equals 0·84 joule. (Maser Optics, Inc., Boston, Massachusetts.) The laser head was mounted on an optical bench with an external lens system. Lenses used chiefly were a single convex lens with a focal length of 10·2 cm, a double convex with a focal length of 6·0 cm and a double convex with a focal length of 1·2 cm. A centring disk and also a special mount were used on the optical bench to hold the area to be exposed. A red light was attached to the head to aid in centring the beam. Protective glasses were used by operating personnel.

The skin specimens examined included white skin of rabbits and man, coloured skin of animals and man, pigmented baso-squamous papillomas (so-called seborrhœic keratoses), superficial hæmatomas in the skin of man, white skin of man covered with lamp black, indian ink, tars, and

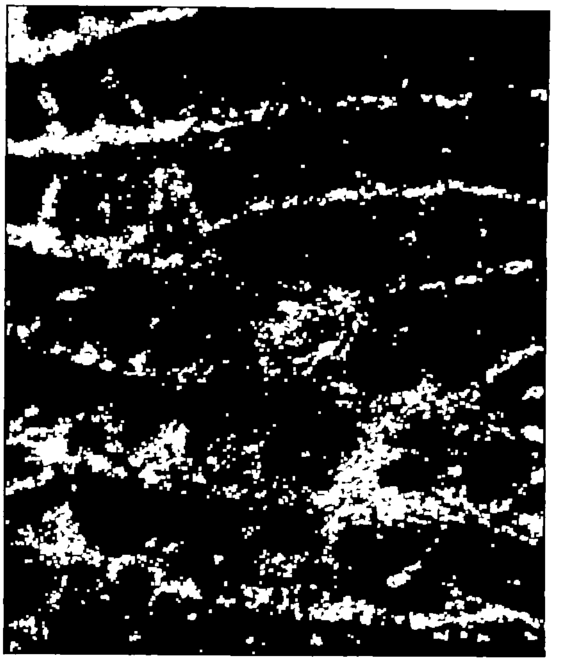

Fig. 1. Surface of skin of palm coloured by dihydroxyacetone and exposed to laser. Superficial crusted area is the area of impact (black-and-white picture from 'Kodachrome', × 50)

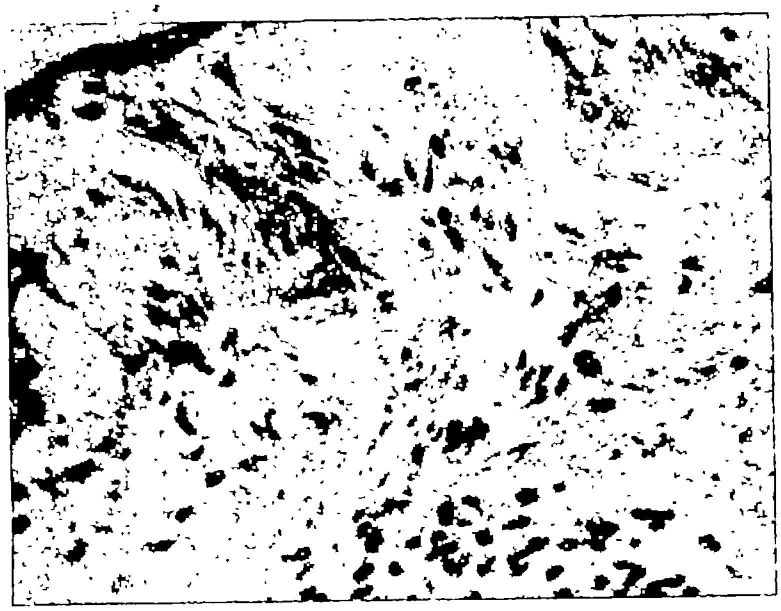

Fig. 2. Coloured skin after exposure to laser showing marked epidermal change (hæmatoxylin–eosin, × c. 300)

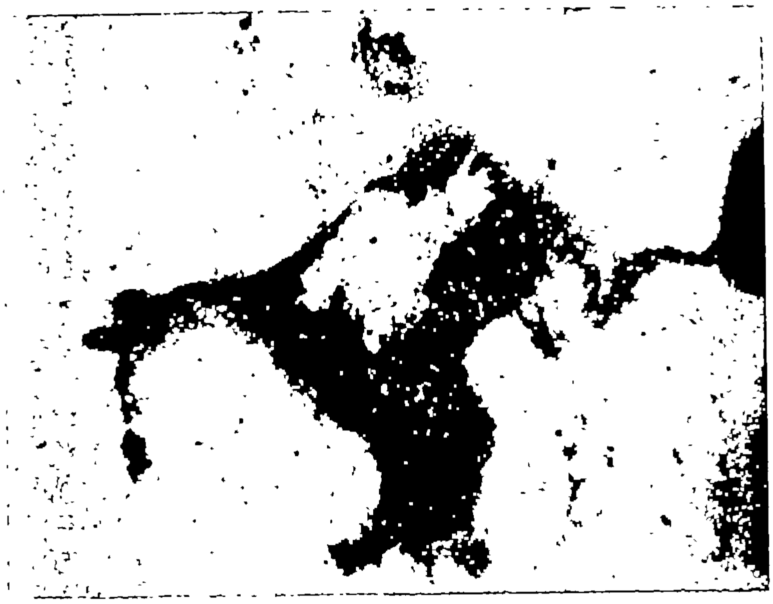

Fig. 3. Cytological preparation from pigmented basal cell carcinoma. Tissue fragments of red cells and tumour masses on slide exposed to laser, then stained with May–Grünwald Giemsa. White area represents area of impact (× c. 39)

coloured by dihydroxyacetone. Curretted material from a pigmented basal carcinoma and from calcinosis cutis were also used. Studies of tattoo marks are not included in this series.

In addition to the skin the following biological materials were also exposed to the laser beam: black widow spider, cockroaches, caterpillars, fresh human cytological preparations from buccal and vaginal mucosa, pigmented and non-pigmented basal cell carcinomas, melanomas, basosquamous acanthomas, molluscum contagiosum, wart, tissue cultures of HeLa cells, cultures of human chromosomes, cultures of bacteria and fungi. In this communication only skin pathology will be described.

The exposed area of skin was examined grossly and examined also with stereobinocular skin microscope at ×20 and × 40 and photographed in colour at × 1 and × 40. In a number of experiments the impact area was photographed in colour at the instant of impact of the beam.

Skin sections were fixed in formaldehyde, stained by hæmatoxylin–eosin and with sulphydryl stains. The interval time between exposure of the skin and the time of excision biopsy was recorded.

With the light-weight laser, the lesions in man were superficial even in heavily pigmented skin. As a rule, the deeper the pigmentation, the deeper the involvement of the epidermis. This is due to the absorption of energy of the laser beams in black substances. On white skin no pathology could be observed. Even application of lamp black or indian ink to the surface of white skin produced no evident lesions of keratin after exposure to the laser. The impact area of the laser was represented by a whitened zone, 1·0–1·5 mm in diameter, in the covering material. This, we believe, was caused by vaporization of the black covering. An effort was made to get black particles deeper into the epidermis by means of partial stripping of the skin with 'Scotch' tape. Then lamp black was rubbed in well and the surface cleaned off. Again, white area of impact of the laser beam was limited only to the dark particles. Later, with the formation of a blackish-brown crust, exposure to the laser beam produced a whitish area limited only to the crust and the laser beam was without any apparent effect on the smooth red skin under the dried crust.

In the coloured skin, the laser produced superficial lesions with fragmentation and splitting of the stratum corneum. Sulphydryl stains showed no changes of keratin adjacent to the impact area. In more severe lesions of coloured skin, cells of the upper spinous layer were eosinophilic and pycnotic; basal cells showed hydropic degeneration with cellular contraction, eosinophilia and pycnosis. In some areas the changes in basal cell varied in severity. Œdema was found in epithelial cells adjacent to the impact area. The dermis showed œdema and perivascular infiltrate. In some areas the perivascular infiltrate included eosinophils which had migrated also into the epidermis. This was observed in a biopsy taken 4 h after exposure. Finely granular, golden-brown pigmented areas were found in the dermis concentrated most heavily within the zones of perivascular infiltrate. The capillaries were dilated with the endothelial cells œdematous. In the lower dermis no changes were found in the eccrine glands. The intensity of the inflammatory infiltrate varied with the time-interval after biopsy.

On skin and hair of albino rabbits no lesions were evident. In the black pigmented skin of rabbit ear through and through tissue destruction was produced. Sections of the ear taken 1 h after exposure to the laser beam showed deep fissured area through epidermis, dermis and into cartilage. The dermis showed compression and increased density of collagen with a moderately thick border of neutrophilic infiltration about the areas. The adjacent dermis showed an œdema and a diffuse infiltration of lymphocytes and histiocytes. This biopsy was taken 1 h after exposure. The epidermis adjacent to the impact area showed œdema and loss of melanin pigment.

An urticating caterpillar, identified as a *Nygmia phaeorrhea*, had a black head, mostly black body with brown mid-zone and brownish-black setæ. Exposure to laser beam produced a deep reddish discoloured zone of the head area. After fixation in Davidson's formaldehyde fixative, hæmatoxylin–eosin stained sections were examined. These showed sharply demarcated areas of brown-coloured coagulation necrosis. Blackish-brown setæ exposed to the laser beam developed a golden-yellow colour.

An examination of lesions produced by higher powered lasers with the skin of man, with animals and with arthropods is still in progress.

Preliminary investigations, then, with a light-weight type solid laser with a ruby crystal showed that superficial destructive lesions may be produced in the skin of man. The more intense the colouring of the skin the deeper the reaction. Deep necrotic lesions were produced in pigmented areas of the rabbit ear.

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RADIOBIOLOGY

Chemical Protection for the Incorporation of Phosphorus-32 into Nucleic Acids of Lymphatic Cells against γ-Irradiation

Honjo *et al.*[1] found that the lowered viability of Ehrlich ascites tumour cells due to continuous β-irradiation with yttrium-91 could be effectively protected by repeated additions of reduced glutathione during irradiation. This may be ascribed at least partly if not wholly to the protection for the synthesis of nucleic acids. Thus, we are interested in whether or not the incorporation of precursors into nucleic acids can be chemically protected against ionizing radiation. Henke *et al.*[2] observed the protective effect of cystein given intraperitoneally for the incorporation of phosphorus-32 into intestinal deoxyribonucleic acid of X-irradiated rats. In *in vivo* experiments, however, uncontrollable factors can scarcely be excluded; for example, migration of certain cells into, or out of, the organ concerned may occur after irradiation. Therefore, we carried out the *in vitro* experiment with the lymphatic cells, which are known to be more radiosensitive than Ehrlich ascites tumour cells in terms of nucleic acid synthesis[3,4].

Lymphatic cells, which had been obtained from appendices and Peyer's patches of rabbits and suspended in calcium-free Tyrode solution (*p*H 7·3), were irradiated with cobalt-60 γ-rays at room temperature either immediately before or 5–10 min after the addition of protectors in various concentrations and thereafter were shaken with phosphorus-32 at 37° C for 4 h. Deoxyribonucleic acid, ribonucleic acid and organic acid-soluble phosphate were isolated[3], and the specific activity of each fraction was determined as counts/min/μg phosphorus. Each value of specific activity is the mean from the duplicate samples. Chemical protectors used were cysteamine and *S*,2-aminoethylisothiuronium·Br·HBr (AET) (Nippon Shinyaku Co., Kyoto).

The inhibitions of incorporation referred to various γ-doses are shown in Fig. 1. If two curves representing the inhibitions for deoxyribonucleic acid and ribonucleic acid are extrapolated, both of them intersect the ordinate at 0 per cent of inhibition, corresponding to 10 r. on the abscissa. This may mean that the threshold for the response of the lymphatic cells to γ-radiation, measured by the incorporation of phosphorus-32 into nucleic acids, is about 10 r.

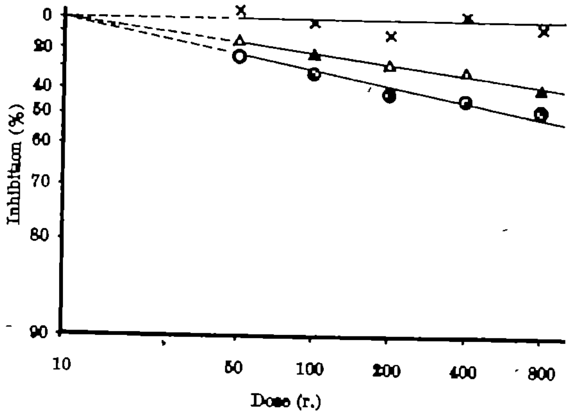

Fig. 1. Dose-effect relation in log–log plots. ○, Deoxyribonucleic acid (37·6); △, ribonucleic acid (146·0); ×, organic acid-soluble phosphate (1,258). Figures in parentheses indicate the specific activities of the unirradiated control. 3 × 10⁶ cells per tube were used. Cf. ref. 4 for the conditions of γ-irradiation and incubation in detail

The incorporations into nucleic acids were not significantly impaired by cysteamine alone, up to the concentration of 0·2 mg/ml., but they were severely affected with 2 mg/ml. (inhibition for deoxyribonucleic acid : 86·4 per cent). AET proved to be much more noxious to these cells. The incorporation into deoxyribonucleic acid was inhibited by about 50 per cent at 0·2 mg/ml., being equivalent to 0·055 mg/ml. of cysteamine on molar basis. Since AET is converted to 2-mercaptoethylguanidine·HBr at neutral *p*H (ref. 5), the inhibition may be owing to the latter agent. Of interest is the fact that the incorporation into organic acid-soluble phosphate was either thoroughly unaffected or somewhat enhanced by cysteamine, at least up to 2 mg/ml., while the incorporation was inhibited by more than 90 per cent by AET in this concentration.

The impairments for the incorporation were not remarkably effaced even if the chemical agents were removed by washing the cells before the incubation.

Some protective effect could be observed when the lymphatic cells were irradiated after addition of cysteamine (Table 1). The effect became much more distinct in higher concentrations, though the incorporation activity was affected remarkably by cysteamine alone as stated before. As shown in Table 1, cysteamine seems to be

Table 1. Effects of Cysteamine and AET on the γ-induced Inhibition of the Incorporation of Phosphorus-32 into Lymphatic Cells

Protector	Concentration of protector (mg/ml.)	Specific activity in percentage of the control which added with protectors but not irradiated			
		Deoxyribonucleic acid		Ribonucleic acid	
		Protectors added before irradiation	Protectors added after irradiation	Protectors added before irradiation	Protectors added after irradiation
Cysteamine	0·02	67		63	
	0·20	63		67	
	0·45	66	45	84	56
	1·00	83	42	112	49
	2·00	100		97	
AET	0·10	53	52	49	55
	0·20	58		54	

Concentrations of protectors indicated are those at the time of irradiation, they are reduced by half at the time of incubation, because of addition of phosphorus-32 solutions. γ-Ray dose applied is 400 r. throughout the experiment. Specific activities of the irradiated samples (without protectors) in percentage of the unirradiated control are 47–51 for deoxyribonucleic acid and 52–57 for ribonucleic acid.

effective, when present at the time of irradiation. On the contrary, AET failed in exertion of protective action in the concentration so far as examined, irrespective of whether it was added before or after the irradiation.

The discrepancy between our result and others reporting the effectiveness of AET *in vivo* has remained unsolved. It may be probable that cysteamine and AET do not necessarily play the same part in protection *in vivo*. In this connexion, the marked difference between cysteamine and AET in their noxious effect on the incorporation into organic acid-soluble phosphate is worthy of note.

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Changes in the Excretion of Sodium by Saliva after Whole-body Irradiation in Rats

We have investigated the excretion of sodium in the saliva of irradiated rats by means of the radioisotope of sodium-22. The following method was used. White male rats of the laboratory strain weighing 150–200 g were divided into three groups: the first group containing 10 rats formed controls, the second and third groups consisted of 20 rats each. The animals were fed by a standard diet and accepted water *ad libitum*.

All the rats were administered intraperitoneally 25 μc. of sodium-22 in 1 cm^3 of physiological solution per kg of the live-weight. After 24 h, the second and third groups of rats were irradiated with a dose of 1,000 r. by an X-ray Macrophos apparatus at 200 kV., 15 m.amp, focus distance of 120 cm. The time exposure amounted to 105 min; 0·5 mm aluminium and 0·5 mm copper were used as filter. The dose was controlled by means of a Mecapion dosemeter. After the exposure 3 animals survived 2 days, and 6 animals 3 days. Ten animals in the last group were used as controls.

Samples of saliva were taken from the first and third groups 96 h after exposure and from the second group 72 h after exposure. Just before saliva was taken, the whole-body radioactivity was measured by means of a whole-body counter with 6 Geiger–Müller tubes which was designed in our Institute[1]. Saliva was taken for 20 min according to Benarde *et al.*[2] under thiopental anaesthesia after the administration of 'Pilocarpin'. Thiopental was applied at a dose of 45 mg/kg intraperitoneally, 'Pilocarpin' at a dose of 10 mg/kg of the live-weight, subcutaneously. The samples obtained were weighed and using a standard the radioactivity was determined by an end-window tube for measuring β-radioactivity.

As the rats excrete the administered radiosodium in urine and faeces at differing rates, it was necessary to correct the results of radiosodium concentration in saliva accordingly. If the initial activity administered to one rat amounted to A_0(μc.), the activity of the same rat, when saliva was taken, measured by the whole-body computer, was A(μc.), the concentration of radiosodium obtained in saliva c(μc./g), then the corrected value of concentration $c_{\text{corr.}}$ (μc./g) was computed from the following equation:

$$c_{\text{corr.}} = c\,\frac{A_0}{A}$$

The results are plotted in Fig. 1.

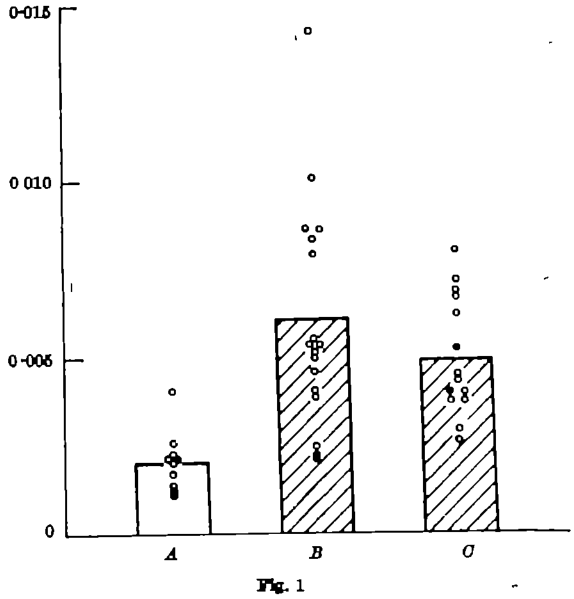

Fig. 1

The corrected concentration of radiosodium in μc./g of saliva is plotted on the abscissa. The height of column A represents the average value of the concentration in controls. The individual points describe the individual values of radiosodium concentrations in saliva. Similarly column B represents the average value and individual values of concentration in rats 72 h after exposure, and column C in rats 96 h after exposure.

There is a significant increase of radiosodium concentration in saliva of rats exposed to a dose of 1,000 r. after 72 and 96 h as compared with controls. The probability of the insignificance of differences of both experimental groups in comparison with controls is less than 0·5 per cent.

It has been shown[3] that the 24-h period between the administration of sodium-22 and the first action in the organism (exposure) is sufficient time to produce equilibrium between the administered radioisotope and the exchangeable sodium in bodies of rats. Therefore the observed relative changes of the radioisotope concentration may be evaluated similarly as changes in the total sodium concentration in saliva. According to the results in the literature, on about the 3rd and 4th day, changes of concentration of sodium in blood[4,5] are not so great as to cause the changes in saliva which we observed. It may lead to the conclusion that the values obtained are caused directly by a disturbed mechanism of saliva excretion.

We might find the explanation in a disturbance of the active sodium transport in the cells of chicks in the columnar epithelium of ducts.

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BIOLOGY

Synthesis of Enzymes during the Germination of Fungus Spores

THE hypothesis that the development of an organism is mediated by alterations in its enzyme systems is generally accepted. Illustrative data, however, are scarce, though some changes in rat liver enzymes have been reported[1,2]. In the fungi, the development from the resting spore to an active growing mycelium is a very dramatic phenomenon. Usually, under proper conditions, the spore swells, produces a germ tube which elongates, branches, and forms a mature mycelium. If any marked changes in the enzyme content of the fungus occurs, one would expect such alterations at some period during germination, before the germ tube was fully formed. A change of this kind was thought to occur during the activation of ascospores of *Neurospora crassa* by heat[3], but this effect could not later be confirmed[4]. Indirect evidence that the enzymes of a spore can be different from those of mature mycelium can be deduced from the catabolism by *Tilletia caries* of glucose variously labelled with carbon-14. The spores utilized glucose through the Entner Douderoff pathway, while the mycelium catabolized the sugar through the Embden, Meyerhof, Parnas system and hexose monophosphate shunt[5]. Results presented here show that in one fungus, *Ustilago zeae*, specific qualitative and quantitative changes in enzymes content occur during the germination of a spore.

Smut spores were obtained from diseased field corn and sieved to a homogeneous mixture. They were germinated in 100 ml. of 5 per cent molasses in a 500-ml. Erlenmeyer flask on a reciprocal shaker for various periods. Samples were taken at intervals up to 12 h, when only about 25 per cent of the spores had formed incipient germ tubes about half the diameter of the spores. If germinated for longer periods, about 75 per cent of the spores produced germ tubes. Assays for all enzymes of the Embden, Meyerhof, Parnas system, the hexose monophosphate shunt, and citric acid cycle were made on spores that were washed three times with distilled water, then ground with sand and suspended in buffers. Standard assay procedures were followed[6]. The only enzyme assays on which no certainty could be placed were those for pyruvic dehydrogenase, and α-ketoglutarate dehydrogenase; these enzymes were very difficult to detect even in growing mycelia and therefore the results have not been included. Oxygen consumption was measured on whole spores in the presence of 50 μmoles glucose in Warburg respirometers. Protein synthesis was studied by determining the amount of incorporation of phenylalanine-1-^{14}C into the smut spores. The spores were incubated with the amino-acid in molasses medium, then treated with sulphuric acid to hydrolyse protein. Phenylalanine was separated by paper chromatography, eluted, and the c.p.m. measured.

Results in Table 1 show that in resting spores some enzymes which are needed for glucose metabolism are either absent, or are present in such minute amounts as to be undetected. Missing in the hexose monophosphate shunt are glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, transketolase and transaldolase. Missing citric acid cycle enzymes are aconitase, succinic dehydrogenase, and fumarase. Even after 6 h incubation in the molasses medium, detectable quantities of these enzymes had not been synthesized. However, they were present after 12 h germination, the period when the germ tubes were first beginning to form. All enzymes of the Embden, Meyerhof, Parnas pathway for glucose catabolism were present even in the resting spores. During the germination process, not only were hitherto undetected enzymes synthesized but the specific activities of those enzymes originally present in resting spores were also increased. The examples in Table 1 show increases within 6 h, and further increases at 12 h.

Table 1. COMPARISON OF SPECIFIC ACTIVITIES OF ENZYMES IN TELIOSPORES OF *U. maydis* AT DIFFERENT PHASES OF GERMINATION

Enzyme	Specific activities units/mg protein		
	0 h	6 h	12 h
Triose phosphate dehydrogenase*	0·015	0·03	0·10
Isocitric dehydrogenase*	0·006	0·009	0·014
Malic dehydrogenase*	0·046	0·051	0·241
Triose PO$_4$ isomerase*	0·32	0·83	1·2
Ribose-5-PO$_4$ isomerase†	9·0	17·0	32·0
α-Glycerol-PO$_4$ dehydrogenase*	0	0	0·009
Glucose-6-PO$_4$ dehydrogenase*	0	0	0·006
6-Phosphogluconic dehydrogenase*	0·014	—	0·027
Transketolase‡	0	0	22·8
Transaldolase‡	0	0	6·6
Aconitase§	0	0	160·0
Fumarase§	0	0	240·0
Succinic dehydrogenase*	0	0	0·005

* One unit equals one μmole substrate transformed per min.

† A unit is that amount of enzyme which gives a change in optical density of 0·01/h at 540 mμ.

‡ A unit is that amount of enzyme which gives a change in optical density of 0·01/h at 405 mμ.

§ A unit is that amount of enzyme which gives an initial rate of increase in optical density of 0·001/min at 240 mμ.

Weak respiratory activity of resting spores, even in the presence of glucose, is shown by their low oxygen consumption: Q_{O_2} is 1. With increasing periods of incubation the spores consumed oxygen at a greater rate until just at germ tube formation the Q_{O_2} is 20 (Fig. 1). If one accepts the incorporation of phenylalanine-1-^{14}C into the spore as indicative of protein synthesis, then there is a concomitant increase in enzyme protein (Fig. 1).

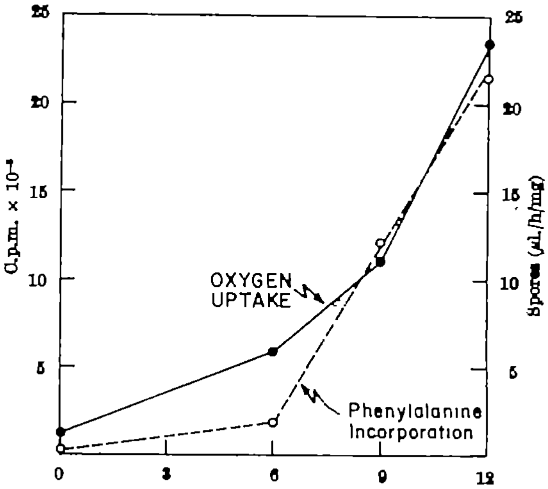

Fig. 1. Incorporation of phenylalanine-1-^{14}C into protein and oxygen consumption during the formation of *Ustilago maydis* teliospores

Comparing the three criteria on a time-scale reveals a strong coincidence among them. Before 6 h, the increase in protein and oxygen consumption is relatively small, and none of the missing enzymes had been synthesized. After that time, when enough of the missing enzymes have been made to allow the ready metabolism of glucose, the oxygen utilization rose sharply and continued at that rate at least until the appearance of the germ tubes at 12 h. Protein synthesis, as measured by phenylalanine incorporation, follows the increase in oxygen consumption very closely. Thus, in *U. zeae* the development of the organism can be linked with the formation of enzymes that are needed in carbohydrate metabolism.

The oxygen consumption by resting spores of *U. zeae* is very low, as might be expected in the absence of enzymes of the hexose monophosphate shunt and citric acid cycle; complete oxidation of carbohydrates or fats would not be possible. The presence of all components of the Embden, Meyerhof, Parnas pathway should have allowed the functioning of the glycolytic system, yet there was no evidence of carbon dioxide production under anaerobic conditions.

From these investigations it is also evident that spore germination cannot be conceived simply as germ tube formation. The morphological manifestation of development appears long after physiological mechanisms begin to operate. The synthesis of needed enzymes and the oxidation of carbon provide the energy and intermediates that are necessary for germ tube formation.

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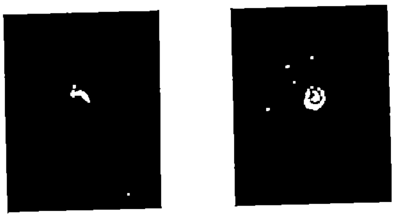
Effect of *Pseudomonas tabaci* on the Metabolism of Starch in Tobacco Leaves

IN the host tissues infected by various plant pathogens, including viruses, bacteria and fungi, as a rule starch accumulates around the infection area[1-3]. By contrast, the toxin-induced chlorotic halo formed in White Burley tobacco leaves infected by *Pseudomonas tabaci* (Wolf and Foster) Stevens fails to show any starch reaction (Fig. 1). The low starch content of the affected area was also shown by quantitative chemical determination[4] (Table 1). The assays were made 7 days after needle inoculation.



A B

Fig. 1. Parts of a tobacco leaf after needle-inoculation with *Pseudomonas tabaci*. *A*, Untreated; *B*, stained with iodine

The decrease in starch content could also be induced by the injection of bacteria-free culture filtrate (Table 1). Czapek–Dox nutrient medium incubated with *Ps. tabaci* for 48 h was used. Treatments were carried out on intact plants by injecting one half of the leaves with the culture filtrate and the other with Czapek–Dox nutrient as control. Measurements were made 3 days after injection.

Table 1. EFFECT OF *Pseudomonas tabaci* ON THE STARCH CONTENT AND PHOSPHORYLASE ACTIVITY OF TOBACCO LEAVES

Treatment of leaves	Starch content* (mg/g fresh weight)	Phosphorylase activity* Synthesized amylose (mg/g fresh weight/1 h)	Phosphorylase activity* PO_4^{3-} liberated (mg/g fresh weight/1 h)
Control†	2·6	4·5	1·8
Needle inoculated	1·5	1·2	0·6
Control‡	5·2	4·4	1·6
Injected with culture filtrate	2·3	2·3	0·9

* Values from representative experiments.
† Unaffected areas of the same leaf.
‡ Half-leaf injected with Czapek–Dox nutrient.

To elucidate the possible mechanism responsible for decrease in starch content the activity of the two major enzyme systems involved in starch metabolism was measured. A negligible and equal amylase activity was found in the extracts from control and culture filtrate treated leaves. The activity of phosphorylase, however, exhibited striking differences. The extent of amylose synthesis from glucose-1-phosphate was measured as follows: 500 mg tissue was extracted in the cold in 2·5 ml. acetate buffer *p*H 6·0. The reaction mixture contained 1 ml. crude extract (without centrifugation), 10^{-2} M sodium fluoride, 0·2 ml. 1 per cent dextrin as primer, and $1·7 \times 10^{-2}$ M glucose-1-phosphate in 2·3 ml. final volume. After incubation at 30° C for 30 min the reaction was stopped by adding equal amounts of 10 per cent trichloroacetic acid (controls were treated with trichloroacetic acid at zero time). The starch (amylose) content was determined colorimetrically in centrifuged samples by adding 0·2 ml. of an iodine solution containing 60 mg iodine/100 ml. A standard curve was prepared from soluble starch. Alternatively the orthophosphate set free during starch synthesis was measured[5].

As shown in Table 1 phosphorylase activity, as compared with the controls, was markedly less both in the halo areas of the infected leaves and in the tissues treated with culture filtrates.

The *Ps. tabaci* strain used in these experiments does not hydrolyse starch. The culture filtrate added to phosphorylase-containing plant extracts *in vitro* did not diminish the activity of phosphorylase. Therefore, it can be stated that the decrease in phosphorylase activity is due to an indirect effect of *Ps. tabaci* on the metabolism of the host. This effect is specific because the activity of some other enzymes, for example, glucose-6-phosphate dehydrogenase, increases in the tissues surrounding the infection area. The parallelism between the decrease in starch content and inhibition of phosphorylase activity suggests a causal relationship.

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Optimum Leaf Area Index in the Potato Crop

IN his review of the effect of competition for light on crop growth Donald[1] notes the existence of but two published accounts presenting evidence to support the generally adopted conception of an 'optimum leaf area index' as the ratio of leaf area to ground covered at which the balance of photosynthesis over respiration is at a maximum value. For kale crops in southern England the figure lies between 3 and 5·4 (ref. 2). In Australia, subterranean clover was found to reach its highest net assimilation rate when the leaf area index lay between 4 and 5 (ref. 3). More recently, Stern and Donald[4] have shown that the optimum leaf area index for clover in Australia varies with the amount of light present, particularly the amount which penetrates to the lower leaves, rising from 3·5 at a radiation-level of 50 g cal/cm²/day to a nearly constant 5 above 150 g/cal/cm²/day. Donald[1] calls for more evidence for other crops and different regions.

In an experiment on the effect of applying four levels of a 12 per cent N : 12 per cent P_2O_5 : 18 per cent K_2O fertilizer to a crop of Majestic potatoes at Boghall, Midlothian, in 1961, measurements were made of leaf area and the dry matter of leaves, stems, roots and tubers at four dates each approximately three weeks apart. The plants which received 2·5 and 5 cwt. per acre rates of fertilizer (Fig. 1) show a steady rise in leaf area index to 4·4 and 5·3 respectively at the third sampling date, declining thereafter to 3·9 and 4·9. The plants given the 7·5 and 10 cwt. per acre treatments rose swiftly through the level of leaf area index 5 to reach maxima of 7·0 and 8·3, afterwards declining to 5·5.

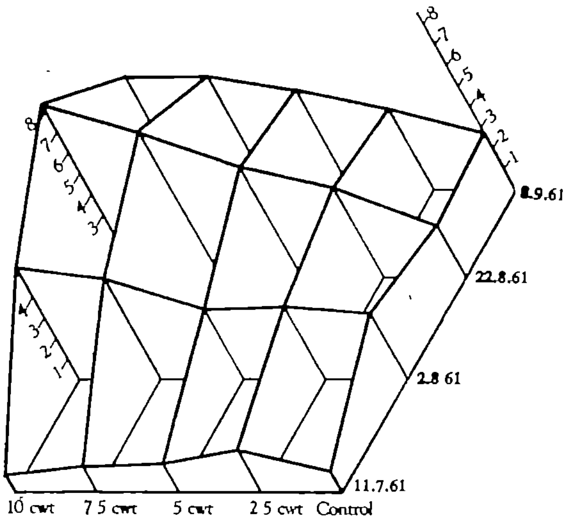

Fig. 1. Leaf area index at four dates of Majestic potatoes treated with four levels of fertilizer. Treatments: 10 cwt., 7·5 cwt., 5 cwt., 2·5 cwt. Control. Sampling dates: 11.7.61; 2.8.61; 22.8.61; 8.9.61

Table 1. Net Assimilation Rates in g/m²/week of Majestic Potatoes treated with Different Levels of Fertilizer

Sampling	Control	2·5 cwt.	5 cwt.	7·5 cwt.	10 cwt.
I–II	37·68	32·30	39·64	53 94	49·13
II–III	9 65	35·60	50·80	40 68	38·30
III–IV	17·98	11 79	10·46	0 72	−6·57

Table 2. Rate of Assimilation in g/m²/week of Material devoted to Tuber Production (Economic Assimilation Rate) in Potatoes treated with Varying Levels of Fertilizer

Sampling	Control	2·5 cwt.	5 cwt.	7·5 cwt.	10 cwt.
I–II	27·64	23·23	23·71	25·56	19·47
II–III	6 74	33·01	41·08	33·19	24·79
III–IV	18 67	12·91	11 98	4 78	2·31

The maximum net assimilation rate (Table 1) achieved by the high fertility-level plants occurs between samplings I and II when the leaf area index is below 5. On the low fertility-level plots maximum net assimilation rate occurs between samplings II and III at leaf area index values between 3·5 and 5·3. Between sampling dates III and IV the net assimilation rate of plants receiving the highest level, 10 cwt., decreased below zero because the loss of lower leaves, which had turned yellow, withered and dropped off, was greater than the increase in tubers. This process of leaf shedding was observed on all plots and is confirmed by the drop in leaf area index values (Fig. 1). The values found at this stage in crop development do not therefore give a true net assimilation rate and no way can be found to collect the lost leaf material in the field in order to make a correction. To overcome this difficulty it was decided to consider only that part of assimilation devoted to tuber formation by substituting dry weight of tuber per square metre of leaf area for total dry weight of plant material per square metre in the calculation. It is proposed to call this quantity the economic assimilation rate. In general, economic assimilation rate (Table 2) is, in the later stages of crop development, highest in the low fertility level plots and lowest in the high fertility level plots. Curves for economic assimilation rates against mean leaf area index between the sampling dates (Fig. 2) show that optimum leaf area index for tuber production lies around 2·6 in the early stages of development and around 4·3 later in the season. The drop in tuber yield on plots to which high levels of fertilizer have been applied[4] may be explained by the production of excess foliage which so shades the lower leaves that they cannot produce sufficient carbohydrate to meet their own requirements for respiration.

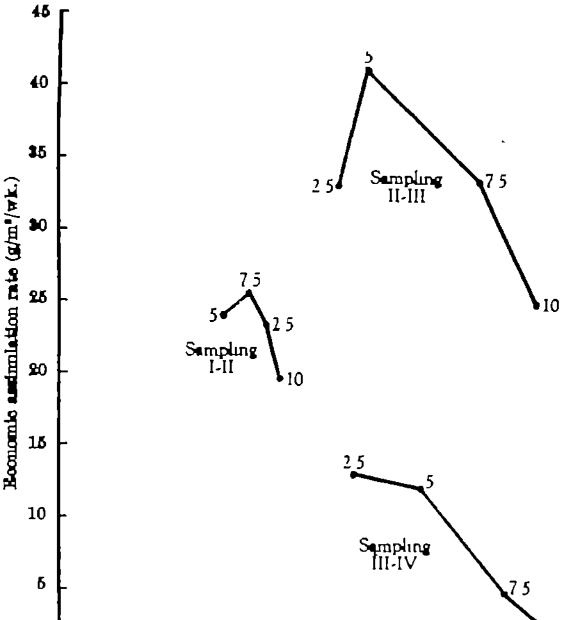

Fig. 2. Curve of economic assimilation rate on mean leaf area index between sampling dates

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Prevalence and Importance of Viable Spores of *Septoria* spp. in Samples of Commercial Celery Seed

In a recent communication Mukhath and Wood[1] suggest that celery seedlings more frequently become infected by mycelium carried within the seed than by spores from pycnidia on the seed.

Viable spores have been found in an appreciable proportion of infected samples during recent surveys of celery seed received for testing for purity and germination at the Official Seed Testing Station.

Marshall (ref. 2 and unpublished work) successfully germinated spores from 6 of 30 samples examined during 1957–58 and from 7 of 53 samples examined during 1958–59. During 1960–61 pycnidia on three samples were observed to exude spores during the standard visual blotter test for their presence, and for two of these samples the presence of viable spores was demonstrated by several techniques, including a seedling inoculation test[3]. In 1961–62 the seedling inoculation test, being developed as a means of checking declarations of efficient hot-water treatment[4], confirmed the presence of viable spores in 9 of 19 samples tested from a total of 34 showing visual infection.

During storage there is a fairly rapid decline in the number of pycnidia which contain viable spores (even so several samples have given infective washings in the August following the presumed year of harvest). Some of the samples tested were of over-yeared seed, and therefore the results are an underestimate of the viable infection present in the seed of any one harvest year.

Diseased seedlings have been grown from infected seed placed on blotters[3], but were not produced from all the viable seeds with oozing pycnidia. Diseased seedlings are rarely obtained from infected seeds grown under glasshouse conditions; perhaps disease initiation by spores depends on the existence of very humid conditions, or even free water.

The low viable infections normally encountered make it difficult to test the efficiency of seed treatments and to obtain confirmation or otherwise of hypotheses concerning infection. But it is evident that viable spores are common enough to be an important potential source of disease outbreaks.

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Heterothallism in *Ustilago cynodontis* (Pass.) Henn.

INFLORESCENCE smut, *Ustilago cynodontis* (Pass.) Henn., is a common parasite of perennial Bermuda grass, *Cynodon dactylon* (L.) Pers. The disease is manifested by the production of black, sooty, smut sori in the spikes of the host inflorescence (Fig. 1). One of the most interesting aspects of the life history of this smut, and one that has received no attention from biologists in the past, is that of heterothallism and sex. Mehta[1] demonstrated seedling infection of Bermuda grass originating from seed-borne teliospores of *U. cynodontis* and reported that the smut perennates in the host from year to year. However, he did not report any observations pertaining to sexuality in the fungus. A comprehensive survey of heterothallism in the Ustilaginales by Whitehouse[2] likewise made no mention of *U. cynodontis*, although more than 30 heterothallic smut species were discussed.

Teliospores of *U. cynodontis* were observed to germinate on 3 per cent potato–dextrose agar, producing four-celled promycelia (basidia) and lateral sporidia (basidiospores). Monosporidial cultures were obtained with the aid of a Chambers micro-manipulator by isolating individual sporidia from very young colonies arising from single germinated teliospores. Each monosporidial line thus originated from a separate teliospore. Eighteen monosporidial lines were isolated in this manner and afterwards were mated on potato-dextrose agar in all possible combinations with each other. The matings were examined after 24 h incubation at 25° C. Two types of compatibility reactions were noted: positive or + reactions denoting sporidial fusions and negative or − reactions indicating incompatibility.

Compatibility relations between pairs of monosporidial lines of *U. cynodontis* were determined by use of the Bauch test. Bauch[3] found that, in *Ustilago violaceae* (Pers.) Roussel, mating types could be determined from the general appearance of the paired cultures. When the mating types were different, considerable white aerial mycelium (Süchfaden) developed, whereas only sporidial or non-aerial hyphae (Wirrfaden) were evident when the cultures were of the same mating type. In studying *U. cynodontis*, the authors found the Bauch test to be a rapid and reliable method of determining compatibility among monosporidial lines of this fungus. As shown in Fig. 2, compatible matings (*A*, *B*, *D*, *E*) produced a white mycelial mat of aerial hyphae, whereas non-compatible matings (*C* and *F*) remained as sporidial mixtures. Lack of anastomosis among non-compatible sporidial matings was confirmed by microscopic examination. The compatible matings were demonstrated to be pathogenic by inoculating seed of Bermuda grass and afterwards observing the emergence of smutted inflorescences.

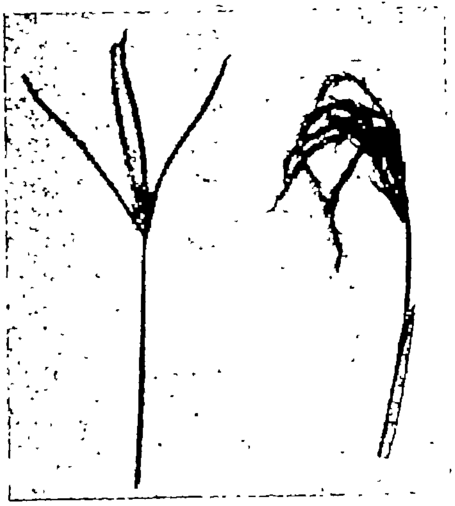

Fig. 1. A healthy inflorescence of Bermuda grass (left), and an inflorescence showing smut sori (*Ustilago cynodontis*) in the spikes (right)

The results of a diallel cross of 18 monosporidial lines (162 matings) of *U. cynodontis* are shown in Table 1. Based on the premise that sporidia of the same compatibility type do not anastomose, the results clearly indicate two intra-sterile, cross-fertile compatibility groups. Thus monosporidial lines 2, 4, 6, 7, 9, 10, 12 and 16 are placed in group I, whereas lines 1, 3, 5, 8, 11, 13, 14, 15, 17 and 18 are placed in group II. The presence of these two compatibility or mating groups is evidence of the existence of heterothallism in *U. cynodontis*. When only two mating types are observed among pedigreed sporidia of smut fungi, the heterothallism is described as bipolar.

The evidence presented here is based on four identical experiments each confirming simple bipolar sexuality in *U. cynodontis*. However, when monosporidial lines from widely separated geographic areas were mated, the

Table 1. COMPATIBILITY PATTERN OBTAINED BY MATING 18 MONOSPORIDIAL LINES OF *Ustilago cynodontis* IN ALL POSSIBLE COMBINATIONS

		Monosporidial line No.																	
		2	4	6	7	9	10	12	16	1	3	5	8	11	13	14	15	17	18
Group I	2	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	6	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	7	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	9	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	10	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	16	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
Group II	1	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	3	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	5	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	11	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	13	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	14	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	15	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	17	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	18	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

Positive compatible matings as evidenced by sporidial fusions and the production of aerial hyphae are indicated as + reactions; incompatible matings as − reactions.

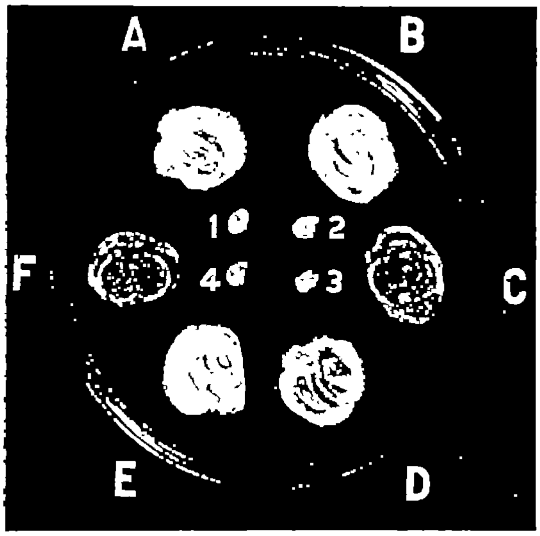

Fig. 2. Compatibility relationships among monosporidial lines of *Ustilago cynodontis* as indicated by the Bauch test reactions. The plate shows four monosporidial lines (small centre colonies) mated in all possible combinations (six larger outer colonies). Colonies *A* (1 × 2), *B* (1 × 4), *D* (3 × 2), and *E* (3 × 4) are covered by white mycelial growth denoting compatibility, whereas colonies *C* (2 × 4) and *F* (1 × 3) have remained as sporidial mixtures indicating incompatibility

evidence suggested more than two compatibility groups in this fungus. According to Whitehouse[2,4], the expression of multiple compatibility groups in smuts is predicated on the existence of 'sterility factors'. Therefore, what may appear to be multipolar sexuality in *U. cynodontis* could be relegated to simple bipolar sexuality on the basis of the effect of these factors.

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Inhibition of Flowering in Poinsettia by Gibberellic Acid

The formation of flowers and decorative bracts in poinsettia (*Euphorbia pulcherrima* Willd.) is induced by short photoperiods[1], the plants remaining vegetative in long day-lengths. Among short-day plants the perennial *Fragaria* spp.[2] and *Kalanchoe blossfeldiana*[3] are inhibited from flowering by gibberellin. This is in contrast to the short-day annuals typified by *Perilla nankinensis*[4,5], *Xanthium pennsylvanicum*[4], *Chenopodium amaranticolor*[4] and *Nicotiana* spp.[6], where flower induction seems usually to be unaffected by this chemical[7]. The strawberry also differs from the short-day annuals in that flower induction seems to be promoted by lowering the temperature during induction[8,9]. As poinsettia exhibits the same negative temperature response[10], it seemed interesting to test the effect of gibberellic acid on flower induction in this species.

In the preliminary experiment poinsettia plants were transferred on January 1, from long day-lengths to short day-lengths for flower induction. Gibberellic acid was then sprayed in aqueous solutions at either 20, 10 or 5 p.p.m. twice weekly. After five applications the concentrations were reduced to half in each case. Control plants were unsprayed. After a further eight applications, treatment was stopped and all plants were returned to long day-lengths to check further photoperiodic induction. Coloured bracts were forming in control plants (unsprayed) by this time, and the plants were in full flower by early April. Flowering was much delayed in the plants which had been sprayed with gibberellic acid. Those which had received sprays of 20 (later 10 p.p.m.) did not flower until late summer and then only produced a few cyathia and poor coloration of bracts. With lower rates an intermediate response was obtained. All rates of application increased stem length.

In the second experiment plants were sprayed weekly with gibberellic acid in aqueous solution at 40, 20, 10, 5 or 2·5 p.p.m. during an induction period of 6 weeks' duration, commencing on October 25. Two sets of control plants, one subjected to short-day induction and the other retained in long day-lengths, were unsprayed. On November 23 the experimental plants were returned to long day-lengths as before. By this time differences between plants treated with gibberellic acid and the induced control plants were already obvious.

The plants were retained in the glasshouse over winter and inspected for flowering from time to time. Again, as in the first experiment, flower induction was not completely inhibited but all rates of application of gibberellic acid delayed flowering, reduced the number of cyathia which developed and reduced the density of coloration of bracts as well as promoting stem elongation. By the end of February the induced control plants and those sprayed with 10 p.p.m. or less were in flower, but not those sprayed with 20 or 40 p.p.m. Counts made at this time showed that the numbers of developing cyathia were reduced from

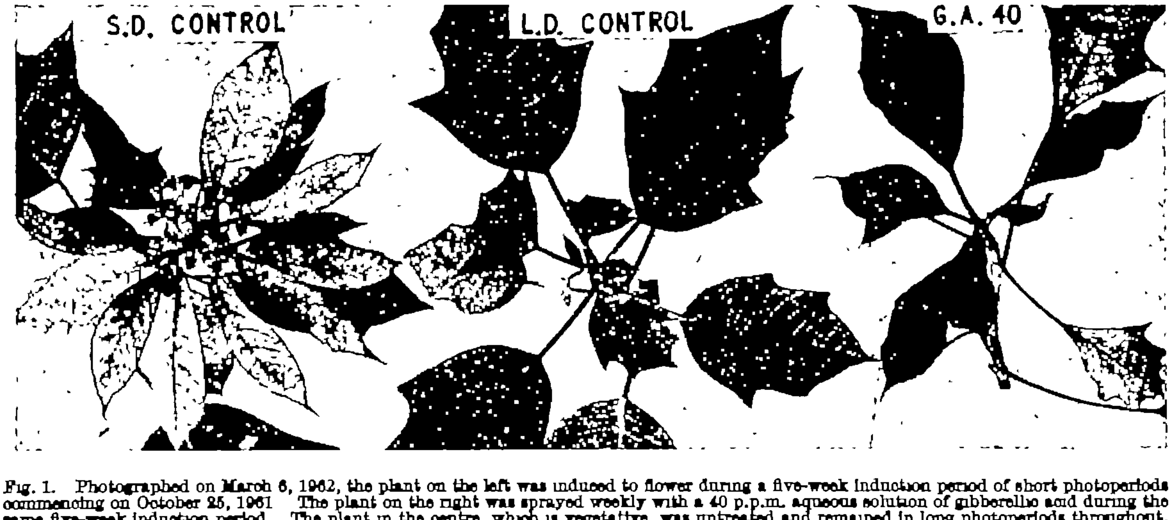

Fig. 1. Photographed on March 6, 1962, the plant on the left was induced to flower during a five-week induction period of short photoperiods commencing on October 25, 1961 The plant on the right was sprayed weekly with a 40 p.p.m. aqueous solution of gibberellic acid during the same five-week induction period The plant in the centre, which is vegetative, was untreated and remained in long photoperiods throughout. Only the upper portion of each plant is shown

a mean of 18·5 in control plants to 5·3, and these greatly retarded, in plants treated with 40 p.p.m. solution. By mid-May a proportion of the plants treated with 40 p.p.m. were still not in flower and it was obvious that the stimulus to flowering in these plants was extremely small. The second set of control plants, which had been retained in long day-lengths throughout the experiment, remained vegetative (Fig. 1).

A slow development of cyathia can also be obtained by partial induction, that is, subjecting the plants to inductive day-lengths for only one or two weeks. The side effects of gibberellic acid on tissue elongation, however, tend to mask superficial resemblances between partially induced plants and plants retarded by gibberellic acid.

It is possible that complete inhibition rather than retardation could be obtained by building up the level of gibberellic acid in the plant before exposure to short day-lengths.

Fragaria spp., *Kalanchoe blossfeldiana* and *Euphorbia pulcherrima* thus seem to differ from the aforementioned short-day annuals in their flowering response to gibberellic acid, and by inference, differ in some way in the biochemical processes by which photoperiodic perception is transformed into flowering responses.

The gibberellic acid used in these experiments (*YF* 4961 formulation) was kindly supplied by Plant Protection, Ltd.

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Neuraminidase in Mammalian Brain

CONTAMINATION of serum with neuraminidase-producing bacterial cells promptly produces changes in the electrophoretic pattern. The mobility (surface charge) of the α_1-globulins (orosomucoid and α_1-antitrypsin), of some α_2-globulins (for example, haptoglobin) and of the β_1-globulin, transferrin, decreases on release of sialic acid from the molecular surface[1]. Vulpé[2] has published electrophoretic protein patterns of rabbit sera before and during development of experimental encephalomyelitis induced by injections of a brain suspension in combination with Freund's adjuvant. The changes in serum presented are similar to those produced by neuraminidase. Furthermore, the transferrin of human cerebrospinal fluid is electrophoretically heterogeneous[3], indicating that part of it has lower sialic acid content than the most common transferrin type of Caucasians. These two observations prompted the present investigation of mammalian brain for neuraminidase. This enzyme is common among micro-organisms, and has been found in chick-embryo chorio-allantois but not in mammalian tissue[4].

Freshly obtained guinea pig, bovine and human brains were freed from their meninges. Weighed samples of the cerebral hemispheres were then homogenized with twice their weight of physiological saline. It was presumed that the sialic acid-rich mucolipids of the brain suspension would serve as substrate for the postulated brain enzyme. To detect the presence of neuraminidase, samples of homogenized brain were incubated at 37° C for variable time-periods; but usually 1 h, with three times their volume of 0·1 M buffer of varying *p*H.

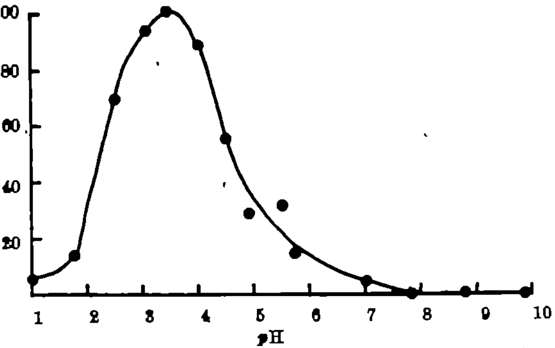

Fig. 1. Neuraminidase activity of brain suspensions in relation to *p*H

The samples were afterwards centrifuged for 3 min and sialic acid measured in the supernatant solution by the thiobarbituric acid method of Warren[5], appropriate correction being made for the presence of 2-deoxyribose in the solutions. This method measures free, but not bound, sialic acid. For each test a blank sample, treated in the same way as the test but centrifuged without previous incubation, was also assayed for sialic acid. The total sialic acid content of the brain homogenates was determined by heating the homogenates with 0·1 N sulphuric acid for 1 h at 80° C, centrifuging and measuring sialic acid in the supernatant by Warren's method.

The release of sialic acid depended markedly on the *p*H of the incubating solution (Fig. 1). In the three species the optimal *p*H was 3·5–4·0. Little or no sialic acid was released when the *p*H was below 2·0 or above 5·5. When the brain homogenates were incubated at *p*H 4·0 for varying time-periods progressive release of sialic acid occurred. This release was almost linear during the first 2 h, by which time, in bovine brain, 15 per cent of the total sialic acid present had been released, but after 2 h the rate of release decreased and after 24 and 48 h 35 and 47 per cent, respectively, had been released.

Heating the brain homogenates from the three species in a boiling water bath for 3 min completely prevented the release of sialic acid during subsequent incubation at *p*H 4·0 and 37° C. After such heating, however, partially purified pneumococcal neuraminidase released sialic acid just as quickly from the brain as from unheated brain homogenate.

In order to check that the material released during incubation and being measured by the thiobarbituric acid assay procedure really was sialic acid, bovine brain homogenate was incubated at 37° C with acetate buffer, *p*H 4·0, for 48 h. Penicillin and streptomycin had been added to prevent bacterial growth. The time-concentration curve for sialic release followed the same principal course as in the short period experiments. The homogenate was then dialysed against several batches of distilled water. The dialysates were concentrated and purified by the method of Svennerholm[6]. The final product after crystallization three times from ethanol, ether, and petroleum ether was found to give the colour reactions typical of sialic acid with thiobarbituric acid, orcinal, Ehrlich's reagent and diphenylamine, and on paper chromatography in 4 solvent systems and high-voltage electrophoresis to have the same R_F value and electrophoretic mobility as authentic *N*-acetyl-neuraminic acid (NANA). In addition the infra-red spectrum was identical with that of NANA[7,8].

These results show that NANA is released from guinea pig, bovine and human brain when they are homogenized and incubated at 37° C. The fact that the release occurs much more quickly at *p*H 3·5–4·0 than at more acid or alkaline *p*H and that it is prevented by prior heating of the brain to 100° C is strong evidence that the release of NANA is enzymatically catalysed. Whether this enzyme is a 'neuraminidase' as defined by Gottschalk[9], in that it catalyses "the hydrolytic cleavage of the glycosidic bond joining the keto group of neuraminic acid to D-galactose of D-galactosamine and possibly to other sugars", is not known. The pH optimum, 3·5–4·0, is different from that found for bacterial neuraminidase (pH 5–6) and from that found by Warren and Spearing[10] for the enzyme present in plasma glycoprotein preparations (pH 5·5), which resembled the bacterial enzyme in this respect. The pH optimum was, however, closer to that of the enzyme present in chick embryo chorioallantois[4] (pH 4·5).

We thank Dr. R. Larsson, Institute of Chemistry, University of Lund, for performing the infra-red spectroscopic examination.

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Growth of Juvenile Red-eared Turtles as influenced by Gravitational Field Intensity

PREVIOUS investigations have shown that, although sufficiently high gravity as simulated by continuous centrifugation can retard growth of certain organisms, a moderate increase in the gravitational field intensity can sometimes slightly enhance growth[1–3]. A similar pattern is reported here for juvenile red-eared turtles (*Pseudemys scripta elegans*). However, a more dramatic growth enhancement was obtained during moderate centrifugation than had been obtained with other forms.

Accelerated growth of other forms under the influence of continual centrifugation has been noted. Gray and Edwards[1] found that growth of wheat (*Triticum vulgare*) coleoptiles during the first 24 h of exposure to 10*g* was accelerated. Wunder[2] noted accelerated growth of fruit-fly (*Drosophila melanogaster*) larvæ at 500*g*. The femur bones of white mice display accelerated growth and rounder cross-sections at 4*g*, despite the fact that their total body-mass decreases[3]. Preliminary results in this laboratory indicate that accelerated growth of mice may occur at 1·5*g*. These findings would lend credence to the hypothesis that for any given organism there may be a specific gravitational field intensity most conducive to growth. The fastest growth would occur at the field in which the growth-stimulating effects of gravity are greatest in comparison with the other gravitational effects which normally retard growth.

Turtles were chosen for these investigations because they have a unique structure and because they are aquatic. The shell of the turtle would tend to serve as a natural 'anti-*g* suit'. An aquatic environment would offer buoyant support which would partially counteract the influence of high gravity.

These turtles, which at the beginning of an experiment measured approximately 34 mm in length and 9 g in weight, were exposed to centrifugal fields ranging from 5 to 28*g* for periods of 5–9 weeks. Throughout the experiment, continual 24 h a day lighting and temperatures of approximately 27° C were maintained. Turtles were removed each day for a 20-min period for feeding and cleaning of cages. They were removed for a 6-h period each week for growth measurements. In this way, it was possible to keep the animals active throughout the winter, a time when hibernation normally occurs. Turtles were kept in plastic refrigerator boxes (7·75 in. × 5·5 in. × 4 in.) containing approximately 500 ml. of water. As many as 6 turtles were kept in the same container. The diet consisted of lean chopped beef containing enough beef-bone dust to constitute 10 per cent of the total mass of food. The criterion for growth reported here was change in carapace-length; however, preliminary analysis of results indicates that changes in body-mass were comparable with changes in body-length.

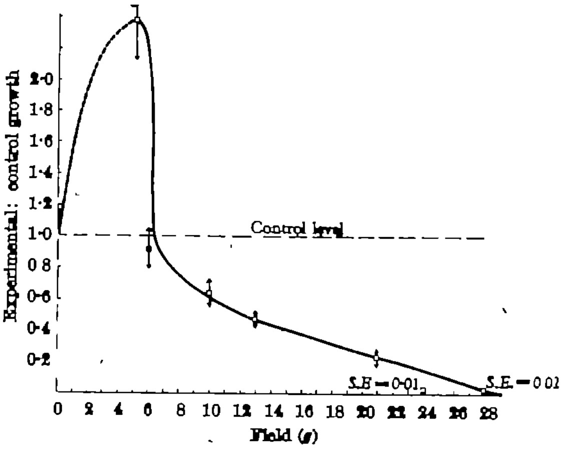
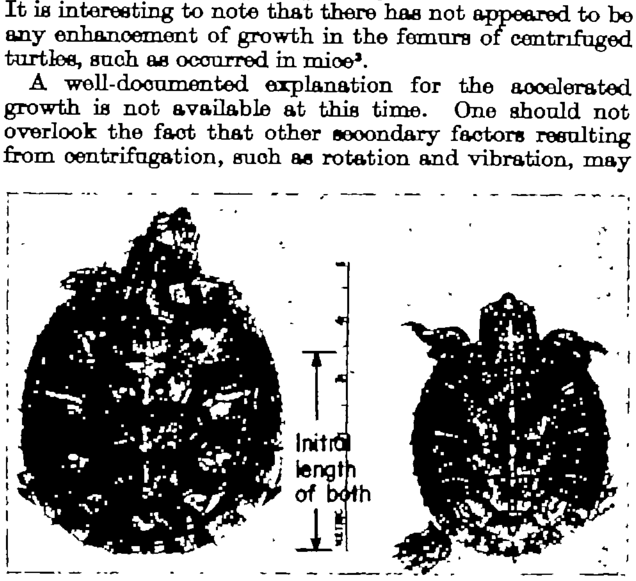


Fig. 1. Average rate of linear growth relative to the paired control turtle ± *S.E.* Over a five-week period, length for both experimental and control turtles is nearly a linear function of time. Control rates are of the order of 1 mm per week. However, this varies markedly with both the season of the year and the exact age of the turtles

As can be seen in Fig. 1, experimental animals exposed to 5*g* grew approximately twice as fast in length as their controls during five weeks of exposure. Growth at 6*g* did not differ significantly from that of the controls. With further increases in field, there occurred a progressive growth decrement of the type reported with other forms.

With some experimental animals, there was both an absolutely and a relatively greater shell mass in turtles exposed to 5*g* as compared with the 1*g* controls. Such an accelerated growth in shell mass would be expected according to Wolff's Law[4], which proposes that mechanical force acting on a skeletal structure will enhance growth of the stressed portion. In the case of turtles, the shell constitutes the primary, functional skeleton. It is interesting to note that there has not appeared to be any enhancement of growth in the femurs of centrifuged turtles, such as occurred in mice[3].

A well-documented explanation for the accelerated growth is not available at this time. One should not overlook the fact that other secondary factors resulting from centrifugation, such as rotation and vibration, may



5*g* for 9 weeks   Control

Fig. 2. Experimental and control turtles after nine weeks of exposure to 5*g*. Note the larger size of the experimental animal and its wider growth lines

be of significance in the accelerated growth of turtles. These turtles withstand maintained centrifugation much better than do mice of comparable size. Perhaps such factors as buoyancy, shell, and lower body temperature protect the turtle from the deleterious results which have been demonstrated for mice and certain other warm-blooded animals. If this were so, the net developmental acceleration would accordingly be more pronounced.

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Long-Distance Migration of Salmon

IT was reported recently[1] that a salmon tagged as a smolt in the estuary of the River Usk in Monmouthshire was caught some eighteen months later near Sukkertoppen on the west coast of Greenland.

Information has now been received that in October, 1962, another English salmon was caught in the same area. This fish was tagged as a smolt in the River Wye at Brockhampton, near Hereford, on April 5, 1961.

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ENTOMOLOGY

Insects attacking *Striga* in Kenya

WILLIAMS and Caswell[1] have reported *Smicronyx* sp. (Curculionidae; Coleoptera) attacking *Striga* sp. in Nigeria. Murthy[2] has given an account of the attack of *Smicronyx albovariegatus* Faust. on *Striga* in India and has listed other insect pests of *Striga* in that country.

Striga hermonthica Benth. (Scrophulariaceae) is an important root parasite of sorghum, the principal cereal crop of the low-lying areas near Lake Victoria in Nyanza Province, Kenya. Maize and wild grasses are also attacked. In 1960 and 1962 *Striga* plants were examined for insect damage. In both years the commonest pest was found to be a small agromyzid fly, which has been identified as a new species, *Ophiomyia strigalis* Spencer[3]. Attack commences when *Striga* begins to flower in late June; and by the beginning of August more than 90 per cent of plants have been attacked. The eggs are inserted singly into the soft epidermis in the axils of leaves 1–1·5 in. above soil-level. The larva makes a narrow mine, stained purplish-black, just underneath the epidermis. The mine goes almost straight downwards to below soil-level, where it then pursues a more tortuous course under the epidermis of the root to a depth of 3–4 in. The mature larva returns up the mine and pupates at soil-level where the site of the puparium can be detected as a small blister of the epidermis. Up to 10 puparia have been found in a single plant; the mean number found in August 1962 was 2 puparia per plant. The maximum interval which has been observed between the caging of infested *Striga* material and the emergence of adult flies is 25 days. Attack causes the root epidermis to rot and peel off, and reduces the number of lateral roots, but no debilitation of the aerial parts of the plant, or reduction of numbers of flowers or fruits has been observed.

O. strigalis is attacked by a number of hymenopterous parasites. In 1960 a collection of infested *Striga* material yielded 22 *O. strigalis*, 26 *Eucoilidea* sp. (Cynipoidea), 2 *Opius liogaster* Szepl. (Braconidae) and 2 *Herberia* sp. (Pteromalidae). In 1962 the incidence of these parasites was lower. Some *O. strigalis* eggs have been found to contain Hymenopterous pupæ.

In 1960 about 10 per cent of *Striga* plants at Kibos were found to bear one to three stem galls, usually on the main stem about 3 in. above soil-level. The primary gall-former appeared to be a *Eurytoma* sp. (Eurytomidae; Hymenoptera). Other insects obtained from the galls were a cecidomyid (Cecidomyidae; Diptera) the larvæ of which appeared to feed on fungus in the cavities of the galls a *Bracon* sp. (Braconidae; Hymenoptera) and a *Ceraphron* sp. (Ceraphronidae; Hymenoptera).

Gall-bearing plants had fewer mature fruit than non-infested plants, but it could not be determined whether this was an effect of attack or whether only late-maturing plants were attacked. Only one galled plant has been found in 1962.

Le Pelley[4] records *Platyptilia taprobanes* Fldr. and *Stenoptilia zophodactyla* Dup. (Pterophoridae; Lepidoptera) from *Striga* in Tanganyika. No *Smicronyx* sp. have been found on *Striga* in Kenya.

I thank the Director, Commonwealth Institute of Entomology, for identifying the insect species concerned, and the Director of Agriculture, Kenya, for permission to publish this communication.

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Effect of Synthetic 'Queen Substance' (9-oxodec-*trans*-2-enoic acid) on Ovary Development of the House-fly, *Musca domestica* L.

RECENT investigations[1] have shown that when 'queen substance' (9-oxodec-*trans*-2-enoic acid) is distributed throughout the colony of honey bees, it partially inhibits the development of ovaries in workers and influences their behaviour by inhibiting queen-cell construction. Similar effects of crude extracts of queen honey bees have been demonstrated on other groups of social insects, for example, *Formica fusca* L.[2] and *Kalotermes flavicollis* F.[3] This non-specificity for orders of social insects suggests that 'queen substance' might have similar inhibitory effects on ovary development even of non-social insects. This hypothesis was tested by examining the effect of synthetic 'queen substance' on ovary development in the house-fly, *Musca domestica* L.

In the house-fly the ovaries of newly emerged virgin females are undeveloped (Table 1) and female flies require a protein diet such as milk for egg development. When newly emerged female flies were 6–8 h old, samples of twenty flies each were injected in the thorax with 5 μl. per fly of a 2 mg/ml. of dilute ethanolic solution of synthetic 'queen substance' with a 50 μl. Hamilton micro-syringe, and injections were repeated at the time-intervals shown in Table 1. Higher concentrations of synthetic 'queen substance' were found to be toxic and lower concentrations did not show any appreciable effect on the inhibition of ovary development in the house-fly. The treated and control flies were allowed to feed on a 1 : 1 (v/v) mixture of condensed milk and water, replenished daily, for a total period of 96 h. Then the flies were dissected and their ovaries measured.

Table 1. EFFECT ON OVARY DEVELOPMENT AND MORTALITY OF THE HOUSE-FLY, *Musca domestica* L., OF INJECTIONS OF SYNTHETIC 'QUEEN SUBSTANCE' (9-OXODEC-*trans*-2-ENOIC ACID)

	Treatment of female flies (20 flies per test) Material injected*	Times of injection (h)	Mean dimensions of ovaries (mm)† Length	Width	Percentage of mortality after 96 h
1	None	—	0·7	0·4	100
2	'Queen substance'	0	1·7	1·1	5
3	Distilled water	0	3·1	2·0	0
4	'Queen substance'	0, 24	1·4	0·9	5
5	Distilled water	0, 24	3·1	2·1	5
6	'Queen substance'	0, 48	1·2	0·8	10
7	Distilled water	0, 48	3·1	2·0	10
8	'Queen substance'	0, 24, 48	1·0	0·7	10
9	Distilled water	0, 24, 48	3·1	2·1	0

* 5 μl./fly of a solution of 2 mg/ml. of synthetic 'queen substance' (9-oxodec-*trans*-2-enoic acid) dissolved in 5 per cent ethanolic solution was injected in flies in tests 2, 4, 6 and 8. Equivalent volume of distilled water was injected in tests 3, 5, 7 and 9. All flies except in test 1 were fed on a mixture of milk and water (1 : 1, v/v) for a total period of 96 h.

† Means are based on measurements of ten flies randomly selected from each of five replicated tests involving twenty flies.

The effect of synthetic 'queen substance' on the ovary development of the house-fly is shown in Table 1. Similar results were obtained with an equivalent dosage of the sodium salt solution of synthetic 'queen substance'.

These results show that the continuous presence of a certain amount of synthetic 'queen substance' in the insect body is necessary for complete inhibition of ovary development in the house-fly. Synthetic 'queen substance' appears to be metabolized or excreted by the house-fly rather rapidly, and thus it must be supplied at regular intervals or its inhibitory effect on ovary development wears off. This effect of synthetic 'queen substance' appears to be similar to that on honey bees where worker bees must receive a continuous supply of 'queen substance' from the queen honey bee, to inhibit their ovary development. Failure to demonstrate any inhibitory effect of 'queen substance' on ovary development in the house-fly in the work of Mitlin and Baroody[4] can also be explained from these observations. They washed 'queen substance' from the bodies of honey bee queens and injected the material obtained into house-flies or fed it to them in milk. It seems likely that the concentrations of 'queen substance' present in their preparations were too low to show any appreciable inhibitory effect on ovary development of the house-fly.

Further investigations on the effects of synthetic 'queen substance', by both feeding and by injections, on the sexual processes in the house-fly, the German cockroach, *Blattella germanica* L., and other insects, are being conducted.

These results and some unpublished data suggest the possibility of investigating the potentiality of synthetic 'queen substance' (9-oxodec-*trans*-2-enoic acid) as a 'female insect chemosterilant'.

I thank Dr. R. K. Callow, National Institute for Medical Research, London, for a gift of synthetic 'queen substance' (9-oxodec-*trans*-2-enoic acid) (synthesized by Glaxo Laboratories, Ltd., for experimental use only) and Prof. A. J. Thorsteinson and Dr. S. C. Jay of the Department of Entomology, University of Manitoba, for many helpful suggestions. This work was done during my tenure of a National Research Council of Canada post-doctoral fellowship.

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CYTOLOGY

Aneuploids in Oat Varietal Populations

THE cultivated oat *Avena sativa* ($2n = 42$) normally forms 21 bivalents at meiosis, but on rare occasions a bivalent fails due either to asynapsis or desynapsis. The irregular transmission of the resulting univalents may lead to the formation of gametes deficient for a chromosome, or with an extra chromosome. If such aneuploid gametes are fertilized by euploid gametes, monosomic or trisomic offspring will arise.

As *Avena sativa* is a hexaploid, there is considerable duplication of genetic material and, in consequence, relatively high levels of aneuploidy are tolerated. Monosomics and nullisomics have been reported to be viable and, to some extent, fertile[1]. Thus once an aneuploid has arisen in a population, its progeny will add to the proportion of aneuploids in succeeding generations. However, a proportion of the progenies of monosomics and trisomics will also revert to euploid. The balancing of these two effects, together with the origin of aneuploids from euploids, will lead to the maintenance in a population of a low, but constant, level of aneuploidy.

The objects of the present investigation were to confirm the report of Riley and Kimber[2] that aneuploids could be extracted from populations within a variety, and to assemble a collection of monosomics in an attempt to obtain a complete series deficient in turn for each chromosome of the haploid complement. Seed of the variety Sun II was obtained from the National Institute of Agricultural Botany, Cambridge (N.I.A.B.), from the Swedish Seed Association, Svalöf (S.S.A.), where the variety was bred, and from a commercial seed merchant. The three stocks were screened for aneuploids by checking the somatic chromosome numbers of young seedlings. Chromosome counts were made on temporary squashes of root-tips stained by the Feulgen technique. Plants giving aneuploid counts were grown to maturity, and their chromosome constitutions were confirmed either at meiosis or by checking the chromosome numbers of samples of their progenies.

The percentage of aneuploids obtained varied from 1·2–1·9 in the samples of the three populations studied (Table 1). Monosomics occurred with a greater frequency than other classes of aneuploid, but nullisomics, trisomics and plants with one or two telocentric chromosomes were also recorded. A supposed 44-chromosome plant failed to survive to maturity, and consequently could not be confirmed as an aneuploid.

Table 1. ANEUPLOID FREQUENCY IN THREE POPULATIONS OF *Avena sativa* VAR. SUN II

Stock	Chromosome constitutions 42	40	41	41 + telo	40 + 2 telos	43	Per cent aneuploid
N.I.A.B.	1,842	4	17	1	1	3	1·4
S.S.A.	1,095	2	17	–	1	1	1·9
Seed merchant	516	–	6	–	–	–	1·2
Total	3,453	6	40	1	2	4	1·5

It is apparent that a proportion of the variety is in fact aneuploid. In addition it should be emphasized that the recorded percentage is minimal, since inevitably, in a study of this kind, some aneuploids would have been discarded after being mistakenly identified as euploids. Also morphologically abnormal types had been rogued out of the populations in the immediately preceding generations. Consequently the frequencies of aneuploids recorded are not necessarily those to be expected in stabilized populations.

The observed frequency of aneuploids may be of importance for two reasons. First, it demonstrates that the occurrence of a proportion of genetically aberrant forms is probably a feature of oat varietal populations, due to the origin and maintenance of cytological abnormalities.

The significance of this in relation to the morphological and physiological purity demanded of oat varieties depends on the phenotypic expression of aneuploid conditions. The limited evidence so far obtained suggests that the euploid phenotype may be considerably modified by aneuploidy. If this is so it is not reasonable to expect the frequency of off types to be less than the aneuploid frequency of the variety.

Secondly, the frequency of monosomics in Sun II suggests there is a possibility that a complete series of monosomics can be assembled by screening varietal populations. At present there is no reason for supposing that certain monosomics occur at a greater frequency than do others. The usefulness of the mass screening technique for isolating a complete monosomic series in oats can only be assessed when the inter-relationships of those monosomics already assembled have been determined.

We thank Dr. G. D. H. Bell for his interest in this work, also the Association of Universities of the British Commonwealth for the grant of a scholarship to one of us (J. B. H.).

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VIROLOGY

Increase of Plant Virus Infection by Magnesium in the Presence of Phosphate

THE increase of tobacco mosaic virus (TMV) infection induced by 0·1 M solutions of ammonium, potassium or sodium mono-hydrogen orthophosphate salts already has been discussed[1,2]. This phenomenon was defined as the 'phosphate effect' and has been interpreted as an effect on the host[3]. The action of the 'phosphate effect' is here considered to be both an effect on the virus and on the host[4].

The enhancing action of magnesium on infection has been observed with poliovirus[5] and with the yield of *r*II mutants of bacteriophage *T*4 (ref. 6). Magnesium in combination with glycine has been reported to stabilize potato yellow-dwarf virus[7], but not barley stripe mosaic virus[8]. Magnesium has also been shown to stabilize the helical regions of the ribonucleic acid of TMV[9]. Phosphate buffer (0·01 M) without magnesium was found to cause dissociation of yeast ribonucleoprotein into two components whereas no such dissociation occurred in the presence of magnesium[10].

Investigations were made to determine whether magnesium could increase infection of several plant viruses. The principal viruses used were the following: a strain of alfalfa mosaic virus (AMV) maintained in *Nicotiana tabacum* L. var. *xanthi*, n.c.; a strain of cucumber mosaic virus (CMV) maintained in young cucumber cotyledons as local lesions (used at 6 days after inoculation) (*Cucumis sativus* L. var. National Pickling); a strain of tobacco necrosis virus (TNV) maintained as local lesions on the primary leaves of blackeye cowpea (*Vigna sinensis* (Torner) Savi); and a strain of tobacco rattle virus (TRV) maintained in *N. tabacum* var. *xanthi*.

The primary leaves of blackeye cowpea were used principally for local lesion assay of the foregoing viruses. Primary leaves of pinto beans (*Phaseolus vulgaris* L.) were used to a lesser extent for assay of purified TMV. Inoculations were made by rubbing the upper surface of 'Carborundum'-dusted leaves of the assay host with a poster brush dipped in inoculum. The inoculum in most cases (except for TMV) consisted of crude virus suspension in water, magnesium, phosphate or phosphate containing magnesium. Although water was reported to inhibit infection[11], all leaves were quickly washed under tap water after inoculation in order to protect the inoculated leaves from salt injury.

Equivalent samples (usually 1 ml.) of freshly ground and appropriately diluted (with distilled water) infected-tissue suspensions were added to equal volumes of water, 0·01 M $MgCl_2$, 0·1 M KH_2PO_4–K_2HPO_4 buffer (*p*H 8·0), and to a 0·1 M magnesium chloride–phosphate buffer solution (*p*H 8·0). For the latter solution, 1 ml. of magnesium chloride was added to 9 ml. of 0·1 M phosphate buffer containing virus rather than in the reverse order to prevent precipitation. The *p*H remained constant for both buffer suspensions. Each preparation was inoculated to at least 24 cowpea leaves. The lesions were counted 3–4 days after inoculation.

Table 1. INCREASED INFECTION BY MAGNESIUM CHLORIDE IN THE PRESENCE OF PHOSPHATE

Virus	Supplement to inoculum* Control (water)	Magnesium chloride (0·01 M)	Phosphate (0·1 M, *p*H 8·0)	Phosphate + magnesium chloride (0·1 M, *p*H 8·0)
AMV	8·0	3·7	30·5	53·3
CMV	18·3	10·4	161·4	279·7
TRV	10·6	10·9	79·8	102·7
TMV	3·3	13·5	120·1	123·8
TNV	9·2	5·2	106·0	107·0

* Numbers represent average number of lesions from cowpea leaves from four separate experiments except for TMV and TNV which were assayed on pinto bean.

Table 2. EFFECT OF CALCIUM CHLORIDE ON THE PHOSPHATE EFFECT

Inoculum	Molar concentration (mM)	Average number of CMV lesions
H_2O	—	5·7
$CaCl_2$	10·0	3·0
Phosphate	100·0	129·1
Phosphate + $CaCl_2$	0·1*	108·9
,,	1·0	72·8
,,	10·0	20·7
,,	100·0	9·7

* Concentration of calcium chloride. The concentration of phosphate remained 100 mM throughout.

The results (Table 1) indicate that there is a decrease in infection from inoculum containing only 0·01 M magnesium chloride. It was noted, however, that the 'phosphate effect' was found to be enhanced in the presence of magnesium chloride. The average increase of the 'phosphate effect' by magnesium chloride for AMV, CMV, and TRV was about 53 per cent. In one experiment using CMV, magnesium increased the 'phosphate effect' to about 140 per cent. A lesser effect of the magnesium–phosphate complex was observed when relatively stable viruses such as TMV and TNV were used.

The effect of other cations was tested in the presence of HPO_4^{-2} by using 0·05 M *tris*(hydroxymethyl)aminomethane adjusted with 0·05 M phosphoric acid to *p*H 8·0. Monovalent cations such as potassium and sodium in the presence of this buffer tended to decrease TMV lesion numbers whereas magnesium chloride gave an increase. Divalent cations such as calcium could not be substituted in place of magnesium without the formation of calcium phosphate precipitates at such concentrations. With increasing concentrations of calcium chloride in phosphate buffer (calcium chloride to buffer, v/v, 1 : 10), the concentration of CMV in the supernatant decreased and there was no increase of the 'phosphate effect' (Table 2). Since it has been reported that sour cherry necrotic ringspot virus and barley stripe mosaic virus are adsorbed on the calcium phosphate precipitate only at low phosphate buffer concentrations[8,12], it is believed that in our case the CMV is trapped in the intermicelles during the *in situ* formation of the calcium phosphate precipitate at these higher concentrations.

It is likely that the action of magnesium is on the virus since greater increase of the 'phosphate effect' is observed with less-stable viruses than with more stable ones. In several trials using identical assay hosts, CMV showed greater infection by the magnesium–phosphate complex than did TNV. Other preliminary evidence indicates that AMV in the presence of the complex is found to remain active longer than virus in phosphate. Therefore, magnesium may act by stabilizing the virus.

The purified preparation of TMV obtained from R. Reichle is gratefully acknowledged. This work was supported by grant G9820 from the U.S. National Science Foundation.

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Loss of the Hæmagglutinating Capacity of Fowl-plague Virus after Culture on HeLa Cells

CERTAIN viruses undergo changes in their hæmagglutinating capacity, when propagated in certain cultures. Green *et al.*[1] and Mogabgab *et al.*[2] discovered, by examining strains of influenza virus, that the hæmagglutinating capacity is modified when passing from one cellular system to another.

Recently, Moscovici *et al.*[3,4] described the fact that some strains of ECHO virus lose their hæmagglutinating capacity when cultivated on HeLa cells, whereas they retain such capacity if cultivated on monkey kidney cells. Moreover, the virus which has lost its hæmagglutinating capacity does not regain it when cultivated again on cells of monkey kidney.

More recently, Johnson *et al.*[5] showed that loss of hæmagglutinating capacity occurs when Coxsackie *A*-21 virus is cultivated on cells of cancerous origin (HeLa and *KB* strains), whereas the capacity is maintained when cultivating the virus on primary human embryonic epithelial cells and semi-continuous diploid human embryonic fibroblastic cells.

It has been also demonstrated by Cassel *et al.*[6] that on serial passage in the Ehrlich ascites tumour loss of hæmagglutinating power occurs in three vaccinia virus strains. Tumorous ascitic fluid was removed from mice, and a cell-free tumour fluid with hæmagglutinin-inhibition power obtained.

We have observed the loss of the hæmagglutinating capacity of fowl-plague virus cultivated on HeLa cells, whereas it retains its capacity when cultivated on chick embryo cells.

The maintenance medium used is the same for both types of cells. It consists of: Hanks's balanced salt solution, 85 per cent; lactalbumin hydrolysate (5 per cent), 10 per cent; calf serum, 3 per cent; penicillin, 100 U/ml.; streptomycin, 100 mg/ml.; nystatin, 20 U/ml.; phenol red (0·2 per cent), 1 per cent; $pH = 7{\cdot}2$. The cells are cultivated in hexagonal flasks of 'Pyrex' glass with a total capacity of 225 ml., containing 10 ml. of culture medium.

Starting from virus cultivated on chick embryo, 20 passages were performed with fowl-plague virus through chick embryo cells; the hæmagglutinating titre and egg lethal dose 50 per cent of each passage were determined.

It is possible to observe a rapid adaptation of the virus to the cellular culture, with stabilization of the evolution period of the infection, and appearance of cytopathic effects 24–48 h after inoculation of the virus.

The hæmagglutinating titre decreases from the first passage to the fourth, increasing afterwards up to a considerable value which is stable with small oscillations. The 50 per cent egg lethal dose undergoes a slight variation during the experiment, with an evolution apparently similar.

At the same time, 14 passages were made of fowl-plague virus on HeLa cells. For each one, samples were taken from the culture medium during the time of the passage; the hæmagglutinating titre and egg lethal dose 50 per cent were fixed. The adaptation of the virus is slower than in the preceding case; it becomes stable, but the evolution period of the infection is longer and the cytopathological effects appear later. The hæmagglutinating titre decreases rapidly, disappearing at about the third passage. However, the cytopathological effects continue to appear during all the following passages.

The existence of virus in the culture medium of infected HeLa cells was detected by electron microscopy; its morphological aspect is similar to that of the virus cultivated on chick embryo cells. Moreover, tests of complement fixation with antigen obtained from virus propagated on HeLa cells and with sera obtained from the same virus present a positive reaction reciprocally and in the presence of antigens obtained from virus propagated on chick embryo and chick embryo cells.

It has been shown that virus cultivated on chick embryo cells hæmagglutinates to a different extent from human type O, sheep and chicken erythrocytes, whereas virus cultivated on HeLa cells hæmagglutinates none of them.

Moscovici and La Placa[4] conclude that this phenomenon is due to an unknown virus-modifying factor, present in the human tumour cell and released at the time of infection, which causes a permanent modification of the surface of the virus. Johnson suggests the possibility that perhaps this is not an uncommon phenomenon among human enteroviruses, and that the loss of hæmagglutinating capacity is due to two types of infectious virus particles: positive and negative hæmagglutinating. When cultivated on malignant cells, they undergo a selection which favours the propagation of negative hæmagglutinating particles, whereas on non-malignant cells the selection is on the contrary.

Our experiments were performed in order to determine the possible existence of a hæmagglutination-inhibitory agent inside HeLa cells. Having dismissed the possibility of influence by the serum constituent of the culture medium (3 per cent calf serum)—since this is the same medium used for both types of HeLa and chick embryo cells—we now believe that we have discovered a new factor and are trying to determine its characteristics. This factor, obtained from free culture medium cell extracts, might possibly act inside the cells on the synthesis of virus hæmagglutinins, preventing their formation or otherwise neutralizing them afterwards.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 4

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. A. Morgan Jones: "The Coronary Collateral Circulation".*

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Peter Haggett: "Coastal Deforestation in Brazil".

Institution of Electrical Engineers, Electronics Division P.G.E3 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Evaluation of Technologies for Fabricating H.F. Transistors" opened by Dr. J. R. Tillman, Mr. P. Beale, Mr. P. Charman and Dr. R. W. A. Scarr.

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. H. G. Conway and Mr. M. Hartland Thomas: "Engineering Problems of the Decimal and Metric Systems".

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Dr. P. W. Brian: "Two Biologically Active Fungal Metabolic Products. 1: Griseofulvin".*

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "New Materials which Could Play an Important Part in Railway Engineering".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. B. K. Blount: "Science in Latin America". (Last of three Cantor Lectures on "Latin America—World of Tomorrow".)

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. J. Pickthall: "About Perfumery"; Dr. B. Dudley Sully: "Perfumery Chemicals from Pinene".

Tuesday, March 5

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. V. R. Sastry and Dr. J. W. Lynn: "Analogue Investigation of the Stability of a Graphite Power Reactor".

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Dr. P. W. Brian: "Two Biologically Active Fungal Metabolic Products. 2: Gibberellic Acid".*

University of London (in the Large Geology Lecture Theatre, Imperial College of Science and Technology, Royal School of Mines, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Dr. D'Arnaud Gerkens: "Seismic Noise and Resolving Power".*

Institution of Mechanical Engineers, Process Engineering, Refrigeration, Ventilation and Vacuum Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Mechanical Aspects of Mixing of Solids".

Institute of Plastics (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. T. C. Moorshead: "Static Electricity".

Wednesday, March 6

University of London (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.7), at 5 p.m.—Dr. R. W. Riddell: "The Bacteriological Investigation of Hospital-acquired Infections".*

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. W. Crook: "Developments in Elasto-Hydrodynamic Lubrication".

Institution of Electrical Engineers, Power Division P.G.P8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. Roullier: "Testing of Mechanical Joints in Insulated Aluminium Conductors".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Prof. R. S. Scorer· "Convection".

British Institution of Radio Engineers, Electric Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. S. C. Brown: "Travelling Wave Crystal Amplifiers".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at the College of Technology, Chesterfield), at 6.30 p.m.—Mr. H. W. B. Gardiner, Mr. E. A. Heywood and Mr. W. J. Worsdell: "Design of Machines for Electric Lamp Manufacture".

Royal Institute of Chemistry, London Section—(joint meeting with the Chelsea College of Science and Technology Chemical Society, at the Chelsea College of Science and Technology, Manresa Road, London, S.W.3), at 7 p.m.—Mr. R. A. Saunders: "Recent Advances in the Mass Spectrometry of Organic Compounds".

Royal Microscopical Society (at the Edward Lumley Hall, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2), at 7 p.m.—Soiree and Exhibition of Modern Microscopes and Ancillary Equipment.

Thursday, March 7

Zoological Society of London (in the Meeting Room, Regent's Park London, N.W.1), at 10 a.m.—Symposium on "The Mammalian Epidermis and Its Derivatives".

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. W. K. Taylor: "Cortico-Thalamic Organization and Memory"; Mr. D. E. Broadbent and Miss Margaret Gregory: "Evidence Concerning a Recent View of the Mechanism of Human Attention".

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 5.30 p.m.—Prof. G. F. Lorentzen: "The Freezer Chain—Problems and Possibilities".

Institution of Electrical Engineers (joint meeting with the Institutions of Civil and of Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. R. S. Edwards: "Objectives and Management in Nationalized Industry" (Ninth Graham Clark Lecture).

University of London (in the Beveridge Hall, Senate House, London, W.C.1), at 6 p.m.—Sir Frederick Brundett: "The Neglected Sea" (Haldane Memorial Lecture).*

Institute of Metals (at 17 Belgrave Square, London, S.W.1), at 7 p.m.—Mr. T. Williams: "Practical Aspects of Explosive Forming".

Friday, March 8

Institute of Fuel (in collaboration with the City and Guilds of London Institute, at the Borough Polytechnic, Borough Road, London, S.E.1), at 10 a.m.—Conference on "Education and Training in Fuel Technology in Relation to National Needs".

Society for Analytical Chemistry (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 2.15 p.m.—Annual General Meeting. 2.45 p.m.—Dr. A. J. Amos: "The Society Yesterday, Today and Tomorrow".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. E. W. Arndt: "Automation in the Laboratory".

Saturday, March 9

London County Council (at the Horniman Museum, London Road Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Malcolm Lyall-Watson: "Animal Behaviour in Zoos".*

Monday, March 11

Institution of Mechanical Engineers, Industrial Administration and Engineering Production Group (at 1 Birdcage Walk, Westminster, London, S.W.1)—All-day Discussion on "Metallurgical and Engineering Problems in the Machining of Materials".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4 p.m.—Symposium on "Noise in Weaving Machinery".

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Emotional Reactions to Physical Illness".*

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. W. Sciama: "The Unity of the Universe".

Institution of Electrical Engineers, Electronics Division P.G.E8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. Lewin: "Microwave Theory or Microwave Green Fingers ?"

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Reinhold Baer (Frankfurt am Main): "The Reduced Rank of a Group".*

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Lords of the Forest" (Colour film).

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m.—Dr. A. R. Flint· "The Channel Bridge Project".

Monday March 11—Tuesday, March 12

Society of Chemical Industry, Chemical Engineering Group; the Institution of Chemical Engineers, South-Eastern Branch; and the Institute of Petroleum (at the Royal Commonwealth Society's Assembly Hall, Craven Street, London, W.C.2)—Symposium on "Fluidization".

Monday, March 11—Thursday, March 14

Oil and Colour Chemists' Association (at the Royal Horticultural Society's Old and New Halls, London, S.W.1)—Fifteenth Technical Exhibition.*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer (with a good honours degree) in Applied Mathematics—The Registrar, King's College (University of London), Strand, London, W.C.2 (March 9).

Lecturer or Assistant Lecturer (graduate in civil engineering or physics) in Soil Mechanics—The Registrar, The University, Manchester (March 9).

Assistant Lecturer (with an honours degree in biochemistry or equivalent) in Agricultural Biochemistry—The Secretary, The University, Aberdeen (March 15).

Lecturer in the Computing Laboratory—The Secretary of University Court, The University, Glasgow (March 16).

Research Biochemist (medical or science graduate, preferably with experience in the field of steroid chemistry) in the Department of Medicine, to undertake biochemical investigations as a member of a team engaged in clinical research—The Registrar, The University, Sheffield (March 16).

Professor (with considerable experience in research and university lecturing in either physics, chemistry, zoology or botany and preferably some administrative experience, including handling of staff and students) to head the Department of Science, University College of Cape Coast, Ghana—The Assistant Registrar (Overseas), University College of Ghana, 15 Gordon Square, London, W.C.1 (March 18).

Research Student (with a good honours degree or diploma in technology in mechanical engineering or in an applied science) in the Department of Mechanical Engineering, to undertake basic research into the thermal strains which occur in machine tool frames in practice—The Secretary, Royal College of Advanced Technology, Salford 5, Lancs, quoting Ref. MB/34 (March 22).

Lecturer (with special interests and qualifications in mineralogy and petrology) in Geology in the Faculty of Science—The Registrar, The University, Manchester 13 (March 23).

Librarian—The Registrar, University of Kent at Canterbury, c/o The Education Department, Springfield, Maidstone, Kent (March 23).

Assistant Lecturer or Lecturer (with a special interest in inorganic chemistry) in Chemistry at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 30).

Assistant Lecturer in Animal Production—The Secretary, Wye College (University of London), near Ashford, Kent (March 31).

LECTURER (permanent or temporary) IN THE DEPARTMENT OF PHYSICS at the University of the Witwatersrand, Johannesburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, March 31).

SENIOR LECTURER or LECTURER (medically qualified with appropriate training and experience) IN CHEMICAL PATHOLOGY at the University of Ibadan—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (March 31).

LECTURER IN PHYSICAL OR INORGANIC CHEMISTRY; and a LECTURER IN THEORETICAL CHEMISTRY—The Secretary, The University, Edinburgh (April 1).

LECTURER (with an honours degree, preferably in chemical engineering with research and/or industrial experience) IN CHEMICAL ENGINEERING at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 15).

LECTURERS or ASSISTANT LECTURERS (3) IN PHYSICS at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

READER IN MEDICAL MICROBIOLOGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 15).

SENIOR LECTURER (preferably with interests in the field of terrestrial or freshwater ecology) IN ZOOLOGY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, April 15).

AGRICULTURAL CHEMIST (male, between 22 and 45, with an honours degree in agricultural chemistry or chemistry, with two years postgraduate experience in agricultural chemistry) in the Ministry of Agriculture, Kenya, to take charge of research projects into soil and plant nutrition problems of coffee—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting Ref. T/RD.213/80/03.

ASSISTANT LECTURER (with a good honours degree) IN BIOCHEMISTRY—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

MASTER TO TEACH MATHEMATICS throughout the school, including Sixth Form; and a MASTER TO TEACH CHEMISTRY, largely in the Sixth Form—The Headmaster, Bolton School, Bolton, Lancs.

POSTDOCTORAL FELLOW IN PHYSICAL CHEMISTRY, for work concerned with the application of radioactive tracer methods to the study of the solid state under the direction of Dr. J. N. Sherwood—Prof. P. L. Pauson, The Royal College of Science and Technology, George Street, Glasgow, C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University of London. Imperial College of Science and Technology. Shorter Postgraduate Courses, Spring Term, Session 1962–63. Pp. 17. (London: Imperial College of Science and Technology, 1962.) [81

Meteorological Office. Geophysical Memoirs, No. 106: A Meso-Synoptic Analysis of the Thunderstorms on 28 August 1958. By D. E. Pedgley. (M.O. 711). Pp. 74. (London: H.M. Stationery Office, 1962.) 12s. 6d. net. [81

University of Cambridge: School of Agriculture. Memoir No. 34: A summary of papers published by the members of the Staff of the School of Agriculture and its Associated Research Organisations during the period Oct. 1st, 1961—Sept. 30th, 1962. Review Series No. 17: Soil Microbiology in the School of Agriculture. Pp. 28. (Cambridge: School of Agriculture, The University, 1962.) [81

The Training of Graduate Science Teachers in England, Wales and Scotland. (A Section of Part II of the Science and Education Report issued by the Science Masters' Association and the Association of Women Science Teachers. Pp. 32. (London: John Murray, 1963. Published for the S.M.A. and A.W.S.T.). 1s. net. [81

Science Teaching Techniques—X: The Transfer of Heat. Worksheets: Guide to Individual Studies. Pp. 64. (London: John Murray, 1963. Published for the Association of Teachers in Colleges and Departments of Education; The Association of Women Science Teachers; and The Science Masters' Association.) 4s. net. [81

British Medical Bulletin, Vol. 19, No. 1, (January, 1963): Respiratory Physiology. Pp. 1–96. (London: The British Council, 1963.) 30s. [81

Transactions of the Royal Society of Edinburgh. Vol. 65, No. 5, 1961–62: Further Observations on the Margin of the Laxfordian Complex of the Lewisian near Loch Laxford, Sutherland. By Dr. J. Sutton and Dr. Janet Watson. Pp. 89–106. (Edinburgh: Royal Society of Edinburgh, 1962.) 8s. 6d. [81

Electrical Research Association. Technical Report No. Z/T125: A New Theory of Electrical Discharge Propagation and of Magnetic Storms. By Dr. C. E. R. Bruce. Pp. 8. (Leatherhead: Electrical Research Association, 1960.) 7s. 6d. [81

National Parks and Access to the Countryside Act, 1949. Thirteenth Report of the National Parks Commission for the year ended September 30, 1962. Pp. vi+80+8 plates. (London: H.M. Stationery Office, 1962.) 6s. 6d. net. [151

The Institution of Professional Civil Servants. Affiliation to the T.U.C. and Co-operation with other Bodies—Report by the National Executive Committee. Pp. 11. (London. The Institution of Professional Civil Servants, 1963.) [151

Continental Paint and Resin News, Vol. 1, No. 1, (January, 1963). (A monthly digest of articles, patents and economic news drawn from the countries of the Continent of Europe.) Pp. 6. Subscription: 40s. or 6 dollars a year; 60s. or 9 dollars airmail. (London: Translation and Technical Information Services, 22 Manston Road, S.E.15). [151

Building Research Station Digest, (Second Series). No. 30: Aluminium in Building—2: Finishes. Pp. 4. (London: H.M. Stationery Office, 1963.) 4d. [151

The Leverhulme Trust. Fourth Report of the Leverhulme Trustees, 1959–1961. Pp. 61. (London: The Leverhulme Trust, 1962.) [151

Agricultural Research Institute of Northern Ireland. Thirty-fifth Annual Report, 1961–1962. Pp. 52. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1962.) [151

The Biochemical Journal: Suggestions and Instructions to Authors. Pp. 8. (London: *The Biochemical Journal*, 1963.) 1s. 6d. [151

Other Countries

Australia. Commonwealth Scientific and Industrial Research Organization. Division of Building Research Annual Report, 1961–1962. Pp. vi+7 Ore-Dressing Section Annual Report for the year ending June 30, 196 Pp. iv+6. Physical Metallurgy Section Annual Report for the year endin June 30, 1962. Pp. 6. (Melbourne: Commonwealth Scientific and Industri Research Organization, 1962.) [8

Inter-American Tropical Tuna Commission. Annual Report for th year 1961. Pp. 171. (La Jolla, California. Inter-American Tropical Tun Commission, 1962.) [8

Smithsonian Contributions to Astrophysics. Vol. 5, No. 14: The Spac Density of Atmospheric Dust in the Altitude Range 50,000 to 90,000 Fee By Paul W. Hodge and Frances W. Wright. Pp. ii+231–238+1 plat (Washington, D.C.: Government Printing Office, 1962.) 30 cents. [8

The University College, Ibadan. Calendar, 1962–63. Pp. 126. (Ibadan University College, 1962.) [8

Eastern Nigeria: Ministry of Agriculture. Annual Report of the Fores Division, 1961–1962. Pp. 22. (Enugu: Government Printer, 1962.) [8

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United States Department of the Interior: Geological Survey. Bulleti 1087–J: Geology of the Lost Creek Schroeckingerite Deposits, Sweetwate County, Wyoming. By Douglas M. Sheridan, Charles H. Maxwell an John T. Collier. Pp. v+391–478+plates 35–42. Bulletin 1099–B: Uranium Bearing Coal in the Eastern Part of the Red Desert Area, Wyoming. B Harold Masursky. Pp. vii+152+10 plates. Bulletin 1107–F: Distributio of Elements in Sedimentary Rocks of the Colorado Plateau—a Preliminar Report. By William L. Newman. Pp. viii+337–445. Bulletin 1122–B Geology and Fluorspar Deposits of the Levias-Keystone and Dike–Eato Area, Crittenden County, Kentucky. By Robert D. Trace. Pp. iii+26+ plates. Bulletin 1144–B: An Improved Method for the Determination c FeO in Rocks and Minerals including Garnet. By Laura E. Reichen an Joseph J. Fahey. Pp. iii+5. Bulletin 1147–B: Uranium in Some Rock of Pennsylvanian Age in Oklahoma, Kansas and Missouri. By Harold J Hyden and Walter Danilchik. Pp. iv+82+5 plates. (Washington, D.C. Government Printing Office, 1962.) [8

United States Department of the Interior: Geological Survey. Water Supply Paper 1544–C: Methods of Flow Measurement in Well Bore By Eugene P. Patten, Jr. and Gordon D. Bennett. Pp. iv+28. Water Supply Paper 1571: Quality of Surface Waters of the United States, 195 Parts 1–4: North Atlantic Slope Basins to St. Lawrence River Basi Prepared under the direction of S. K. Love. Pp. xv+773. Water-Suppl Paper 1607: Reconnaissance of Ground-Water Resources in the Easter Coal Field Region, Kentucky. By W. E. Price, Jr., D. S. Mull and C. Kilburn Pp. iv+56+9 plates. Water-Supply Paper 1619–V: Alluvial Aquifer i Northeastern Louisiana—a Large Source of Water. By A. N. Turcan, J and B. B. Meyer. Pp. iv+28. (Washington, D.C.: Government Printin Office, 1962.) [8

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Optical Masers. (*Applied Optics*, Supplement No. 1). Pp. 142. (Washington, D.C.: Optical Society of America, 1962.) 5 dollars. [15

University of California Publications in Botany. Vol. 33, No. 4: Comparative Morphological Studies in Theaceae. By Hsuan Keng. Pp. 269 384 (7 plates). (Berkeley and Los Angeles: University of California Pres 1962.) 2.50 dollars. [15

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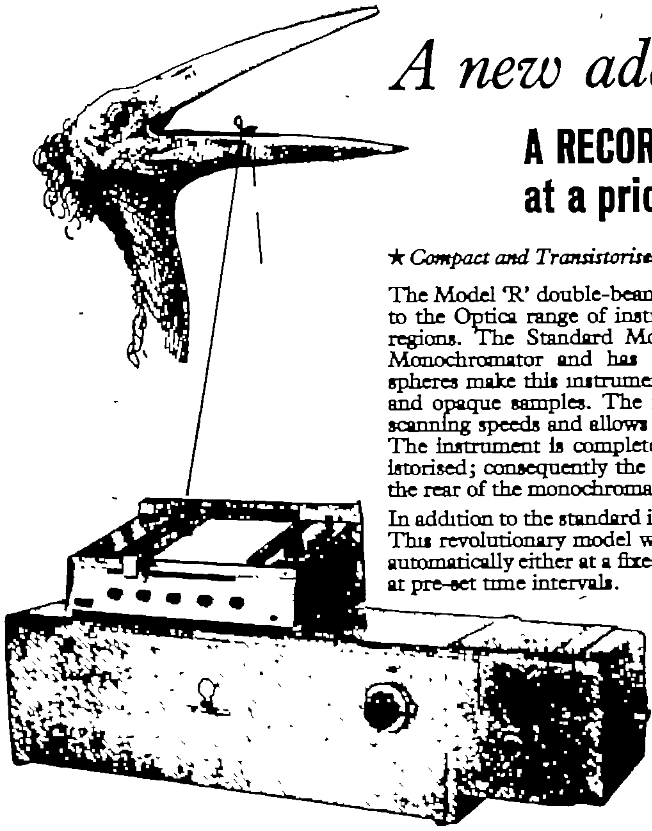

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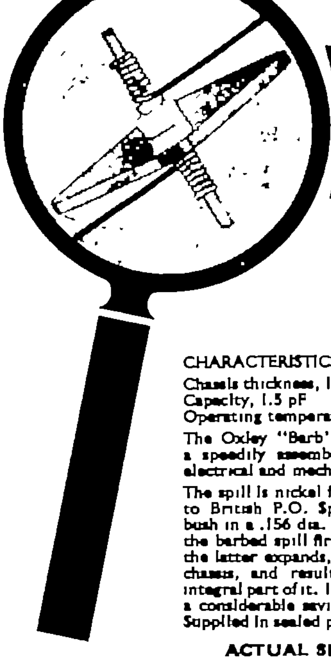

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Application Forms may be obtained from the Nova Scotia Civil Service Commission, P.O. Box 943, Provincial Administration Building, Hollis Street, Halifax, Nova Scotia.

7678

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
(UNIVERSITY OF READING)

Applications are invited for a post in the Statistics Section of the Institute. Candidates should have an honours degree in mathematics, with preferably an interest in biology. Duties include consultation and research on design and analysis of experiments, and co-operation with the research staff of the Institute in the statistical aspects of the work. Appointment in Scientific Officer (£768 to £1,271) or Senior Scientific Officer (£1,396 to £1,720) grade, according to age and experience.

Applications, naming two referees, to Secretary, N.I.R.D., Shinfield, Reading. Quote ref 63/6.

SCOTTISH MARINE BIOLOGICAL ASSOCIATION
OCEANOGRAPHIC LABORATORY
EDINBURGH

Scientific Officer or Senior Scientific Officer for research on marine plankton, hydrography and fisheries. Opportunities to develop statistical techniques in ecology. Fifteen years data and computer facilities available. Salary, depending on age and experience, in range £768 to £1,271 (Scientific Officer) or £1,396 to £1,720 (Senior Scientific Officer) but special consideration might be given to more senior candidates. F.S.S.U.

Further particulars and application forms from the Officer-in-Charge, Oceanographic Laboratory, Craighall Road, Edinburgh, 6.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF RADIOPHYSICS

EXPERIMENTAL OFFICER–DATA ANALYST

Applications are invited for appointment to a position of Data Analyst with the Organization's Division of Radiophysics located in the grounds of the University of Sydney, N.S.W.

The duties will involve the analysis of and assistance in the interpretation of the results of cloud seeding and similar experiments and participation in the statistical design and physical assessment of the experiments

Applicants should possess either a degree or a diploma in Physics and Mathematics and have some interest in statistics, or similar qualifications in Statistics and have some interest in Physics.

SALARY : Dependent upon qualifications and experience within the ranges :

£A1,376 per annum to £A2,231 or £A2,331 to £A2,631 per annum.

Fares paid for the appointee and his dependent family. Further particulars of conditions, etc., supplied on application to :

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 780/295), should be addressed by March 30, 1963.

THE INTERNATIONAL NICKEL COMPANY (MOND) LIMITED

requires

SENIOR ASSISTANT

in the

TECHNICAL INFORMATION SECTION

DEVELOPMENT AND RESEARCH DEPARTMENT, LONDON

Duties will include abstracting, indexing, translation and literature searches. Candidates, who should preferably not be older than 35, must offer a degree (or equivalent) in metallurgy, the physical sciences or engineering and have a sound knowledge of French. Some Spanish or Italian would also be useful.

Pension and assurance schemes are in operation and, in appropriate cases, assistance can be given with housing.

Apply giving full particulars of qualifications, experience, age, salary required etc., to :

The Technical Information Officer,
Development and Research Department,
The International Nickel Company (Mond) Limited,
Thames House, Millbank, London S.W.1.

Mark envelope " Confidential M. 13."

MARQUETTE UNIVERSITY SCHOOL OF MEDICINE

MILWAUKEE 3, WISCONSIN

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

Invites application of Lecturer in Medical Microbiology and Immunology. The lecturer is to participate in teaching medical students during one semester of the academic year in co-operation with the staff of the Department. The remaining time of the year can be devoted to research. Modern, adequate research facilities. Annual salary £3,200, or higher, depending on rank and experience.

RESEARCH. VACANCY EXISTS FOR Qualified Assistant to work in the field of Powder Metallurgy, including Hot Pressing. Some experience in carbide would be an advantage. Candidates should possess a degree or equivalent qualification, and preferably some industrial research experience. Conditions of employment are good and include pension and bonus schemes. Salary will be commensurate with qualifications and experience.—Applications, giving full particulars to Personnel Officer, The Carborundum Company Ltd., Trafford Park, Manchester 17, quoting reference R D.2.

McGILL UNIVERSITY

MONTREAL

A permanent teaching and research appointment is available in the Department of Civil Engineering and Applied Mechanics for a man with interests in the broad field of engineering hydrology. Required qualifications are Ph.D. or equivalent research experience. Extensive soils and fluids laboratories are available for independent research. Teaching load is moderate. Initial appointment as Assistant Professor, salary range $7,000 to $9,000 plus benefits, to be made September 1.

Inquiries to Professor J. O. McCutcheon, Chairman, Department of Civil Engineering and Applied Mechanics

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PHYSICIST TO LEAD RESEARCH GROUP in the field of thermo-electricity required by an expanding organization specializing in the production of the rare metals and semi-conductor materials. Minimum qualifications Ph.D. or equivalent and preferably at least two years' experience in industrial research. The post presents great opportunity to a scientist of the right character. Good working conditions in the country but close to London.—Apply Box No. 688, T. G. Scott and Sons, Ltd., 1 Clement's Inn, London, W.C.2.

QUEEN'S UNIVERSITY

KINGSTON, ONTARIO, CANADA

EXPERIMENTAL PHYSICIST

Applications are invited for an appointment in Experimental Physics from September 1, 1963. Fields of Research in which candidates will be considered are Radio Astronomy, Nuclear Physics, and Solid State Physics Salary and rank will depend upon qualifications and experience. The minima of the salary scales are as follows: Professor, $12,000 ; Associate Professor, $9,000 ; Assistant Professor, $7,000 per annum. The new three-million-dollar physics building with a research wing is on schedule for completion in 1963. One purpose of the appointment is to strengthen postgraduate work. This is an excellent opportunity for a research physicist with a good publication record to find satisfaction in the combination of teaching and research.

Applications and inquiries should be addressed to the Head of the Department of Physics.

UNIVERSITY COLLEGE OF CAPE COAST, GHANA

DEPARTMENT OF SCIENCE

Applications are invited for

LECTURESHIPS
and
ASSISTANT LECTURESHIPS

in the following subjects: 1. Chemistry. 2. Zoology. 3. Botany. 4. Physics. 5. Mathematics. Applicants must have good science degrees with, preferably, experience in teaching. Teaching experience up to Sixth Form or " A " Level may be considered. It would be an advantage for candidates to have the following special interests, for Zoology: Marine Zoology or Physiology or both or Vertebrate Zoology; for Botany: Morphology or Mycology or Taxonomy (field work). Research facilities will be available in due course. Unusual opportunities exist for Biologists. Salaries: Lecturer, £G.1,050 by £G.50 to £G.1,400 by £G.75 to £G.1,850; £G.1.900 per annum. Assistant Lecturer, £G.900, £G.950 per annum. Twenty per cent addition for non-Ghanaians. Entry at appropriate point. Appointment normally five years in the first instance. Outfit allowance £G.60 (where applicable). Car maintenance allowance £G.150 per annum. Family allowance for non-Ghanaian £G.50 per annum for each child in West Africa or, if under 21 years of age and being educated outside West Africa £G.100 per annum (maximum five children). Accommodation with basic furniture. Rent not exceeding 7.5 per cent of salary. F.S.S.U. policies maintained if already held or superannuation on similar basis. Passages for appointee and family on appointment and normal termination payable. Annual leave with three return passages in every four years for non-Ghanaians and one in every three years for Ghanaians.

Applications (six copies), giving details of qualifications, etc., and naming three referees, should be sent before March 18, 1963, to the Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1, from whom further information may be obtained.

ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY

GLASGOW

POST-DOCTORAL FELLOWSHIP IN PHYSICAL CHEMISTRY

Applications are invited for the above post. The work will be concerned with the application of radioactive tracer methods to the study of the solid state and will be under the direction of Dr. J. N. Sherwood. The appointment will be for a period of two years from October 1, 1963. The salary will be £1,000 per annum plus F.S.S.U. benefits

Applications, including the names of two referees, should be sent to: P. L. Pauson, Professor of Chemistry, George Street, C.1, as soon as possible.

UNIVERSITY OF IBADAN

Applications are invited for (a) Senior Lectureship or (b) Lectureship in Chemical Pathology, from medical., qualified candidates with appropriate training and experience. Salary scales: (a) £2,650 by £100 to £2,950 per annum. (b) £2,200 by £100 to £2,600 per annum, both carrying honorary consultantships to University College Hospital. Passages paid for appointee, wife and up to five children under 11 years, on appointment, approved overseas leave and termination where applicable. Children's and car allowances. F.S.S.U. Part-furnished accommodation at rent not exceeding 7 per cent of salary.

Detailed applications (ten copies), naming three referees, by March 31, 1963, to Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1, from whom further particulars may be obtained.

BACK ROOM BOYS NEED NOT APPLY—

for we have no back room in which to accommodate you.

Our Research Laboratory in the Home Counties is an integral part of our business in the food industry and has contributed very substantially to our successful record of growth. The work of our Research Scientists brings them into contact with all Departments connected with the development and marketing of our well-known range of products—production, sales, market research and raw materials buying.

Although the Research Scientist operates from the Laboratory (which is amongst the best equipped in the industry) his influence on the Company's progress goes far beyond the ability to conduct successful research : he is a member of our management organisation and his success is measured as much by his effectiveness in contributing to the overall development of a project as by his capacity as a Research Scientist.

As evidence of this, we could quote a number of examples of men who have joined our organisation as Research Scientists and have subsequently been promoted into other Divisions of the Company, e.g. brand management. You could well develop along similar lines, although, of course, there is nothing to preclude you from developing your career within the Research Laboratory if your interest lies more in this direction

Our expansion has created the vacancy for an additional Research Scientist and if you would like to learn more about us, we should be pleased to hear from you. You should have a good honours degree in pure science and a higher degree is desirable We would prefer you to have had experience of research work, preferably in industry and you should be in the age range 25–33.
The initial salary will be in the range

£2,000—£2,300 p.a.

and we operate a non-contributory pension, sick pay and life assurance scheme In the event of a move of house being necessary, the Company will assist in defraying the cost of the removal and will help in obtaining a mortgage

Please address your application in confidence to Personnel Manager, Box 693, T. G. Scott and Son, Ltd , 1 Clement's Inn, London, W.C.2

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF ANIMAL HEALTH

RESEARCH BIOCHEMIST

Applications are invited for appointment to a position of Research Biochemist with the Organization's McMaster Animal Health Laboratory, situated in the grounds of the University of Sydney, with which a close association is maintained

The appointee will be required to undertake research on some aspects of chemical pathology particularly in relation to the effects of parasitism.

Applicants should possess an Honours degree in science or a degree in Veterinary Science, with postgraduate training and research experience in Mammalian Biochemistry

SALARY : Dependent upon qualifications and experience within the range :

£A1,541 to £A2,296 per annum.

Salary for a woman will be £A188 per annum less than corresponding rates for men.

Promotion within C.S.I.R O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made

Fares paid for the appointee and his dependent family Further particulars supplied on application to :

Mr. P. F Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, to whom applications (quoting Appointment No : 202/214), should be addressed by March 30, 1963.

Gas Chromatographers

SWISS subsidiary of the leading U.S. Gas Chromatography company is seeking a qualified Technical Representative as manager for its Sales and Service office in the U.K. The applicant should have a background in Organic Chemistry, two or more years of practical gas chromatographic experience and some basic knowledge of instrumentation. A pleasant personality and interest in meeting people is necessary. Actual sales or management experience is desirable but not essential.

The initial salary, which will be commensurate with qualifications, will be in the region of £2,000

Detailed application indicating present salary to Box 692, T G Scott and Son, Ltd , 1 Clements' Inn, London, W C 2

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

Applications are invited for the recently instituted Chair of Physical Oceanography. The Chair is intended to provide opportunities for a new approach in the application of the physical sciences to oceanography. It will offer scope for the development, in association with the existing department of Marine Biology, of an integrated programme of teaching and research at undergraduate and postgraduate levels. Applicants should have a wide basic knowledge of physical oceanography, or some field of applied mathematics, physics, geology, or chemistry, which is related to oceanography. The appointment will date from October 1, 1963, or from some other date to be agreed. The salary will not be less than £2,900 per annum, and in addition there will be F S.S U. benefits and family allowances.

Further particulars may be obtained from the Secretary and Registrar, The University College of North Wales, Bangor, to whom twelve copies of the application should be sent not later than March 25, 1963.

ROYAL MILITARY COLLEGE OF SCIENCE

Requires Senior Lecturers or Lecturers in Mechanical Engineering and Mathematics. Duties: Teaching at University level. The successful candidate will lecture to degree courses and specialized courses and, for the post in Mechanical Engineering, assist with laboratory classes. There are extensive well-equipped laboratories. Research is encouraged and work will be suitable for publication and submission for higher degree. Qualifications· First- or second-class honours degree or equivalent. For the Mechanical Engineering post at least two years' experience in teaching, industry, or in research establishments is also required and special experience in the field of theory of mechanics or machines would be an advantage. Salary: Senior Lecturer, £1,396 to £1,720 ; Lecturer, £768 to £1,271. Starting pay may be above the minimum in both cases ; credit for postgraduate experience and National Service. Superannuation under F.S.S U Prospects of permanency. Residential accommodation for single man, prospects of house for married man.

Further particulars and forms from the Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire. (5)

UNIVERSITY OF ESSEX

WIVENHOE PARK, COLCHESTER

CHAIR OF CHEMISTRY

Applications are invited for the First Chair of Chemistry. Salary not less than £2,900 per annum (new scale) with family allowances

Applications should be received not later than March 11, by the Registrar (University of Essex, Wivenhoe Park, Colchester), from whom further particulars may be obtained.

DEVELOPMENT AND EXPLOITATION OF SCIENCE IN THE PUBLIC INTEREST

IN view of the attention which has been given in Parliament and elsewhere in the past year or so to the use of the development contract, it is scarcely surprising that the latest annual report of the National Research Development Corporation* should give some space to discussing the Corporation's activities generally and the extent to which the Corporation is achieving its purposes. Quite apart from the emphasis placed on the work of the Corporation in recent reports from the Council for Scientific and Industrial Research and the co-operation now established between the Corporation and that Department, the Minister for Science has on several occasions recognized the value of the Corporation's work and indicated a readiness to reconsider whether the Corporation is appropriately placed under the Board of Trade. This is being examined by the Trend Committee; but in the meantime the functions of the Corporation received some comment in the report, *Science in Industry*, published last November by the Conservative Political Centre (see *Nature*, 197, 629; 1963).

The Corporation, which was set up in 1949 under the Development of Inventions Act, 1948, was broadly intended, first, to provide a single public body to exploit commercially those patent rights derived from inventions made in the research establishments of Government Departments and the Research Councils. Secondly, it was held that the liaison between centres of research such as the universities and the colleges of technology, on one hand, and industry, on the other, should be strengthened by a public body which could assume the responsibility for undertaking the work of patenting and exploiting inventions. Thirdly, it was believed that a public body should be formed to ensure the full exploitation of the inventive talent of the nation, which, it was sometimes alleged, was frequently frustrated by lack of support for the individual inventor without industrial connexions.

The original Act gave the Corporation the permissive function of securing "where the public interest so requires" the development and exploitation of inventions from publicly supported research and also of inventions from other sources which lack a sponsor. These functions were extended under the 1954 Act, which empowered the Corporation to sponsor research likely to lead to inventions of practical importance. This exploitation activity has been carried out on a considerable scale, and more than 1,000 licence agreements have already been made with industry, of which more than 900 were with United Kingdom firms. The resulting revenue totals about £1·5 million, and payments to individual inventors from these receipts total nearly £300,000; but the Corporation has found no trustworthy evidence to support the belief that inventions are being suppressed because of their effect on the position of established products. The latest report is particularly encouraged by the increase in the number of approaches made by university inventors, and although it is disappointed at a decrease in these coming from Government Departments and Research Councils, one important consequence of the formation of the Corporation is that the medical profession's tradition of refraining from patenting medical discoveries has been reversed and the Corporation is now developing and exploiting in the public interest a number of discoveries in the medical field.

While for financing much technological development the United Kingdom relies, and must continue to rely, on industry, the Corporation has helped industry with a number of projects which appeared worth while from a national point of view. Invariably, such projects required a long development period or resources beyond those available to the firm concerned, or the invention was applicable to no existing industry or seemed unlikely to offer sufficient return to the developer. In these activities the Corporation has gained a unique experience in making the specialized arrangements required with industry and inventors and has evolved its own methods of handling individual projects based on a commercial approach. It is believed that the experience gained will be of much value in handling future projects, but fundamentally the Corporation's obligation to try to pay its way is not readily reconcilable with the obligation to act in the public interest.

The main difficulty is the prolonged period usually required before returns come in on any substantial scale. As much as fifteen or twenty years may elapse between the financing of a project and any substantial return, and in addition the risks are greater than normal. At the end of June 1962, the Corporation's indebtedness to the Board of Trade was almost £6 million, of which just more than £5 million was represented by advances and £920,000 by accrued interest. Its borrowing powers from the Board of Trade at Treasury rates of interest is limited to £10 million and the power to make advances up to this ceiling expires in June 1969.

The report recapitulates five commercial disabilities under which the Corporation at present operates. Overall, the sponsorship of inventions is notoriously speculative, and especially when the Corporation is concerned with inventions which industry itself is not prepared to sponsor on its own account. While, however, the development of an invention is essentially a risk-taking activity, the Corporation is not provided with risk capital. It operates on loans which cannot be written off and on which interest accumulates, notwithstanding that the money may become locked up in long-term development projects, which if successful will produce no income until industry has taken over, or if unsuccessful will be irrecoverable. Thirdly, while industry normally pays for development out of the trading profits it earns, the Corporation is not permitted, except in special circumstances, to enter into trading. It has thus no bread-and-butter business or trading profits to use for financing new projects, establishing reserves, or paying for unsuccessful ventures.

Fourthly, much work and money are involved in assessing the 1,000 inventions submitted every year. Costs are heavy in terms of overheads, staff salaries, payment for independent experts' reports, experimental work, etc., but although a high proportion of each year's submissions is discarded, the assessment activity is very important if inventors are to have a fair deal and no invention of importance to the community is to remain neglected. Finally, in the nature of the business heavy costs are incurred in patenting inventions at home and overseas, to protect the export trade of United Kingdom manufacturers. The statutory requirement to accept and exploit all inventions offered to the Corporation by Government Departments calls for large outlays on patent costs, and while much of this expenditure bears fruit, a considerable part proves to be abortive.

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* National Research Development Corporation. Report and Statement of Accounts for the year 1st July, 1961, to 30th June, 1962. Pp. ii+34. (London: H.M.S.O., 1963.) 2s. 3d. net. See also p. 960 of this issue of *Nature*.

The Corporation is particularly disturbed that most of its present income is derived from a small number of the inventions in its portfolio. Although it might happen in the future that a single invention would yield a revenue sufficient to transform the whole of the Corporation's financing, in the absence of any such windfall the prospects of the Corporation being able to balance its accounts on the basis of interest-bearing capital with no power to write off are remote. Since the Corporation operates solely on interest-bearing loans without any risk or equity capital in the notoriously hazardous field of invention, its members are forced to conclude that, with its existing capital structure, the prospects of the Corporation achieving its statutory objective of paying its way must be remote.

Nevertheless, the members of the Corporation are in no doubt that the continued existence of the Corporation is justified. Bearing in mind the vast Government expenditure on research and development in other spheres of activity, they consider the public receives exceptional value for money from the activities of the Corporation. It has played a major part in establishing industries of great potential importance, and in introducing important products or processes in others, for relatively small outlay by the community. Much of this outlay of £6 million, including interest charges, will prove to be recoverable. Moreover, and this is of special importance, the Corporation's methods of operation encourage the establishment of industries and processes capable of standing on their own feet rather than relying on continuing Government subsidies.

Specifically, the Corporation obtains substantial revenue, much of it from abroad, from inventions arising in the United Kingdom; it provides unique facilities for inventors who seem, by and large, to consider that they are fairly treated; it bridges the gap between research carried on in public research establishments, the universities, etc., and the practical application of the results of such research; it works in increasingly close co-operation with industry's own research establishments and seems to be gaining steadily the confidence of industry itself; it has taken the initiative in providing co-ordinating machinery for investigating and sponsoring projects of common interest to more than one field of industry or to different firms within the same field, frequently projects which otherwise would never have been started. It has also helped to develop inventions in the surgical and pharmaceutical field primarily for their humanitarian rather than profit-earning value; it fosters projects calculated basically to be of value to the nation where the prospects of return to the Corporation are slender or non-existent; and it has acted in a catalytic role in introducing research results to industry again with no resulting financial reward.

These judgments are substantiated from the present report and from earlier annual reports, and the conclusion that, with its strict control of expenditure and its small, loyal and now uniquely experienced staff, the Corporation has a part of increasing practical value to play in the nation's affairs that cannot be dismissed or discounted as *ex parte*. It is fully supported by comments in reports from the Advisory Council on Scientific Policy, the Council for Scientific and Industrial Research and by the observations in the report *Science and Industry* from the Carr Committee of the Conservative Party, which on political grounds at least might be expected to be critical of an institution created by a Labour Government. That Committee, however, fully recognized the value of the Corporation's work and that its main difficulty has been to meet its statutory obligation to pay its way. While it considers that the commercial slant should be retained, both for its value in maintaining the morale of the Corporation's staff and in ensuring that the projects are assessed and reviewed, it suggests that extra support should be given, possibly by some form of direct grant, to those projects to which a special degree of risk is attached.

This proposal could well involve difficulties in view of the exceptionally long time that is likely to elapse before any substantial returns are received on advances for projects of this character; reconsideration of the place of the Corporation in the Government organization for civil research and development as well as of the basis of its finance may be advisable. The latter could well fall outside the terms of reference of the Trend Committee, but the Carr report also recommends further consideration of a recommendation of the Radcliffe Committee on the Working of the Monetary System. This was for an Industrial Guarantee Corporation which would "guarantee for a commission, an agreed proportion of loans made by existing financial institutions to borrowers wishing to finance novel processes or the manufacture of new types of product". Implementation of this proposal might enable the National Research Development Corporation to obtain the extra support needed by insuring its more risky projects with this body.

The Carr Committee urges that this recommendation of the Radcliffe Committee should be adopted. However, although it considers that the National Research Development Corporation has not so large a part to play in financing certain kinds of invention as was thought when it was first established, it does not discuss the question how far the Corporation could be used for other purposes, such as placing of development contracts. It was in this context that the Minister for Science indicated that he had asked the Trend Committee to look specifically at the functions of the Corporation and its position under the Board of Trade. Both the Carr report and the Corporation's own annual report demonstrate that the Corporation has a real part to play in financing invention and they should stimulate further thought as to the most appropriate means by which the necessary finance should be provided. Such consideration should now be proceeding as it is unlikely that the Trend Committee will feel free to make recommendations on that aspect of the Corporation's work.

What might equally receive attention, however, is the more positive function opened up for the Corporation under the 1954 Act, through which it is continually surveying the needs of industry for new techniques and products, for improving existing ones, and for developing new knowledge resulting from present-day research. The report sites its investigations in the cryogenics field which have led to the sponsorship of the cryogenic engineering project and its efforts to encourage in the United Kingdom the establishment of adequate industrial facilities in this field. Its investigations in relation to the mechanical properties of solids are also noted. A conference sponsored at the University of Cambridge in July 1961 to formulate proposals for research and development in this relatively new field aroused considerable interest. The tempo of work generally was increased and programmes have been expanded to take note of the recommendations of this conference, and steps are being taken to co-ordinate the work being carried out in the public research establishments.

It may be for consideration how far the National Research Development Corporation should function in this way and whether it is not here entering on activities which the Council for Scientific and Industrial Research should be fostering. If the Corporation through its experience has a vital contribution to make, activities of this kind provide one strong reason for placing it in closer relation to the Department of Scientific and Industrial Research and within the survey and responsibility of the Minister for Science rather than the Board of Trade. Some clear thinking on these and related issues is imperative when the whole structure of Government organization for civil research and development comes under review.

RADIOBIOLOGY'S EXPANDING FUNDAMENTALS

Fundamentals of Radiobiology

By Z. M. Bacq and Peter Alexander. Complete revised second edition. (International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 5.) Pp. xii + 555. (London and New York: Pergamon Press, 1961.) 70*s*. net.

In the preface of the first edition of this book, the authors remarked on the diversity of disciplines involved in radiobiological work, and radiobiologists do indeed need to have an acquaintance, at least, with a wide variety of subjects. The difficulties for beginners in this field can easily be imagined, and a brief but adequate survey of the basic facts and present working hypotheses is very necessary for them. The 350 pages of the first edition proved to be a very useful text for this purpose, but the second edition runs to more than 500 larger pages and the tiro's task has thus increased. Much new material has been incorporated and the increase in size is in many ways an indication of the enormous number of radiobiological publications during the six years between the editions. An increase in the amount of data available, however, should not necessarily mean an increase in the 'fundamentals' of a subject. As the authors have resisted the temptation to change their title when they changed publishers, it is hoped that they will resist the temptation of turning their volume into a large text-book. Perhaps this second edition is merely suffering from the adiposity of adolescence, in which case we can look forward to the third and fourth editions regaining the slimness of the youthful *Fundamentals*.

The book is, however, still unique in its general usefulness and no other covers the scientific aspects of the subject so well. Although primarily intended for beginners, more experienced workers will, no doubt, refer to it for guidance in those aspects of the subjects outside their own field. These latter users will find a good deal of extra information and some shifts of emphasis in the new edition. The nature of the initial chemical lesion is stressed, the chapter on radiation effects on macromolecules has almost doubled in size and the section on "alteration of permeability in cells" is replaced by one on "the enzyme-release hypothesis". The chapters on the processes of restoration and the physiopathology of radiation sickness are greatly expanded and there are new chapters on delayed effects and the interaction between cells and tissues following irradiation. As one would expect from these authors the chapter on chemical protection is particularly useful. Genetic effects of radiation are only very briefly described.

This is not an impersonal book, and it is pleasant to note how the authors are ready to become expansive when they reach their own interests and how, after marshalling a number of theories, they are ready to offer summaries and conclusions. There is sometimes a lack of clarity in the writing and in some parts peculiar phraseology is used; for example, "profound trouble in cholesterol synthesis" (p. 399). Misprints, even when acknowledging illustrations (for example, Figs. 20–2 and 20–3), and errors such as "polynucleated" instead of "polymorphonucleated" and "picnotic" instead of "pyknotic" are not uncommon. Numerous references follow each chapter, but occasionally they are not mentioned in the text, and in one instance experiments are attributed to an author which he did not do and a conclusion which he did not draw.

E. V. Hulse

CLASSICAL PROBLEMS IN PHYSICS

Cavendish Problems in Classical Physics

Edited by Prof. A. B. Pippard. Compiled by the Staff of the Cavendish Laboratory, Cambridge. Pp. x + 51. (Cambridge: At the University Press, 1962.) 5*s*. 6*d*. net; 1 dollar.

In the preface to this excellent collection of problems, Prof. Pippard mentions the situation that faces university teachers of physics. It is necessary to move with the times, and to spare no efforts to make physics, and a career in physics, attractive to students. 'No amount of exciting new presentations of the basic ideas is going to make it easy for the student to acquire professional competence If he cannot make a good showing at these classical problems all his expertise in the realm of ideas is so much vanity; for they are examples of what will continually confront him in the practice of his art." What he is really saying was said long ago, that there is no royal road; and as improved teaching takes the drudgery out of learning, the discipline must still remain. The observations in the preface are wise, and relevant to physics teaching at all levels nowadays; though one feels that Prof. Pippard is inconsistent in saying that the conversion of electrical units is responsible for more error than anything else in physics, and also chiding as folly the views of those who settle for avoiding conversions.

The problems are varied and challenging. Some are straightforward calculations, a few are standard theorems, many involve the two- or three-stage application of elementary principles and would be within the scope of a scholarship candidate; others, not too difficult in principle, require a mature mathematical approach. They find the chinks in the armour, too ; of two equally innocent-looking examples, I did one in my head and spent a fruitless hour on the other. In general, the forty or so questions on thermodynamics seem to be more exacting than the rest—perhaps because they offer the inquisitor more scope, or it may be that one meets this type of question less regularly and is less able to judge.

A selection of classical problems naturally includes examples gross as Earth; and besides satellites and rockets there is one about the building of the Great Pyramid.

one on the interantipodeal tunnel, and one about the Eiffel Tower. It also covers the classical physics of the microscopic world—free electrons, α-particles, protons and electrons in atoms. The correspondence principle, the Schrodinger equation, and Planck's constant appear; these are properly classical too. (There ought to be alternative answers to the problem about engraving the Lord's Prayer on a pin-head, since it comes in two lengths, differing by about 8 per cent.)

Many of the problems originated as questions in Part 1 of the Natural Sciences Tripos. Behind them lies an inventive genius that can design examples to exercise specific techniques, and build in both standard difficulties and unusual subtleties; with an occasional sparkle of wit. I have not sampled all the problems myself; but I am sure that they will develop the competence of students at this level, while maintaining their interest and enthusiasm.

G. R. Noakes

TECHNOLOGY AND SCIENCE OF CERAMICS

Progress in Ceramic Science

Vol. 2. By Dr. J. E. Burke. Pp. vii+245. (London and New York: Pergamon Press, 1962.) 80*s*. net.

Science of Ceramics

Vol. 1: Proceedings of a Conference held at Oxford, 26–30 June 1961, under the auspices of the British Ceramic Society and the Nederlandse Keramische Vereñiging. Edited by G. H. Steward. Pp. 334. (New York and London: Academic Press for the British Ceramic Society, 1962.) 65*s*.

TEN years ago anyone wanting a book on either the technology or science of ceramics would have had a rather limited field from which to choose, but so rapid has been the development of interest in non-metallic inorganic materials in the past few years that the supply of substantial literature can barely meet the demand. Technological pressure in the field of materials research has opened up new horizons both for the applied scientist and for the fundamentalist in solid state physics, and the two books reviewed here illustrate both aspects of this.

The book *Progress in Ceramic Science* is the second of a series put out by the publishers to provide a continuing review under the general editorship of Dr. J. E. Burke of the General Electric Co. Research Laboratory, Schenectady, and contains just four major contributions from American authors. The first, on dislocation etch-pits in non-metallic crystals, provides a useful survey of theory and techniques which should offer some encouragement to workers who are apt to complain that their materials are so full of holes anyway that crystal defects can scarcely matter! The second article deals with the catalysed crystallization of glass, and comes from the laboratory (Corning) in which the 200-year-old observation of Réaumur has been turned into a brilliant practical reality. The theory of crystallization in glasses is outlined, and nucleation by the use of oxide and metal catalysts and by the action of ultra-violet light or X-rays on photosensitive glasses is discussed. Next comes a chapter on radiation damage, an important question in view of the increasing interest in the use of oxides and carbides, rather than elemental metals, in nuclear engineering. The last chapter is an examination of the problem of thermal conductivity in ceramic dielectrics and provides a wealth of new experimental information on a wide range of oxide materials and glasses.

The book *Science of Ceramics* is quite different in character and is in effect a supplementary volume to the *Transactions of the British Ceramic Society*. It is the outcome of a joint effort between the British and Netherlands Ceramic Societies to co-operate in providing a European forum for the exchange of papers on the 'basic' science of ceramics, and it is a record of the proceedings of a conference held in Oxford in the summer of 1961—the first, one understands, of a series of such conferences. Apart from the very useful and authoritative review of sintering by Prof. J. White with which the book opens, the other twenty-two papers are accounts of various researches in progress, ranging from studies on clays themselves to the properties and performance of end-products as diverse as building ceramics, high-permittivity dielectrics and fluoride-based ceramics.

Both books are very well produced and contain some fascinating optical and electron micrographs. With such a rapidly expanding subject, it would be pointless to comment on the probable permanent value of either volume, but there is no doubt of their timeliness and immediate value.

N. F. Astbury

ORGANIC SEMICONDUCTORS

Organic Semiconductors

Proceedings of an Inter-Industry Conference. Editors: James J. Brophy and John W. Buttrey. Pp. xi+243. (New York and London: The Macmillan Company, a Division of the Crowell-Collier Publishing Company, 1962.) 68*s*., 9.00 dollars.

THE recognition that conjugated organic substances can behave as intrinsic semiconductors by virtue of thermally excited π-electrons only dates from 1948. Over the ensuing years a few papers appeared every year, until around 1958 contributions started to appear in various scattered conferences. By this time the main types of compound, phthalocyanines, polyaromatics, charge-transfer complexes, etc., had been recognized, and in 1959 there appeared reports of semiconductivity in polyacrylonitrile. This activity had developed in such widely scattered countries as England, the U.S.S.R. and Japan, and around 1959 several large American industrial firms decided to proceed on from literature surveys to active experimental work. The result has been an ever-increasing volume of papers which have given considerable stimulus to the subject. The present volume is entirely concerned with these American contributions. The first three papers, by R. G. Kepler, O. H. LeBlanc, jun., and M. Silver, are concerned with the experimental and theoretical aspects of determination of charge carrier mobility in highly purified single crystals of anthracene. The pulse techniques advocated by these authors may be expected to be widely adopted by workers in the field and form a contribution of great importance.

Further papers deal with charge-transfer complexes in single crystal form, and there are a variety of papers dealing with d.c. measurements on compressed powders, of which the two papers by H. A. Pohl on semiconduction in polymers are worth special mention. There is a very substantial contribution by C. A. Klein on the electrical properties of pyrolytic graphites, and the volume concludes with a not too illuminating "management appraisal". Those academic scientists interested in the purely scientific aspects of organic semiconductors will be able to find a good deal of the present work available in fairly accessible journals. But industrial scientists requiring a fairly up-to-date collection of papers prepared with industrial research in mind will find this volume very useful. I myself have long held the opinion that industrial developments in this field have been held up by the need to secure collaboration between electrical and chemical industries. Conferences such as the present one should help to break down the dividing barriers, and deserve every encouragement.

D. D. Eley

FINGER-PRINTS, PALMS AND CHROMOSOMES*

By Prof. L. S. PENROSE, F.R.S.
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Historical Notes

THE analysis of finger-prints from the point of view of personal identification is well known. Early examples can be taken from Chinese documents. In modern times the method is used by police and immigration officials. The principle involved is that the ridges formed by the raised apertures of sweat glands have unique detailed formations which have arisen almost by pure chance. The large-scale pattern of ridges, however, is much less fortuitous in origin. There are anatomical regularities[1]. There are also normal variations, which represent hereditary differences between members of separate populations, members of the same population or members of the same family.

The contrast between general pattern and detail is made fairly clear by the study of identical twins. The general configurations of ridges agree, as hereditary traits should, on the hands of such pairs, and there are usually only slight differences in arrangement; but the details do not agree and are individually specific.

All configurations are laid down permanently at a very early period of development, during the third month of fœtal life. Best known are those on the finger-tips.

Description of Triradii, Main Lines and Creases

The systematic classification of ridge patterns, which can be used as a preliminary in personal identification, was first proposed by Galton[2]. It depended on the recognition of 'deltas', now known as 'triradii'. These are natural centres, and the radiant ridges form the main lines of the patterns or configurations.

A triradius can be defined as the meeting point of three spokes which demarcate three regions, each containing a system of almost parallel ridges. One triradius on a finger-tip always accompanies a loop pattern (Fig. 1) and two produce a whorl. In the absence of any triradius, the pattern is known as an open field, arc or arch.

On the palm there are normally four triradii, one at the base of each finger, called *a*, *b*, *c* and *d*, and another, known as *t*, near the base of the fourth metacarpal bone or at some point on its axis.

By starting at the triradii, the configurations of the main lines of the dermal ridges can be marked out (Fig. 2). The patterns produced on different hands fall into natural groups and the most prominent lines are named, for example, *A* and *D*.

At this point it may be well to emphasize that the dermal ridge main lines are of an entirely different character from the flexion creases which have such an attraction for fortune-tellers. The flexion lines on the palm, traditionally named after the head, the heart and life, and the creases on fingers certainly show interesting variations, but the ancient interpretations have to be revised.

Physiological Variations

These large-scale patterns of lines and creases are all strongly, but not exclusively, influenced by heredity. On the corresponding hands of identical twins, dissimilarities can be attributed to environmental modifications; they differ about as much from one another as do normally the left and right hands of a single person.

Just as the sexes differ in stature and other physical traits, so also are there characteristic peculiarities of dermal ridges to be found in males and females by careful analysis. All these variations can be considered to be physiological, like the fact that female stature is less than male stature; their presence sometimes obscures pathological changes in pattern.

Tests of Inheritance of Dermatoglyphic Traits

Analytical investigations of hereditary influence can best be made by quantitative methods which involve counting ridges between specified points or measuring angles. One well-established result is that obtained by measuring the size or 'intensity' of the finger-tip patterns. The measurement is made by counting the ridges crossed

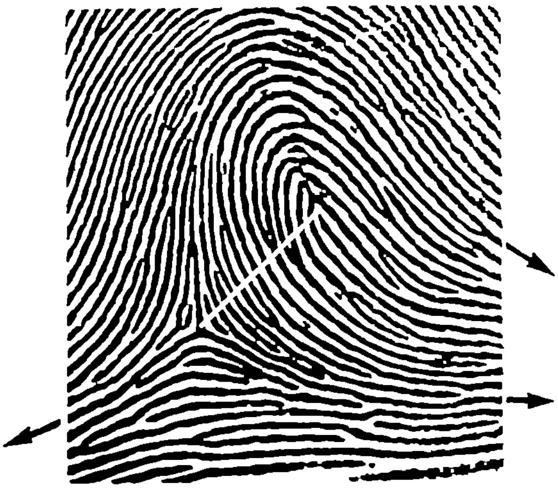

Fig. 1. Loop pattern on a finger: the arrows mark continuations of the three ridge lines which radiate from the delta or triradius. The white line joining the delta to the pattern core crosses 18 ridges

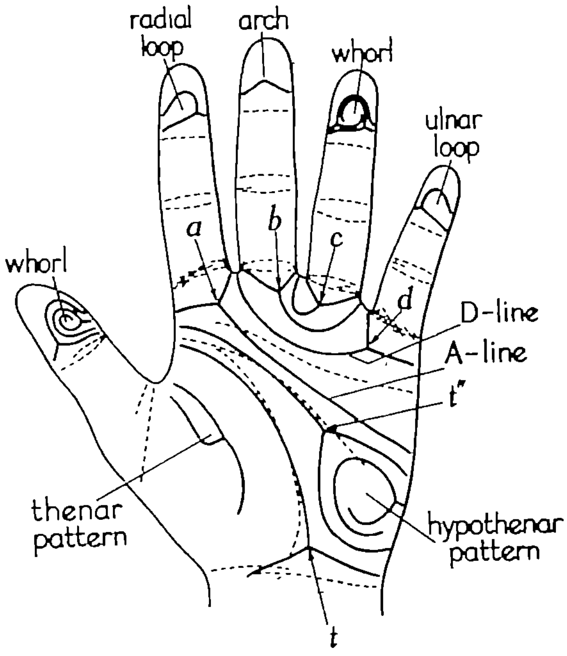

Fig. 2. Dermatoglyphics on palms and finger-tips showing nomenclature

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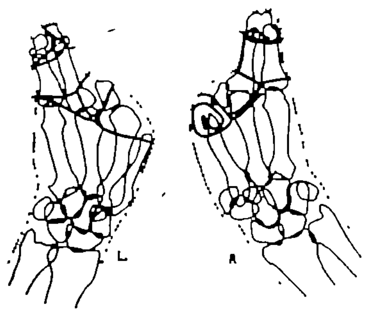
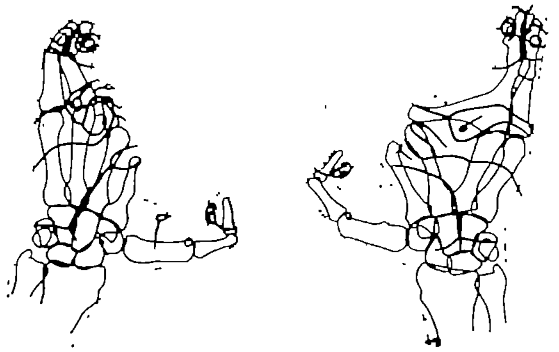
* Substance of a lecture delivered at University College, London, on January 15.



IV 31 (E P.). Left and right hands.



VI. 13 (J F A) Left hand. VI 13 (J F A) Right hand

Fig. 3 Dermatoglyphic distortion caused by a single gene for ectrodactyly

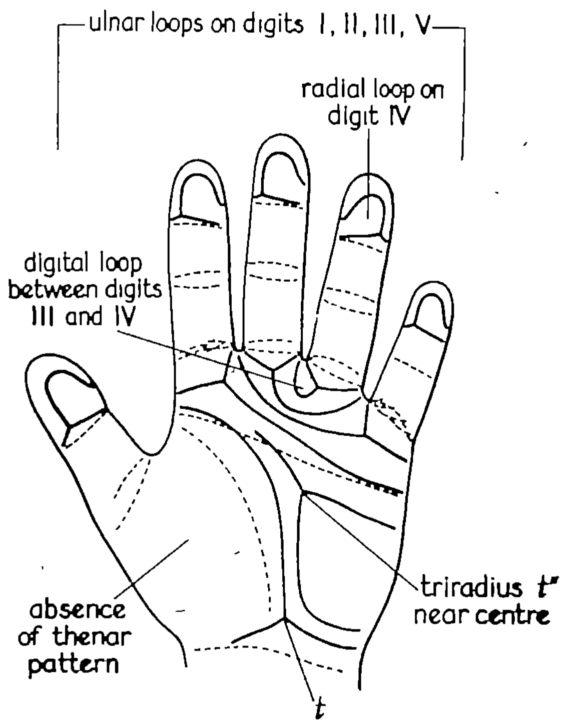

ulnar loops on digits I, II, III, V
radial loop on digit IV
digital loop between digits III and IV
absence of thenar pattern
triradius t″ near centre
t

Fig. 4. Stereotype pattern found in mongolism

by a straight line drawn from the triradius to the core of the pattern[3]. An arch scores zero and a loop 12, on the average: usually whorls score still higher, about 19 (only the larger of the two numbers obtained in a whorl is recorded). The total score for all ten fingers averages about 145 in males and 127 in females. It is a trait which, like stature, is determined by many genes with a combined additive action. This is demonstrated by analysis of families, in the traditional manner, using correlation coefficients to measure hereditary likeness. The characteristic value, in a random population, for parents and their children and for sib pairs is 0·5; and this is very closely approached by observations on the total finger-ridge count[4]. Equality of inheritance from father and mother to sons and daughters excludes sex-chromosomal influence.

Other traits, such as the position of *D*-line exit, the third interdigital pattern[5], the number of ridges between *a* and *b*, the presence or absence of thenar[6] patterns and the maximal *atd* angle[7], can all be shown to have strong genetical determination; but these features are more capricious than the total finger pattern quantity and more influenced by early fœtal environment. Some of them, like angular measurements, can alter with age.

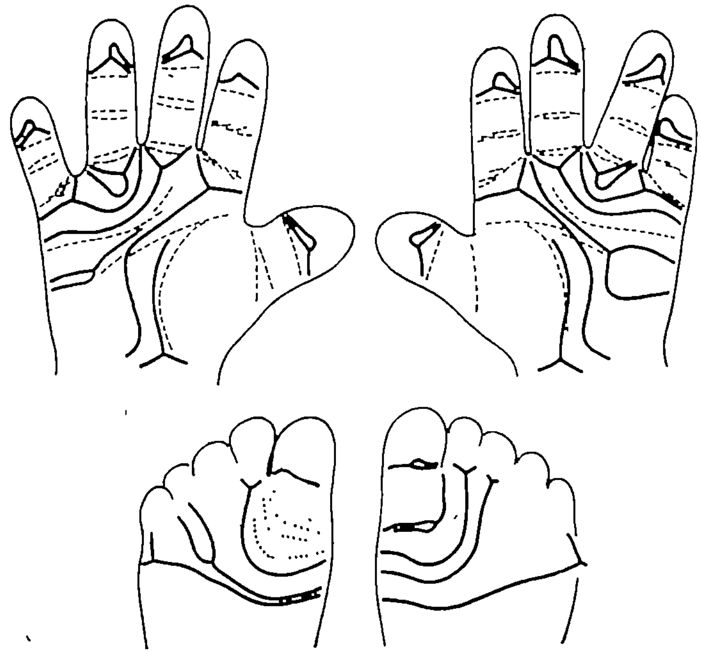


Fig. 5. Main lines on prints of palms and soles from a mosaic mongol female child whose cells are about 1/3 trisomic and 2/3 normal. Note the open field on left hallucal area (scale for hands and feet doubled)

Distortions caused by Growth Abnormalities

Any early disturbance of growth which affects the hands r the feet is liable to cause distortions of the natural lermatoglyphic patterns. The classical example is olydactyly, in which an extra digit is often associated vith an extra triradius. Conversely, ectrodactyly[8], or oss of digits, and also syndactyly, fusion of digits, leads o loss of triradii or alterations of arrangements which nay be very extensive (Fig. 3). These distortions can be orrectly said to be caused by abnormal genes, but they re secondary effects; the underlying developmental listurbances are the more direct results of abnormal gene ction. Some distortions are quite obscure in origin, uch as the very rare vertical alignment found in some alms and usually connected with digital deformity.

Distortions caused by Chromosomal Aberrations

Many curious and unexpected distortions of pattern on alms and soles occur in connexion with chromosomal berrations. The common types indeed present highly haracteristic peculiarities. I will describe them in turn.

(1) *Mongolism.* This condition, in which there is a nultiplicity of anomalies, was first recognized nearly a hundred years ago by Down. It has, of course, nothing to do with Mongolian peoples specifically. Recently its origin in chromosomal aberration has been proved. The hands were first examined dermatoglyphically by Cummins[9] in 1936. Before this the flexion creases had interested clinicians, and the transverse palmar crease, or simian line, played an important part in diagnosis. According to the students of cheiromancy, this crease is equivalent to the fusions of the lines of the head and the heart; it is a sign of concentrative mental power. Other alterations in flexion lines of mongols include a marked crease between first and second toes and occasional absence of the distal creases on the fifth fingers[10]: these have passed unnoticed by cheiromancers.

On the hands, one noticeable feature of the mongol stereotype is the strong tendency for every finger to possess a loop pattern rather than a whorl or an arch (Fig. 4). Moreover, the distribution of radial loops on digits IV and V in mongolism is unusual as compared with normal hands. The total finger-ridge count is less variable than among normal people[11]. Very characteristic is the central position, on the palm, of the triradius *t* which sub-tends an angle of about 80° with the points *a* and *d* (and in this position it is called *t*″) instead of the normal 45°. Hypothenar patterns are common and thenar patterns rare. A distal loop is nearly always present between *b* and *c* (the third interdigital region). The ridges themselves are often poorly formed.

On the hallucal area of the sole, patterns are infrequent and an open field is typical[12].

One of the recent important discoveries in human cytology is that more than one genetical cell type can take part in the formation of the same individual. A person in whom this occurs is called a mosaic. A person can be a partial mongol in that only a proportion of his cells are abnormal. These abnormal cells are usually distributed fairly evenly over the whole body and thoroughly intermingled. It has, for many years, been observed that some people exist who have a few characteristics of mongolism but that they are otherwise perfectly normal. There are also mentally sub-normal patients who are incomplete mongols. Some of these people are in fact mongol-normal mosaics in varying degrees. Their dermatoglyphs are interesting in that they show some of the features of the mongol stereotype (Fig. 5)[13]. The degree

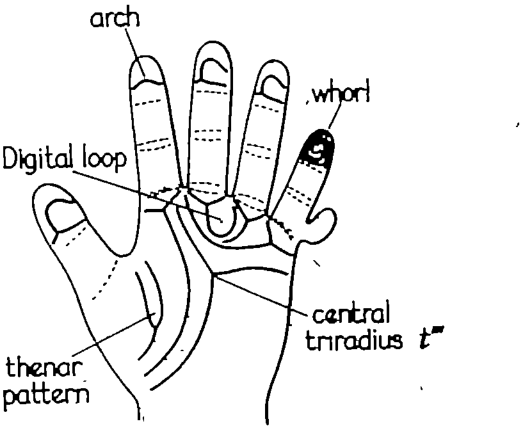


Fig. 6. Palmar pattern stereotype found in trisomy 18

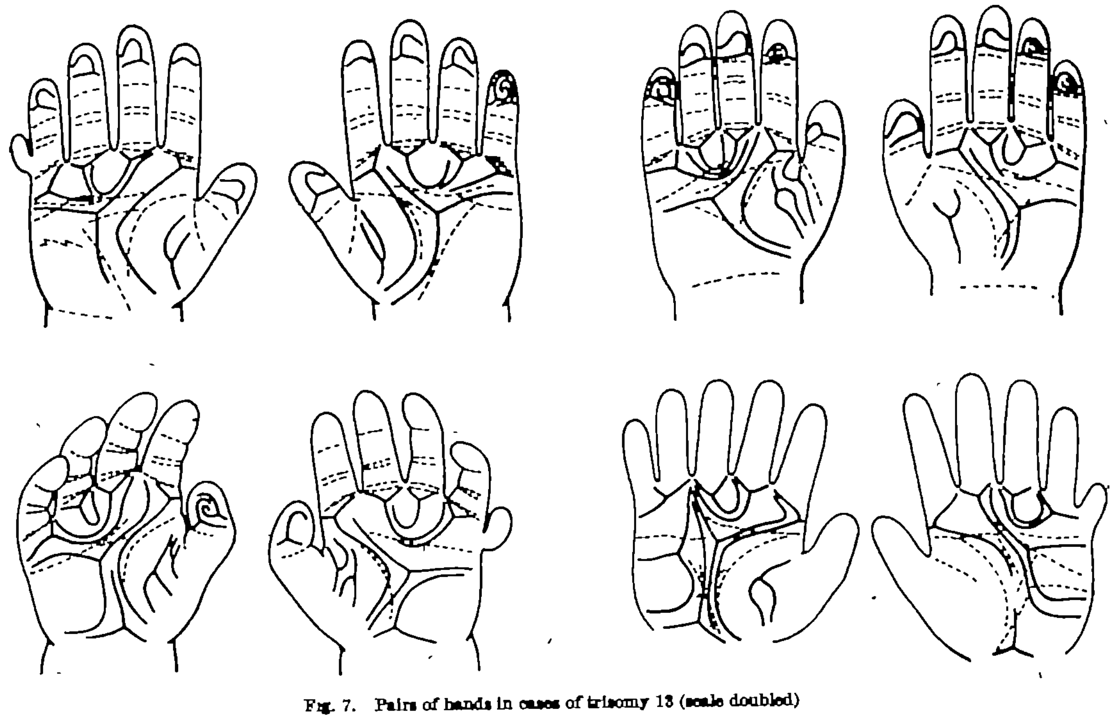


Fig. 7. Pairs of hands in cases of trisomy 13 (scale doubled)

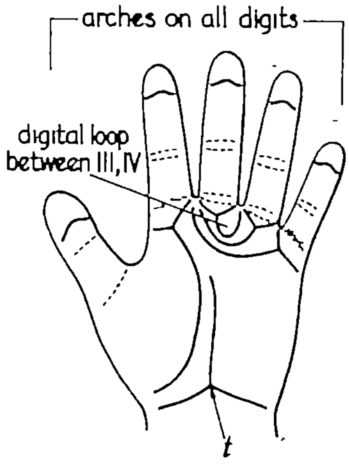


Fig. 8. Pattern stereotype found in trisomy 17 or 18

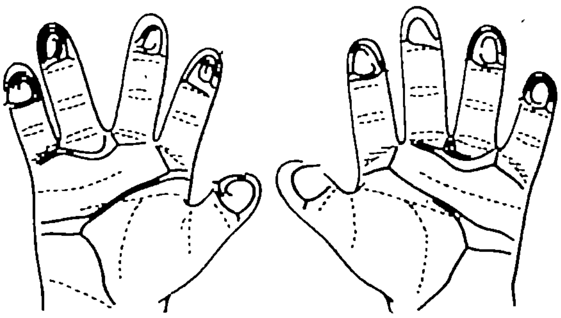


Fig. 9. Typical dermatoglyphics in Turner's syndrome

of distortion is not closely related to the proportion of abnormal cells, as demonstrated in cultures, nor to the level of mental development attained.

(2) *Trisomy* 13. An entirely different group of peculiarities characterizes other trisomic conditions. Those connected with trisomy of chromosome No. 13 (it may be No. 14 or No. 15—we are not quite sure) are striking. Deformities of the eyes, lips, palate and ears are part of the clinical picture. Polydactyly may be present and this itself is likely to upset the dermatoglyphic pattern. The stereotype includes a very distal axial triradius, the position of which, as measured by the angle *atd*, is distorted more than it is in mongolism and could sometimes be called t''' (Fig. 6). The extra patterns, however, tend to be in the thenar, not the hypothenar, region. Unlike the mongols, their fingers show both arches and whorls as well as loops (Fig. 7). On the soles of the feet there are few triradii and peculiar loops or open fields are the rule[14]. These observations are obtained with considerable difficulty since most of these cases fail to survive more than a few weeks and the hand-printing even of normal new-born infants is far from easy.

(3) *Trisomy 17 or 18*. The third kind of well-established autosomal aberration concerns trisomy of either chromosome No. 17 or No. 18, it is uncertain which. The head is long, ears are 'low set' and the chin is small. Again there is a stereotyped distortion of dermal ridge patterns along with a group of physical peculiarities (Fig. 8). The hands are specially difficult to examine because the fingers are held permanently flexed but with the distal joint extended. There is absence of the distal flexion creases on most fingers and the tips only rarely have any triradii on them and nearly all the patterns are arches[14]. In contrast to the mongols and trisomics No. 13. the palms show no obvious abnormality. The hallucal areas of the soles, however, have somewhat unusual configurations with fairly wide open fields and there are arches on the toes.

(4) *Turner and Klinefelter types*. When we come to sex chromosomes there is a wealth of material for study, and some surprising results have been derived. First of all, Turner's syndrome, in which there are only 45 chromosomes in each cell nucleus because there is one X and no Y present, shows various peculiarities. The patients are females with short stature and are liable to have webbing of the neck and slight limb deformities. There is no very stereotyped dermatoglyphic pattern but a general tendency to certain types of arrangement (Fig. 9). The pattern intensity on the finger-tips is high, large whorls are common and the total ridge number is, on the average, greater than normal. On the palms, the axial triradius is found nearer the centre than is usual in controls: on the average it is in the position t' (Fig. 10). Thenar patterns are not uncommon. The upper region of the palm is

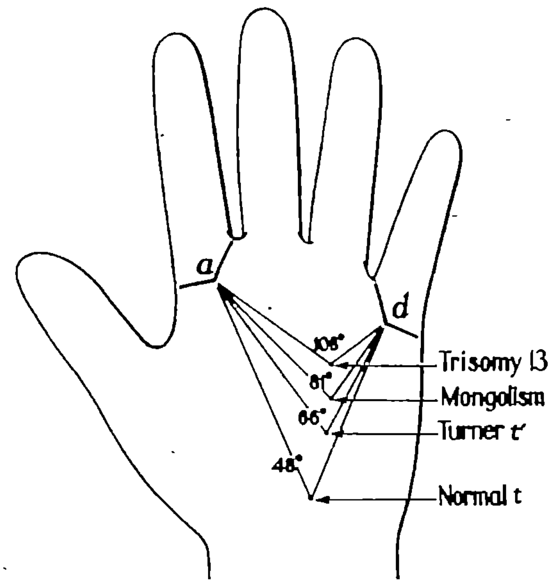


Fig. 10. Mean position of most distal triradius t in children up to 4 years of age

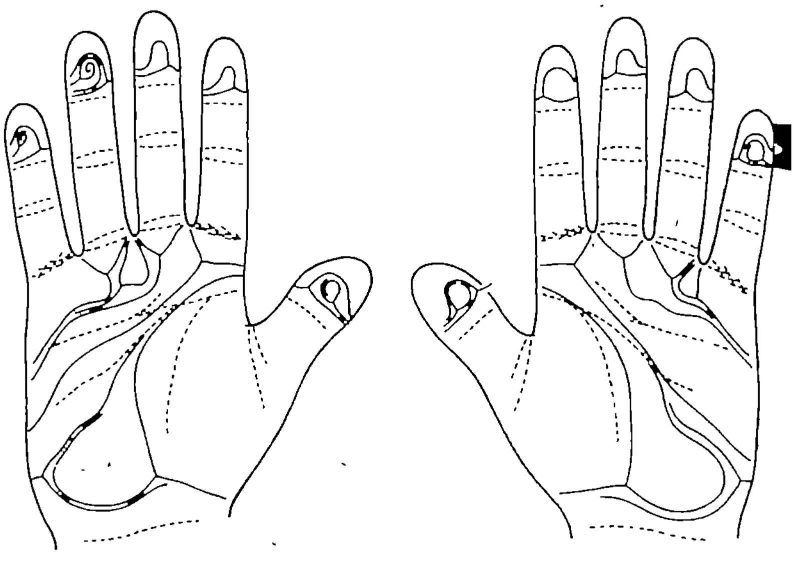


Fig. 11. Example of dermatoglyphics in a male with $XXXXY$ sex chromosomes

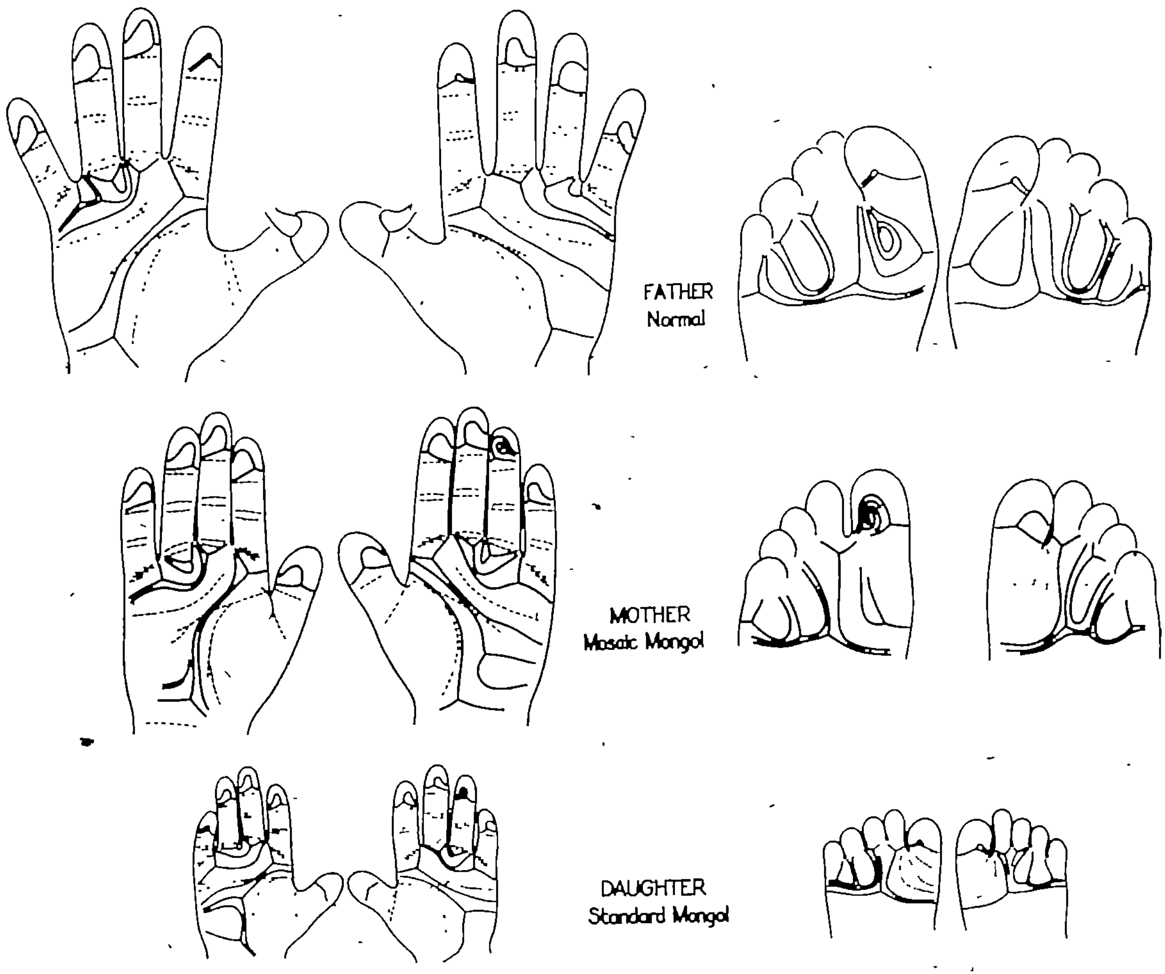


Fig. 12. Palms and soles in a family with mosaic mongol mother and standard mongol daughters

often peculiar in that triradius *b* tends to move towards the ulnar side of the hand. Correspondingly, the distance between *a* and *b* and the mean number of ridges between them is increased.

Mosaics composed of Turner and normal cells show the same features as in Turner's syndrome and, also, other conditions, where one of the *X* chromosomes in a female is grossly aberrant morphologically as, for example, when it forms a ring and has suffered deletion.

One might suppose that a person who has too many *X* chromosomes, instead of two few, would show the opposite characteristics of the Turner's syndrome. The triple-*X* females, however, show very little deviation from the normal dermatoglyphs. The tendency for high ridge scores to occur on finger-tips in a Turner is replaced by a normal, and perhaps a slightly lowered, count. Males, with additional *X* chromosomes giving rise to the syndrome of Klinefelter, are tall rather than dwarfed and they have, in association, rather long hands and feet. Finger-prints show a slight general tendency towards patterns with low counts and arches are quite frequently observed. The axial triradius, on the average, is low down in the palm, probably a little lower than in normal hands, especially when there are more than two *X* chromosomes[15], as occasionally happens (Fig. 11); there is, however, no obvious stereotype connected with the possession of too many *X* chromosomes. There is insufficient information to say what effect is produced by having too many *Y* chromosomes.

(5) *Miscellaneous anomalies.* Many miscellaneous examples are known where chromosomal aberrations have been found and where enthusiasts have printed the hands and feet in the hope of finding characteristic variations. Sometimes observers are lucky and sometimes not. The key factor seems to be to what extent the chromosomal change induces a disorder of limb growth for, as we have seen, any such change is likely to distort the normal patterns. Since many of the aberrations observed are unique, the evaluation of the findings is difficult. Usually the most useful information obtained is that the patient's dermal ridge patterns do, or do not, resemble a known stereotype associated with known trisomics. The observation may then be used to aid the cytologist in sorting out which chromosome is involved in a particular aberration. For example, if the hands of a patient trisomic for a small chromosome do not show any traits of mongolism, there is a strong likelihood that the chromosome concerned is not the usual one, for example, No. 22, not No. 21.

Genetical Studies on Distortions

Somewhat more definite results can be obtained when groups of close relatives are studied. The correspondence between a chromosomal aberration and a dermatoglyphic pattern can be followed through more than one generation. Here are some examples.

(1) *Mongolism with mosaicism.* It was possible, over a period of years, to examine the inheritance of the position of the axial triradius in relation to mongolism before anything could be known about its chromosomal origin. It became clear that mothers of mongols, especially if they had more than one affected child, were more

likely than control females to have centrally placed axial triradii[7]. No explanation then seemed satisfactory. However, if the mother should, as is now known to happen, suffer from an aberration involving chromosome No. 21, this could both tend to cause mongolism in her children and produce some features of the mongol stereotype on her own hands and feet (Fig. 12). The best example of this occurs when the mother is herself a mongol or a mosaic mongol[16].

(2) *Mongolism with translocation.* Another type of situation occurs when the mother transmits what is called a translocated chromosome, caused by breakage followed by the rejoining of the wrong ends. In one section of a certain family, every individual who carried an abnormal chromosome after it had arisen by translocation also had a central axial triradius like the two mongols who occurred among its members[17].

(3) *Other unbalanced-autosomal complements.* Equally striking are some cases of abnormal children with aberrations of unusual kinds affecting the larger chromosomes and whose hands showed remarkable dermatoglyphic patterns which included strong thenar configurations[18]. Unbalanced in chromosomal complement they showed, as in mongolism, evidence of growth disturbances and mental retardation. In two families the father, though physically and mentally normal, carried the same translocation as the abnormal children in a compensated form; in each case, his palms exhibited less marked but similar peculiarities to those of his offspring.

Conclusion: Information about Location of Genes from Dermatoglyphs

It would be natural to infer, from observations like those on the families just described, that the gene for this or that dermatoglyphic pattern was located on the aberrant chromosome concerned. One must, however, be wary of all such inferences unless supported by family investigation. Some people have rashly supposed that, because Turner patients are short and Klinefelters are tall, the genes for stature must occupy loci on the X chromosome. Previous observations on the inheritance of stature, however, have shown it to be autosomal, not sex-linked. Exactly the same reasoning precludes us from attributing the observed changes in finger-ridge and a–b ridge counts in Turner's syndrome to the X chromosome genes. Similarly, the stereotypic peculiarities in trisomics, like triple-21 (mongolism) and triple-13, must not be attributed perfunctorily to genes located on No. 21 or No. 13, respectively. The problem of gene location involves extensive long-term investigations. In the meantime let us take pleasure in the fact that even the obscure study of dermatoglyphic patterns is, in spite of its inherent difficulties, at last beginning to play a significant part in the ever-widening field of human genetics.

I thank a great number of colleagues who have helped me to obtain material for dermatoglyphic studies. In particular I thank Prof. P. E. Polani, Dr. J. Lindsten and Dr. M. Fraccaro for many hand-prints from cases of Turner's syndrome, Dr. J. H. Edwards, Dr. C. E. Blank, Dr. W. J. Mellman, Dr. O. J. Miller and Dr. A. Shapiro for obtaining prints from patients with other chromosomal aberrations, Dr. Sarah B. Holt for making available to me the results of her measurements, and Mr. A. J. Lee for his drawings.

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AUTOMATIC COMPARISON OF FINGER-RIDGE PATTERNS

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THE usefulness of finger-ridge patterns in personal identification was established by the investigations of Galton[1–3], after earlier work by Herschel[4] and Faulds[5]. Among many other results, Galton demonstrated that individual finger-ridge patterns are permanently and uniquely characterized by the occurrence and arrangement within them of certain common local details or 'minutiæ' (Fig. 1). This result led to world-wide adoption of the practice of comparing identified and unidentified finger-ridge patterns with regard to the types, orientation and locations of their minutiæ, as an ultimate test of personal identity. The difficulties of automating such comparisons are apparent in the demands they now make on human abilities, not only in detecting and typing the minutiæ in varied ridge-line backgrounds, but also in comparing their locations, for the latter are considered in relation to overall ridge-line topology in order that their comparison be valid as between a finger-ridge pattern recorded at one time and the same pattern, scaled and distorted by physiological and environmental changes, observed at a later time.

It is the purpose of this article to present, together with some evidence of its feasibility, a method by which decentralized automatic identity verification, such as might be desired for credit, banking or security purposes, can be accomplished through automatic comparison of the minutiæ in finger-ridge patterns. The novelties in the method are its use of: (1) photosensitive minutia detectors, which act automatically; (2) 'relative' co-ordinates of minutia location, which are unaffected by affine pattern transformations, supplanting much topological complexity; (3) a tolerance on the agreement required of minutia locations, which allows for detector inaccuracies and for slight departures of actual pattern transformations from strict affineness. For concreteness, the method will be presented in the form of an operational description of an identity verification system. Throughout, patterns will be assumed plane, as when pressed against a flat surface, and the only minutia types utilized will be the ridge end and valley end.

The prospective user of the identity verification system must initially undergo a non-automatic registration

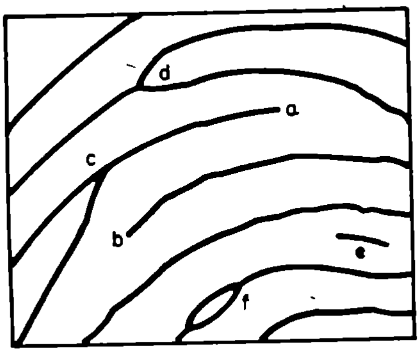

Fig. 1. Portion of fingerprint pattern (diagrammatic, enlarged) after Galton, showing minutiæ. *a* and *b* are ridge ends, *c* and *d* are ridge branchings or valley ends, *e* is an island, and *f* is an enclosure. The ridge end and valley end are the principal minutia types, accounting for almost all minutia occurrences

process wherein data on certain of his minutiæ are taken and stored against the times when claims shall be made on his identity. In this registration process, a set of three non-collinear minutiæ, W, X and Y, designated as 'reference minutiæ', and a number of others, P_i, designated as 'test minutiæ', are selected in one of the registrant's patterns. The registrant places his finger in position in a simple mechanical guide, where the co-ordinates and orientations of W, X, Y and the P_i are measured relative to rectangular co-ordinate axes the origin of which is denoted by O, fixed in the guide. The data stored by the system for each of the three reference minutiæ, W, X and Y, are: its type, its rectangular co-ordinates, its orientation, and its situation relative to one or two immediately neighbouring minutiæ. The data stored for each of the test minutiæ P_i are: its type, its orientation and, not its rectangular co-ordinates, but its 'relative' co-ordinates ξ_i, η_i in a system based on the reference minutiæ, which are computed from its rectangular co-ordinates and those of the reference minutiæ by means of the pair of simultaneous equations $\overrightarrow{OP_i} = \overrightarrow{OW} + \xi_i \overrightarrow{WX} + \eta_i \overrightarrow{WY}$.

When, at a later time and possibly a distant place, a claimant appears and requests that his identity be verified as that of a certain registrant, the system automatically retrieves its above-described stored data on that registrant, for comparison with data to be obtained by automatic observation of the claimant's minutiæ. The claimant places his finger in a guide identical to that used in registration, and having identically fixed rectangular co-ordinate axes the origin of which is denoted by O', whereupon automatic minutia detectors such as that of Fig. 2 scan his pattern, detect his minutiæ and observe their types, their rectangular co-ordinates and their orientations.

As a ridge end detector, each ray and the central disk can distinguish between fully light and not-fully light states. The rays and the disk are connected to circuitry which signals the occurrence of a target illumination state wherein, simultaneously, (*a*) the central disk is fully light, (*b*) there are exactly two unbroken sequences of fully light inner rays, and (*c*) there is exactly one unbroken sequence of fully light whole rays. This corresponds to the presence of the image of a ridge end approximately as shown in Fig. 2*B*. The point marked with an *X*, circumferentially midway in one sequence of fully light inner rays and radially midway in an outer ray, is taken to be the observed location of the detected ridge end. The greatly enlarged image of the whole finger ridge pattern is systematically scanned over the target to detect all its ridge ends. Operation as a valley end detector is entirely analogous.

Now, if the claimant is *bona fide*, the automatically observed rectangular co-ordinates of his reference minutiæ and orientations of his reference and test minutiæ will roughly approximate the corresponding retrieved registrant's data, owing to the guide-imposed similar placement of patterns in the rectangular co-ordinate systems of the registration and verification processes, to the moderateness of ordinary intervening pattern changes, and to the high expected detector accuracies. Though this approximation may be a crude one, inadequate to the full task of identity verification, it can be depended on to preserve the approximate orientations of all the minutiæ (a feature used further on) and, of interest here, enable the system to identify correctly the three reference minutiæ, which were at W, X and Y in the registrant's pattern, in their transformed observed locations W', X' and Y' in the claimant's pattern.

Having identified the reference minutiæ in their transformed observed locations W', X' and Y' in the claimant's pattern (correctly for a *bona fide* claimant or falsely by coincidence for an occasional impostor) the system proceeds to compute, for each of the registrant's test minutiæ P_i on which 'relative' co-ordinates ξ_i, η_i have been retrieved, the rectangular co-ordinates of its predicted transformed location Q_i' in the claimant's pattern, using the pair of simultaneous equations $\overrightarrow{O'Q_i'} = \overrightarrow{O'W'} + \xi_i \overrightarrow{W'X'} + \eta_i \overrightarrow{W'Y'}$, the retrieved ξ_i, η_i and the observed rectangular co-ordinates of the reference minutiæ.

It can be shown that if the claimant is *bona fide*, if the transformation his pattern has undergone since registration is perfectly affine (that is, is a translation plus a linear transformation, which latter includes differential scalings

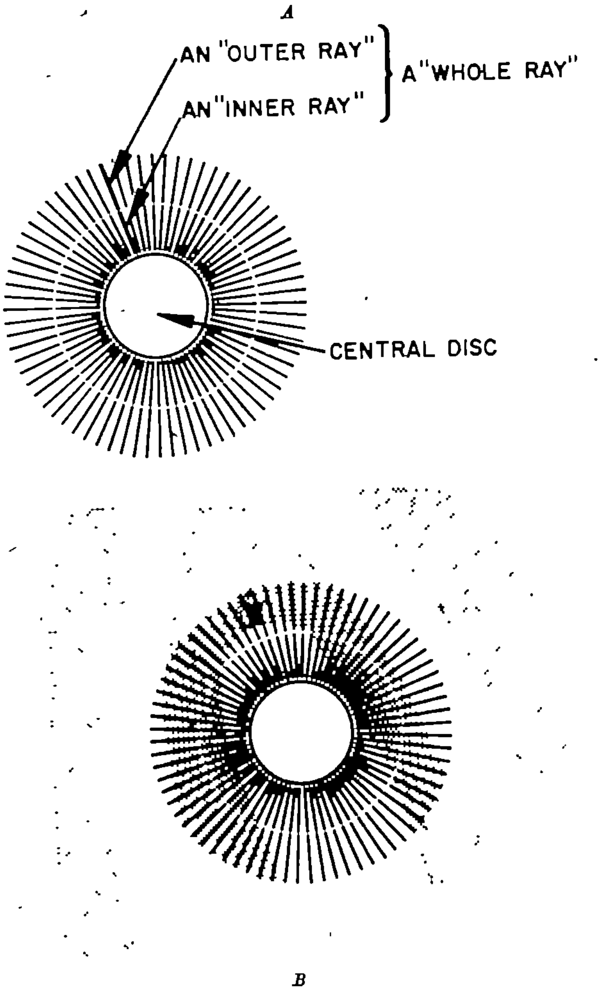

Fig. 2. Minutia detector. *A*, Minutia detector target, an array of photosensitive rays and a photosensitive disk; *B*, target with greatly enlarged image of pattern fragment superimposed

along different directions), and if the claimant's minutiæ are located by the detectors with perfect precision, the locations Q_i' in the claimant's pattern predicted as described will be perfectly correct, and the system will, in fact, observe the transformed test minutiæ to be at the computed rectangular co-ordinates, and observe their types and approximate orientations to agree with retrieved test minutia data.

The situation is only slightly altered in the more practical case of a near-affine pattern transformation and fairly precise minutiæ detection. It can be shown that if the claimant is *bona fide*, if his pattern transformation nowhere deviates by more than a from perfect affineness, if the detectors' errors in locating minutiæ are no greater than ϵ, and if all the registrant's test minutiæ have been chosen within the triangle WXY of reference minutiæ, the system will observe the transformed test minutiæ in the claimant's pattern at locations within a distance $2\epsilon + a$ of those predicted as here for them and, as before, the observed minutia types and approximate orientations will agree with retrieved test minutia data. This result provides the desired practical grounds for comparison of the registrant's and claimant's patterns. On these grounds, the system verifies a claim as genuine if, and only if, for each of the registrant's test minutiæ P_i on which data are stored, it detects a minutia in the claimant's pattern the observed location of which matches, to within $2\epsilon + a$, the transformed location Q_i' predicted for P_i, and whose observed type and approximate orientation match those retrieved for P_i.

Such a system will not reject *bona fide* claimants, as demonstrated. Only by rare chance will an impostor's pattern agree so well with that stored for the claimed identity that his claim will be falsely verified. To estimate this chance, the reasonable-appearing assumptions are made that: (a) approximately, the locations of minutiæ in a pattern are random, the two minutia types are equally likely, the two utilized approximate minutia orientations ('clockwise' and 'counterclockwise') are equally likely, and the location, type and approximate orientation of a minutia are independent of one another and of other minutiæ; (b) the 'wave-length' (width of one ridge plus adjacent valley) varies by at most 2:1 over any individual pattern; (c) the local ratio of ridge-to-valley width is never outside the range 1:3–3:1; (d) normal pattern variations are so nearly affine that a of the foregoing discussion is never greater than half the average pattern wave-length; (e) detectors such as those described are feasible, for which the errors ϵ of the foregoing discussion are no greater than half the local ridge or valley width. On these assumptions, it can be shown that, for registrant's patterns wherein N test minutiæ have been chosen for data storage and comparison purposes, and false claimants whose pattern areas display s minutiæ per square mean pattern wave-length and who are free to use the 'best' of their ten patterns, the probability of false identity verification is about $10r(9\pi s/16)^N$, where r is the probability that satisfactory reference minutiæ appear in the false claimant's pattern.

To obtain numerical values for s and N in the foregoing expression, observations were made on a series of twenty finger-ridge patterns, all adult male, mid-finger right hand, ulnar loop. First, considering these as false claimant's patterns, the area of minimal minutia density was deleted from each. The largest observed value for s in a remaining area was found to be 0·11 minutiæ per square average wave-length. Next, considering them as registrant's patterns, a quadrilateral was drawn in each pattern with vertices at prospective reference minutiæ (this presumes the separate use of two three-reference-minutiæ 'relative' co-ordinate systems). The least number of test minutiæ found in such a quadrilateral was 16; this, reduced to allow for the small sample of patterns and for possible malformed minutiæ, suggests that N may conservatively be taken as 12. The final constant, r, can at present only be guessed at; the value 1/100 is felt to be conservative. On substitution of the cited values for s, N and r, the expression given here yields, as a rough estimate of the false verification probability for a combination of 'weak' registrant and 'strong' impostor, 4×10^{-10}, or 1 chance in 2,500,000,000. In a simpler mode of operation in which it makes use only of valley ends, and hence has no need for auxiliary means of eliminating the false ridge end pairs occasionally created by breaks and creases in the pattern, the system's false verification probability, similarly estimated, becomes no greater than 10^{-7}, or 1 chance in 10,000,000.

There have also been examined the demands that a system of the kind herein described will make on its users and on its internal data storage, computation and communication facilities, with results which indicate its technical feasibility, speed (a few seconds per claim), and convenience of use.

[1] Galton, Sir F , *Nature*, 38, 201 (1888).
[2] Galton, Sir F., *Finger Prints* (Macmillan and Co., 1892).
[3] Galton, Sir F., *Nature*, 66, 606 (1902).
[4] Herschel, W. J., *Nature*, 23, 76 (1880).
[5] Faulds, Henry, *Nature*, 22, 605 (1880).

MODIFICATION OF THE DESIGN OF THE PROPOSED BENELUX RADIO TELESCOPE

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RECENTLY, the design of the Benelux radio telescope which was described by Christiansen and Högbom in *Nature*[1] has undergone drastic revision. The previous design involved several cylindrical parabolic reflectors with apertures of 5 km. It appeared to be entirely feasible technically, but several considerations have led us to adopt an even more ambitious proposal. The first of these was on the basis of operating frequency. The previous design was for a 400-Mc/s instrument. We have not yet obtained satisfactory protection of this frequency from terrestrial interference. Since the 1,400–1,425 Mc/s is now well protected, it appeared wise to utilize this higher frequency, if possible. The available funds appeared sufficient to consider seriously an instrument satisfying the original goal of 1-min resolution in spite of the closer tolerances and higher cost per unit receiving area necessitated by the higher frequency.

The growing recognition of the importance of polarization and spectral studies has equally influenced our plans. The stretched-wire reflectors of the previous design would have been intrinsically polarized, and any change of polarization would be impossible. A change of operating frequency for spectral studies would have required modification of approximately 20 km of line feed. This would have been practically impossible, and we have been led to adopt a design involving parabolic dish-type reflectors with point feeds. Although approximately

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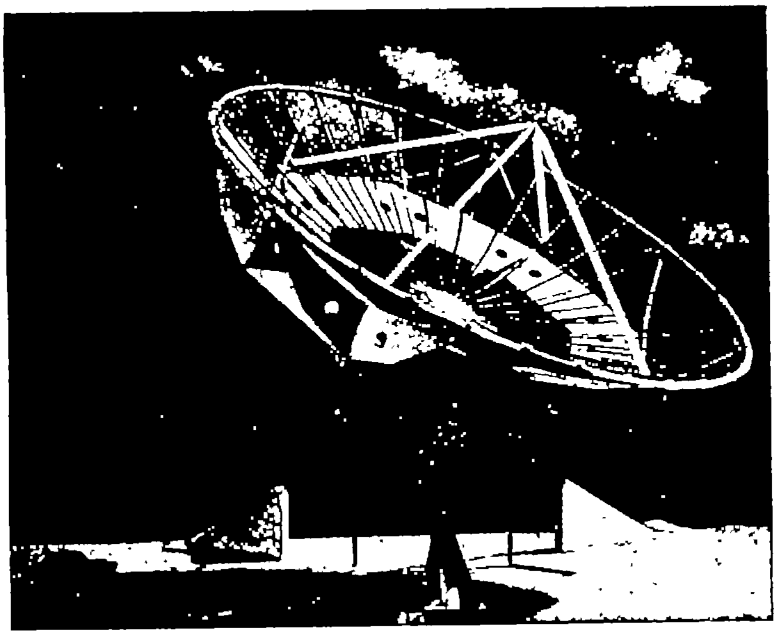

Fig. 1. A model of one of the 30-m reflectors

100 reflectors will be required, modification of their feed systems might be accomplished in a matter of weeks. Therefore, polarization and spectral studies become feasible.

In addition, the examination of the 21-cm line has been stressed in the Netherlands during the past decade. The 1,420 Mc/s operating frequency will allow these studies to continue with unequalled resolving power.

Previous studies were limited to Mills cross-type configurations utilizing receiving elements of various types. The foregoing considerations have now dictated the use of parabolic dish-type elements, but various configurations other than the Mills cross design have been considered. However, it appeared that a cross-type design would be the most suitable system also at the new design frequency of 1,420 Mc/s. A full report concerning the present plans is to be published[2].

The new proposal involves approximately 100 equatorially mounted reflectors, each of 30 m diameter. A model of one of the reflectors is shown in Fig. 1. Sky coverage will be from the north celestial pole to +5° declination. The total collecting area will be much less than that of the previous design, but the telescope will be able to follow an object for about 4 h in right ascension, and this longer integration time gives the instrument a sufficiently high sensitivity. The Benelux Cross will therefore not be basically a survey instrument; it will be used mainly for high-resolution studies of limited regions of the sky.

A rather curious arrangement of the reflectors has been developed. It is an extension of the method of suppressing the unwanted grating responses of the north–south arm proposed in the previous design[1]. The situation is more complicated in the new proposal, since grating responses must be suppressed in both directions, east–west as well as north–south, and it must

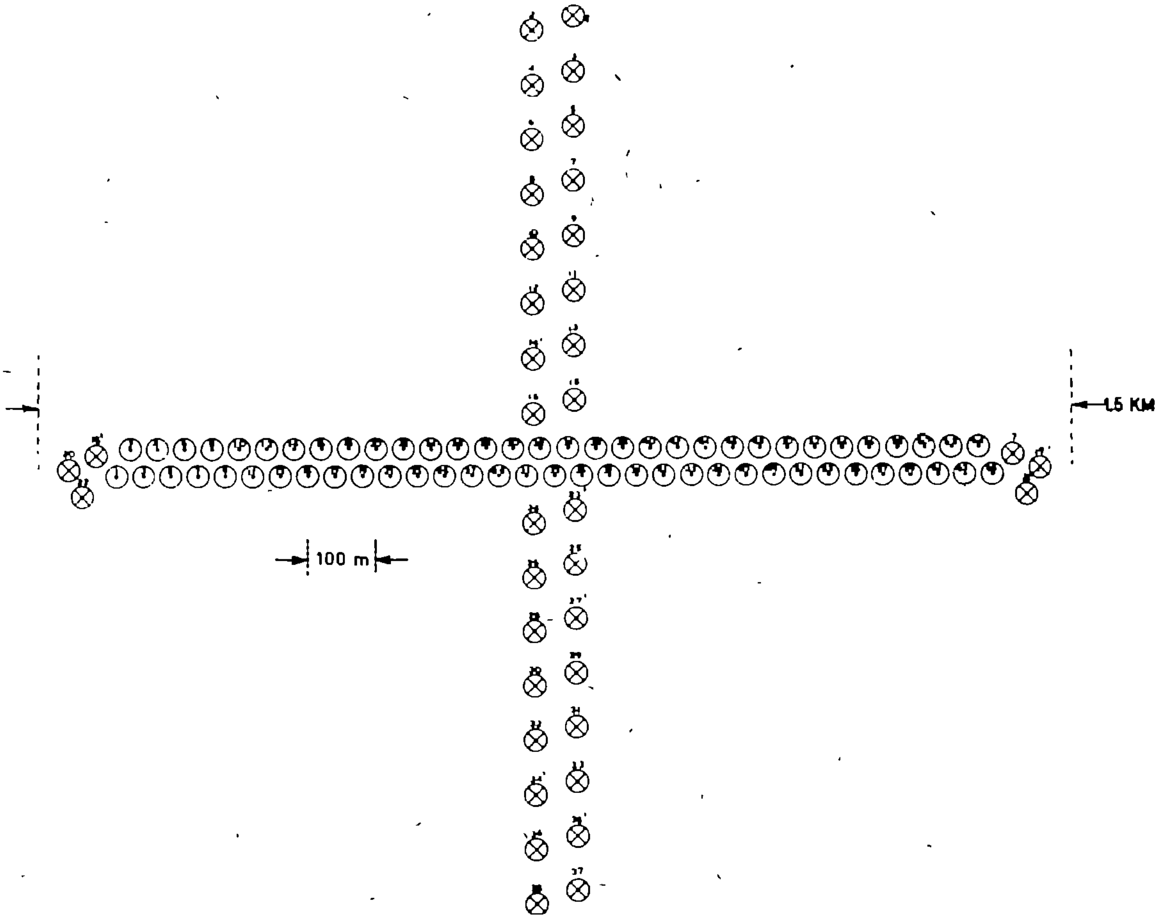

Fig. 2. The proposed arrangement of the reflectors

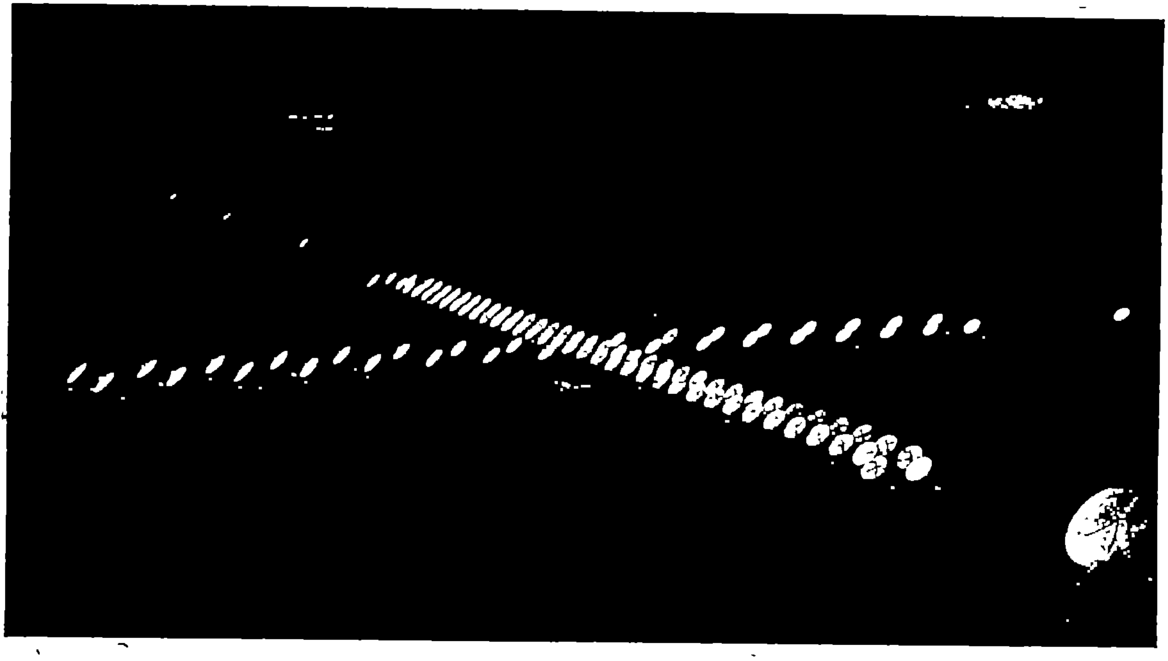

Fig. 3. An impression of the completed radio telescope

be possible to steer the reflectors without having them 'shadow' each other. The arrangement shown in Fig. 2 fulfils these requirements.

A new method will be used to overcome the geometrical difficulties in the central region of the cross where the two arms overlap. As shown in Fig. 2, three reflectors belonging to the north–south arm are placed at each end of the east–west arm. These gather most of the information which is lost by the omission of two north–south arm reflectors which would conflict with the east–west arm at the centre. Some information is still missing, but this will be gathered by a single, large reflector of about 70 m diameter. Therefore, the design involves a single large reflector as well as the cross of 30-m reflectors. No correlated components are needed between the large reflector and the cross. It could be located at a different site and run at different times. In fact, it can gather the information required for restoration of the components missing from the cross in a fraction of the cross's observing time. Therefore, this large reflector will be available most of the time for independent investigations as well as for operation as a 'finding instrument' for the delineation of interesting regions which will be subjected to detailed study by the cross. Fig. 3 gives an impression of the appearance of the antenna when completed.

The design of the electronic system is very similar to that of the previous proposal. Amplifiers will be distributed throughout the antenna, and multiple beam operation[3] is still planned. Somewhat greater use of the methods of aperture synthesis[4] is envisaged. That is, rather than physically connecting the outputs from all the reflectors in a number of differently phased combinations, the phase and amplitudes of the correlated signals between various pairs of reflectors will be measured. This information will then be processed by a digital computer to form a map of the area of sky under observation. Many directions in the sky will therefore be measured simultaneously, and this makes the effective sensitivity of the instrument comparable with that of the corresponding filled aperture antenna.

Funds for the design studies were provided by the Organization for European Co-operation and Development Committee on Scientific and Technical Personnel, the Netherlands Organization for Pure Scientific Research, and the Belgian Ministère de l'Instruction Publique.

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[2] Christiansen, W. N., Erickson, W. C., and Högbom, J. A., *Proc. Inst. Rad. Eng.* (Australia) (in the press).
[3] Blum, E. J., *Ann. d'Astrophys.*, 24, 359 (1961).
[4] Ryle, M., and Hewish, A., *Mon. Not. Roy. Astro. Soc.*, 120, 220 (1960).

HISTORY, SAND WAVES AND NEAR-BED CURRENTS OF LA CHAPELLE BANK

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It has rightly been urged that those who study the shifting sands beneath shallow near-shore waters should make direct observations of water movements virtually on the sea-bed. This can easily be done.

Now that the presence of sand ripples beneath very great depths of water has been revealed by submarine photography, it is of considerable interest to discover what speeds of water movement can occur very close down to the deep ocean floor at such places. One way of measuring such deep near-bed currents from an unanchored ship with little trouble and at small cost has recently been described[1]. Though the single observations provided by that simple method are easily repeatable, they cannot yet be carried out hourly beneath great depths of water.

Particular interest is attached, therefore, to places where much is known about convolutions of the sea-bed thanks to detailed operations with echo-sounding sets of high quality and modern echo-ranging technique, and where the depth is small enough for it to be possible to repeat the easy single bottom current measurements at hourly intervals. Such a possibility means, of course, that continuance of the observing 'round the clock' takes account of changing tidal streams.

For a variety of reasons La Chapelle Bank is eminently such a place. Centred at a point 100 miles from Ushant Light in the direction 240° true, the shoalest part of the bank is shown on the French chart No. 4735 as a small area of soundings ranging from 151 to 158 m. The bank lies very near to the edge of the continental shelf which has a general direction of south-east to north-west over a wide extent in the Bay of Biscay. The declivity towards south-west is such that the isobaths for 200 and 4,000 m are separated often by no more than 25 miles. A French fishery chart which embodies the results of surveys made from 1956 to 1960 and which portrays the bottom topography by isobaths drawn at 200-m intervals shows that, proceeding in a direction about 210° true from the region of the 150-m soundings, a depth of 3,600 m is reached in about 16½ miles. This is equivalent to an overall slope of 1 in 9 over that distance measured down what is in effect quite a marked canyon. A bathymetric chart of the shelf edge west of Ushant prepared by my colleague Miss L. Hadley shows that over a distance of 23 miles along a course trending south-west from the shoalest part of La Chapelle Bank, the depth increases to 2,000 fathoms—which implies an overall slope of about 1 in 12 over that greater distance in a more westerly direction. When Commandant L. Beaugé surveyed the bank in summer 1935 he moored a buoy at the shoalest point sounding 150 m and ran lines of soundings in all directions from it. He recorded that the bottom fell away to more than 1,000 m in less than two miles towards south; it did so in fact towards all directions between east-south-east and west-north-west. Thus, near to the bank, he found bottom declivities of the order of one in four.

There has recently become available an underwater relief model of the North Atlantic Basin on the scale of 1 : 5½ M with an exaggeration of 20 : 1. This, prepared by the U.S. Naval Interpretation Center in December 1960 and marked *NPIC* 910/60–*U*, shows La Chapelle Bank located very near the edge of a virtually vertical 'cardboard cliff' the foot of which is marked for the depth of 2,500 fathoms.

So far, because of its close bearing on what follows, we have been concerned to discuss the bed macrotopography of the region in some detail.

It is relevant now to mention that the region has quite a considerable history in terms of vigias. This Spanish word literally signifying 'watch' or 'look-out' was customarily placed on charts to convey a navigational warning in respect of possible pinnacles, rocks or shoals of uncertain position, and it is on record that Le Sieur La Chapelle Richard claims to have seen there, at 4 p.m. on the Shrove Tuesday of 1695, a rock standing 15 ft. proud of the water at pistol shot distance from his ship. It is recorded that he had a nearby sounding made without finding bottom at "130 brasses", that is, at some 130 fathoms. On a chart of 1766 the rock was marked because of an alleged re-sighting of it in 1764, and the repetition was made on a later chart. The detailed surveys made by Beaugé in our times have revealed no trace of any rock at the stated position. He found merely an '*éminence légère*' carrying 151 m of water with surrounding depths of 160–170 m. Because the position of this shoalest point was the same as that where the rock was allegedly seen in 1695, it was given the name of La Chapelle Bank. In 1822 discoloured water was reported at the position, and a depth of 4 ft. was reported in 1842 and one of 2 or 3 ft. in 1857.

I mention these vigias here as likely evidence of the occurrence at times of surface turbulence at the position of the bank notable enough to have made old-time mariners report the existence of shoals.

E. Le Danois has written much about the Chapelle Bank area in his two books *Les Profondeurs de la Mer* (Paris, 1948) and *L'Atlantique: Histoire et Vie d'un Océan* (Paris, 1938). He deals lightly with the vigia question already mentioned, and, after telling a good deal about the constitution of the bed, elects to explain the presence of the characteristic carpet of coarse sand by regarding it as "le cône de déjection de l'ancien estuaire de la Seine". He gives a map depicting his views of the drowned river drainage system and of the aforetime coastlines of the area now covered by the Celtic Sea. The ancient débouchure of the Seine is shown lying between the positions of the Castor and Parson banks. Because of the existence of other banks of insular nature to the west of La Chapelle, Le Danois sees in this assemblage of such shoal areas the former islands of the Cassiterides. He points out that a north/south line drawn on the chart between the richest tin-yielding areas of Wales and Spain passes through the region, and he discusses the subsidence to be held accountable for the drowning of the presumed former islands beneath their present water cover of the order of 150 m and less.

The matters so far discussed are all of relevance when an explanation for the convolutions of the sea-bed which will be described has to be advanced. Le Danois makes a statement which bears on one aspect of sediment movement when he writes: "Entre le banc du Castor et le banc Parson et sur le banc de La Chappelle s'effectue en effet du printemps jusqu'en août un véritable déversement d'eaux continentales provenant du Canal de Bristol et de la Manche. Au delà du plateau ces eaux froides et peu salées continuent leur course et s'enfoncent jusqu'au dela de 800 mètres". This process would nowadays go under the label 'cascading'.

Turning now to the sand formations on La Chapelle Bank and its immediate environs where the tidal range at springs is 3 m, the first reference must be to a short paper by Cartwright and Stride about five years ago[2].

An echo-sounding survey over the bank revealed to them the presence of a patch of sand waves about 12 miles long which ended abruptly at the edge of the continental shelf but which had a rather ill-defined eastern limit. The patch had a lateral extension of at least 5 miles, and the sand wave crests were on the average 25 ft. high with a maximum height of 40 ft. Though they found considerable variation in the distance between crests, the average separation was about 2,800 ft. The crest trend was from 290° to 110° true, which put their direction parallel to the general line of the shelf edge. On sampling the bottom both on a ridge and in a trough sand was found with some shell gravel, the median grain size being of 0·5 mm diameter. In a paper by Berthois to which reference will later be made, additional information is given regarding the nature of the bottom in the area.

Cartwright and Stride naturally inferred that the presence of the large sand waves required the existence of bottom currents on La Chapelle Bank sufficiently strong to transport along the floor the rough sand and gravel which they had found there. A slight measure of asymmetry of the ridge profiles was taken to indicate the existence of a net westwards transport of sediment, and the abrupt western limit of the sand ridges at the edge of the shelf showed that once the material reached there it was presumably carried over into deeper water—perhaps periodically. As bearing on this last expectation the cascading effect dwelt on by Le Danois is relevant, and so too are my own deep-current measurements made on the slope which were reported earlier[1]. It is of interest to mention at this stage that Stride has since discovered sand waves of comparable inter-crestal distance (though less high) on the shallower Parson Bank about 50 miles away to the north-east and so well behind the shelf edge. Others of similar wave-length have been mapped by him still farther towards the English Channel entrance.

These latter had profiles indicating oceanwards movement but the lines of their crests were not straight but convex towards the Channel. It seems that there may be something unusual about the height of the very large sand ridges on La Chapelle Bank, and, seeking to produce an adequate explanation for their formation, Cartwright[3] worked out a theory by which they could be considered generated as a result of stationary internal waves in the layer of sharp temperature gradient seasonally found between the warm surface water and colder water near the bottom. Such internal waves can be due to the tidal currents being disturbed as they travel landwards over the edge of the shelf. On Cartwright's theory, the sand waves built in summer would not be altered in height during the winter and spring when the thermocline is absent. A harmonic analysis of the sand wave profiles agreed fairly well with the proposed theory.

Independently, Dr. C. S. Cox of the Scripps Institution of Oceanography (California) had found some indications that internal waves of tidal periods are closely coupled with irregularities of the sea floor. The stationary internal waves adduced by Cartwright in explanation of the large sand waves on La Chapelle Bank would be equivalent to lee-waves found on the down-wind side of a mountain. Internal waves (not necessarily stationary) which develop in the thermocline as the tidal currents moving landwards feel the constraining influence of the shelf are the only possible explanation of the abnormal oscillation of a scattering layer later reported on by Stride and Tucker[4]. These observers were using this Institute's narrow-beam fish-finding transducer on La Chapelle Bank from R.R.S. *Discovery II* at the end of July 1960 while proceeding on a course approximately normal to the known crest direction of the large sand waves, when, at a distance some 8 miles within the oceanwards flank of the bank, they observed undulations in the scattering layer of length and height about the same as those of the stationary internal waves predicted by Cartwright from his theoretical studies.

This new information which seemed to support Cartwright's theory has since been followed by another line of supporting evidence. My colleague, Mr. R. Bowers, has since made runs over La Chapelle Bank while towing from R.R.S. *Discovery II* a thermistor chain having six sensing elements which reached 0, 15, 23, 31, 46 and 62 fathoms depth respectively at a steaming speed of 3 knots. Every 5 sec a temperature plot at each of these depths was made on a 'Mufax' recorder, and 48 h of continuous record were obtained over the bank. The records revealed undulations of the isothermal 'surfaces' having a wave-length such as to afford further support to Cartwright's theory, although it is still not known for certain how far the latter waves may be regarded as stationary.

An obvious requirement was to know everything possible about the tidal currents over the bank. Prior to 1961 all that was known was the information printed on the French chart No. 4735 to which reference has already been made. This information, which is in the form of the usual table of hourly speeds and directions for both springs and neaps, relates to the position 47° 40′ N.–7° 10′ W. The tidal currents at springs are shown as running fastest over La Chapelle Bank (at 1 knot) during 4 h straddling the time of high water at Brest. They are then directed towards between 50° and 130° true. During 6 h straddling low water Brest they are shown running towards between 228° and 340° true. The surface tidal stream data in question, which would not be claimed to be of the high quality produced by modern tidal stream surveys, came from observations made as long ago as 1891 when a French surveyor repeatedly measured the direction and speed of the drift away of his ship from an anchored buoy. Allowances for windage had necessarily to be made.

The cited table does, however, show that the surface tidal currents over the bank often head in a direction more or less normal to the shelf edge. The other long and curved sand waves mapped by Stride nearer to the English Channel lie beneath tidal currents which attain their fastest speeds at surface in the north-east/south-west directions.

In November 1961 Messrs. Cartwright and Woods (A. J.) made current observations over La Chapelle Bank working from R.R.S. *Discovery II* (unanchored) with a couple of Kelvin–Hughes direct-reading current meters. The work occupied almost three days, and the operation details will be reported elsewhere. Their observing depths were 40 ft. below surface and 30 ft. above bottom, but not enough observations were made at the deeper level.

From the results obtained, Cartwright produced a smoothed M 2 ellipse which shows the main directions of surface current flow to have been 30° and 210° true. These are more or less normal to the above-stated direction of the sand wave crests as would be expected if they reflect the water movements near bottom.

Picking out the fastest speeds actually measured at the nominal depth of 40 ft. one finds: 1·8 knots towards N. 17° E. true and 2·1 knots towards S. 17° W. true.

Thus, the latest information on near-surface water movements shows that the fastest rates were normal to the sand wave trend—with the greater speed in favour of direction towards the depths, that is, off the shelf. Cartwright's current measurements were made when the tides were about half-way between average and springs, that is, at a time when the coefficient was 80 to quote from the French tide tables. It is, of course, very useful to have the tidal current information gathered by Cartwright in order to know at what speeds the uppermost water can flow over the line of the shelf edge, but an urgent need had remained for actual measurements of the near-bed currents on the bank. These would be expected to be of major importance even if not powerful enough actually to erode the bed themselves. Their function would be to transport the sand from the places where the internal waves exert their major excavating power and deposit it along lines beneath the crests of the internal waves where these cause least movement of the bottom water.

Measurements of the currents close to bottom were made on La Chapelle Bank on November 7 and 8, 1962, from the research vessel *Sarsia* belonging to the Marine Biological Association of the United Kingdom. I had the assistance of my colleagues Messrs. L. A. Baxter and C. L. Gulliver in making seventeen single observations 1 h apart with my leaning-tube bottom current indicator ('Pisa'). These observations, which absorbed the entire ship time available, were all made as nearly as possible to the position 47° 40′ N.–7° 14′ W. under depths of water varying little from 155 m, and it can be taken that they relate on the average to a distance of about 10 in. above the sea-bed. The ship's officers were able always to move the vessel suitably between observations to keep to the shoalest part of the bank. In respect of bottoming gear the operations were as already described[1] but the indicating unit used in the 'Pisa' tube was not the directional inclinometer then used. Instead, the original 'Pyrex' bottle filled half with jelly and half with coloured castor oil was used. This, containing the usual compass disk suspended on a terylene thread from the bottom tip of an axial rod within the bottle, differed but slightly from the form first used[5]. Though this initial version of the 'Pisa' cannot be used down to depths much in excess of 1,400 m, its use has advantages at lesser depths which permit hourly repetitions. One merit is that the bottle indicator can give evidence of the presence of turbulence because, when the bottom current is not laminar, a clear-cut interface between the set jelly and the oil will not result even though the 'frozen-in' compass may give an acceptable overall direction as proved by experiments conducted in turbulent mill streams.

The results obtained were first plotted against clock hours in the form of two curves drawn for the north–south and east–west components respectively. These curves were then smoothed somewhat and supplied with new ordinates relating to Brest high water and hours from that time.

The components were then read off and re-compounded to relate to the tide times at Brest. Table 1 presents the results which start at about 1400 h G.M.T. on November 7 and end at about 0600 h the next day. Speeds are given in hundredths of knots and the directions are magnetic (declination 10° W.). The plus and minus figures denote hours after and before Brest high-water respectively.

Table 1

+2	+3	+4	+5
42 S. 21° W.	33 S. 16° W.	42 S. 13° W.	55 S. 20° W.
+6	−6	−5	−4
50 S. 50° W.	40 S. 81° W.	30 N. 78° W.	29 N. 18° W.
−3	−2	−1	H.W.
39 N. 18° E.	42 N. 37° E.	23 due E.	30 S. 23° E.
+1	+2	+3	+4
41 S. 10° W.	37 S. 45° W.	46 N. 63° W.	49 N. 61° W.
+5			
36 S. 16° W.			

No explanation is attempted why the directions for the second values at +3 and +4 have veered a dozen points.

The directional spread of the near-bottom current vectors is best seen from the accompanying plot wherein the single plus and minus signs have the same meaning as in Table 1. The four double plus signs mark the second observations made at the concerned tidal hours, and the circles measure tenth-knots successively from two to six proceeding outwards.

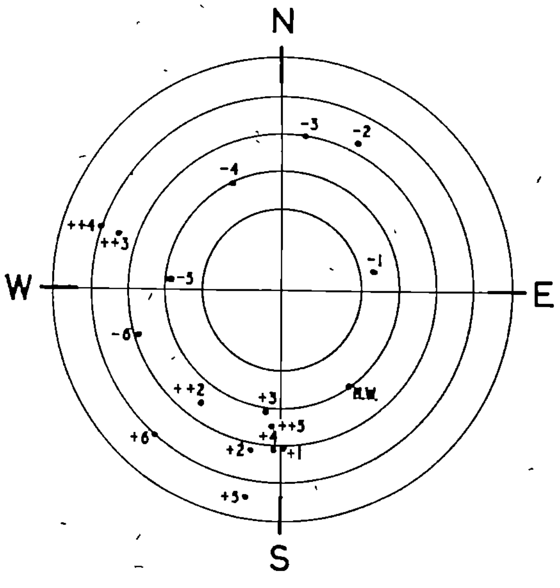

Fig. 1

We were working at a time when the tides were about half-way between neaps and average—at a time when the coefficient was about 65 to quote again from the French tide tables. Thus, the upper water observations made by Cartwright were carried out when the tide had a range about one-quarter greater than at the time of our measurements made just off bottom.

The briskest near-bed water movement measured was towards 190° true at 0·55 knot and the fastest about 6 h later was towards almost a reciprocal point at the rate of 0·42 knot. Thus, the fastest current was normal to the known direction of the crest line of the sand waves. Several other features of the results are noteworthy. The most remarkable thing about them is the absence of any current weaker than about 0·25 knot. The overall average speed is 0·40 knot and, although there is a large spread of direction with a good deal of near-bed water movement into the north-west quadrant, flow towards south and west much outweighs that towards the two opposite directions.

On all occasions save one the interface between the set jelly and the oil was clear-cut. The odd occasion was when the current was heading north-west, that is, in a direction parallel to the shelf edge. Perhaps the surge of water flowing at the time out of the deep funnel of the inner Bay of Biscay was pressed against the steep shelf by Coriolis effect sufficiently to thrust up the canyon of which we wrote earlier and cause an overspill of turbulence reaching somewhat landwards of the shelf edge. Cooper has called such a happening a submarine eagre and much of what he has written about "eagre spurts" of canyon water into shelf water is suggestive in this connexion[6].

The satisfaction of having been able to make actual measurements of near-bottom currents on La Chapelle Bank is all the greater when one considers the difficulties experienced by L. Berthois[7] in attempting to explain the large sand waves there. Agreeing with Cartwright and Stride[8] as to their size and crest orientation Berthois concluded that the big sand waves cannot have been produced by present-day currents but must be fossil features produced by much more powerful water movements existing in times long past. This conclusion he founded on some rather extraordinary decisions that the near-bottom tidal current probably does not reach one-fifth of the speed of that at the surface, and went on to argue that when it is in the "most favourable" direction. that is, normal to the ridge orientation, its speed will not exceed 0·16 knot. He came to this conclusion after applying some surprising ratios to the surface speeds as he knew them. This latter knowledge was restricted to the table on French chart No. 4735 already mentioned. yet Berthois declares that possession of the said table amounts to knowing the surface tidal streams over the region of the sand waves with precision. In spite of the fact that the speeds in knots which the table gives for the surface streams must be very imprecise, Berthois converts them into m/sec speeds expressed to three places of decimals.

All in all, the subject of the La Chapelle sand waves and their causation is of such interest as to prompt the desire for future expeditions there. One of these should be at the time of spring tides in September and the other similarly in February. That would provide for observations when the stationary internal waves invoked by Cartwright would be respectively at their greatest and least development for vertical density gradient reasons; but difficulty is naturally seen in arranging for the observing to be at exactly the same place both times. On both occasions the sand waves should be carefully measured and observations to reveal any undulations in scattering layers carried on. Besides measuring the near-bottom currents hourly, the towed thermistor chain should be used for reason already shown. What is also advocated is that, along courses normal to the ridges, sand traps should be used as frequently as possible just above bottom in order to learn something about the amount of sand actually in movement above different parts of the sand waves.

Doubtless, in these days, however, the sediment burden in the water just off bottom could be more speedily assessed by other means than the use of sand traps.

I thank Dr. F. S. Russell for the use of the research vessel *Sarsia*; Captain C. A. Hoodless and his ship's company for help; my colleagues Messrs. L. A. Baxter and C. L. Gulliver for sharing the work at sea; and Messrs.

D. Cartwright and A. H. Stride for helpful discussions during the writing of this article.

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DIFFERENTIAL RESPONSES OF *PARAMECIUM AURELIA* TO CIGARETTE COMPONENTS

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ALTHOUGH the use of *Paramecium* in cigarette research is not readily conceivable, the idea is nevertheless encouraged by two findings that the organism is highly sensitive to traces of metallic ions[1] and it reacts specifically to proteolytic enzymes of diverse origins[2]. A programme was thus started with its initial phase aimed primarily at determining whether *Paramecium* can also respond differentially to isolated cigarette components. This article presents observations concerning the protozoon's responses to cigarette toxins with special emphasis on the specificity of manifestation patterns this organism is capable of eliciting under the present experimental conditions.

A kappaless strain of *Paramecium aurelia* (stock No. 51, courtesy of Dr. T. M. Sonneborn) was used. Pure-line clone cultures of this organism were raised in sterilized Petri dishes, using 10 raw grains of rice for each culture. A culture was considered 'ripe' when the population in a single micropipette drop (1/40 ml.) consisted of several hundred organisms. Water used for all purposes was obtained from a Stokes distiller, model 171–*E*.

From six domestic and one imported brands of non-filter cigarettes, four components were separated for experimental analyses: tobacco, mainstream smoke, ash of whole cigarette, and ash of its paper alone. Cigarettes were smoked by means of a simple device—a suction-filter flask attached to a tap. Hydraulic pressure was adjusted so that a cigarette was consumed in 3½ min, during which time the mainstream smoke was directed to bubble through 20 ml. water in a test-tube placed inside the flask. It was estimated on the basis of cigarette smoke solubility in water and the present method of getting it dissolved that less than 10 per cent of the smoke so produced actually went into solution. The resulting ash was collected in 20 ml. water, filtered after 1 h with occasional shaking. A second cigarette was separated into tobacco and paper. The former was placed in 20 ml. water, filtered after 1 h. The cigarette paper was burned on a Bunsen flame for 1 min, the resulting ash (white) made into a 20-ml. solution in like manner. I designated these test solutions, each in 20 ml., as having an arbitrary concentration 1·0, which was found to be the most suitable as well as critical (see below) for our experiments.

For each experiment 1-ml. test solution was transferred to a depression slide (3 in. × 1½ in. with a central 1⅛-in. concavity) into which two drops of a culture were introduced with the micropipette. Continuous observation was made on these organisms from this point until their death. The concentration of the test solutions was regarded as critical because only at that concentration (1·0) could meaningful quantitative and qualitative data be obtained. The former represents an overall toxicity of the solution and is measured as 'survival time', or time required to kill a whole population. To estimate the percentage of the population killed at spaced intervals was almost impossible. The latter are manifest in motility and morphological changes undergone by the organism before death occurred. In general, as might be expected, higher concentrations shortened survival time. For example, when the concentration of a test solution was tripled, almost instant death to the organisms resulted, and the differentials in survival time of the four components normally obtained at concentration 1·0 (Fig. 1) became nullified. Conversely, at concentration 0·3 or lower, the components were no longer lethal.

Another important factor was temperature. Our experiments were carried out at a range of 23°–31° C within which tobacco and smoke were each completely lethal to *Paramecia* as contrasted to cigarette ash, whole or of the paper alone, which was incompletely lethal, that is, always leaving survivors behind. The latter may amount to 10–20 per cent of a population. Again, it is to be noted that this differential between tobacco and smoke, on one hand, and ash on the other, disappeared at higher temperatures, that is, 38° C and upwards. This phenomenon of survival is interpreted as probably denoting a low toxicity; but apart from this, it has raised a very interesting question. Is the resistibility exhibited by these individuals the result of somatic superiority of some kind over the others, which succumb, or is it the result of a germinal variation? Experiments are now being conducted to resolve this problem. The data showing the effect of temperature at concentration 1·0 are presented in a graph (Fig. 1). It was ascertained that *p*H was not a factor. The *p*H of the four components ranged from 6·0 (tobacco) to 8·1 (whole ash), which is well within the range of tolerance by *Paramecium*[1].

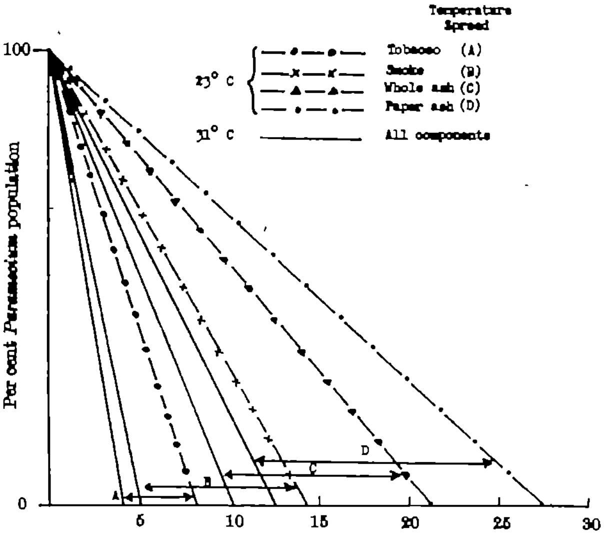


Fig. 1. Survival time of *Paramecium aurelia* in cigarette components at 23° and 31° C. Graph is based on data from all seven brands of cigarettes used: average of 20 experiments for each component

The remaining observations deal with changes in motility and morphology of *Paramecium*. All photomicrographical illustrations used in this article were from fresh preparations neither fixed nor stained (Figs. 2–9). It should be emphasized that these changes constitute patterns which are component-specific under our experimental conditions and that they are definitely not subject to alteration or modification by either temperature or concentration, or both. For these reasons, they are assumed to be qualitative rather than quantitative expressions of the toxicities under consideration. That is, most likely they are caused by the action of specific chemicals present in each of the components isolated[8]. If death represents terminal action of a toxin, the events described here are those which lead to the death of the organism as a result of impairment or disruption of some vital function. However, there is no apparent relation between the nature of responses and the overall toxicity of the solution on the basis of survival time. In other words, the events leading to death appear to bear no direct influence on the time required for evident lethality.

Tobacco. The action of this component was one of powerful tranquillization. The animals became motionless and died in the solution within a few minutes (Fig. 2). They appeared to have suffered from no gross deformity of any kind apart from a slight shrinkage, and the dead organisms remained intact, little disintegrated if at all. A unique phenomenon, however, occurred to most of them. The protrusion of a single ectoplasmic blister occurred at one pole of the animal. It is noteworthy that this happened in no other components studied.

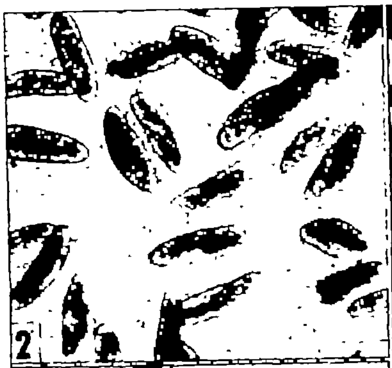


Fig. 2. Dead *Paramecia* showing typical manifestation pattern on 1 0 concentration tobacco solution at 23° C, 25 min after immersion. Note polar ectoplasmic blisters and a mild body shrinkage

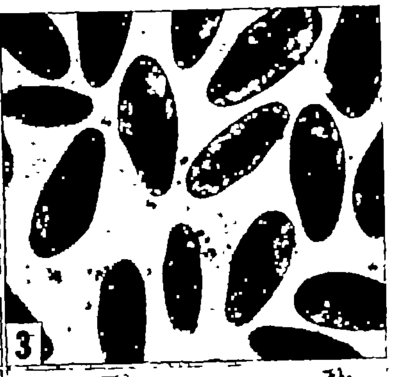


Fig. 3. *Paramecia* killed in 1·0 concentration smoke solution at 23° C, 25 min after immersion, revealing flattened swollen but intact bodies. Two contractile vacuoles were clearly shown in some of them

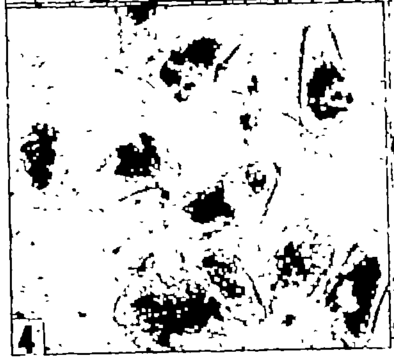


Fig. 4. Dead and partly burst *Paramecia* in 1·0 concentration cigarette ash (whole) solution at 23° C 10 min after immersion. Note clumping and centrally aggregated endoplasmic granules (dark), and slightly deformed bodies. Point of break-through marking the beginning of post-mortem disintegration was clearly indicated

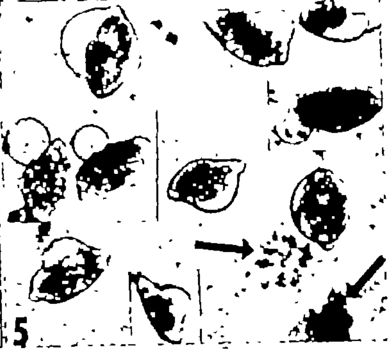


Fig. 5. *Paramecia* in 1·0 concentration cigarette paper solution at 23° C, 12 min after immersion. The photograph has incorporated 3 rectangular inserts taken from the same print in order to show greater varieties of blistering and body deformation. The organism in the insert at right has a polar endoplasmic blister, the rest all having ectoplasmic blisters. Arrow points to debris left over by disintegrated *Paramecia* which had succumbed earlier. These four illustrations portray the specificity of manifestation patterns evoked by the four cigarette components. They are taken at the same magnification (× c. 110)

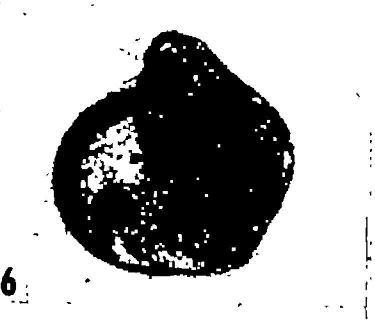
Mainstream smoke. Smoke solution also forcefully tranquillized *Paramecia* and quickly halted motility. No blistering was evident, but instead a considerable degree of body swelling coupled with some endoplasmic clumping took place (Fig. 3). The contrast in size of animals shown in Figs. 2 and 3 (same magnification) is evidence for the opposite effect of tobacco and smoke on plasma membrane permeability. Post-mortem disintegration, if any, was also mild. Thus, the death of *Paramecia* in both tobacco and smoke solutions was quiet and uneventful.

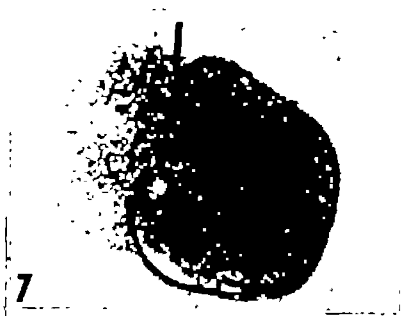
Ash of whole cigarette. Unlike tobacco and smoke, it stimulated the organisms to greater activity. The effect began almost from the moment of contact. Several motility patterns, all deviating far from the normal, followed in this order: first, an accelerated 'avoiding reaction'[4], changing to a spiral forward motion with either end of the animal whirling a circle, ending up with stationary circling of the body as if the latter were firmly anchored at its centre. After a while they settled down and died with a characteristic translocation and aggregation of endoplasmic granules (Fig. 4). The latter phenomenon so noticeable from Fig. 4 is believed responsible for the death of the animal. Moderate disintegration followed, which, however, was not preceded by any deformation or blistering such as invariably occurred following the action of cigarette paper ash.

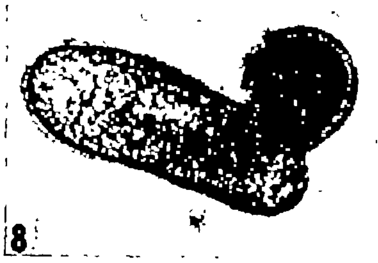
Ash of cigarette paper. The effect it has on the motility of *Paramecia* is closely comparable with that exerted by the ash of whole cigarette. But the morphological manifestations evoked by it are most fascinating to watch under the microscope. Attendant to active motility, blistering and bizarre types of deformation set in (Figs. 5, 8 and 9). The most severe cases, for example, sausage- or banana-shaped bodies with additional irregular protrusions involving both ecto- and endo-plasm, together with one or more blisters, were frequently observed (Figs. 8 and 9). Sooner or later, they slowed down and succumbed with an exploding type of disintegration (Figs. 6 and 7). Characteristically, the body burst at one point, resulting in collapse of the blisters and reduction of the cell to a mass of debris (arrows, Fig. 5). In fact, most of the scattered dots and particles seen in this photomicrograph represent this debris from completely disintegrated (cytolysed) *Paramecia*. The physiology of body deformation and blistering will be treated elsewhere; it probably involves changes of sol–gel relationships of the cell surface (cortex of *Paramecia*) brought about by specific chemical action.

The reason for the difference in effect between whole ash and that of cigarette paper alone may be sought in terms of combustion temperature[5,6]. In our experiments the cigarette paper was burned at a temperature much higher than that of smoking the cigarette on the artificial device, or naturally. In line with this, we found that when a paper was burned for a few seconds to a black (instead of white) ash, its solution was scarcely toxic to *Paramecia*. It is further assumed that certain combustion products, which are responsible for the toxicity phenomenon characteristic of the paper ash, may be masked or inhibited by certain organic compounds. The amount of the latter, of course, would decrease with increasing combustion temperatures. Another observation in this connexion seems significant: the paper solution, even after filtering, was slightly cloudy owing to the suspension of a fine particulate matter. On standing, the latter slowly settled out as a precipitate, which became partly afloat and partly smudging the wall of the container. Coincident with this precipitation, the solution lost toxicity appreciably. These observations strongly suggest that the active causative agents here involved may be polycyclic aromatic hydrocarbons[3,6].

In conclusion, this work has shown mainstream smoke to be the most toxic of the cigarette components. This is true of all the brands tested (so are all other findings of this work, with a variability among the brands that is well expected). The fact that this finding is in agreement with the literature constitutes an endorsement of *Paramecium aurelia* as a useful assay organism. The ultimate realization of the significance of this fact comes from the consideration that mainstream smoke is what should directly concern the smoker. Within the past decade or so, evidence for deleterious effects of cigarette smoke on the epithelial cells of the respiratory tract has been fast accumulating from both *in vivo* and *in vitro* experiments[7-13]. *Paramecium* is a ciliated unicellular animal, and as such it is likely to bear certain physiological resemblance to human respiratory epithelium[14,15]. On this ground alone the use of this organism in future research may be viewed as a desirable new approach to cigarette toxicity problems.







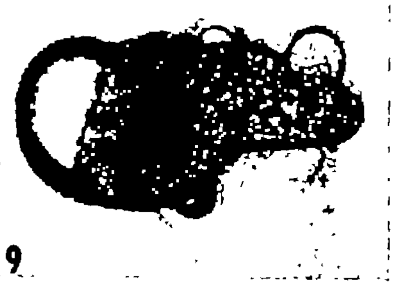


Fig. 6. A deformed *Paramecium* bearing an endoplasmic blister almost the size of body in 1·0 concentration cigarette paper solution at 25° C 15 min after immersion

Fig. 7. The same organism in Fig. 6, 3 min. later showing the blister collapsing and releasing its contents following a break-through some distance above (arrow)

Fig. 8. Another deformed *Paramecium* with a typical sausage-shaped body with a round protrusion near one end; the latter is not a blister since it was filled with endoplasm from the start. Later, however, blisters may be formed anywhere along the body surface. Note deranged cilia. This organism was in 1·0 concentration cigarette paper solution at 25° C, 11 min after immersion

Fig. 9. A 'monster'-like *Paramecium* afflicted by 1·0 concentration cigarette paper solution at 31° C, showing a combination of body deformation and blistering. At this stage all its blisters were of ectoplasmic kind, although the one at top right-hand corner of this figure was just about to be converted into an endoplasmic blister. (× c. 330)

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CORAL GROWTH AND GEOCHRONOMETRY

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ABSOLUTE age determinations of points on the geological time-scale, based on radioactivity, are generally accepted as the best approximations now known, even though they rest on a series of assumptions, any one of which may be upset at any time. At present there is no means of confirming or denying the accuracy of these determinations by independent methods. Can palæontology provide anything by way of verifying the pronouncements now emanating, at very considerable expense, from the black boxes? Can palæontology give any support to the shaky chronometric creation of the geophysicists and astronomers?

Except for providing a relative geochronology, palæontology has thus far offered no data on absolute geochronometry. Several geochronologists have noted that there is no fossil evidence concerning variations in the length of the day[1], the rotation of the Earth, or diurnal variations, in the geological past—variations that are linked to the passage of time, whereas the changes of the seasons have left indelible records in such things as tree rings, fish scales, stalactites, anhydrite bands, and other kinds of varves, all of which have little or no bearing on the problem of age or position in geological time. Organic evolutionary rates provide no evidence for they are wholly dependent for their values in isotopic age estimates.

It seems not unlikely, however, that if we lower our sights on suitable units of time for expression of age from the generally used year to the humbler day, we might find data in palæontological material for estimates of absolute age. How nice it would be if instead of paying a large sum for an isotopic analysis we could examine a fossil and estimate directly, with luck, not only its relative, but also its absolute age—every palæontologist a geochronometrist; every fossil a geochronometer!

The two chief approaches to geochronometry are based on radioactive isotopes and on astronomical data. The

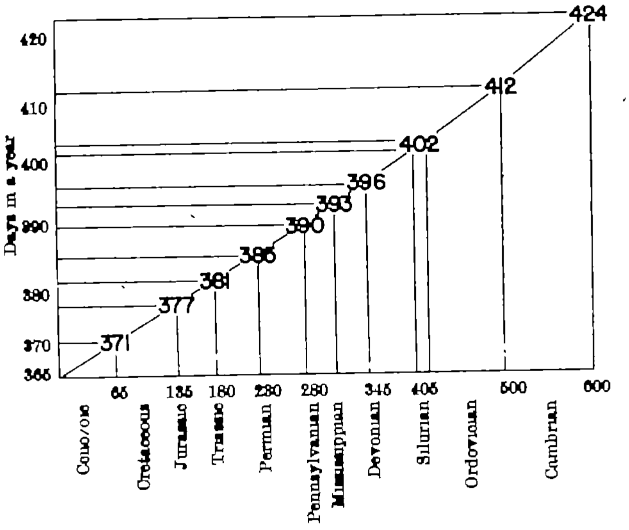

Fig. 1. Relation between days in each year and geological time (radioactive age data from Kulp, 1961)

most recent estimates[1] of geological time based on the rates of radioactive decay give the Cenozoic a length of 65 million years, the Mesozoic 165 million years, and Palæozoic 370 million years, and so on. The beginning of the Cambrian is placed about 600 million years ago. To accept these figures is an act of faith that few would have the temerity to refuse to make. The other approach, radically different, involves the astronomical record. Astronomers seem to be generally agreed that while the period of the Earth's revolution around the Sun has been constant, its period of rotation on its polar axis, at present 24 h, has not been constant throughout Earth's history, and that there has been a deceleration attributable to the dissipation of rotational energy by tidal forces on the surface and in the interior, a slow-down of about 2 sec per 100,000 years according to the most recent estimates. It thus appears that the length of the day has been increasing throughout geological time and that the number of days in the year has been decreasing. At the beginning of the Cambrian the length of the day would have been 21 h (ref. 1). After the first, a second act of faith is easy, and we can develop a simple relation between the geological time-scale and the number of days per year (Fig. 1).

Accepting this relation, in the absence of evidence to the contrary, it now follows that if we can find some means of determining the number of days per year for the different geological periods, we have a ligation between the results of geophysical and astronomical deductions. If we could determine, for example, that the number of days in the Cambrian year was of the order of 420, it would seem to confirm an indicated age of 600 million years based on tidal friction and in turn that since isotopic methods give the same result they too are in harmony. Is it possible to obtain estimates of the number of days per year from data preserved in recent and fossil organisms ? I would like to think so.

Among the several groups of organisms which have an additive mode of skeletal formation, the corals—tabulates, rugosans and scleractinians—are fairly well known, and there is a good deal of empirical information on rates of growth of recent forms, and a very little on the factors governing growth.

The earliest notice of annual fluctuations in coral growth is fairly recent. In 1898, R. P. Whitfield[3] described a large colony of the reef coral *Acropora palmata* from the Bahamas that showed regular undulations on the upper surface of the flat, spreading branches and suggested that these represented annual growth increments which he ascribed to seasonal temperature changes. The late T. Wayland Vaughan's experiments[4] a few years later with the same species in the Bahamas gave virtually the same result: 40–100 mm per year compared with 50–100 mm one gets from measurements of the undulations shown in Whitfield's specimen.

It has since been speculated that the growth annulations so common on many Palæozoic corals (Fig. 2) were the result of yearly fluctuations in growth produced by seasonal temperature change, variations in supplies of nutrients, or periodic reproductive activity. Beginning in 1934, T. Y. H. Ma[5] undertook investigations of variation of annual growth increments in recent and fossil corals as a means of determining the temperatures and latitudes of

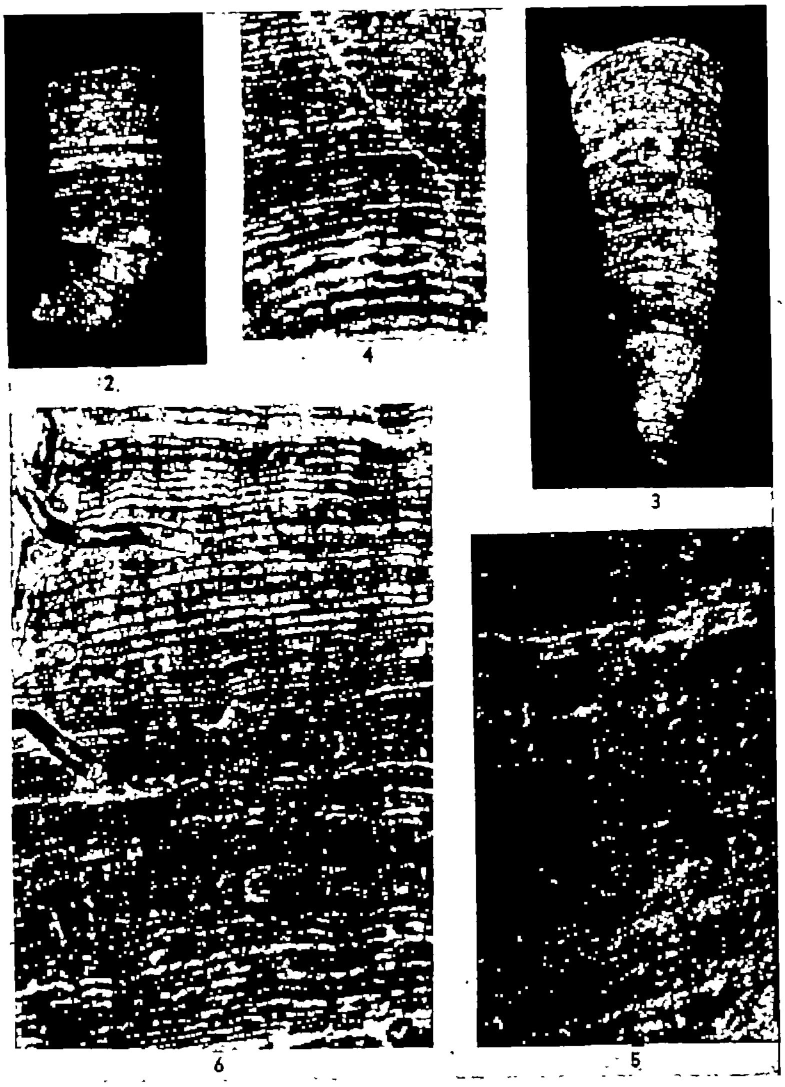

Fig. 2. *Heliophyllum halli*, Middle Devonian, western New York, showing 18 annual growth increments (× c. 0 5) Figs. 3 and 4 *Holophragma calceoloides*, Lower Silurian, Gothland · (3) corallum about 240 days old, showing growth-lines on epitheca (× c 2 7), (4) growth-lines on epitheca (× c. 13). Fig. 5. *Manicina areolata*, Recent, Dry Tortugas, Florida, growth-lines on epitheca (× c. 40). Fig. 6. *Eridophyllum archiaci*, Middle Devonian, western New York growth-lines on epitheca (× c 21)

the ancient seas, and assembled an appalling mass of very raw data on which he has leaped, with the agility of Raphael Pumpelly's mouflon, from assumption to assumption to heights of speculation where few would care to follow. But Ma's basic idea that annual growth-rates can be determined for many fossil corals appears to be sound.

Others have concluded that annulations or related internal variations in some Palæozoic corals have an annual basis, but no experiments have been undertaken on living corals to determine directly whether or not there is a causal relation between such annulations and annual variations in temperature or other factors. I do not know why such an obvious and simple test has not yet been attempted. There are some experimental data on allometric and volumetric growth-rates but these have little bearing on the problem of annulations, nor do these data tell, or are likely to tell, much more than: (1) that reef corals grow more rapidly in warmer waters; (2) that some corals grow faster than others. There is some evidence that fluctuations in nutrient supplies and reproductive activity have little or no effect on the overall growth-rate of corals.

We must now make an act of faith and assume that the major annulations on corals represent annual growth increments or varves. This, however, is of no direct value in geochronometry, even though it can thereby be shown that some large fossil coral colonies endured for a couple of hundred years, and that some living colonies of reef corals have been growing for the past thousand years or so. There is nothing in these values to orient them in time. A closer look at certain coral structures is necessary.

A number of people have noticed the fine ridges (Figs. 3 and 4) on the surface of the coral epitheca, a fundamental skeletal structure in tabulates, rugosans, and scleractinians, the basic material not only of the exterior of the corallum but in various guises as septa and dissepiments. Whenever epitheca is present and unmodified these ridges are present, although in many fossil specimens they are obliterated by post-mortem wear. The ridges are parallel to the growing edge of the corallum and are tacitly accepted as growth increments variously termed striæ, ridges and growth-lines. I cannot find, however, that anyone has concerned himself with their significance in growth-time. They clearly indicate regular variations in the rate of calcium carbonate secretion, but what is the relation of these to the passage of time? Hourly, daily, circadian, weekly, lunar monthly, periodic reproductive activity, yearly?

I submit that they indicate daily or circadian variation in skeletal deposition, and there is some slender evidence in this direction. Physiological studies on calcium carbonate secretion in recent corals, first made some years ago by Kawaguti and Sakumoto[6], and recently and more elegantly by Goreau[7], who used the isotope calcium-45, show that in reef corals the rate of calcium carbonate uptake in coral tissues falls at night or in darkness and rises during the day, but the reflexion of this in the skeleton has not yet been investigated. A much less-sophisticated approach would be to compare the number of ridges with the annual growth-rate; but this has not been directly tried on living corals in the field. I have tested it indirectly on one or two recent corals the annual linear growth-rate of which is fairly well known, and to my gratification found that the number of ridges on the epitheca of the living West Indian scleractinian *Manicina areolata* (Fig. 5) hovers around 360 in the space of a year's growth. This strongly suggests, subject to experimental confirmation, that the growth-lines are diurnal or circadian in nature. It may be noted in passing that they may provide a much more sensitive caliper for measurement of annual growth-rates than the larger yearly annulations.

The next step, of course, is an attempt to determine the number of growth-lines per annum in fossil corals. Here, as is usually the case, hypothesis is easier than practice. Few fossil corals are sufficiently well preserved to show clearly the supposed diurnal growth-lines, and it is not easy to determine the annual rate. In epithecate recent corals the growth-lines are commonly abraded or corroded even before death of the polyp. The best of the limited fossil material I have examined so far is from the Middle Devonian of New York and Ontario, especially specimens of *Heliophyllum*, *Eridophyllum* (Fig. 6) and *Favosites*. Diurnal and annual growth-rates vary in the same individual, adding to the complexity, but in every instance there are more than 365 growth-lines per annum, usually about 400, ranging between extremes of 385 and 410. It is probably too much, considering the crudity of these data, to expect a narrower range of values for the number of days in a year in the Middle Devonian; many more measurements will be necessary to refine them.

A few more data may be mentioned: *Lophophyllidium* from the Pennsylvanian (Conemaugh) of western Pennsylvania gave 390 lines per annum, and *Caninia* from the Pennsylvanian of Texas, 385. These results imply that the number of days a year has decreased with the passage of time since the Devonian, as postulated by astronomers, and hence that values of the isotopic dates of the geophysicists agree well with the astronomical estimates of the age of the Earth. It is not claimed that coral growth proves that either is right; but it is suggested that palæontology may well be able to supply a third stabilizing, and much cheaper, clue to the problem of geochronometry, and that further search for diurnal or circadian records in groups other than corals may result in strengthening this weak anthozoan prop.

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OBITUARIES

Prof. H. Graham Cannon, F.R.S.

HERBERT GRAHAM CANNON, Beyer professor of zoology in the University of Manchester, died in St. Bartholomew's Hospital on January 6 at the age of sixty-five. He had been in poor health for some time.

Born in London, Graham Cannon was educated at Wilson's Grammar School, Camberwell, and Christ's College, Cambridge. Having suffered compound fractures of his wrists during a fall in his youth, he was declared unfit for military service, and on graduation in 1918 became biologist to the then Board of Fisheries. A brief period of research at Cambridge soon followed, and in 1920 he was appointed demonstrator, and later lecturer, in zoology in the Imperial College of Science and Technology. In 1926 he became professor of zoology in the University of Sheffield, and in 1931 Beyer professor of zoology in the University of Manchester. He was elected a Fellow of the Royal Society in 1935.

Graham Cannon's early zoological work, influenced by Doncaster, concerned the spermatogenesis of lice and the nature of the centrosomal force, but he soon turned to an active interest in the Crustacea, which was to last for the greater part of his life. At first his work consisted of embryological and anatomical studies on Cladocera and Conchostraca, but in 1926 he published a paper on the feeding of an ostracod, *Pionocypris*, which was the first of a long series of detailed investigations into the complex filtratory feeding mechanisms found in a wide range of Crustacea. He recognized clearly the essential functional components of these mechanisms, describing current producing, filtering, scraping, and transporting limbs, and illustrated them with minute accuracy. The small size of many of these animals, and the difficulty of direct observation of feeding currents, compelled him to rely in large measure on deduction based on the details of structure. His strong interest in functional morphology generally clearly arises from these researches. Papers on feeding mechanisms were followed by a return to internal anatomy, and outstanding papers were those on the ostracods *Doloria* and *Gigantocypris* and the cirripede *Lithotrya*. In spite of the injuries to his wrists, Cannon showed great skill in fine dissection, and became a zoological artist of distinction. His early papers show the steady development of his technique, and by 1930 his illustrations could truly be described as unequalled. Furthermore, he had the ability to communicate his skill to others, and zoologists generally profited greatly by his publication of a book on methods of drawing and illustration.

In 1948 Graham Cannon was president of Section D (Zoology) of the British Association for the Advancement of Science, and his presidential address on undergraduate zoology marked the beginning of a new interest. From this time on he frequently wrote and spoke on educational matters, and rebelled against increasing specialization in teaching in schools and universities. He turned this interest to practical account by serving on the Northern Universities Joint Matriculation Board, and played an active part in the development of the University of Keele.

Towards the end of his life Cannon became increasingly occupied with thinking and writing on the relationship between Lamarckian and Mendelian inheritance, an interest which clearly had its origin in his work on crustacean feeding mechanisms. He came to hold the view that the fundamental property of protoplasm is its ability to adjust itself to changes in the environment always in the manner optimum to its survival, and that inheritance by the mechanism of the Mendelian gene complex was to be regarded as a later addition to this fundamental property.

In addition to his research and teaching, Graham Cannon played a full part in University administration. He undertook in Manchester periods of office as dean of the Faculty of Science and as pro-vice-chancellor, and was for many years chairman of the Manchester Museum. He served on the Council of the Marine Biological Association, and was well known as an examiner in the University of London. His range of interests was wide, and embraced such diverse interests as music, angling in his early years, cookery, and practical building. But his overriding passion was his love of Japanese objects of art, particularly swords and their furnishings. He was always ready to talk on this topic, publicly or privately, with enthusiasm and understanding.

As a man, Graham Cannon was direct in speech and expressed his views forcibly. He did not easily accept compromise. But those who knew him well saw a genial and kindly scholar who took a real interest in his students and staff. They will remember him with gratitude and affection. Their sympathy and that of his friends will go out to his wife and sons and daughters. R. DENNELL

Mr. A. D. Cotton, O.B.E.

ARTHUR DISBROWE COTTON, who for nearly sixty years was closely connected with the affairs of the Royal Botanic Gardens, Kew, died, after a long spell of ailing health, at Hertford, on December 27, 1962, at the age of eighty-three. He was born in London on January 15, 1879, and received his early education at King's College School, London. During his schoolboy days he developed a strong interest in the British flora, and he built up a considerable collection of pressed plants. His first botanical tuition was received from lectures arranged at Chelsea Physic Garden by J. B. Farmer, and these may well have fostered his liking for gardens and garden plants, an attachment which remained and indeed increased throughout his career. At one time Cotton seems to have determined on a career in horticulture, and he undertook three years' practical training which led to a first-class certificate in the Royal Horticultural Society's general examination.

Later, he decided to follow botany as a vocation, and to this end he spent three years at the Royal College of Science, London, where, under the influence of J. B. Farmer, he specialized in the study of the lower plants. In 1902 he obtained his first post at Owens College, Manchester, where he became an assistant lecturer and, under the influence of O. V. Darbyshire, became interested in fungi and did some research on orchid mycorrhiza. Two years later he entered Government service as an assistant in the Herbarium at Kew under George Massee, the distinguished mycologist. His general duties and research were mainly concerned with the algae, but he also worked with the fungi and lichens, and during this period he published several papers on these groups including his very important contribution to algal ecology in the Clare Island Survey. In 1915, Cotton was placed in charge of advisory services and research on plant diseases in a new laboratory installed at Kew, and indeed he was the first plant pathologist to be appointed in Government service in Great Britain. The laboratory which he initiated was later transferred to Harpenden and has become the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food. Cotton's continuing interest in mycology is reflected in his sixty years' membership of the British Mycological Society of which he was president in 1913.

Cotton returned to Kew in 1922 when he succeeded Otto Stapf as the keeper of the Herbarium and Library, a post he held until his retirement in 1946. This was a period of active development in the Herbarium with an ever-increasing flow of material to be identified, incorporated and distributed, and about a million specimens were added to the collections during Cotton's keepership. While he retained his interest in the algae and fungi, he turned more to the study of flowering plants. He became an expert on the cultivated lilies, published a number of papers on this attractive group, and largely wrote the botanical text of the *Supplement to Elwes' Monograph of the Genus Lilium* of which latterly he was joint author. For a short time he was editor of *Curtis's Botanical Magazine*, and for this and other services to horticulture he was awarded the Victoria Medal of Honour by the Royal Horticultural Society. Cotton attended the meeting of the British Association in South Africa in 1929 and during his return journey visited Kilimanjaro. The extraordinarily bizarre vegetation of the alpine slopes fired a new interest and he undertook a critical study of the arborescent species of *Senecio* of the East African mountains. One species which he collected on Kilimanjaro bears his name.

He became a Fellow of the Linnean Society in 1902, served for several periods on the Council and was president during 1943–46. In 1934 he was awarded an O.B.E. Cotton was a man of gentle, unassuming manner who will be remembered gratefully by all who had the pleasure of his friendship as a genial, kind and obliging man.

GEORGE TAYLOR

Prof. Karel Hrubý

PROF. KAREL HRUBÝ, professor of genetics in the Charles University in Prague, died in a motor-car accident in Jihlava on December 10, 1962. He was one of the few outstanding Mendelian geneticists in eastern Europe.

Karel Hrubý was born in Prague on September 30, 1910. He received all his education in that city, studying genetics at the Charles University under Prof. B. Němec and Prof. A. Brožek. In order to broaden his genetical outlook, he worked for eight months as a Rockefeller Fellow at the John Innes Horticultural Institution in London in 1938; this greatly influenced all his later work and strengthened his regard for classical genetics when the Lysenko wind became most severe. After the Second World War he again visited Britain, this time on invitation by the British Council, to study the progress of genetics in the Western World during the War. He participated in the genetics congresses in Stockholm (1948) and Montreal (1958), and was at his death member of the Genetical Section of the International Union of Biological Sciences.

Prof. Hrubý lectured in genetics at the Charles University, at which he succeeded Prof. Brožek, first as *docent*, in 1937, and later as professor, in 1945. He also lectured in his subject for some time after the War at Brno and Bratislava, but otherwise all his active academic life was spent at his own Alma Mater.

Prof. Hrubý was an ardent worker in different fields of genetics, in animals and plants and even humans, but he also made significant contributions to the taxonomy of certain plant groups. He was the author of about 170 papers. In addition, he published four books which were widely distributed in his own country. The last of these, *Genetika*, published in 1961, is a masterly treatment of classical and modern Mendelian cytogenetics, the only recent publication of its kind in the Soviet part of the world. Naturally, it is widely used in academic teaching in Czechoslovakia and neighbouring countries, and it is being translated into Russian.

Though very active in scientific research and academic teaching, Prof. Hrubý also found time to take part in many outside activities. At his death he was a member of scientific committees of several academic and practical institutions, on the editorial staff of several journals and the editor of the horticultural publications of the Czechoslovak Academy of Sciences. He was also president of the large Czechoslovak Botanical Society and the chief organizer of its very successful symposium on experimental taxonomy held at its fiftieth anniversary jubilee meeting in July 1962, with several hundred participants from home and abroad.

Prof. Hrubý is survived by his wife, Anna, a son and four daughters. He is remembered as an energetic supporter of scientific cytogenetics during times of bureaucratic suppression of Mendelian ideas, as a scientist of unusual ability, but above all for his character and personality and untiring helpfulness to others.

Áskell Love

Dr. Thomas Bedford, O.B.E.

Dr. Thomas Bedford died on January 24 in his sixty-ninth year. His sudden passing will have come as a great shock to a wide circle of friends and colleagues.

Dr. Bedford had been engaged on problems of occupational hygiene for the past 44 years, and he will rank as one of the pioneers in this important field. He was appointed to the staff of the then Industrial Fatigue Research Board in January 1919. A year later he joined forces with the late Dr. H. M. Vernon, and their conjoint working continued until the latter's retirement in 1933. During this period a wide range of investigations were concerned with problems relating to rest pauses and accident rates in many industries, and the influence of atmospheric conditions on the working capacity and the comfort of workers was also studied.

From 1933 Dr. Bedford began to specialize in the field of thermal studies. His researches into the physiological reactions of the human being to thermal stresses established basic principles and methods of measurement and evaluation which became accepted as standard practice internationally. As director of the Environmental Hygiene Research Unit of the Medical Research Council he attained great eminence and became an outstanding authority in his particular field. In 1953, by which time Dr. Bedford had long felt that a society should exist in Great Britain to represent those engaged in the field of occupational hygiene, his personal effort and enthusiasm were responsible for the founding of the British Occupational Hygiene Society. This now flourishing body will continue to be a fitting tribute to his memory. Although he retired from the service of the Medical Research Council in 1959, when he was awarded an O.B.E., Dr. Bedford maintained his many contacts on Government and other official committees. Personally, Tom Bedford was of a quiet and unassuming nature. His dedication to work was always blended with qualities of marked benevolence and kindness that endeared him to all who had the good fortune to be associated with him, privately and professionally. Many will remember with gratitude the help that he gave them so generously in dealing with their problems.

So has ended a full life of distinguished contribution to the science of occupational hygiene, in which Tom Bedford's guiding principle was always service before self.

C. G. Warner

NEWS and VIEWS

Deputy Principal at the University of Birmingham: Dr. T. Alty

Dr. Thomas Alty, retiring vice-chancellor of Rhodes University, South Africa, has been appointed deputy principal of the University of Birmingham as from May 8. Dr. Alty was appointed master of Rhodes University College in 1948 and became first principal and vice-chancellor of Rhodes University on its establishment in 1951. Dr. Alty was educated at the University of Liverpool, where he was Oliver Lodge Fellow in 1921, and at the University of Cambridge. He was a lecturer in physics at the University of Durham from 1924 until 1925, and in 1925 was appointed to the chair of physics at the University of Saskatchewan, Canada. He held this chair until 1932 when he was appointed research professor of physics in the same University. In 1935 he returned to Britain when he was appointed to the Cargill chair of applied physics at the University of Glasgow. This he held until 1945 and thereafter the Cargill Chair of Natural Philosophy in the same University until 1948. From 1958 until 1960 Dr. Alty was chairman of the Association of Universities of the British Commonwealth. From the date of Dr. Alty's appointment as deputy principal the office of vice-principal will be known as the office of pro-vice-chancellor and vice-principal.

Electronic Engineering at Leeds: Prof. P. J. B. Clarricoats

Dr. P. J. B. Clarricoats has been appointed professor of electronic engineering in the University of Leeds. He was educated at the Imperial College of Science and Technology, where he obtained the degree of B.Sc.(Eng.) in 1953 and five years later was awarded the degree of Ph.D. Following graduation, Dr. Clarricoats was employed by the General Electric Company at Stanmore until 1958, when he was appointed lecturer in the Department of Light Electrical Engineering in the Queen's University of Belfast. There he created a microwave research group and arranged contracts with the Department of Scientific and Industrial Research and the Ministry of Aviation, which provided apparatus and grants. In 1962, he was appointed lecturer in the Department of Electrical Engineering at the University of Sheffield. He was awarded two premiums by the Institution of Electrical Engineers for his work on microwave ferrites. He has been a consultant with Messrs. Short Bros. and Harland since March 1961. He was awarded a senior visiting fellowship of the Organization for European Co-operation and Development by the Swedish Natural Science Research Council in December 1962.

Director of Dounreay: Mr. R. R. Matthews

Mr. Roy R. Matthews, formerly deputy director, reactor design in the Reactor Group of the United Kingdom Atomic Energy Authority, has been appointed to succeed Dr. R. Hurst as director of the Dounreay Experimental Reactor Establishment. Dr. Hurst is leaving the Authority to become director of research of the British Ship Research Association. Mr. Matthews was educated at Lindisfarne College, Westcliff-on-Sea. A scholar of Queen's College, Cambridge (1940–42), he graduated with honours in mechanical sciences. In 1942 he joined the Royal Navy and served on H.M.S. *Indefatigable* in the Atlantic and the Pacific until 1946 as a temporary lieutenant (E.), R.N. In 1947, after a short period with E. S. and A. Robinson, Ltd., Bristol, he joined the London Office of the Anglo-Iranian Oil Co. Ltd. In 1948 he went to Abadan, Iran, for three years, holding various posts in the maintenance and development engineering departments. Returning to England in 1951, he joined Monsanto Chemicals design department as a senior project engineer. He joined the Atomic Energy Authority in 1955 as assistant chief engineer in the Fast Reactor Design Office, becoming deputy chief engineer in 1956 and chief engineer in 1957. In 1961 he was appointed deputy director of the Reactor Design Branch. His qualifications include Associate Membership of the Institutions of Mechanical, Chemical and Electrical Engineers.

Marine Nuclear Propulsion

In a speech in the House of Commons on the Brussels negotiations on the European Economic Community on February 11, the Prime Minister said that Britain had always been anxious not merely to build a nuclear engine which could propel a merchant ship but to get a nuclear reactor which is economically attractive. This point was now near at hand and the Minister of Transport would very soon be discussing with all those concerned the arrangements for the construction, ownership and operation of such a vessel. In a written answer on February 13, Mr. E. Marples stated that those talks had now begun, but it was too soon to say what type and size of vessel would be selected if the Government decided that one should be built, nor was it possible at this stage to say how the order would be placed. If suitable financial and other arrangements could be agreed with industry, Mr. Marples hoped that a firm decision whether a ship should be built would be taken before the end of the year.

In a further written answer (in the House of Commons) on February 15, the Minister of Transport stated that since December 20 research had progressed to a point where it became clear that it would be possible to decide before the end of the year whether a prototype nuclear ship would be built. In these circumstances, the Government had decided that the time had come to have discussions with the shipping and shipbuilding industries about the arrangements for such a ship. It was expected that it would be possible to decide before the end of the year which reactor system should be installed in a prototype nuclear ship, if one is built. The Civil Lord of the Admiralty, Mr. C. Ian Orr-Ewing, added that the Admiralty had no present plans for building a nuclear-powered surface vessel, but was keeping in close touch with the latest developments and applying the knowledge gained from them to studies of possible future warships.

Teaching Machines and Programme Learning

In an adjournment debate in the House of Commons on February 12, Mr. R. Gresham Cooke and Mr. G. M. Thomson raised the question of teaching machines and urged the support of further research into programmed learning and the use of such machines. In replying for the Government, the Parliamentary Secretary to the Ministry of Education, Mr. C. Chataway, welcomed the debate and said that this development was being very carefully studied. Although almost all the research on programmed learning was being carried out in the United States, there was in Great Britain a shortage of thoroughly validated programmes. The Government particularly welcomed the work being undertaken by the Association for Programmed Learning—a professional body mainly of practising teachers. Research was still proceeding into the efficiency of the linear type of programmed learning and of the multiple choice variety, and so far the results were rather inconclusive. Mr. Chataway thought that Mr. Cooke had overestimated the amount of research so far carried out. The first significant research in Great Britain was in the Psychology Departments of the Universities of Aberdeen and Sheffield, supported by grants from the Department of Scientific and Industrial Research, and was directed mainly to the industrial application of programmed learning. However, within the past few months research into educational aspects of the subject had commenced in the Department of Education at the University of Sheffield. Work had also commenced at Sheffield in October 1962, on methods of using programmed learning in secondary school mathematics up to the Ordinary Level of the General Certificate of Education, with the view ultimately of Advanced Level. This was made possible by a grant from the Ministry of Education of £12,000 over three years. The Ministry had also offered a grant of £1,000 a year for three years to support research under the Department of Education, University of Leicester, with simple linear machines and programmes suitable for use by primary school children, especially in arithmetic and reading. Replies to an inquiry of all local education authorities, colleges of advanced technology and institutes of education in October indicated that only 20 authorities out of 146 had anything positive to report. Three colleges of advanced technology and the College of Aeronautics at Cranfield were experimenting with machines, and in several institutes of education programmed learning was beginning to play a part in teacher training.

River Authorities and Land of 'Special Interest'

At the report stage of the Water Resources Bill in the House of Lords on February 14, the Government accepted an amendment moved by Lord Hurcomb which inserted a new clause. Under this clause the Nature Conservancy, when of the opinion that any area of land not managed as a nature reserve but which is of 'special interest', and which may at any time be affected by schemes, activities or operations of a river authority, has the duty of notifying the river authority that the land is of special interest by reason of its flora, fauna, geological or physiographical features, etc. It is already the duty of the Nature Conservancy to notify the local planning authority of any area which in its opinion is of special scientific interest, and, for the Government, Earl Jellicoe agreed that such early information should help to avoid possible clashes of interest between the river authorities and the Conservancy. He wished to consider further, without prejudice, whether it would be reasonable for the Minister to give a directive to the river authorities requiring them to consult the Conservancy at the earliest possible stage about any scheme, operations or activities likely to affect the areas of which they have been notified. An amendment moved by Lord Conesford and proposing to insert specific reference to the use of sea-water and extracting the salt as a means of augmenting water resources was, by leave, withdrawn on Earl Jellicoe pointing out that the matter was covered more comprehensively elsewhere in the Bill under a clause which made the Water Resources Board responsible for considering desalination along with other means of augmenting water supplies.

Emperor Tandem Van de Graaff Accelerators

AN *Emperor* tandem Van de Graaff accelerator, costing 3 million dollars, for the University of Rochester has been financed by the largest single grant ever made by the U.S. National Science Foundation. The accelerator is being made by the High Voltage Engineering Corporation, Burlington, Mass. This is the fourth *Emperor* recently built by the Corporation. All the machines will be used to conduct research on the structure of the atomic nucleus. The most precise, powerful and versatile instrument yet developed for the study of nuclear structure, the *Emperor* tandem accelerates protons and heavy atomic nuclei to energies of 20 million eV and velocities close to the speed of light. The highly stabilized and homogeneous accelerated ion beams, at currents rated up to 25 μamp, will permit many experiments to be carried on simultaneously. Two of the 187-ton accelerators were recently purchased by the U.S. Atomic Energy Commission to be installed at Yale University and the University of Minnesota. A third will go to the Chalk River Nuclear Laboratory of Atomic Energy of Canada, Ltd., in Ontario. Other large radiation machines for physics research have been supplied by the Corporation to West Berlin, the Laboratoire Joliot-Curie de Physique Nucléaire, Orsay, France, and the Universities of Washington, Pittsburgh, Virginia and Kentucky.

Science and the Welfare of Mankind

IN a lecture, "Science, State and Society", delivered at Dynevor School, Swansea, on October 18, 1962, Prof. B. H. Flowers dealt with the potentialities of science for good and ill on lines that are strikingly reflected in Dr. C. P. Haskins's subsequent presidential report to the Carnegie Institution of Washington (see *Nature*, 197, 729; 1963). Beyond the possibilities which the development of nuclear energy, of synthetic materials, of satellites, of biotechnology have put in our reach, science has profoundly affected the political and sociological atmosphere of the world through radio and television, as well as through the use of electronic computers. Many of the technical problems of world government are disappearing and the time-interval between scientific discovery and full-scale use is now so short that the scientist himself can be involved from the moment of discovery to the final application. Further, scientific endeavour now absorbs a very large proportion of the intellectual and industrial skill of the State, so that it is a matter of public importance whether one venture is undertaken rather than another. Endorsing Sir John Cockcroft's comment to the British Association about wrong priorities in the context of space research, defence and the under-developed countries, Prof. Flowers urged that it is not enough for universities simply to provide the scientists and technologists needed and to carry out the fundamental research on which progress in technology depends; they must also prepare a far larger fraction of the population for intelligent participation in the democratic way of life and must foster the study of man himself. Copies of the lecture (Pp. 16) are obtainable from Dynevor School, Swansea, price 1*s*. 6*d*.

The Southern Regional Council for Further Education

THE fifteenth annual report of the Southern Regional Council for Further Education expresses concern at the additional accommodation problems which may beset colleges where more workshop space is required to implement some of the new engineering courses, and has already recommended to the National Advisory Council that some financial help should be given by the Ministry to local education authorities through an additional minor projects allocation specifically for engineering workshop accommodation (Pp. 38. Reading: Southern Regional Council for Further Education, 1962). A conference of teachers and representatives of the engineering industry was organized in July to discuss the organization of the new courses. Full-time students in the 1961–62 session numbered 8,034 compared with 7,437 in 1960–61; for part-time day students the corresponding figures are 46,996 and 37,329, and for part-time evening students 41,214 and 33,357. Notes are included on progress and developments in the colleges in the area, and the Regional Academic Board has expressed to the Ministry of Education the view that some new agency is needed to give financial assistance in the development of research in technical colleges. The report includes also the accounts for the year ended March 31, 1962, lists of chairmen and changes in membership of the Council, the Regional Academic Board, Executive Committee and Advisory Committees as at September 30, 1962, evidence submitted to the Robbins Committee and a list of 42 research projects notified by colleges in the region during the session 1961–62.

Fellowship Programme in Nuclear Science

THE Fellowship Programme of the International Atomic Energy Agency has, since the inception of the fellowship activities of the Agency in April 1958, provided with the Exchange Programme a variety of training opportunities relating to the peaceful uses and applications of nuclear energy and its technology. The Fellowship Programme operates through the award of several types of fellowship to qualified applicants and is financed from three different sources: (*a*) member States making contributions to the Agency's General Fund, allocations of which are made every year to the Fellowship Programme by the General Conference; (*b*) direct from the member States which make fellowships available to the Agency for training at their national institutions and universities; (*c*) the Expanded Programme of Technical Assistance of Unesco according to the usual procedure of country programming. Stipends and other allowances are determined mainly by the scale of monthly stipends agreed on by Unesco in consultation with its specialized agencies. Travel expenses of the candidates are expected to be borne by the nominating Government. A brochure entitled *The Fellowship Programme in Nuclear Science* deals with the following aspects of the Programme: types and periods of training; training opportunities; application and nomination of candidates; documents required for submission to the Agency; selection of candidates and award of fellowships; financial considerations; insurance; reports and control; vacation leaves; extensions; obligations of Governments; statistics; general list of institutions; opportunities at the International Atomic Energy Agency Laboratories. Copies of the brochure and further information can be obtained from the International Atomic Energy Agency, Karntner Ring, Vienna 1, Austria.

New Research Project for Mental Health

In 1962 Mrs. Rees-Thomas donated £50,000 to the National Association for Mental Health. It has now been decided to use the income from this donation in the first instance to finance a research project concerning the adequacy of present training methods for teachers working in training centres for the mentally handicapped. The Association at present runs courses of training for these workers and its diploma is so far the only recognized certificate of competence for these workers. The Scott Committee has made certain recommendations about these trainings and its report is at present before the Minister of Health. No validation of present teaching methods has so far been undertaken and research is generally felt to be desirable. This might cover not only the suitability of present training schemes but also the most suitable methods of approaching the children at various stages of their development. The donation now enables the National Association to appoint research workers in this important field. It is hoped that a university department will be prepared to co-operate with this inquiry. The successful applicant will be expected to undertake detailed and systematic surveys of work at present in progress. Further information can be obtained from the National Association for Mental Health, Maurice Craig House, 39 Queen Anne Street, London, W.1.

Fellowships for Scientific Research

The Department of Scientific and Industrial Research awarded a total of 92 postdoctoral research fellowships during the year 1962–63, almost twice as many as in the previous year. The awards enable scientists and technologists to study abroad, either through their own scheme of awards or through the science studentship and fellowship scheme of Nato, including 48 D.S.I.R. fellowships, and 44 awarded by the Department under the Nato scheme. Twenty-five of this year's Nato fellowships were for study abroad and 19 to enable British scientists who had been studying in North America to return to Great Britain. Thirty-four postgraduate studentships were granted by the Department of Scientific and Industrial Research under the Nato scheme, these being held in nine different European countries, the United States and Canada. The awards, which are tenable for two to three years at universities, colleges and other approved institutions in any part of the world, can be either at postgraduate studentship or postdoctoral level. Details of these awards and methods of application are given in a new edition of the booklet, *D.S.I.R. Studentships and Fellowships, 1963*, published by H.M.S.O. (price 3*s*.), which also gives the particulars of such awards tenable in Great Britain.

Endeavour Prizes

Prizes totalling 100 guineas are offered by Imperial Chemical Industries, Ltd., publishers of the international scientific review *Endeavour*, for essays submitted on scientific subjects. In addition to the cash prizes, the prizewinners will receive invitations to attend the whole of the British Association meeting at Aberdeen during August 28–September 4. Accommodation will be provided in Aberdeen, and travelling expenses within the United Kingdom will be paid. As the primary purposes of these awards are to stimulate younger scientists to take an interest in the work of the British Association and to raise the literary standard of scientific writing, the competition is restricted to those whose twenty-fifth birthday falls on or after June 1, 1963. Five prizes will be awarded, namely: a first prize of 50 guineas; a second prize of 25 guineas; a third prize of 15 guineas; two special prizes of 5 guineas each for competitors who have not passed their eighteenth birthday on June 1, 1963. The subjects for the essays are as follows: (1) Space research: its scientific significance. (2) Response to viral infection. (3) Photosynthesis. (4) The mechanism of muscular contractions. (5) High-temperature chemistry. (6) The concept of aromaticity in chemistry. The essays, which must be in English and typewritten, should not exceed 4,000 words in length, and only one entry is permitted from each competitor. All entries should be addressed to the Deputy Secretary, British Association for the Advancement of Science, 3 Sanctuary Buildings, Great Smith Street, London, S.W.1, and the envelope should be clearly marked 'Endeavour Prize Essay'. The latest date for receipt of entries is June 1, 1963. The essays must be submitted without signature, and the competitor's full name and address and date of birth should be disclosed in a sealed covering letter attached to the essay and addressed to the Deputy Secretary of the British Association, who will acknowledge all entries.

Announcements

Prof. W. H. Pearsall and Dr. Sidnie Manton have been awarded Linnean Gold Medals as an expression of the Society's estimate of their services to science. Mr. J. E. Lousley has been awarded the Society's Bloomer Award in recognition of important contributions to biological knowledge made by an amateur naturalist.

Monsieur P. M. J. Ailleret, deputy director-general of Electricité de France, has been awarded the Faraday Medal of the Institution of Electrical Engineers for his contributions to the techniques of the high-voltage long-distance transmission of electric power and the economic integration of national and international electricity supply networks. The Rt. Hon. Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd., has been elected an honorary member of the Institution.

Dr. K. P. Oakley, of the British Museum (Natural History), has been awarded the Prestwich Medal of the Geological Society of London, for his researches on fossil man and the development of physical and chemical methods for the dating of fossil mammalian bone.

A conference on "Inert Gas Compounds" will be held at Argonne National Laboratory during April 22–23. Further information can be obtained from Herbert H. Hyman, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois.

A one-day colloquium on "Electrical Properties of Flames", organized by the Committee of the British Section of the Combustion Institute, will be held at the College of Advanced Technology, Birmingham, on April 19. Further information can be obtained from Dr. W. G. Parker, Department of Chemistry, College of Advanced Technology, Gosta Green, Birmingham 4.

Under the sponsorship of NATO a summer school will be organized at the University of Ghent during July 29–August 9. The topics to be treated will be concerned with the surface properties of semi-conductors. Further information can be obtained from Prof. W. Dekeyser, Laboratorium voor Kristallografie en Studie van Vaste Stoffen, Rozier, 6, Ghent, Belgium.

The College of Advanced Technology, Birmingham, is arranging the following courses in metrology and quality control: metrology and quality control (April 29–May 10); advanced metrology (May 20–24); statistical quality control and acceptance sampling (September 9–27). Further information can be obtained from the Secretary, College of Advanced Technology, Gosta Green, Birmingham.

The second bionics symposium on "Information Processing by Living Organisms and Machines", sponsored jointly by the Aeronautical Systems Division and the Aerospace Medical Division, United States Air Force Systems Command, will be held in Dayton, Ohio, during March 19–21. Further information can be obtained from the Information Office, Aeronautical Systems Division, Wright-Patterson AFB, Ohio.

U.S. EDUCATIONAL SERVICES INCORPORATED

EDUCATIONAL Services Incorporated of Watertown, Mass., a non-profit educational research organization which originated as the Physical Science Study Committee under the auspices of the Massachusetts Institute of Technology in 1956, has been awarded a grant of 1,000,000 dollars by the Ford Foundation.

Responding to the need for improving curricula in American schools, Educational Services Incorporated has initiated the improvement of projects in many disciplines; it also participates in various educational programmes overseas, including the new Indian Institute of Technology, at Kanpur, and in emergent countries in tropical Africa.

Curriculum reform programmes are now being conducted in the teaching of science in the elementary grades and in developing a social studies and humanities curriculum applicable at all grade levels. In addition to these and other programmes in the primary and secondary schools, Educational Services Incorporated is assisting in the development of new techniques and methods of teaching science and engineering in the universities. The organization also offers administrative support to the National Commission on Engineering Education.

Educational Services Incorporated was founded in 1958 to handle the expanding Physical Science Study Committee programme and to provide administrative guidance and support for other course content improvement projects. The Committee course, formed in 1956 by Prof. Jerrold R. Zacharias, professor of physics in the Massachusetts Institute of Technology, and the late Prof. Francis L. Friedman, also a professor of physics in the Institute, has revolutionized the teaching of high-school physics. The course is now being taught to about 135,000 students in the United States, or approximately 30 per cent of those taking high-school physics.

Physicists and educators at many universities contributed to this course, including groups from the University of Illinois, Cornell University, Bryn Mawr College, Educational Testing Service of Princeton, New Jersey, Harvard University, the Massachusetts Institute of Technology, and other schools. Material supplied by the course has been translated into Swedish, Norwegian, Italian, Japanese. Hebrew, Spanish and Portuguese. It has also been translated into French for use in Canada. Further translations are being prepared in Danish and Turkish.

The Physical Science Study Committee course is notable not just for the text-book which stresses basic concepts of physics and carries high-school students into modern physics, but also because of the coherence and variety of the accompanying teaching aids, including films, examinations, laboratory apparatus and laboratory experiments. So far, fifty-three films made by the course have been produced by Educational Services Incorporated with many of the nation's leading physicists serving in principal roles in demonstrating some of the basic concepts of physics. Some of the films are now being adapted for college use.

The Science Study Series, published by Doubleday Anchor Books with the editorial counsel of Educational Services Incorporated, provides collateral reading for science students. This series of paperbacks includes biographies of famous scientists and their roles in the development of fundamental physical concepts. Teacher-training institutes for high-school physics teachers in the United States and in many nations overseas have also been organized.

In addition to the Physical Science Study Committee, Educational Services Incorporated is now developing programmes in the following areas.

Elementary Science Study. Physicists, chemists, biologists, mathematicians and scientists from other disciplines are participating in the preparation of material for the elementary grades. This material includes text, films, the development of experiments for classroom use, and the inauguration of teacher-training programmes to introduce a study of science in the elementary school curriculum. Some of the topics being studied this year in experimental classes at selected schools in New Jersey, Pennsylvania, Delaware, Oregon, New York and the District of Columbia, include micro-ecology, plant growth, elementary physics, and laboratory science. More than one hundred scientists and educators in the United States and overseas are associated in the preparation of preliminary material.

Kanpur Indo-American Programme. The Indian Institute of Technology at Kanpur, about two hundred miles south-east of New Delhi, is being developed by the Government of India into a major centre for education and research in engineering and science. Now in its second year of operation, the Institute offers undergraduate programmes in physics, chemistry, mathematics, and civil, electrical, chemical, mechanical and metallurgical engineering. It is expected that graduate courses will be given in the next few years.

At the request of the Government of India, nine leading American universities are assisting in the development of the Indian Institute. Because the resources of a single American university would be severely overtaxed if it were to furnish the sustained effort required to carry out the United States commitment at the Institute, a consortium of universities was formed in April 1962. Educational Services Incorporated administers the project contract which is financed by the United States Agency for International Development. The participating universities are California Institute of Technology, Carnegie Institute of Technology, Princeton University, the University of Michigan, the University of California, Purdue University, Ohio State University, Case Institute of Technology, and the Massachusetts Institute of Technology. Twelve American staff members are now teaching at the Institute. It is expected that this number will be doubled towards the end of 1963.

Indian plans for the Institute, which now has a staff of 40 and approximately 200 undergraduates, provide for 1,600 undergraduates, 400 graduate students, and a staff of about 250.

African Education Study. Educational Services Incorporated, following two major conferences in Africa at Accra, Ghana, in 1961, and at Entebbe, Uganda, in 1962, is engaged in a broad programme designed to help emergent countries in tropical Africa to assess their educational needs in the future in mathematics, in the sciences, in languages, and in the social studies and humanities.

Text material in mathematics, prepared by Educational Services Incorporated, including representatives from the Stanford University Arithmetic Programme, is being used in preliminary trials in many African schools now beginning.

The African Education Programme is developing as a partnership between the African educationists and those from the United States and Great Britain. In this endeavour Educational Services offers especially the organization's experience with course content improvement programmes.

Commission on Engineering Education. Members of this Commission include many leading representatives from industry, Government, and education. The Commission was formed at a meeting at Boulder, Colorado, in 1961.

The headquarters of the Commission in Washington function as a point of co-ordination for the members. The Commission is developing educational resources of particular relevance to fundamental fields of engineering, including all types of educational resources and procedures which involve both students and faculty in the areas of design, data processing and decision making.

Semiconductor Electronics Education Committee. The teaching programme of this Committee—now being used for the first time, in its preliminary form, at eleven universities in the United States and Great Britain—demonstrates introductory semiconductor physics, the principles of semiconductor devices, and the most important features of electronic circuit design and construction which use semiconductor devices. It is intended that the final material produced by the Committee will be used in either the junior or senior year of college.

The Committee's presentation includes text material in the form of a series of six paperback books on the principles and applications of semiconductors; the development of home laboratory experiments; take-home laboratory kits; a series of formal laboratory experiments; and a set of films both complementary and supplementary to the texts.

National Committee for Fluid Mechanics Films. The films in fluid mechanics produced by this Committee are being used at more than sixty universities and Government institutions in improved teaching of aeronautical and astronautical engineering, and in chemical, civil, mechanical and marine engineering. The films are also being used in the instruction of meteorology, applied mathematics, and in medicine. The first two major films produced, *The Fluid Mechanics of Drag* and *Vorticity*, are being used at universities in the United States, Ghana, Great Britain, Australia, Canada, Israel and Holland.

Social Studies and Humanities. This programme evolved from a conference sponsored by Educational Services Incorporated at Dedham, Massachusetts, in the summer of 1962. Participants at the conference represented the humanities and the social sciences, including literature, history, art, music, Latin and Greek, as well as sociology and psychology. The consideration of the possible revision of the present social studies and humanities curricula is the aim of this programme, which is not yet fully implemented. Investigations are being made of the individual humanistic disciplines with the view of focusing the major ideas of the past and present into a presentable, coherent framework which may then, using some of the techniques developed by the behavioural scientists, be taught most effectively.

SOLVENT EXTRACTION CHEMISTRY

A SYMPOSIUM on "Solvent Extraction Chemistry" was held at Gatlinburg, Tennessee, during October 23–26, by invitation of the Oak Ridge National Laboratory. The purpose of the symposium was to bring together persons engaged in investigating solvent extraction and to provide for the interchange of information, not merely through the papers presented, but also in an open session and by informal discussions.

The presentation of thirty-two papers submitted from laboratories in the United States, Great Britain, France, Sweden and Italy in a programme which filled four days is a measure of the expansion of work in this field since the one-day symposium (*Nature*, **172**, 149; 1953), arranged by Atomic Energy Research Establishment, Harwell, in 1953. In the intervening period, the chemistry of solvent extraction has been included in conferences of wide scope, such as the International Conferences on the Peaceful Uses of Atomic Energy at Geneva in 1955 and 1958; in these, the chemical aspects have generally taken second place to flowsheets and processing problems.

Dr. F. L. Culler, head of the Chemical Technology Division, Oak Ridge National Laboratory, in his opening remarks pointed out that the progress achieved by research in the past decade had made solvent extraction an important and powerful technique for solving the separation, purification and recovery problems that occurred in the field of atomic energy. Solvent extraction had moved forward from the use of diethyl ether to separate uranium from contaminating metals to being a versatile technique applied to the separation of closely related inorganic compounds in a variety of acid media: separations could if necessary now be achieved in a matter of seconds rather than minutes. Dr. Culler foresaw the time when its use for separations of biological substances would be greatly extended.

Organo-phosphorus compounds, which were grouped as 'neutral' or 'acidic', formed the subject of several papers presented by the members of the Argonne National Laboratory, Atomic Energy Research Establishment, Harwell, Oak Ridge National Laboratory and the General Electric Co. Laboratories at Hanford. The neutral class extract metals and acids: the former mainly by solvation $\equiv P{=}O \rightarrow M$, there being replacement of aquo groups from the co-ordination sphere; the latter by hydrogen bonding. The extractive power of these compounds has been correlated with their basicity, which is reflected in a number of properties such as the frequency of the infra-red stretching vibration of the PO group: the extraction order is phosphine oxides > phosphinates > phosphonates > phosphates.

The phosphates, though relatively weak as extractants, are ideal for many separation processes as recovery from the organic phase is usually easy: tributyl phosphate (TBP) has been extensively studied on account of its versatility, and the part played by ions and ion-pairs in TBP systems has been recognized. Increased attention has been paid to the influence of the polarity of the diluent, often a hydrocarbon, on the distribution coefficients. Among these neutral compounds, phenyl phosphonates have been found to have enhanced selectivity for the separation of certain metals, while phosphine oxides are suitable for small-scale laboratory separations and for analytical purposes. Other compounds included in the reviews were certain diphosphonates found to be very powerful extractants, and thiophosphates.

The main impression given by the papers dealing with the acidic organo-phosphorus compounds as extractants was the variety of mechanisms that have been found to occur. Thus the metal complexes obtained with the monoacidic phosphates, HX, may contain: (1) the unit X^- as a monodentate or as a bidentate group; (2) the group HX_2^- derived from the dimer of HX; (3) the neutral group, HX, co-ordinated through its phosphoryl oxygen; (4) the anion, for example, NO_3^-, present in the aqueous phase. In view of the interaction of the neutral compounds (for example, TBP) with HX and of their ability to form solvates, it is not surprising that the extracting power of a mixture of a neutral compound and HX is often considerably different from that of HX alone.

Amines and quaternary ammonium compounds have not yet been applied so extensively as organo-phosphorus compounds, but the five reviews devoted to them suggested they will be found increasingly useful for the extraction of acids and for the separation of the platinum group metals and of the transuranium elements. Other papers surveyed developments in the application of solvent extraction to analytical purposes and to the extraction of fission products from the waste liquors that occur from the treatment of irradiated fuels.

The final session was of particular interest to those concerned with the operation of tributyl phosphate processes for the separation of fissile material from irradiated reactor fuels. The papers and the discussion which followed dealt with chemical aspects of the degradation (by thermal and radiolytic reactions) of tributyl phosphate itself and of the hydrocarbon diluent which form the bulk of the organic phase. Complementary studies at the Atomic Energy Research Establishment, Harwell, and at Windscale Technical Works have identified the class of compounds responsible for the retention of fission product zirconium in the organic solvent after it has been in use for several months; the compounds are certain hydroxamic acids, present at less than 10^{-5} M and derived from the nitration of the diluent.

The symposium was organized by a committee under K. B. Brown as chairman. The papers are to be published in *Nuclear Science and Engineering*. J. M. FLETCHER

HYDRAULICS AND FLUID MECHANICS

A CONFERENCE on "Hydraulics and Fluid Mechanics" was held at the University of Western Australia, Nedlands, during December 6–13. It was sponsored by the Faculty of Engineering and is to be the forerunner of a triennial series. These will be held initially in Australia or New Zealand and will be known as Australasian Conferences on Hydraulics and Fluid Mechanics.

The inclusion of both the terms was for the purpose of attracting a wide variety of papers. This aim was fulfilled and the conference assisted in bringing applied mathematicians, physicists and engineers closer together in a most co-operative atmosphere. It also helped to make the attendance sufficient for all activities associated with such conferences to be undertaken. This is a particular problem presented to conference conveners in Australia and New Zealand, where travel distances are so great and specialists in any field so few. In these circumstances, most attenders must be authors to warrant travel subsidy by an employer, and a wide variety of topics must be accepted so that the gathering warrants the outlay to attend.

The open invitation resulted in 57 papers being presented or tabled during the course of the five working days. In each session of one and a half hours three papers were generally presented. Each author had twenty minutes for presentation, which left half an hour for discussion of all papers. Some concurrent sessions were necessary.

The proceedings of the conference are to be published by Pergamon Press and should be available about May 1963. The volume will contain approximately half the papers submitted, together with the inaugural address delivered by Prof. Hunter Rouse, director of the Iowa Institute of Hydraulics Research. It was appropriate that Prof. Rouse should be guest at this first conference of a new series having been inaugurator of the renowned series of Iowa Hydraulics Conferences.

Reference was made to the Iowa series and the different approach made in it and the present conference. For example, the former had specific themes with solicited speakers while the latter covered a wide range of topics with unsolicited papers. Prof. Rouse recommended experimentation in the various facets of organizing the conference. As future gatherings are to be the sole responsibility of the university which chooses to hold it, such variations are likely. The only probable common denominators are the need for a wide variety of papers, for the reasons given here, and the desirability of having as many overseas visitors as possible.

Prof. Rouse's address was entitled "On the Art of Advancing the Science of Hydraulics", emphasis being placed on 'science' as distinct from applications, and 'hydraulics' which connoted a specific branch of fluid mechanics—the sturdy offspring which the original empirical experimentation of hydraulics begot. Speaking on the part played by the university, Prof. Rouse submitted that fundamental research was as essential to it as was higher education. He accepted that product or process development had its creative or instructive sides, but that routine testing, involving no new procedures or concepts, should be reserved for the commercial laboratory or the technical institute. Other requirements in the art of advancing any field of knowledge were free exchange of ideas among research workers and emphasis on good staff above all else—equipment, buildings, maintenance, funds, etc.

The conference papers of more direct interest to engineers have been summarized elsewhere[1,2] and hence only those of general scientific interest will be included here. The topics on which two or more papers were received included flow through porous media, the effect of vortices, waves and guided weapon research.

Porous Media

R. A. Wooding (Applied Mathematics Laboratory of the Department of Scientific and Industrial Research, Wellington, New Zealand) presented a paper entitled "Mixing Layers in Flow Through Porous Media". It treated properties of steady vertical convection from a source of heat, or of heated fluid in a saturated porous medium, when the Rayleigh or Péclet number was very large. The effects of thermal diffusion were important only in zones of mixing between fluids at different temperatures. Flows of this type could be expected in geothermal regions.

J. de Yong (Commonwealth Scientific and Industrial Research Organization, Division of Forest Products, Melbourne) discussed the merits of applying capillary or drag models to flow through media of high porosity. The paper was aimed specifically at paper pulp pads, and emphasized consideration of rheological properties in evaluating permeability. Various theories were described and tests reported, from which it was concluded that the 'drag equations' described the permeability flow-rate relationship most accurately.

Vortices

A paper entitled "Sink Vortices and Whirlwinds" was presented by E. K. Webb (Commonwealth Scientific and Industrial Research Organization, Meteorological Physics Division, Melbourne). Whirlwinds develop only over smooth ground when there is strong thermal convection (ground hotter than air) with a light wind. The criterion for the formation of a whirlwind was developed in terms of inflow and an effective turbulent viscosity. It was only when a significant portion of the inflow shrank into the molecular conduction boundary layer that this criterion was satisfied. The equation was derived which satisfied this latter condition. It corresponded to a wind less than one metre per second over bare ground on a summer's day.

E. Inoue (Division of Meteorology, National Institute of Agricultural Sciences, Tokyo) discussed the "Horizontal Diffusion over the Sea Surface". He applied the techniques of analysing the diffusing smoke puffs and plumes in the atmosphere to similar phenomena in the sea. In this way oceanic turbulence can be gauged. Two types of diffusion were considered; that generated instantaneously and that from a fixed source over time. Equations were presented for the variation of area with time and the vanishing time of the dye patch. These results are applicable to problems of sea pollution, such as ship discharge, sewage outfalls and the marine disposal of radioactive wastes.

A paper from the University of New South Wales, Sydney, entitled, "The Motion of a Two-Dimensional Buoyant Vortex" was presented by I. R. Wood. The theory of the vortex pair was modified to include the effect of walls and for the case of sloping walls the component of the buoyant force. Tests were conducted in a 'Perspex' flume with coloured dye identifying a vortex of salt solution entering a fresh-water medium.

Waves

The paper "An Exact Theory of Simple, Finite Shallow Water Oscillations on a Rotating Earth" approached the problem from the unusual angle of taking a simple form of liquid motion and finding the shape of basin necessary to produce it. The author, F. K. Ball (Commonwealth Scientific and Industrial Research Organization, Meteorological Physics Division, Melbourne), discussed two simple types of motion: "pure displacement" and "pure distension". The former is a lateral displacement without change in depth profile and was found to occur only in a paraboloid. The latter consists of a combination of a uniform, two-dimensional isotropic dilation and a rotation, and could only occur in a paraboloid of revolution if the underlying surface were axially symmetric. The governing equations in both cases were simple linear differentials with constant coefficients.

J. W. Miles (Australian National University, Canberra) presented a review paper on the generation of surface waves by shear flows, especially with regard to wind blowing over water. Theories were cited which involved the direct action of turbulent fluctuations in pressure on the water surface and those concerned with an instability mechanism whereby energy was transferred from shear flow to the surface wave. Observational evidence by Longuet-Higgins[3] was discussed from which it was shown that energy transfer to ocean waves was probably produced through the instability mechanism. Other topics pertaining to transfer of energy were presented.

H. C. Levey (University of Western Australia, Perth) presented a paper entitled "Diffraction in Fluid Mechanics". The acoustic equations that are used to discuss the propagation of small disturbances in a gas are only approximations. They govern, in fact, the first-order terms that arise from an assumed regular perturbation expansion in the maximal disturbance strength. It was shown that in pulse diffraction problems the acoustic approximation always leads to a singularity of a kind unacceptable in gas dynamics in the shadow boundary. It was submitted, therefore, that although such problems were of a singular perturbation type they required different techniques from those usually applicable in wave propagation problems.

Guided Weapons Research

Two papers were submitted on this topic from the Weapons Research Establishment at Salisbury, South Australia.

The first, by H. C. Newbigin, was concerned with aerodynamic data obtained from a wind tunnel from which a comprehensive mathematical model was derived. This model was later corrected with the aid of flight trial data, which could not be adequately analysed until a high-speed computer became available. It was found that important aerodynamic derivatives obtained from the wind-tunnel tests were incorrect. Although these could be readily adjusted from the prototype tests, the reason for the discrepancy had not yet been found.

The second paper, submitted by B. Goodwin, referred to the mathematical modelling of the fuel system of a guided weapon. The fuel flow to the engines of a missile determines its effective range, it must therefore be known to a high degree of accuracy. In one weapon under consideration the fuel flow was a function of the Mach number and ambient conditions. A control system with a number of feed-back loops controlled this flow, and was devised from a comprehensive mathematical model based on an examination of the various physical components.

R. SILVESTER

[1] *Water Power* (London, in the press).

[2] *J. Inst. Eng. Austral.* (in the press).

[3] Longuet-Higgins, M. S., *Proc. Roy. Soc.*, A, 265, 286 (1962).

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

THE annual report and statement of accounts of the National Research Development Corporation for the year ended June 30, 1962 *, surveying the thirteenth year of the Corporation's activities, discusses at some length, in view of the attention which is at present being given in Government support of civil development work in industry, the extent to which the Corporation is achieving its purposes and the main disabilities under which it has to operate. For the rest, the report stresses the progress made in furthering the hovercraft project; four prototypes of the second generation are undergoing trials, including experimental operational use in ferrying passengers on selected routes. However, much development is still required before this new form of transport becomes fully established commercially, and further development would be greatly advanced if commercial or other operators who have specific operational requirements which hovercraft can meet were to place orders now. In the *Dracone* project for flexible barges, development of the larger sizes is still proceeding and commercial sales of the sizes already developed doubled, largely for export. A major advance is noted in the administrative arrangements for the fuel cell project; further development will be supported by a company formed for the purpose, Energy Conversion, Ltd. Commercial interest in cephalosporin c has been fully maintained and the Corporation is even more hopeful that this antibiotic, which is resistant to penicillinase, will play a useful part in medical therapy. Other major revenue-earning inventions listed include the alignment of light projectors; anemometers; anti-carcinogenic compounds; anti-thyroid compounds; Bailey bridge; collet chucks; electrically conductive films; electronic computers; exploders for firing charges electrically; fog detectors; glass fire and heat detectors; gyroscopic apparatus; hecogenin; ignition systems; ionization gas detectors; insecticidal fumer; manufacture of cheese;

* National Research Development Corporation. Report and Statement of Accounts for the year 1st July, 1961, to 30th June, 1962. Pp. ii+34. (London: H.M.S.O., 1963.) 2s. 3d. net. (See also p. 929 of this issue of *Nature*.)

microdensitometer; navigational wireless system; photosensitive cells; polarograph; potato harvester; resonant electronic circuits; selective weed killers, triiodothyronine; universal joints; variable speed induction motors; and visual flying aids.

Between them these major inventions earned £209,662, of which £104,701 came from two only, both concerned with drugs and mostly from the United States. Exploitation receipts by way of royalties, option payments and the like increased to £279,435, compared with £259,902 in 1960–61. The Corporation now holds the rights in 1,064 United Kingdom patents and patent applications and in 2,491 overseas ones, while the Corporation's Development Companies hold a further 705 United Kingdom and overseas patents and patent applications. A further 42 licence agreements with United Kingdom firms brought the total in force to 516, while 6 further licence agreements with overseas firms raised the latter total to 53. At the end of the year the Corporation was providing financial and other assistance for 35 development projects, of which 5 were new, and expenditure on development was £1,099,030, compared with £811,730 in 1960–61, and forward commitments amounted to £850,000. Most of this expenditure was concerned with the hovercraft, electronic computer (*Atlas* and *Emidec*) and *Dracone* projects. Some £140,000 was written off when work was stopped on a number of projects.

Of 911 inventions communicated to the Corporation during the year (compared with 973 in 1960–61), 161 were from universities in the United Kingdom (115 in 1960–61), but only 257 were from Government Departments and Research Councils, compared with 312. For nationalized industries the figure was 19 (2), industrial research association 11 (8), and for private firms and individuals in the United Kingdom 406 (466). At the end of the year 23 potential development projects in a wide range of technological fields were under consideration, more than half of these deriving from private firms and individuals. Of 243 patent rights assigned during the year, 167 were from Government Departments and Research Councils and 69 from universities. The new projects reported for the first time include equipment for the rapid and automatic analysis of raw milk and other emulsions; development of a refrigerator for temperatures in the region 4°–20° K using expansion turbines with helium as the working substance and running on helium bearings; *JASTAC* fastening techniques; multiple fixed speed motors; and water control in arid zones.

THE INSTITUTE FOR NORTHERN STUDIES, UNIVERSITY OF SASKATCHEWAN

THE Institute for Northern Studies, University of Saskatchewan. was authorized and the director appointed in January 1960 to aid original research and academic work on northern problems and to assist the training of humanists, scientists, engineers and others interested in northern problems and in the inhabitants of this part of Canada. The Institute possesses no research staff of its own, and research will be carried out under the supervision of the various University departments.

The first annual report of the Institute* summarizes the work carried out during the summer of 1960 on seven projects: a plant ecology study, with special reference to the forest vegetation; a systematic collection of small mammals and another of biting insects; a study of the history and economics of water transport on the Athabasca–MacKenzie River system and another of some factors in the economics of the Lake Athabasca mining area; a study of the trace elements in the Coronation mine, Flin Flon, and one of a radio-communication system in the very-high-frequency field which, it is believed, will operate even under conditions where electric storms are prevalent.

Further accounts of these projects are included in the second annual report, 1961–62†, as well as ornithological studies in the Lake Athabasca district; the commercial fishing industry of Northern Saskatchewan; Saskatchewan's role in a changing fur industry; an investigation of the sand dunes in the vicinity of the William and McFarlane Rivers on the south shore of Lake Athabasca; and of the deep seismic sounding of the Earth's crust in the vicinity of Flin Flon. A list of publications resulting from work assisted by the Institute is included.

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* Institute for Northern Studies, University of Saskatchewan. First Annual Report, 1960–1961. Pp. 11. (Saskatoon, Canada: Institute for Northern Studies, The University, 1961.)

† Institute for Northern Studies, University of Saskatchewan. Second Annual Report, 1961–1962. Pp. 12. (Saskatoon, Canada. Institute for Northern Studies, The University, 1962.)

MAGNETO-TELLURIC EFFECTS OBSERVED IN DALLAS, TEXAS, FROM THE JULY 9, 1962, HIGH-ALTITUDE NUCLEAR EVENT

By JACK J. BURCH and ARTHUR W. GREEN, Jun.

Texas Instruments Incorporated, Dallas, Texas

THE U.S. Atomic Energy Commission detonated a megaton-range nuclear device over Johnston Island at an altitude of 250 miles on July 9, 1962, at about 09 h 00 min 09 sec, U.T.

It was expected that magnetohydrodynamic waves generated by the expanding blast plasma would propagate in various modes across and down the lines of force of the Earth's field, as well as through the exospheric ducts postulated by Bomke *et al.*[1]. Expected velocities of propagation range from several hundred to several thousand kilometres per second[2]. At the base of the ionosphere, where magnetohydrodynamic wave propagation is no longer possible, some of the wave energy would be converted to ordinary electromagnetic waves which would then propagate around the world at the speed of light. Thus, the electromagnetic waves resulting from the various magnetohydrodynamic modes would be seen by magnetometers and Earth current (telluric) sensors several seconds after the detonation. In addition to these delayed signals, Troitskaya (U.S.S.R.), during the 1958 *Argus* test series, observed direct, speed of light, signals which marked the instants of the events[3]. Because these instantaneous signals suffered great attenuation in travelling as electromagnetic waves through the highly conductive ionosphere, they were seen only on very sensitive Earth current systems.

Fig. 1 shows magnetic field and electric field variations at Dallas, Texas, produced by the event of July 9, 1962. The magnetic field was measured with an optical pumping magnetometer (metastable helium type) and the electric field was measured by orthogonal pairs of Earth current electrodes aligned in magnetic north–south and east–west directions.

The main phase magnetohydrodynamic wave-derived signal which arrived at 09 h 00 min 11 sec, two seconds after the detonation, can be seen very clearly on trace 2, which is the north–south telluric field (bandpassed from 0·01–2·0 c/s), and on trace 3, which is the total magnetic field (bandpassed from 0·3–2·0 c/s). It is interesting to note that the total field record (trace 3) and the east–west electric field (trace 4) correlate quite well. This is to be expected since a bandpassed total field magnetometer measures, to a good approximation, the dot product of the geomagnetic micropulsation vector and the Earth's total field vector; thus in a region of horizontal homogeneity, and for vertically incident waves, the magnetic wave components are in direct proportion to the orthogonal electric wave components, and a total field magnetometer will measure the north–south horizontal magnetic component times a constant. Since horizontal homogeneity is thought to exist at the Dallas site, traces 3 and 4 probably represent orthogonal *E* and *H* components of the same electromagnetic wave. The nominal period of this wave was about 2 sec.

The 'first arrival' noted on trace 2 of Fig. 1 which propagated at the speed of light, and thus marks the time of the event, may be seen more clearly on traces 1 and 2 of Fig. 2. A similar instantaneous arrival was also noted by Roquet, Schlich and Selzer[4].

Fig. 3 is a display of instantaneous power in the east–west telluric field as a function of both frequency and time for an interval of several hours which includes the nuclear event. The sinusoidal variations before and after the event are typical of the 60-sec period micropulsation

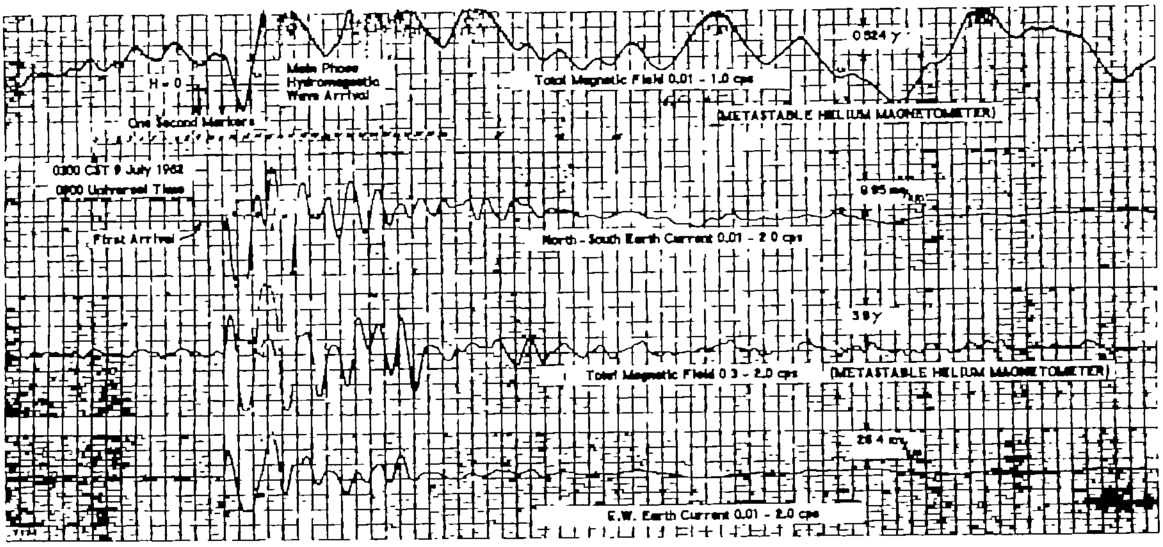


Fig. 1. Magneto-telluric effects of the Johnson Island high-altitude nuclear event of July 9, 1962, recorded at Texas Instruments Geomagnetic Observatory near Dallas, Texas (The direction of increasing magnetic field and positive north and east electrodepotentials is downward)

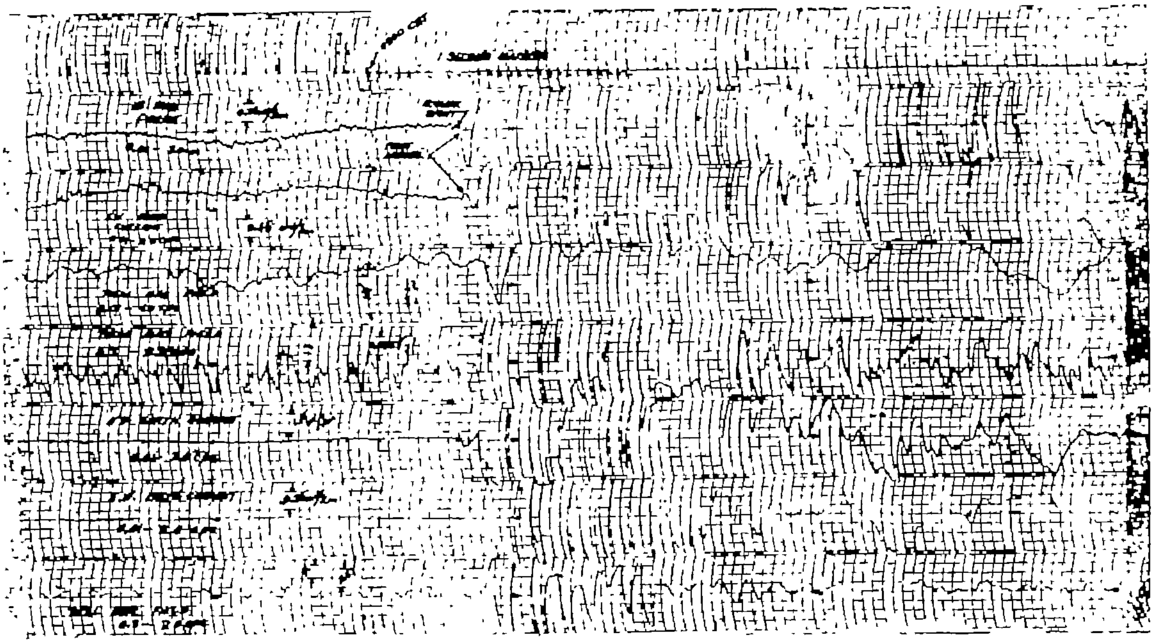


Fig. 2. High-resolution trace showing details of first arrival (speed of light)

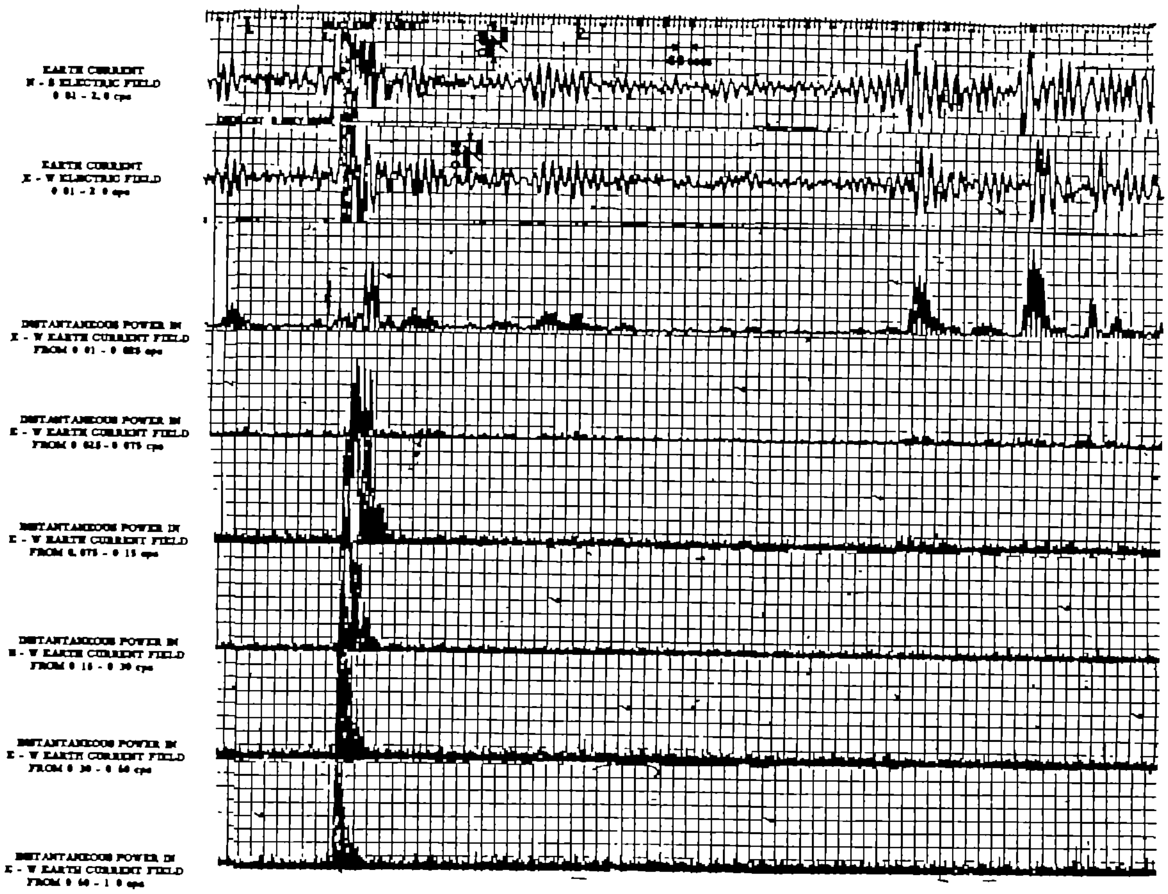
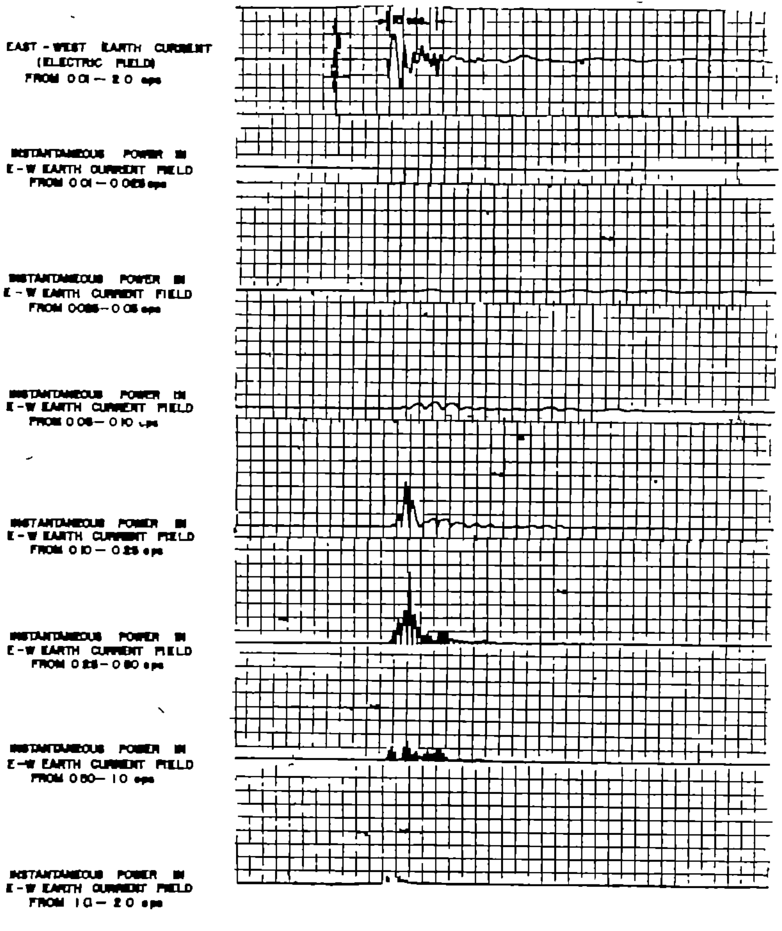


Fig. 3. Instantaneous power as function of frequency and time for an interval including the July 9, 1962, nuclear event and background detail



activity seen in Dallas at night. It may be seen from Fig. 3 that the main spectral elements of the micropulsation activity are concentrated well below those of the bomb signal.

Fig. 4 shows instantaneous power as a function of both time and frequency for the bomb signal only, as seen on the east–west telluric field. The predominant 2-sec period of the magneto-telluric wave is attested to by the large amount of energy concentrated in the band from 0·25 to 0·50 c/s. This tendency toward 2-sec periodicity was also noted by Roquet *et al.*[4] and by Plesset[5].

Irregular magnetic field activity having no apparent electric field counterpart was observed on the low-frequency magnetic record (Fig. 1, trace 1). This trace differs only from trace 3 in that it is bandpassed from 0·01 to 1·0 c/s instead of from 0·3 to 2·0 c/s. Similar activity was also noted by Breiner[6], who reconstructed some rubidium vapour magnetometer records on which the strong signal had exceeded the dynamic range of the recording equipment. Breiner reported oscillations with periods of 5–7 sec, but apparently failed to see the 2-sec period, magneto-telluric wave. This failure was undoubtedly due to saturation of the recorder rather than any fundamental lack of high-frequency response in the rubidium vapour device.

Fig. 4. Instantaneous power as a function of frequency and time for the signal from the nuclear event of July 9, 1962

We thank Mr. Charles E. Lewis and Mr. Charles M. Tilley, who were responsible for much of the recording site instrumentation.

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SEASONAL VARIATION OF TURBULENCE IN THE UPPER ATMOSPHERE

By R. G. ROPER and Dr. W. G. ELFORD

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THE measurement of the motion of the atmosphere at heights between 70 and 110 km by the radio observations of meteor trails is now a well-established technique. Systematic observations have given accurate data on the diurnal and seasonal variation of the air motion at these levels in medium and high latitudes[1-3].

By using one transmitting site and three or more receiving sites the relative motion of three or more points on the same meteor trail can be studied. In the system at present in operation at Adelaide four receiving sites are used; receiving sites 2 and 3 are 4·8 km north and east of receiving site 1, and the fourth is at a distance of 14 km approximately south-east of site 1. The transmitter is 23 km south of site 1. The equipment is a combination of continuous wave and pulse, operating on 27 mc/s.

The orientation of the trail in space is determined by the time differences between the commencement of an echo at the receiving sites 1, 2 and 3, and the velocity of formation of the trail is obtained from the diffraction wave-form associated with the build-up of the echo at any one of the receiving sites. Thus the spacial separation of the four reflexion points can be determined for each meteor trail observed.

The absolute position of one of the reflexion points is measured from site 1, thus fixing the absolute position of the trail in the atmosphere.

During 1961 a turbulence survey was run in conjunction with the routine wind velocity measurement programme which has been in operation since 1952. This wind programme has shown that winds over the height range of 75–105 km are well correlated; the method of data collection and reduction ensures the measurement of a true mean motion. The subtraction of the mean wind component (given as a function of time and height) in the direction of measurement yields four turbulent line-of-sight velocities for each meteor trail observed. Preliminary computations have been carried out using data from the three close-spaced stations. The final analysis will utilize the data from all four receiving sites, and will extend the direct investigation of eddy sizes up to 7 km.

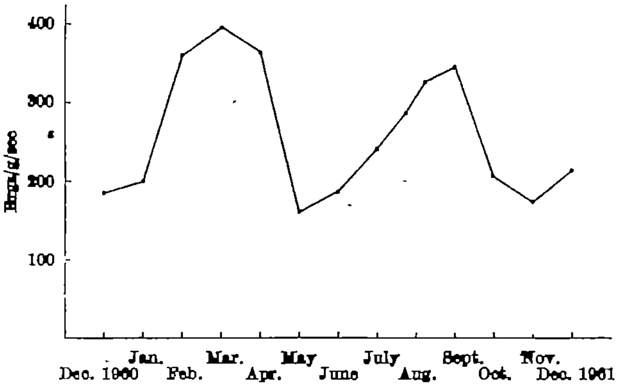

Fig. 1. Seasonal variation of the turbulent dissipation rate at 90 km

The magnitude of the turbulent component of the wind velocity has been found to vary from some 15 m/sec to 60 m/sec, and is, in general, less than 1/3 of the magnitude of the mean wind.

The variation of the differences in velocity at two points on a meteor trail as a function of their separation can be explained in terms of a modified Kolmogoroff spectrum, at least for the scales measured (up to 3 km). The departures from the spectrum of an isotropic inertial region can be explained in terms of a buoyancy sub-range of characteristic scale 0·5–1·0 km. Once allowance is made for this buoyancy sub-range, the velocity differences measured as a function of separation r follow the relation:

$$\overline{[u(x) - u(x + r)]^2} = 4{\cdot}82\alpha(\varepsilon r)^{2/3} \text{ (ref. 4)}$$

where ε is the rate of dissipation of turbulent energy and α is the constant of proportionality in the expression relating the energy spectrum function to the wave-number space vector $\mathbf{k}$, namely:

$$E(k) = \alpha\varepsilon^{2/3}k^{-5/3}$$

To determine an absolute value for ε from the velocity differentials, α has been taken as 1·0.

The turbulent dissipation rates shown in Fig. 1 represent an average for each month over the height-range of 75–105 km. The seasonal variation in the turbulent dissipation is quite marked, ε having a minimum value of some 150 ergs/g/sec in summer and winter, and rising to over 350 ergs/g/sec in autumn and spring. Diurnal variations have also been observed. The magnitude of these diurnal variations is dependent on the season, but as yet no simple daily pattern has been found. Further analysis to examine the turbulent motions as a function of height is contemplated.

The velocity differences at separations less than 500 m are affected most by the action of buoyancy forces. If the buoyancy forces are neglected and the velocity differences at these scales as measured at Adelaide are interpreted in terms of an isotropic inertial spectrum, values of turbulent dissipation of 30–100 ergs/g/sec are forthcoming. These are in excellent agreement with the results obtained from photographic observations of meteor trails[5] and sodium trails[6] which are limited by visibility to scales of < 1 km.

While the mean squares of the velocity differences associated with spacial separation follow the Kolmogoroff 2/3 power law, those associated with height difference, regardless of separation, follow the 4/3 power law characteristic of a turbulence subjected to shear. Zimmerman[7] has pointed out that similar velocity-height differentials measured by Blamont and de Jager follow the shear theory developed by Tchen[8], which predicts a 4/3 power law. Extrapolation of the height shears as measured at Adelaide to a velocity difference equal to the characteristic velocity of the energy-bearing eddies of the Kolmogoroff spectrum yields a vertical correlation distance of 5–9 km, which is quite comparable with the generally accepted value of 7 km.

This work is being supported by the Radio Research Board, Australia.

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FRACTIONATION PHENOMENA IN HIGHLY RADIOACTIVE FALL-OUT PARTICLES

By T. MAMURO, K. YOSHIKAWA, T. MATSUNAMI, A. FUJITA and T. AZUMA

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HIGHLY radioactive fall-out particles were found throughout Japan, resulting from the series of large-scale nuclear test explosions carried out by the U.S.S.R. during September and October 1961 [1-3]. It was revealed by γ-ray spectrometry that radiochemical compositions of these particles were different from those of smaller fall-out particles. This was considered to have been caused by the so-called fractionation effect[7-10]. In order to express the extent of the fractionation effect, a fractionation factor f for a given nuclide A is defined in the following way:

$$f = \left[\frac{N(\mathrm{A})}{N(^{95}\mathrm{Zr} + {}^{95}\mathrm{Nb})}\right]_{\mathrm{exp}} \times \left[\frac{N(^{95}\mathrm{Zr} + {}^{95}\mathrm{Nb})}{N(\mathrm{A})}\right]_{\mathrm{theor}} \qquad (1)$$

where N is the radioactivity of the nuclide in question. The first bracket contains the experimental and the second the theoretical values as obtained from fission yield values if the age is known[9].

Previously[3] we showed the γ-ray spectrum of the filter-paper on which air-borne dusts were collected on November 8, 1961, and that of a highly radioactive particle which was found in the yard of our Laboratory at that time. On comparison of these spectra, it was found that the radiochemical composition of the highly radioactive particle was much enriched in ^{95}Zr + ^{95}Nb and impoverished in ^{103}Ru, ^{140}Ba + ^{140}La, ^{131}I and ^{141}Ce + ^{144}Ce, compared with that of the dusts on the filter-paper.

Fig. 1 is another example from which the fractionation phenomenon can be seen clearly. Curves (*a*) and (*b*) are the γ-ray spectrum of a highly radioactive particle and that of the dust sample (*b*) which was taken in an air-filter in February 1962, respectively. Curve (*c*) is the γ-ray spectrum of the dust sample (*c*) which was prepared in the following way. The dusts which were taken in the same air-filter were spread uniformly on a gummed paper and this was autoradiographed on an X-ray film (Fuji X-ray Film No. 200) exposing for two days. On the autoradiograph were found many relatively small spots. Only the parts of the gummed paper which did not produce any spot on the autoradiograph were punched out. The dust sample (*c*) was the collection of the punched small pieces of gummed paper. This dust sample, therefore, was not thought to contain any fall-out particles so high in radioactivity as to produce a visible spot on the autoradiograph. On comparison of (*a*) with (*c*), it is clearly seen that the radiochemical composition of the highly radioactive particle is strongly enriched in ^{95}Zr + ^{95}Nb and impoverished in ^{103}Ru and ^{141}Ce + ^{144}Ce. On careful comparison of (*b*) with (*c*), it is seen that the radiochemical proportions of ^{103}Ru and ^{141}Ce + ^{144}Ce in the dust sample (*b*) are somewhat smaller than those in the dust sample (*c*). Photo-peaks of ^{131}I and ^{140}Ba + ^{140}La are not seen in these spectra, which were measured in the middle of March 1962. The peak found at about 210 keV is mainly due to the back-scatter peak produced by the γ-rays of about 750 keV of ^{95}Zr + ^{95}Nb and the photo-peak of 231 keV of ^{95}Nb.

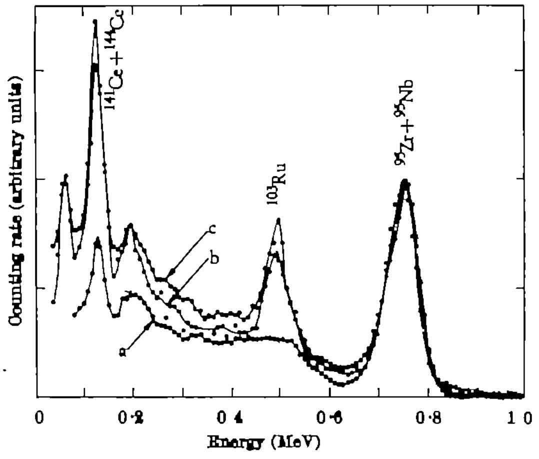

Fig. 1. (*a*) γ-Ray spectrum of a highly radioactive particle (Particle *P* in Table 1), measured on March 13, 1962; (*b*) γ-ray spectrum of the dust sample collected at an air-filter, measured on March 13, 1962; (*c*) γ-ray spectrum of the dust sample which was prepared by excluding larger fall-out particles, measured on March 16, 1962

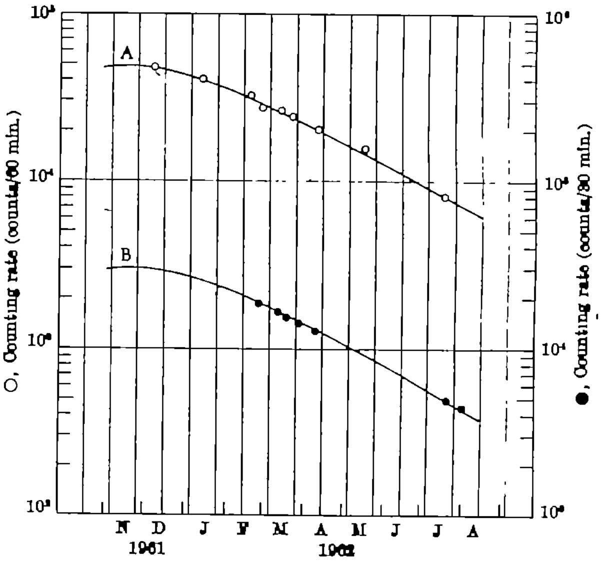

Fig. 2. Decay characteristics of activity of ^{95}Zr + ^{95}Nb. ○, Particle *P*; ●, dust sample.

The decay characteristics of activity of ^{95}Zr + ^{95}Nb were followed for the particle and the dust sample by measuring the photo-peak of about 750 keV, the measured points being expressed as white and black circles in Fig. 2,

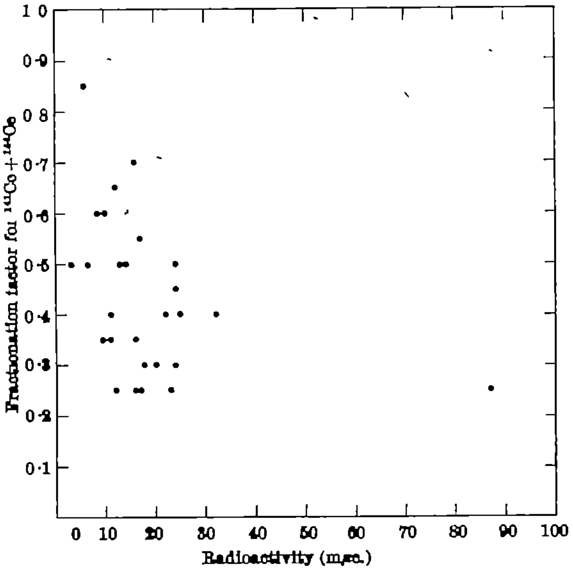

Fig. 3. Relation between particle radioactivity and fractionation factor for ^{141}Ce+^{144}Ce

respectively. Curves *A* and *B* are the decay curves which were theoretically obtained assuming the date of explosion was October 30, 1961. The good agreement between theory and experiment indicates that both the particle and the majority of the radioactive dusts contained in the dust sample were produced on October 30, 1961.

γ-Ray spectra of many highly radioactive particles were measured and fractionation factors defined by equation (1) were calculated. The results are summarized in Table 1. The yield values for fast fission of ^{238}U, as shown in Table 2 (refs. 8 and 11), were used on carrying out the calculation. The date of explosion was assumed to be October 30, 1961, because almost all the particles given in Table 1 were thought to have been produced on that day. The half-lives of ^{131}I and ^{140}Ba + ^{140}La are so short that fractionation factors for them could not be measured. The highly radioactive particles Nos. 1–21 in Table 1 were actually isolated from among non-radioactive dust particles and examined microscopically[9].

Table 1. Fractionation Factors

Sample No.	Estimated β-activity at the beginning of Nov. 1961 (mμc.)	Particle diameter (μ)	Fractionation factor Assumed date of explosion. Oct. 30, 1961	
			^{141}Ce + ^{144}Ce	^{103}Ru
No. 1	24	10·1	0·45	~0
No. 2	25	10 4	0·40	~0
No. 3	16	8·7	0 25	~0
No. 4	87	13·9	0 25	~0
No. 5	18	9·8	0·30	~0
No. 6	13	9·0	0 50	~0
No. 7	22	10·1	0·40	~0
No. 8	20	11 8	0·30	~0
No. 9	6·3	8·4	0·50	~0
No. 10	23	11·3	0·25	~0
No. 11	14	9·0	0 50	~0
No. 12	16	9·2	0·70	~0
No. 13	17	12 1	0 55	~0
No. 14	5·8	6·9	0·85	~0
No. 15	16	10·1	0·35	~0
No. 16	24	10·4	0·50	~0
No. 17	32	10·1	0·40	~0
No. 18	12	8·1	0·65	~0
No. 19	8·3	8·7	0·60	~0
No. 20	10	8·7	0·60	0 02
No. 21	11	10·4	0·35	~0
L	17	—	0·25	0 07
M	11	—	0·40	0 04
N	9·6	—	0·35	~0
O	13	—	0·50	~0
P	24	—	0·30	0·04
Q	12	—	0·25	~0
R	3 0	—	0·50	0 08
The dust sample the γ-ray spectrum of which is given in Fig. 1 (*b*).			0 85	0·45
The dust sample the γ-ray spectrum of which is given in Fig. 1 (*c*)			1·00	0 55

Table 2. Assumed Fission Yield for Fast Fission of Uranium-238

Nuclide	Yield (per cent)
^{95}Zr	5·7
^{103}Ru	6·6
^{141}Ce	5 6
^{144}Ce	4·5

Fractionation factors for ^{103}Ru are smaller than 0·1 for all the particles and those for ^{141}Ce + ^{144}Ce are smaller than 0·6 for almost all of them. Fig. 3 shows the relation between particle activity and fractionation factor for ^{141}Ce + ^{144}Ce, indicating roughly that particles of higher activity are more affected by the fractionation effect. The fractionation factors obtained for the dust sample (*b*) and (*c*) are shown at the bottom of Table 1.

The fractionation factor for ^{103}Ru of the dust sample (*c*) is about 0·6, while that for ^{141}Ce + ^{144}Ce is 1·0, suggesting that the fractionation effect for ^{103}Ru is present even in this dust sample, which contains only fine fall-out particles. If the yield values given in Table 2 are reasonable, this may be possibly explained in the following way. The dust sample (*c*) was taken at a wire-mesh air-filter through which very fine fall-out particles might pass out. The distribution of fall-out particles in the dusts caught by the air-filter might be biased towards larger size. The fractionation effect is not only much more remarkable but also seems to extend to much smaller particles for ^{103}Ru than for ^{141}Ce + ^{144}Ce.

There was a rainfall of 37·6 mm at our Laboratory on September 17, 1961. About 100 ml. of rain-water was collected at the beginning, using a rain collector of 1,000 cm^2 in collecting area. The concentration of radioactivity in this rain-water was abnormally high compared with that in average rain-water. Curve *X* in Fig. 4 is its γ-ray spectrum. The γ-ray spectrum of average rain-water of about 30 l. on the same day was measured and is shown as curve *Y*. An appreciable difference is seen between the two spectra, suggesting that some highly radioactive particles were present in the rain-water collected at the beginning of the rain-fall. It may be generally considered that from a test sample of small quantity in which the fractionation effect is remarkable a higher measured value of the concentration of fall-out may be obtained than that which would be obtained from an average sample of much larger quantity. It may be also possible that rain-water at the beginning of a rain-fall has not only a higher concentration of fall-out than that of average rain-water, but also shows a radiochemical composition different from that of average

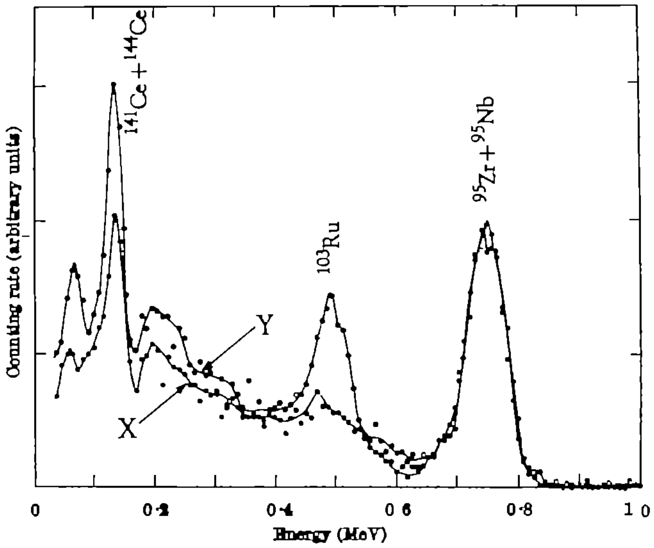

Fig. 4. Fractionation effect observed in rain-water on November 17, 1961, measured on February 22, 1962. ○, Average rain-water; ●, rain-water at the beginning of the rainfall

rain-water, especially just after a large-scale nuclear detonation.

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COMPACTION OF BURIED SANDS

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MAXWELL and Verrall[1] have investigated the consolidation of quartz sand to sandstone under experimental conditions simulating deep burial. Compaction was produced by compressing a sample in a steel cylinder with a tightly fitting piston driven by a hydraulic jack while a second hydraulic system maintained a high hydrostatic pressure inside the cylinder. Cementation was produced by keeping the specimen at high pressure and temperature for several days. Their results for the compaction of sand at room temperature and one atmosphere fluid pressure for various directed pressures show a steadily decreasing fall in the porosity from about 35 per cent at 10,000 lb./in.^{2} to about 25 per cent at 50,000 lb./in.^{2}. At 50,000 lb./in.^{2}, which represents a depth of about 45,000 ft. the rate of decrease of porosity with increase in depth is zero.

Jane Taylor[2] has suggested that for the efficient investigation of the factors producing consolidated sediments each variable must be investigated separately. The behaviour of sand under a range of directed pressures has been examined using a simplified version of the Maxwell and Verrall apparatus. The fluid pressure has been neglected and the compaction of the specimen carried out by a standard hydraulic testing machine (Amsler *SZBDA* 699) which gives a steady rate of deformation with the load shown as the dependent variable. The experiments were designed to show the nature of the deformation process rather than the change in porosity. Borg and Maxwell[3] have examined the nature of the deformed product but have ignored the variation in particle size caused by the various pressures. The simulated weight of overburden has been so great in previous experiments that specimens have been virtually completely deformed although possibly incompletely compacted and cemented.

The stages in the deformation process are illustrated by a typical load versus deformation (piston travel) curve as shown in Fig. 1. This can be divided into three stages; stage I represents elastic compression and the beginning of yield; stage II represents the continued breaking of the sand grains which diminishes as the pores are filled with the fracture product; stage III occurs when the pores are filled and the aggregate becomes effectively a solid mass. The sand was compressed in a 2-in.-diameter steel cylinder by a steel piston. Samples of 100 g were used and various loads were applied up to a pressure of 20,000 lb./in.^{2}. The sand used was quartz from Leighton Buzzard which had been graded so that 95·5 ± 2 per cent passed a No. 16 and was retained by a No. 18 B.S. sieve. Before each test a sieve analysis was carried out to ensure the correct initial grading, the sand was then compressed at a chosen pressure and analysed again. The results of the analyses after deformation, for three particular sizes, are shown in Table 1.

Fig. 2 shows the variation in amount of these three sizes plotted against load. The 18-sieve curve shows a point of inflexion at the load value representing the transition

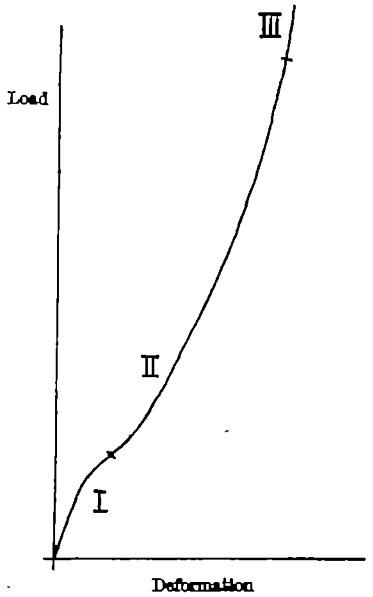

Fig. 1. Shape of load versus deformation curve for sands compacted by direct pressure only

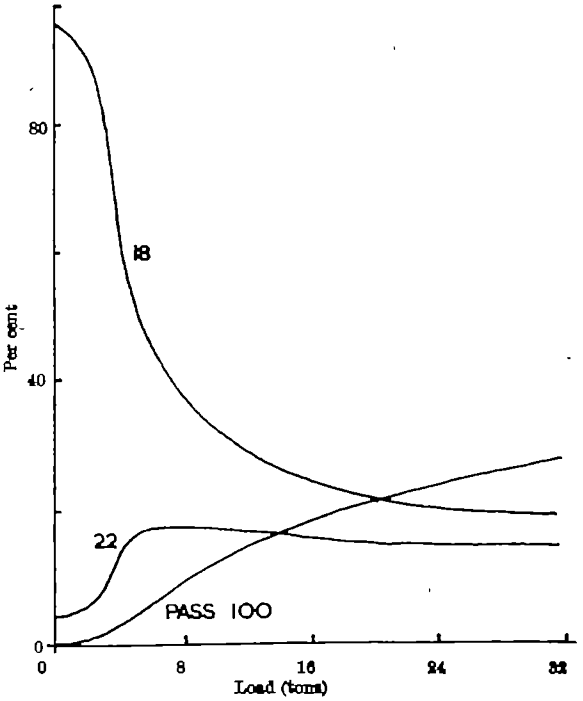

Fig. 2. Variation in particle size percentage in sands compacted at different pressures

Table 1. PARTICLE SIZES IN COMPACTED SANDS

Compacting load (tons)	Compacting pressure (lb./in.²)	% on 18 sieve	% on 22 sieve	% passing 100 sieve
0	0	95·5 ± 2	4·0 ± 2	0
2	1,426	91·0	5·5	0·5
4	2,852	61·0	15·5	2·5
6	4,278	45·5	18·5	7·5
8	5,704	37·0	17·5	10·5
10	7,130	32·0	18·0	12·5
14	9,982	28·0	16·5	17·5
26	18,538	20·5	15·5	25·5
29	20,677	20·5	15·5	27·5

from type I deformation to type II. The pass 100 curve steadily increases. The 22-sieve curve flattens out very early and remains at a fairly constant percentage value. It appears from these curves that the sand particles which break form a very fine fracture product and it is this which fills the pores during stage II of the deformation. Also it appears that 20 per cent is a limiting value of the 18-sieve curve; further increases of pressure will not change the amount of the starting size sand. Assuming that the initial porosity was of the order of 35 per cent measurements of the volume of the specimen after compression show that a porosity of the order of 25 per cent is produced by a pressure of about 20,000 lb./in.².

Tests have also been carried out to see if different starting sizes in the sands cause any noticeable variation in the pressure required for various stages of the deformation process. Table 2 shows the pressure at the I–II transition point in sands with different starting sizes.

Table 2. I–II TRANSITION PRESSURES IN COMPACTED SANDS

B.S. Sieve Nos.	14–16	16–18	22–25	25–30	30–36	44–52
I–II Pressure	2,570	2,670	3,200	3,750	4,050	4,800

There is a steady increase in the I–II transition pressure with decreasing particle size over the range examined.

[1] Maxwell, J. C., and Verrall, P., *World Oil*, 138, No. 5, 106; No. 6, 102 (1954).
[2] Taylor, Jane M., *Bull. Amer. Assoc. Petroleum Geol.*, 35, 701 (1950).
[3] Borg, I. Y., and Maxwell, J. C., *Amer. J. Sci.*, 254, 71 (1956).

A VISCOSITY/DENSITY RELATION FOR SOLUTIONS OF STRONG ELECTROLYTES IN WATER

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THE relative viscosity (η/η_w) of electrolyte solutions can be described empirically by the well-known Jones and Dole equation:

$$\frac{\eta}{\eta_w} = 1 + A\sqrt{c} + Bc$$

where c is the concentration of electrolyte in moles/l., and A and B are empirical constants for a given electrolyte.

Falkenhagen and his collaborators related the term $A\sqrt{c}$ to the electrostatic interactions of the ions. Although the coefficient B has received a great deal of attention from various workers, it has not as yet, in our view, been satisfactorily accounted for. Thus Kaminsky[1] explains it in terms of structure-making and structure-breaking tendencies of the various ions; Robinson and Stokes[2] interpret it through the Einstein thickening effects of hydrated ions; Gurney[3] considers it as the result of a loosening of the water structure in the neighbourhood of an ion.

All such explanations imply that the presence of ions will change the specific volume of the water fraction of the solution. The object of this article is to present a correlation between the volume change of the water fraction, represented by a parameter α, and the viscosity B-coefficient.

The parameter α I define as:

$$\alpha = \frac{\bar{V}_2^\circ - V_s^\circ}{1{,}000}$$

where $\bar{V}_2^\circ$ is the partial molar volume of the salt at infinite dilution, and V_s° is the intrinsic volume of the salt and is calculated from the van der Waals radii of the ions, as given by Pauling[4]. Since both $\bar{V}_2^\circ$ and V_s° are additive properties of the individual ions it is possible to calculate α for the individual ions. Values of α for a number of ions at 25° C have been obtained from published data on partial molar volumes[5]. (The partial molar volumes given in reference 5 were calculated by taking $\bar{V}_2^\circ$ = 18·0 c.c./mole for the chloride ions.) The B-coefficients are also additive. Kaminsky[1] gives the B-coefficients of many ions at 25° C based on $B = -0{\cdot}0070$ for the chloride ion.

Our correlation is shown in Fig. 1. Here the values of α are plotted against values of the term ($B - V_s^\circ/400$). There is a very strong correlation which is independent of the sign and size of the charges on the ions.

Since the line in Fig. 1 passes through the origin the correlation embodies only one empirical constant, equal to the slope of the line. The correlation can be used for calculating the B-coefficient of the viscosity equation for a salt solution at concentrations up to about 1 mole/l. from known values of the partial molar volumes and the van der Waals radii of the ions.

We arrived at the foregoing correlation by making the following four assumptions.

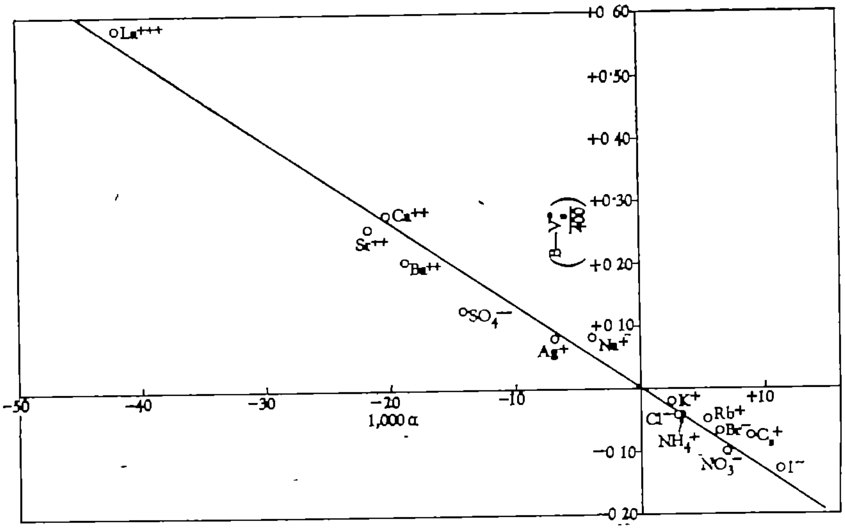

Fig. 1. Correlation between change of volume of water fraction (α) and the viscosity B-coefficient

(1) That the molar volumes of both the salt and the water fraction in a solution can be represented as a function of concentration by the equations:

$$V'_w = V_w\,(1 + \alpha c) \qquad (1)$$

$$V'_s = V_s^{\circ}\,(1 + \beta c) \qquad (2)$$

where V_w is the molar volume of pure water; V_s° is the molar volume of the salt in solution at infinite dilution; α is the expansion coefficient of the water; β is the expansion coefficient of the salt.

Although on purely phenomenological grounds the volume of a solution cannot be split up into volumes occupied by the two components, such a separation is justifiable on the basis of a particular model such as I am using here.

α and β are constant for any given salt. The α-coefficient will take into account changes in the volume of the water fraction caused by such factors as electrostriction, changes in the amount of ice-like structure, etc. The β-coefficient will account for the changes in the volume occupied by the salt in solution caused by such effects as ion-pair formation.

(2) That V_s° is equal to the volume calculated from the crystal radii of the ions. The view that an ion in solution occupies a volume equal to that of a sphere of radius approximately equal to the crystal radius is supported by the refractive index studies of Böttcher[6]. For example, Böttcher calculates a value of 1·82 Å for the radius of the chloride ion in solution, which is in good agreement with Pauling's value of 1·81 Å obtained from the crystal data.

(3) That Einstein's equation can be applied to ions. Robinson and Stokes[2] applied Einstein's equation to calculate the volume fraction φ occupied by the ions from the B-coefficients, assuming the viscosity of the water fraction is unchanged. They concluded that since φ was usually larger than the partial molar volumes, the ions must be hydrated. I differ from Robinson and Stokes in that I do not believe that the whole of the B-coefficient is accounted for by Einstein thickening but that the viscosity of the water fraction η^{*} is changed and is not equal to the viscosity of pure water η_w. In order to calculate the relative viscosity of the water from the B-coefficient I use the intrinsic volume of the ions V_s° (assuming no hydration).

Einstein's equation incorporating the above assumptions becomes:

$$\eta = \eta^{*}\left(1 + \frac{V_s^{\circ}.c}{400}\right)$$

where η is the observed viscosity, and η^{*} is the viscosity of the water fraction. Thus, for a solution:

$$\frac{\eta^{*}}{\eta_w} = \frac{1 + Bc}{1 + V_s^{\circ}.c/400} \simeq 1 + Bc - V_s^{\circ}.c/400$$

The errors introduced by the foregoing approximation are usually negligibly small at concentrations up to 1 mole/l. In the worst case, the approximation introduces an error of only 0·009 into the calculated value of η^{*}/η_w.

Thus at a nominal concentration of 1 mol/l.:

$$(B - V_s^{\circ}/400) = \frac{\eta^{*}}{\eta_w} - 1$$

that is, the factor $(B - V_s^{\circ}/400)$ represents the change in the relative viscosity of the water fraction.

(4) That the relative viscosity of the water fraction is a function only of the amount of expansion or contraction that the water fraction undergoes.

According to equation (1) the relative amount of expansion of the water fraction is given by:

$$\frac{V'_w - V_w}{V_w} = \alpha c \qquad (3)$$

Therefore, at a concentration of 1 mole/l., α gives a direct measure of the relative expansion or contraction the water fraction has suffered.

According to equations (1) and (2) the volume V of a solution containing m_1 moles of water and m_2 moles of salt is given by:

$$V = m_1 V_w\,(1 + \alpha c) + m_2 V_s^{\circ}\,(1 + \beta c) \qquad (4)$$

Equation (4), on differentiating partially with respect to m_1 and m_2, yields the partial molar volumes of the water and the salt, respectively $\bar{V}_1$ and $\bar{V}_2$, in terms of α, β, m_1 and m_2. Taking the limiting values as $m_2 \rightarrow 0$ we obtain the partial molar volumes at infinite dilution $\bar{V}_1^{\circ}$ and $\bar{V}_2^{\circ}$, these are found to be:

$$\bar{V}_1^{\circ} = V_w \qquad (5)$$

$$\bar{V}_2^{\circ} = V_s^{\circ} + 1000\,\alpha \qquad (6)$$

Hence α can be obtained from the partial molar volume data at infinite dilution by using the equation:

$$\alpha = \frac{\bar{V}_2^{\circ} - V_s^{\circ}}{1000}$$

Thus the correlation shown in Fig. 1 is a plot of the change in the relative viscosity of the water fraction against the relative expansion of the water fraction. This interpretation leads to the conclusion that salts which expand the water fraction decrease the viscosity of the water fraction and vice versa.

The correlation can be used for testing the validity of the various theories of the viscosities of solutions.

For example, Robinson and Stokes interpret the viscosities of aqueous solutions of electrolytes in terms of the Einstein thickening effect of hydrated ions. However, my correlation shows that ions which increase the volume of the water fraction (that is, α-positive), lie on the same straight line as ions which decrease the volume (α-negative). This suggests that the same mechanism operates for all ions regardless of whether they increase or decrease the viscosity of the solution. Hence, although Einstein thickening by hydrated ions could explain the viscosity data for ions which increase the viscosity, Robinson and Stokes's theory is incapable of explaining the data for the ions which decrease the viscosity.

On the other hand, Kaminsky supposes that salts which increase the viscosity increase the amount of ice-like structure. They would, therefore, be expected to increase the specific volume of the water fraction. However, my correlation shows that those salts which increase viscosity decrease the specific volume, in direct contradiction to Kaminsky's supposition.

Fig. 1 includes all the ions for which the data were available with the exception of the lithium ion. This point was omitted because it lies outside the normal scatter about the correlation line. α × 1000 for the lithium ion is − 1·6 and $(B - V_s^{\circ}/400)$ is 0·1480. The interpolated value of $(B - V_s^{\circ}/400)$ corresponding to this α value is, from the correlation line, equal to + 0·02. Lithium could be brought into line if it were assumed that its ions are strongly hydrated, and hence that the volume that should be used for the Einstein correction is not that of the bare ion but the volume of the ion plus its solvated water molecules, $V_s^{\circ\prime}$. Hence $(B - V_s^{\circ\prime}/400) = 0{\cdot}02$. From this it is found that $V_s^{\circ\prime} = 51{\cdot}2$ c.c./mole. Since $V_s^{\circ} = 0{\cdot}60$ c.c./mole, this difference is accounted for if 2·8 water molecules are strongly attached to each lithium ion.

I thank Mr. R. P. Bell for advice and criticism.

[1] Kaminsky, M., *Faraday Soc. Disc.*, **24**, 171 (1957).

[2] Robinson, R. A., and Stokes, R. H., *Electrolyte Solutions*, 303 (Butterworths Scientific Publications, 1959).

[3] Gurney, R. W., *Ionic Processes in Solution*, 166 (McGraw-Hill Publishing Co., 1953).

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[5] Moelwyn-Hughes, E. A., *Physical Chemistry*, 851 (Pergamon Press, 1957).

[6] Böttcher, C. J. F., *The Theory of Electric Polarisation*, 279 (Elsevier Publishing Co., 1952).

THYMOL BASIC ETHERS AND RELATED COMPOUNDS: CENTRAL NERVOUS SYSTEM DEPRESSANT ACTION

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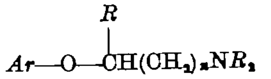
During routine toxicity investigations of the well-known drug β-dimethylaminoethyl thymol ether ('Tastromine') and its homologue β-diethylaminoethyl thymol ether (also known as '*F.929*') conducted in these laboratories in 1958, it was observed[1] that the animals (mice) became sedated. Further pharmacological investigation[1] of these two compounds indicated that they possessed properties characteristic of central nervous system depressant drugs, since they were found to depress spontaneous motor activity, produce hypothermia, potentiate hexobarbitone and alcohol hypnosis and re-induce hexobarbitone sleep. Although these and related compounds have been extensively investigated in the 'pre-tranquillizer era', particularly by Fourneau[2], Bovet[3] and Staub[4], it was thought worth while to re-investigate this series of basic phenol ethers using the more recently developed pharmacological methods for the discovery of central nervous system depressant properties. Recent reports[5-7] of similar investigations prompt us to report such results as we have so far obtained.

A series of basic ethers of the general structure:



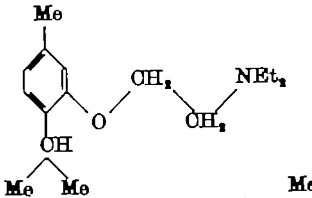
$$Ar{-}O{-}\overset{R}{\overset{|}{\mathrm{C}}}\mathrm{H(CH_2)}_n\mathrm{N}R_2$$

was prepared (usually as crystalline citrates) in which *R* was either hydrogen or a lower alkyl group and in which *Ar*O represents a phenoxide radical. Initially the phenols used for these preparations included: phenol; *o*-cresol; *p*-cresol; *o*-isopropyl phenol; *p*-isopropyl phenol; *p*-tertiary butyl phenol; 6-chloro-2-methyl phenol; thymol; isothymol; 2,4,6-trimethyl phenol; carvacrol; 4-chloro-2-isopropyl phenol; 3-isopropyl phenol; 2,3,5,6-tetramethyl phenol; and 2-*cyclo*hexyl phenol.

It was found that significant central nervous system depressant activity only appeared when the basic ethers were derived from thymol (specifically, 1-methyl-3-hydroxy-4-isopropyl benzene). Compounds derived from isomers of thymol (for example, carvacrol and isothymol) were relatively inactive and there was a very considerable loss in activity (of the order of a twenty-fold diminution) when they were obtained from 2-isopropyl phenol. The alicyclic compound β-diethylaminoethyl menthol ether possessing the same skeleton as the most active thymol ether was quite inactive. Further substitution in the thymol nucleus (specifically 6-chloro, 6-benzyl, 6-amino, and 6-methoxy) also lead to considerable reduction in activity. In the basic ethers derived from thymol itself it was found that maximum activity was obtained when a β-diethylaminoethyl side-chain was attached; if this chain was extended (γ-dialkylamino-*n*-propyl ethers) or the chain was branched (β-dialkylaminoisopropyl ethers) or the N-diethyl groups were exchanged for hydrogen, methyl[8], *n*-propyl or *n*-butyl groups, or the basic side-chain was part of a piperidine ring, there was always a considerable loss in activity. A partial resolution of the asymmetric β-dimethylaminoisopropyl thymol ether, resulting in the preparation of the pure laevorotatory isomer, indicated no detectable difference between this and the racemic form. From this work we concluded that significant activity only appeared in this series when the nucleus was aromatic, the basic ether was ortho to an isopropyl group, the nucleus carried a methyl group para to the isopropyl group, the number of carbon atoms between oxygen and nitrogen was two, the nitrogen was tertiary and the alkyl groups attached thereto were ethyl.

It is interesting to compare these structural requirements with those suggested[9-11] for morphine-like analgesics, when it will be seen that most of these are met in the active basic ether (β-diethylaminoethyl thymol ether, which will hereafter be referred to as '*CR* 127').

The construction of molecular models (Catalin) of '*CR* 127' shows that a strainless conformation can exist which might be expected to fit the hypothetical receptor surface described by Beckett[11] and in which ether–oxygen and nitrogen are in close proximity. In any conformation the oxygen atom should be influenced by the relatively bulky ortho isopropyl group[12], and indeed thymol itself possesses cryptophenolic properties. We considered, therefore, that such juxtaposition of oxygen, nitrogen and the hydrocarbon (isopropyl) group might allow some mutual interaction either in the molecule itself, a metabolite or at the receptor surface and that this might be electronically influenced by the methyl group in para position to the isopropyl group. This was an attractive hypothesis since it would account for the absolute necessity of the 1-methyl group, the general orientation of the molecule and the necessity for a two-carbon chain between oxygen and nitrogen. In this context the work of H. C. Brown[13] on the rate of solvolysis of tertiary cumyl chlorides appeared relevant; these reactions proceed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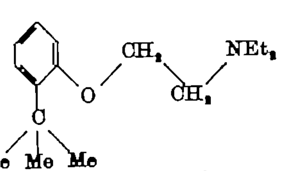
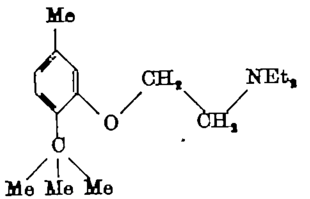
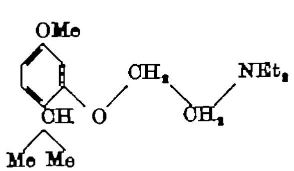
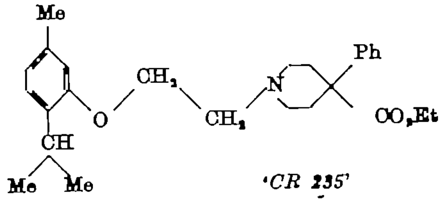

'*CR* 277'


'*CR* 256'


'*CR* 299'


'*CR* 235'

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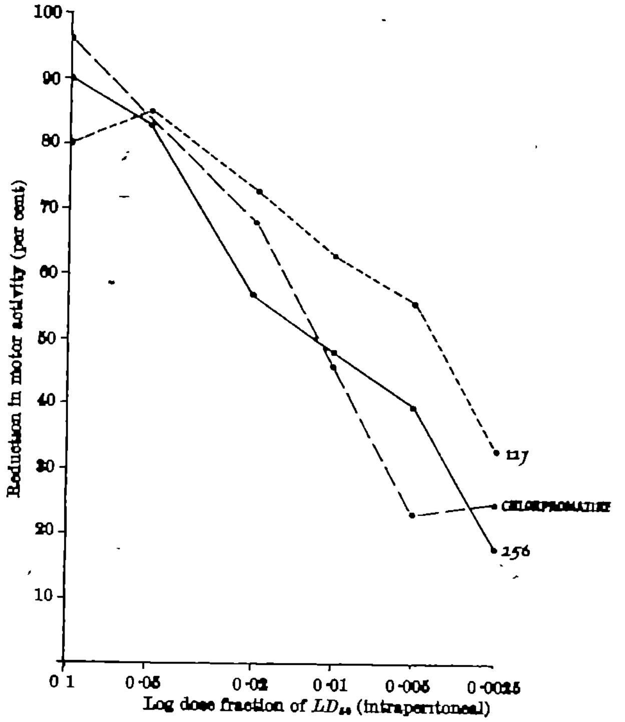

Fig. 1. Spontaneous motor activity. *P* values

Dose Fraction of LD_{50}	'*CR* 127'	'*CR* 256'	Chlorpromazine
0·1	<0·001	<0·001	<0·001
0 05	<0 02	<0 05	—
0·02	<0·001	<0·001	<0 05
0·01	<0 01	<0 001	<0 05
0·005	<0·02	<0 02	N.S.
0·0025	N.S.	<0·05	N.S.

through a carbonium ion and are considerably affected by the nature of the para substituent. These considerations led to the preparation of 1-methyl-3-diethylamino-ethoxy-4-tert. butyl benzene ('*CR* 256')[14] and 1-methoxy-3-diethylaminoethoxy-4-isopropyl benzene ('*CR* 299'). In compound '*CR* 256' the steric hindrance is increased and the molecule more closely fits the requirements for a morphine-like analgesic; in '*CR* 299' the 1-methyl group has been replaced by the more powerfully electron-donating methoxy-group. At the same time, *N*-2'thymoxy ethyl norpethidine ('*CR* 235') was prepared.

Pharmacological examination of these compounds showed that '*CR* 256' was somewhat more active than '*CR* 127' and that '*CR* 299' was inactive. The pethidine derivative ('*CR* 235') possessed no analgesic action. Structural modifications in the tertiary butyl series (as done for the thymol series) again showed the same specificity of structural requirements for central nervous system depressant activity.

A small selection of the pharmacological results obtained in this work is given here; active compounds '*CR* 127' and '*CR* 256' are described and '*CR* 277' is included to show the effect of the para methyl group.

Schneider mice (in groups of five) weighing 18–22 g were used in these experiments, and all injections were given by the intraperitoneal route.

Acute toxicity. Over a 5-day period the following LD_{50} values were obtained (calculated by Karber's method).

Table 1. LD_{50} VALUES INTRAPERITONEAL

Drug	LD_{50} mg/kg
'*CR* 127'	350
'*CR* 256'	332
'*CR* 277'	245
Chlorpromazine	140
Pethidine	150

Reduction in motor activity. Motor activity was measured by the photoelectric cell technique of Siegel[15] as modified by Dews[16]. For measurement of effect on spontaneous motor activity a range of doses from 0·0025 to 0·1 of the LD_{50} was used, the drug being injected 15 min before the mice were placed in the observation cage, where they remained for a period of 30 min (Fig. 1). *P* values refer to differences from controls.

Further tests were carried out at a dose-level of 0·1 LD_{50} in animals made hyperactive by the injection of cocaine hydrochloride (20 mg/kg) or amphetamine sulphate (5 mg/kg) given immediately before the test started. The results are given in Table 2 (figures in parentheses refer to the degree of significance).

Table 2. PERCENTAGE REDUCTION IN HYPER-MOTOR ACTIVITY

Drug	No. gps. mice	Cocaine	Amphetamine
'*CR* 127'	6	40 (N.S.)	57 (<0·05)
'*CR* 256'	13	28 (N.S.)	72·5 (<0·001)
'*CR* 277'	7	26 (N.S.)	0
Chlorpromazine	13	90 (<0 001)	94 (<0 001)

Potentiation of hexobarbitone and alcohol sleep. Drugs were administered at one dose-level (0·1 LD_{50}) at various intervals of time before the mice received an injection of either hexobarbitone (100 mg/kg) or 30 per cent ethanol (0·3 ml./20 g). Regaining of the righting reflex was taken as the end-point of the test. The results are given in Table 3.

Table 3. PERCENTAGE PROLONGATION OF SLEEPING TIME

Drug	Total No. mice		Interval in min between injection of potentiator drug and hypnotic			
			0	30	60	120
'*CR* 127'	60	Hexobarbitone	149 (<0·01)	133 (<0·001)	130 (<0·001)	51 (N.S.)
'*CR* 256'	95		212 (<0·01)	117 (<0·001)	143 (<0 001)	105 (N.S.)
'*CR* 277'	65		47 (<0·02)	10 (N.S.)	48 (<0·01)	5 (N.S.)
Chlorpromazine	80		266 (<0·001)	394 (<0·001)	318 (<0·001)	290 (<0 001)
'*CR* 256'	25	Alcohol	1,137 (<0·001)	188 (<0 01)		
'*CR* 277'	40		0	0		
Chlorpromazine	20		987 (0·001)	1,412 (<0 001)		

Hypothermia. The rectal temperatures of mice were taken by thermistor at intervals of 30 min following the injection of the drug in a dose of 0·2 LD_{50}.

Table 4. EFFECT OF DRUGS ON RECTAL TEMPERATURE

Drug	Maximum fall (°C)	Time of max. effect (min)	Recovery time (h)
'*CR* 127'	5 6	30	5
'*CR* 256'	5·1	30	5
'*CR* 277'	1·0	60	3–4
Chlorpromazine	6 9	90–180	>6

Analgesia. This was measured by the clip test of Bianchi[17]. Drugs were administered in a dose of 0·2 LD_{50}. At this level both '*CR* 127' and '*CR* 256' were equipotent and possessed 1/3 of the activity of pethidine. '*CR* 277' was inactive. All three drugs potentiated pethidine.

In other pharmacological tests the following results were obtained for '*CR* 127' and '*CR* 256'.

(*a*) They exhibit only slight anticonvulsant activity against strychnine, but their effect against leptazol is more marked although still inferior to that of phenobarbitone. Chlorpromazine has approximately the same degree of activity as '*CR* 127' and '*CR* 256'.

(*b*) They reduce the pressor effect of adrenaline in the rat but are weaker adrenolytic agents than chlorpromazine.

(*c*) They cause a sharp fall in blood pressure in the rat and cat and inhibit respiration following intravenous injection. These effects are short-lived.

(*d*) They cause a hypersecretion of corticotrophin from the pituitary gland on initial injection and do not prevent the stimulant effects of an ether stress on corticotrophin. In this they resemble chlorpromazine.

(e) They antagonize only slightly the increased toxic effect of amphetamine in grouped mice, in contrast to the powerful antagonism of chlorpromazine.

(f) '*CR* 127' is not anti-emetic in contrast to chlorpromazine.

(g) They do not antagonize the increased capillary permeability in the rat caused by anaphylactoid agents. Again this is in sharp contrast to chlorpromazine.

There are several points of similarity between '*CR* 127', '*CR* 256' and chlorpromazine among which is the observation that, following an intraperitoneal injection, mice rapidly become quiet and appear to be in a state of catalepsia, in which they can be placed in bizarre positions. The drugs similarly potentiate the effects of hypnotics and reduce spontaneous activity and hyper-motor activity caused by amphetamine. However, they differ in their effects against cocaine excitement and amphetamine toxicity. The lack of effect of '*CR* 127' and '*CR* 256' against cocaine is rather surprising in view of the strong effects of the same dose against amphetamine hyperactivity. Differences in the mode of action of cocaine and amphetamine on the central nervous system evidently exist and the lack of effect of '*CR* 127' and '*CR* 256' against cocaine may be related to the weakness of the adrenolytic properties of these drugs, since it has been shown that at least peripherally cocaine may act by inhibiting the uptake of noradrenaline by the storage organs[18,19].

When '*CR* 127' and '*CR* 256' were tried in man (volunteers) a surprising effect was noted: approximately 1·5 h after taking 10–12 mg (equivalent to 0·125 mg/kg) epigastric pain was noticed, and this developed to severe aching and tenderness in all muscles of the body, but particularly those of the legs, arms, muscles of deglutition and superficial muscles of the scalp; tachycardia was noticed on climbing stairs and these effects persisted for 13 h (that is, to the time of retiring). No evidence of tranquillization was observed at this dose-level, but the volunteers were of course non-psychotic and for obvious reasons larger doses were not investigated. These effects in man resemble the extra-pyramidal reactions shown by certain tranquillizing drugs[20,21] (phenothiazines and reserpine).

The demonstration[22] that dopamine is found in high concentrations in the caudate- and entiform-nucleus has led to the suggestion that reserpine may produce extrapyramidal effects by depletion of this amine; it has also been suggested[20] that phenothiazines cause this effect by interference with dopamine, and Ernst[23] has ascribed hypokinetic rigid syndromes to O-methylation of dopamine. It is interesting that Martin *et al.*[24] have stated that β-diethylaminoethyl thymol ether (that is, '*CR* 127') specifically inhibits dopa-decarboxylase, hence it may be that '*CR* 127' and '*CR* 256' exert their action by interference with the biosynthesis of catechol amines. There is, however, evidence[25] that dopa-decarboxylase is identical with 5-hydroxytryptophan decarboxylase (inhibition of which by phenylalanine has been suggested[26] as a cause of mental disturbance in phenylketonurics).

Quaternary ammonium compounds derived from these basic ethers showed curare-like properties and caused flaccid paralysis with no evidence of initial stimulation. Most of the compounds caused a transient fall in blood pressure and were respiratory depressants. Many of the compounds were potent local anaesthetics. As might be expected, the rigid structural requirements elucidated for significant central nervous system-depressant activity in the tertiary bases were no longer important.

This work is part of an investigation of the relationships between structure and central nervous system-depressant activity in a simple model.

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SYNTHESIS OF ORGANIC COMPOUNDS BY HIGH-ENERGY ELECTRONS

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THE use of ionizing radiation for the synthesis of organic compounds under possible primitive earth conditions was initiated by Calvin *et al.*[1]. These investigators obtained formic acid and formaldehyde by irradiating aqueous solutions of carbon dioxide with 40-MeV helium ions. The reduction of carbon dioxide to formic acid and formaldehyde was confirmed using cobalt-60 γ-rays by Getoff *et al.*[2], who, in addition, observed also the presence of acetaldehyde among the reaction products. Since the foregoing initial observation was made, a number of experiments have been reported[3–7] which show the production of a few amino-acids and other organic molecules by the action of ionizing radiation on simple compounds, or mixtures of compounds, containing carbon, nitrogen, oxygen and hydrogen.

More recently, Palm and Calvin[8], using methane labelled with carbon-14 in aqueous ammonia mixtures and irradiating with 5-MeV electrons from a linear accelerator, observed the formation of glycine, alanine, aspartic acid, several hydrocarbons and a relatively large number of other unidentified compounds. Among these compounds purines and pyrimidines were suggested to be present[9,10].

Further experiments using ^{14}C-methane and 5-MeV electrons have been performed and are presented here. Some experiments were carried out with the irradiated mixture mainly in the gas phase, so that the results obtained may be compared with those obtained with electrical discharges under similar conditions[11]. An experiment was carried out with a mixture of ^{14}C-methane, ammonia and water in the solid phase. It was thought that such an experiment may give some insight on the possible products formed by ionizing radiation from the protosun acting on 'ices'[12] or hydrates[13] of simple compounds of carbon and nitrogen assumed to have been present in the primordial solar nebula. (Such interactions may also have occurred in protoplanetary bodies, and may be occurring at present in comets and other cosmic bodies of the solar system[14,15].)

The results have shown that the pattern of compounds formed by the irradiation of the aforementioned mixtures in the gas phase is similar to the pattern of compounds formed by the action of electrical discharges on analogous mixtures[11]. Secondly, it has also been observed that the irradiation in the solid phase of methane–ammonia–water mixtures leads to the formation of a reasonably large number of non-volatile organic compounds.

Irradiation technique and dosimetry. In general, the irradiation technique, particularly that used in the gas-phase experiments, was essentially that of Palm and Calvin[8]. High-energy electrons (5 MeV) were delivered from a linear accelerator (The linear accelerator was operated by W. Everette and D. Pounds, of the Lawrence Radiation Laboratory, who also carried out the dosimetry. Their help is gratefully acknowledged.) The absorbed dose was measured by means of a cobalt glass dosimeter. For this purpose a tube similar to the irradiation vessel was made (Fig. 1) and small pieces of cobalt glass were located inside the tube every 5 cm along its axis. The tube was partially evacuated, placed in front of the linear accelerator with its axis coinciding with that of the electron beam, and irradiated for a given time. The colour change of the cobalt glasses was measured by means of a spectrophotometer. The corresponding number of rads per microcoulomb was plotted against the distance of each piece of cobalt glass to the window of the linear accelerator. The results are shown in Fig. 2. It can be seen that the dose absorbed at about 25 cm (middle of the tube) is of the order of 1·5 × 10^4 rads per microcoulomb. In general, each irradiation was carried out for a period of 2 h. The current used during this time was 64,800 microcoulombs. Therefore, considering complete linearity, the dose absorbed was of the order 1 × 10^9 rads. It is of interest to note that this measured value agrees with the upper value calculated previously by Palm and Calvin[8]. For individual experiments the approximate values for the absorbed dose are given in Table 1.

Table 1. COMPOSITION OF GAS MIXTURES AND OTHER DATA OF THE EXPERIMENTS WITH HIGH-ENERGY ELECTRONS

	Exp. No.			
	RS–1	*RS*–2	*RS*–3	*RS*–4
Volume, irradiation tube (c.c.)	755	755	8·8	260
CH_4 pressure (cm Hg)	25	9	670	40
$^{14}CH_4$ activity (μc.)	—	370	100	300
$^{14}C_2H_6$ activity (μc.)	680	—	—	—
N_2 pressure (cm Hg)	25	24·5	—	—
H_2O (ml. liquid)	20	5	2	0·2
NH_4OH (moles/l.)	0·5	14	6	6
Average temperature (°C.)	25	80	20	−196
Time of irradiation (h)	2	2	0·4	2·5
Dose (1 × 10^9 rads)	1	0·97	0·12	1·3
Weight of residue (mg)	12	4·4	2	3·2
Activity of residue (μc.)	28	5·5	0·07	14·0
Activity of distillate (μc.)	26	1·2	0·10	3·6
Conversion of ^{14}C reactants to non-volatile products (per cent)	4·1	1·5	0·07	4·6
Conversion of ^{14}C reactants to volatile water-soluble products (per cent)	3·8	0·3	0·10	1·2

Gas-phase experiments. Not all the experiments were performed under the same conditions. The first and the second experiments (*RS*-1 and *RS*-2) were carried out with the hydrocarbons in the gas phase under slightly reduced pressure, and water and ammonia in the liquid and vapour phase. The vessel used in these two experiments was the same tube *B* described by Palm and Calvin[8].

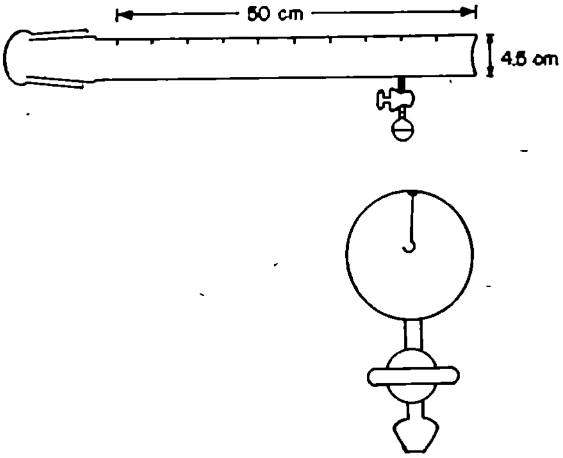

Fig. 1. Diagram of a 'Pyrex' glass tube used for dosimetry. Small rectangular pieces of cobalt glass were placed inside the tube at distances of 5 cm along the tube

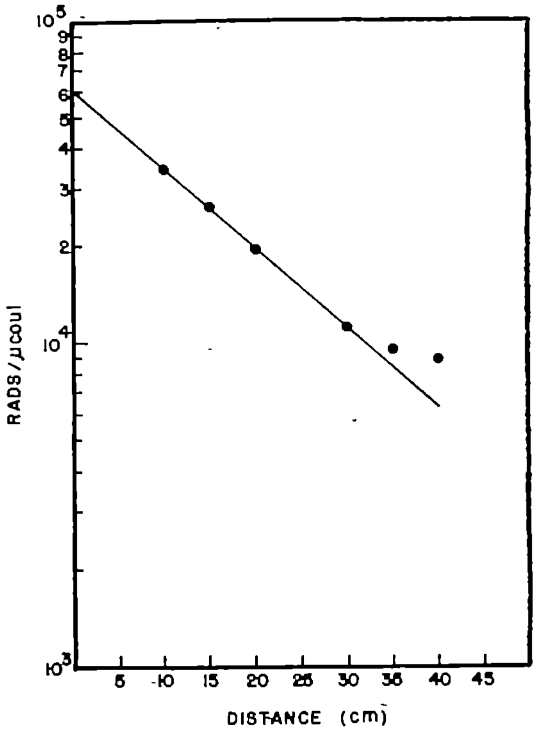

Fig. 2. Semilogarithmic plot of rads/microcoulomb against the distance of each piece of cobalt glass to the window of the linear accelerator

Only a minor modification was made: two permanent stopcocks were attached to the inlet and outlet of the tube.

After evacuation, the radioactive and non-radioactive hydrocarbons, and a solution of ammonium hydroxide were successively introduced into the vessel. The ammonium hydroxide solution was prepared using boiled distilled water, and care was exercised to avoid any bubbles of air going into the system. The tube was placed in front of the linear accelerator and irradiated. In experiment *RS*-2 the bottom well of the tube *B* was heated during irradiation to reflux the water and thus increase the partial pressure of the water vapour. In experiment *RS*-1 this was not done.

Solid-phase experiment. Experiment *RS*-4 was carried out in the apparatus shown in Fig. 3 with the reactants in the solid phase. The reactants were admitted into the horizontal tube at ambient temperature under slightly reduced pressure, and then liquid nitrogen was added into the adjacent Dewar flask. Immediate con-

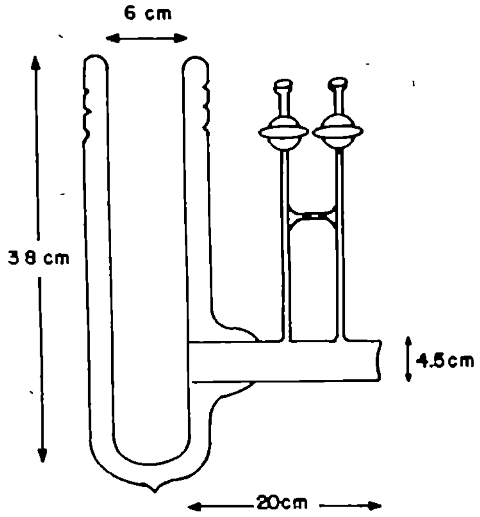

Fig. 3. 'Pyrex' glass vessel used in the solid-phase irradiation experiment. The horizontal tube contained the reactants which did condense in a solid layer on the left window as soon as the Dewar flask was filled with liquid nitrogen.

densation of water, ammonia and methane occurred on the inside of the window facing the liquid nitrogen. The resulting layer of white crystals, approximately 2 mm thick, was irradiated for about 2 h as in the case of the gas-phase experiments. The Dewar flask was always kept full of liquid nitrogen during the irradiation process, so that no volatilization or liquefaction of the solid layer occurred at any time. Visual observation of the integrity of this layer was afforded by a system of mirrors which reflected the image to the outside of the irradiation chamber.

Liquid-phase experiment. Experiment *RS*-3 was performed using a small tube, of 6·8 c.c. of volume, filled partially with water. The methane and ammonia were admitted while the tube was immersed in liquid nitrogen. The tube was then sealed and allowed to warm to room temperature. This resulted in a substantial positive pressure. In consequence, an appreciable portion of the methane was dissolved in water. This tube was irradiated for only 25 min and the current used during this time was 8,100 microcoulombs. The pressure measured after irradiation was of the order of 16 atmospheres.

Analysis of products. The gaseous and highly volatile products present in the gas phase of each experiment were not analysed. At the end of each run the contents in each tube were diluted with water and transferred quantitatively into a small tared flask. This solution was evaporated to dryness in a vacuum rotating evaporator. The water and other volatilized products were trapped in a tube immersed in liquid nitrogen. The flask containing the dry residue was weighed and the residue was dissolved in 0·2–0·4 ml. of 0·1 N hydrochloric acid. A small aliquot of the product from each experiment showing approximately 1×10^4 d.p.m. was analysed by two-dimensional paper chromatography and autoradiography. The chromatographic procedure followed was identical to that used in the previous experiments with electric discharges[11].

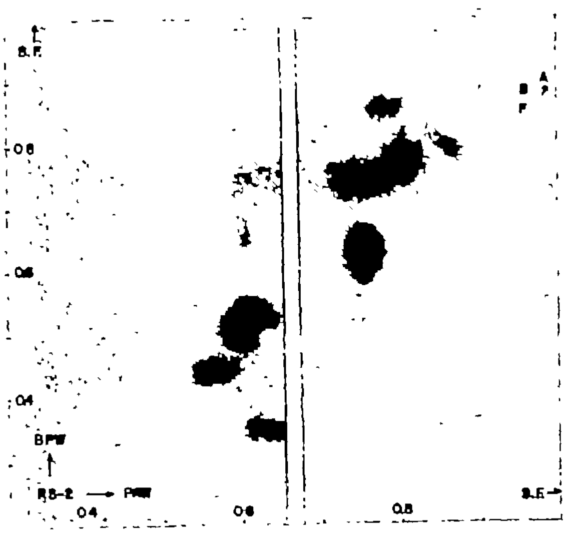

Fig. 4. Autoradiograph of a two-dimensional chromatogram of a gas-phase irradiation experiment (*RS*–2).

An autoradiograph of a two-dimensional chromatogram of experiment *RS*-2 (gas-phase irradiation) is shown in Fig. 4. By comparison with the autoradiograph of experiment *S*-5 (electric discharges[11]) it can be seen that the general distribution of compounds is similar. Since amino-acids, hydroxy acids, and their respective amides and nitriles seem to predominate in the experiments with electric discharges, the tentative conclusion is made that analogous compounds are formed by irradiation with electrons.

In particular, on the left side of the *RS*-2 autoradiograph there appears to be at least five compounds which show rates of migration essentially identical to those of five compounds on the left side of the *S*-5 autoradiograph. Of these five compounds, it is suggested that three more intense lower spots correspond to alanine, glycine and glycinamide. Spots corresponding to the latter compounds can also be observed in the chromatogram of *S*-7 (electric discharges[11]) which was sprayed with ninhydrin.

An analogous correlation can be observed by comparing the right side of *RS*-2 with the right side of *S*-5. At least three compounds appear to have identical R_F in the two chromatograms. This area of the chromatogram corresponds to the area of migration of some of the less polar amino-acids, such as the leucines, and also of some purines and pyrimidines such as adenine, thymine, uracil and cytosine[16].

The autoradiograph of the other gas-phase irradiation experiment (*RS*-1) was somewhat similar to that of *RS*-2. However, additional fast-moving components were also observed. Some of the latter compounds may be less polar or more lipophilic in nature than amino-acids or amino-acid amides.

The existence of a similarity between the products formed by irradiation with electrons and those formed by electrical discharges should not be surprising since both processes involve excitation and ionization phenomena by electrons. In fact, the products obtained in the decomposition of methane by electric glow discharges and low-energy electrons are almost identical and include hydrogen, ethane, ethylene, acetylene and other hydrocarbons[17]. The irradiation of methane by high-energy electrons is also known to produce hydrogen, ethane, ethylene, propane and butane[18].

An autoradiograph of a two-dimensional chromatogram of the products obtained from the solid-phase irradiation experiment (*RS*-4) is shown in Fig. 5. A careful comparison of this autoradiograph with that of *RS*-2 (Fig. 4) will reveal a number of spots with identical R_F. This correlation can be carried back to the autoradiographs of the experiments with electric discharges[11]. A comparison of the same autoradiograph with a two-dimensional chromatogram of standard purines and pyrimidines[16] revealed the presence of radioactive spots in the areas corresponding to purines and pyrimidines. No definite claim about the formation of these compounds can be made until they are identified by co-chromatography with authentic samples in additional solvent systems. (Adenine has been recently identified by C. Ponnamperuma and R. Lemmon[19].)

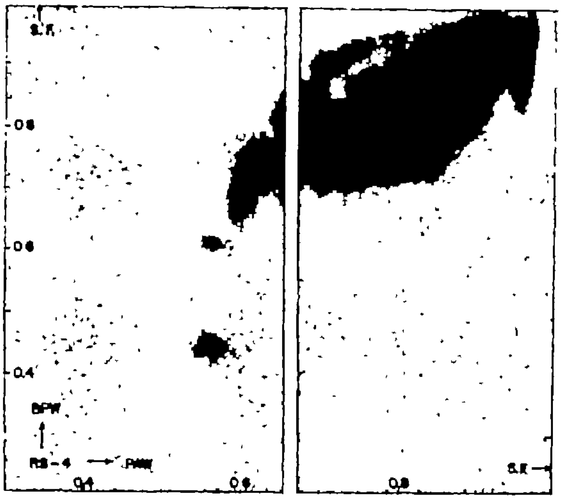

Fig. 5. Autoradiograph of a two-dimensional chromatogram of a solid-phase irradiation experiment (*RS*-4).

The increased number of radioactive spots in the zone of higher R_F's (Fig. 5) may be taken as an indication that the compounds formed in the solid-phase irradiation experiments are of a less polar character than those formed in the previously described fluid-phase experiments (electron irradiation or electric discharges). This is probably what should be expected from processes occurring in the solid phase, where hydrolytic and ammonolytic reactions must be very slow if not completely absent.

The autoradiograph of the irradiation experiment carried out in a predominantly liquid phase (*RS*-3) showed no detectable radioactive spots. Although only $3 \cdot 8 \times 10^4$ d.p.m. could be placed on the paper to carry out the two-dimensional chromatographic analysis, it would seem that if any compound was formed in significant amounts in this experiment it should have left a radioactive spot on the photographic film after five days of exposure. It can also be seen in Table 1 that the fixation of radioactive carbon into the non-volatile products in this experiment was between 1 and 2 orders of magnitude lower than in the other experiments. It is possible that under the conditions of this experiment most of the methane was oxidized to carbon dioxide.

Discussion

(1) Organic compounds are formed in the irradiation by high-energy electrons of mixtures of methane, ammonia and water not only when these reactants are in the gas phase but also when they are in the solid phase.

(2) The autoradiographic patterns of the compounds formed by high-energy electrons are similar to those obtained by electric discharges, although the products obtained in the solid-phase irradiation experiments appear to be of a less polar character. The compounds detected by comparing two-dimensional chromatograms include amino-acids, amino-acid amides, amines, and other molecules of biological significance. The positions of some radioactive spots on the chromatograms correspond closely to those of some purines and pyrimidines. Work is in progress for the identification of these compounds.

(3) On the basis of the results obtained here, and recognizing that the hydrides of carbon, nitrogen and oxygen (in the solid and gas phases) prevail in the universe, it is suggested that the radiation synthesis of organic and biochemical compounds is an important cosmic process. If an appreciable radiation flux prevailed during the early evolution of the primordial solar nebula[14] an extensive synthesis of these compounds probably took place in the protoplanets of the solar system. The synthesis may also occur presently, at lower rates, in the atmospheres of the Jovian planets and in cometary bodies during perihelion orbital travel.

I thank Prof. M. Calvin for providing the stimulus and laboratory facilities to carry out these experiments, and Dr. R. Lemmon for advice.

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EFFECT OF ADENOSINE DIPHOSPHATE ON THE CONCENTRATION OF PLATELETS IN CIRCULATING BLOOD

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WHEN adenosine diphosphate (ADP) is added to blood plasma containing platelets they rapidly adhere to each other to form aggregates[1]; this effect is not brought about by any other naturally occurring nucleotide. In separated plasma the aggregation has been investigated quantitatively by a turbidimetric method[2]. With platelet-rich plasma of pigs and man, the rate and extent of aggregation depend on the concentration of added ADP, and its aggregating effect is inhibited by adenosine monophosphate (AMP) and, even more, by adenosine[2,4].

It has been suggested[3] that ADP is responsible for the initial adhesion of platelets to damaged vascular endothelium and to each other in the formation of hæmostatic plugs and of thrombi. If this were so, ADP might be expected to diminish the concentration of platelets circulating in the blood stream of living animals. This possibility has now been tested in experiments on cats.

When ADP was added *in vitro* to platelet-rich plasma of cats the platelets aggregated (Fig. 1), just as they do in plasma from man and from pigs. However, the plasma of cats differed from the plasmas of pig and man[4]. With

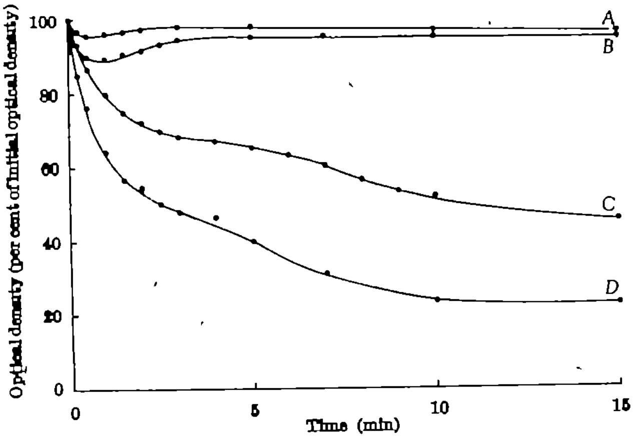

Fig. 1. Effect of adding ADP on the optical density of cat's plasma containing $1·91 \times 10^8$ platelets/ml. The plasma contained sodium citrate (3·0 mg/ml.) and was stirred at 1,000 r.p.m. ADP was added at 0 min to give the following concentrations: 2×10^{-7} M (curve *A*); $3·3 \times 10^{-7}$ M (curve *B*); 5×10^{-7} M (curve *C*), and $6·6 \times 10^{-7}$ M (curve *D*)

cats' plasma: (1) the concentrations of ADP which brought about aggregation were lower; (2) small changes in the concentration of added ADP had much larger effects on the rate and extent of aggregation (Fig. 1); (3) the aggregation produced by ADP was not inhibited by AMP or by adenosine; and (4) the effect of a second addition of ADP was no smaller than that of the first. Cats' platelets were, therefore, highly susceptible to the aggregating effect of ADP; moreover, its breakdown products did not inhibit the effect. This suggested that if there was an effect of ADP on circulating platelets it should be demonstrable in cats.

We infused ADP into the blood stream of healthy cats weighing 2·5–6·0 kg and determined the concentrations of circulating platelets. Each cat was anæsthetized with ethyl chloride followed by chloralose (40 mg/kg) injected through a cannula in the right external jugular vein. Heparin was injected intravenously at a concentration of 120 units/kg. More heparin and chloralose were injected at intervals during the experiment; neither affected the concentration of platelets. A cannula was tied into the trachea and another into the left common carotid artery from which the blood pressure was recorded with a mercury manometer recording on a kymograph. The left femoral vessels were exposed just below the inguinal ligament and a polythene cannula was tied into the first, very small, branch of the artery. The cannula was closed with a pair of artery forceps. For taking samples of blood, the forceps were released; about 0·1 ml of blood was allowed to flow out through the cannula to flush the dead space with fresh arterial blood; a sample of 0·1 ml of blood was withdrawn into a syringe containing 0·1 ml. of 4·5 per cent (w/v) ethylenediamine tetraacetate. The syringe was rapidly emptied into a plastic centrifuge tube containing 1·8 ml. of 4·5 per cent ethylenediamine tetraacetate which prevents aggregation of platelets. The platelets in the diluted blood were counted in duplicate by the method of Nygaard[5] as modified by Davidson and Tomlin[6].

In 19 cats the blood contained $0·9–3·2 \times 10^8$ platelets/ml., with a mean of 1·9. When a cat was left all day except for having blood samples taken from it the concentration of circulating platelets varied little (Fig. 2). Infusions were made through the cannula in the external jugular vein or through that in the femoral artery, at a rate of 0·2 ml./min. The concentration of circulating platelets was not altered significantly by infusing isotonic saline, in which the adenine compounds were dissolved.

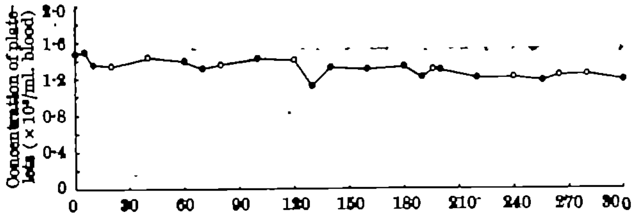

Fig. 2. Concentration of platelets in blood from the femoral artery of an anæsthetized cat weighing 3·0 kg

In four experiments, when ADP in concentrations of 1 and $2·5 \times 10^{-5}$ M was infused into the external jugular vein, the concentration of circulating platelets immediately decreased (Fig. 3). In two of these experiments the mean blood pressure did not change. In the other two the blood pressure fell briefly by 10 and 45 mm mercury respec-

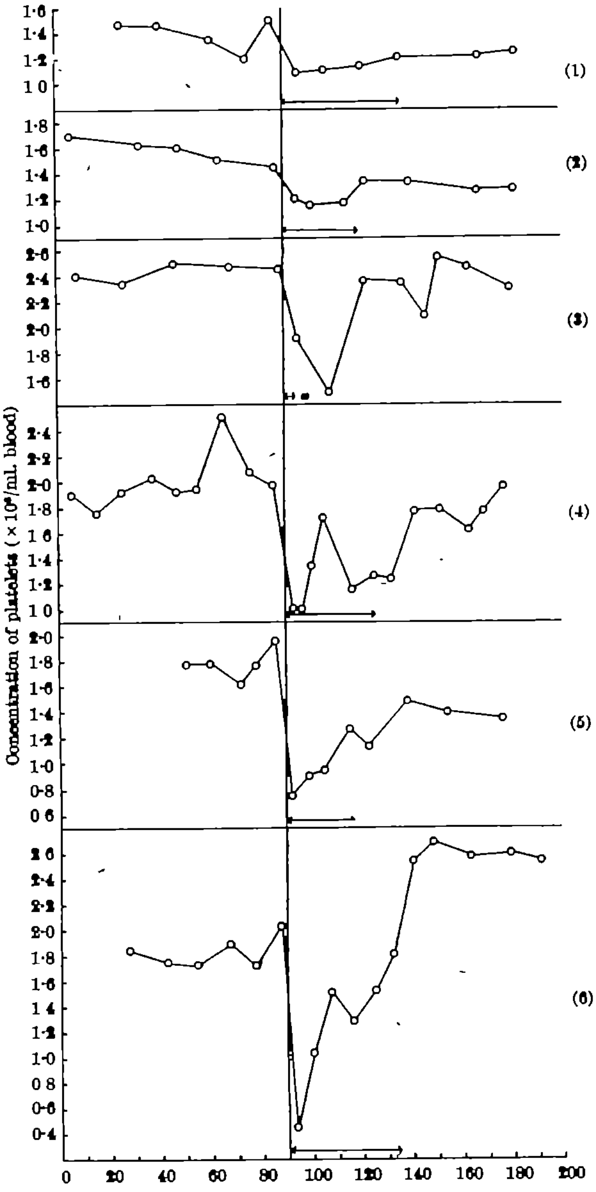

Fig. 3. Concentrations of circulating platelets in blood from the femoral artery of anæsthetized cats. Saline was infused into the external jugular vein from the beginning of each experiment; then ADP from the line at 90 min for times indicated ⟷; and then saline again. The rate of infusion was 0·2 ml./min

Exp. No.	Weight of cat (kg)	Concentration of infused ADP (M)
1	3·1	$1·0 \times 10^{-5}$
2	4·2	$5·0 \times 10^{-5}$
3	3·8	$1·0 \times 10^{-5}$
4	6·0	$1·0 \times 10^{-5}$
5	6·0	$2·5 \times 10^{-5}$
6	2·8	$2·5 \times 10^{-5}$

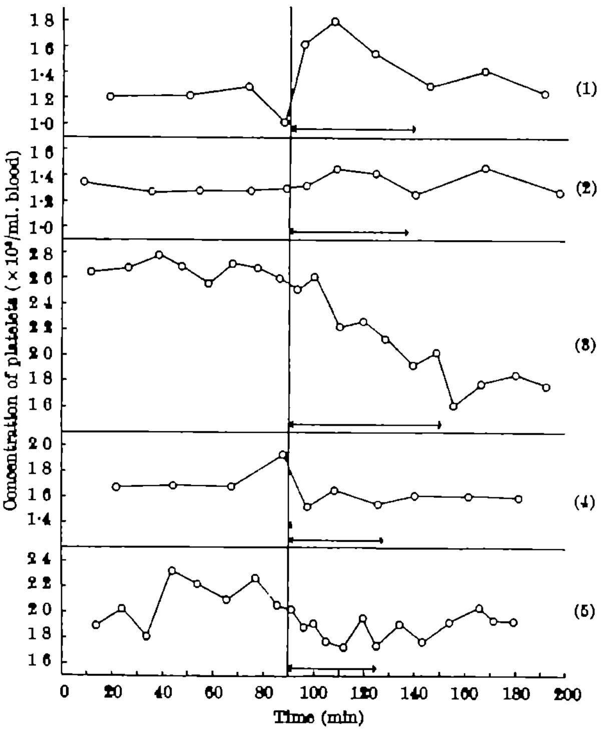

Fig. 4. Concentrations of circulating platelets in blood from the femoral artery of anaesthetized cats. Saline was infused into the external jugular vein from the beginning of each experiment; then adenosine (Exp. Nos. 1–3) or AMP (Exp. Nos. 4 and 5) from the line at 90 min. for times indicated ←→ and then saline again. The rate of infusion was 0·2 ml./min

Exp. No.	Weight of cat (kg)	Infusion Substance	Infusion Concentration (M)
1	3 1	Adenosine	$1\ 0 \times 10^{-3}$
2	4 2	Adenosine	$1·0 \times 10^{-3}$
3	2·8	Adenosine	$5·0 \times 10^{-3}$
4	3·9	AMP	$1·0 \times 10^{-3}$
5	6·0	AMP	$5\ 0 \times 10^{-3}$

tively and then rose to what it had been before the infusion of ADP; at this time the platelet concentration was still reduced. When ADP was infused in lower concentrations the platelet concentration decreased slightly or not at all. In four experiments ADP was infused into the cannula in the femoral artery; the infusions were stopped when blood samples were taken. Again, the platelet count fell when the ADP concentration was 1×10^{-3} M but not when it was 5×10^{-3} M. In some experiments, samples of blood were also taken through a cannula in the uppermost branch of the accompanying femoral vein. Changes in the concentrations of platelets in the venous blood did not follow those in the arterial blood very closely, but large decreases in the arterial blood were accompanied by similar decreases in the venous blood.

When AMP was infused into the external jugular vein in concentrations of 1 and 5×10^{-3} M it had little or no effect on the platelet count (Fig. 4); the lower concentration caused no change in blood pressure, the higher a fall of 36 mm mercury. Adenosine was also infused at concentrations of 1 and 5×10^{-3} M. It had no effect on the blood pressure; its effects on the concentration of circulating platelets were less consistent (Fig. 4) than those of ADP and AMP but in no experiment was there a rapid fall in the platelet count.

The results show that when ADP was infused in high concentrations into the circulation of cats it brought about a rapid decrease in the concentration of platelets; neither AMP nor adenosine had this effect. The effect of ADP on the platelet count seemed to be independent of changes in blood pressure, for two reasons: first, in some experiments ADP reduced the platelet count without affecting the blood pressure; and secondly, AMP reduced the blood pressure without affecting the platelet count.

The diminution in the platelet count could have resulted from the formation of platelet aggregates which remained suspended in the circulating blood but were sedimented with the red cells when the blood samples were prepared for counting[5]. However, it is unlikely that all the aggregates, even the smallest, would have been removed in this way; and no platelet aggregates were seen during counting. Therefore, it seems that ADP brought about a real decrease in the concentration of circulating platelets.

The platelet count was decreased when ADP was infused at a rate of about 4×10^{-7} moles/min kg. From this and the cardiac output of cats[7] it can be calculated that the initial concentration of ADP in the blood which was sampled was about 4×10^{-6} M; this is about ten times the concentration of ADP which brought about aggregation *in vitro*. How the concentration of ADP changed as the infusion went on, because of breakdown, etc., is unknown.

When ADP was added repeatedly to human plasma its effectiveness in causing platelet aggregation progressively decreased. We have suggested[4] that this might be due to the inhibition of aggregation by breakdown products of ADP. This conclusion is supported by the results obtained *in vitro* with cats' plasma: a second addition of ADP was no less effective than the first; and the aggregating effect of ADP was not reduced by AMP or by adenosine, which are the most likely breakdown products.

The way in which ADP brings about the rapid decrease in the concentration of circulating platelets remain to be established. It may be that ADP makes platelets adhere to endothelium and form aggregates which are trapped in the smallest vessels.

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DIFFERENCES BETWEEN PENCILLINASES FROM GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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UNTIL recently, studies on penicillinase have been devoted, almost completely, to the enzymes elaborated by *Staph. aureus* and the *Bacillus* group; furthermore, attention has remained directed largely at those aspects of the properties and production of penicillinases which provide models either for enzyme induction[1] or for the release of enzymes from bacterial cells[2,3]. No systematic enzymological study has been made of the properties of the penicillinases produced by other organisms, and indeed, reports of enzymological studies on even the well-

Table 1

	Gram-negative									Gram-positive		
	A. cloacae 53 at pH 7·4	*A. cloacae* 53 at pH 6 0	*E. coli* 214 *T*	*E. coli* 214 *O*	*E. coli* 419	*A. aerogenes* 418	*A. aerogenes* 366	*A. aerogenes* 402	*A. aerogenes* 415	*Staph. aureus* E3 (*a*)	*B. cereus NRRL* 569 (*a*)	*B. licheniformis*
Absolute rate of destruction of benzylpenicillin (μg/ml./min/10⁹ cells)	158	294	20	0·4	3·8	7·6	1,170	2·8	3·8	696	560	(*b*)
	Per cent rate of destruction of other penicillins taking that of benzylpenicillin as 100 per cent											
Phenoxymethyl- (penicillin V)	60	70	53	63	65	119	110	85	95	110	117	88
α-Aminobenzyl- (ampicillin)	100	119	—	—	—	182	200	220	287	198	156	110
Phenoxyethyl- (phenethicillin)	57	96	—	—	—	85	58	44	50	100	103	64
Phenoxypropyl- (propicillin)	40	44	—	—	—	71	24	17	17	71	56	40
Phenoxybenzyl- (phenbenicillin)	49	64	—	—	—	31	31	22	23	167	91	96
6-Aminopenicillanic acid	—	14	—	—	—	32	31	43	31	—	—	—
2 : 6-Dimethoxyphenyl- (methicillin)	—	—	—	—	—	—	—	—	—	—	—	—
5-Methyl-3-phenyl-4-isoxazolyl- (oxacillin, *BRL* 1400)	20	42	—	—	—	—	—	—	—	—	—	—
5-Methyl-3-*o*-chlorphenyl-4-isoxazolyl- (*BRL* 1577)	—	16	—	—	—	—	—	—	—	—	—	—
5-Methyl-3-*o*-chlorphenyl-4-isoxazolyl- (cloxacillin, *BRL* 1621)	—	—	—	—	—	—	—	—	—	—	—	—
Apparent activation energy (calories/mole)	4,600	4,800	4,600		5,300	3,400	4,000	3,400	3,400	7,200	7,700	8,800
pH optima (95 per cent limits) for benzylpenicillin	4·8–6·4		6 7–7·5		6·3–7·9	5·5–7·2	6·5–8·0	5·0–8·0	5·7–7·5		7·0–7 6 (ref. 4)	

— Rate <10 per cent of that of benzylpenicillin. (*a*) Induced preparations see ref. 9 for details. (*b*) Commercial preparation.

Table 2. RATIO (TO THE NEAREST WHOLE NUMBER) OF PENICILLINASE ACTIVITY AGAINST VARIOUS PENICILLINS OF DISRUPTED TO INTACT SUSPENSIONS OF THE GRAM-NEGATIVE ORGANISMS

Penicillins	*A. cloacae* 53 pH 7·4	*A. cloacae* 53 pH 6 0	*E. coli* 214*T*	*E. coli* 419	*A. aerogenes* 418	*A. aerogenes* 366	*A. aerogenes* 402	*A. aerogenes* 415
Benzylpenicillin	18	25	10	4	4	11	3	4
Penicillin V	8	10	7	3	4	11	2	2
Ampicillin	8	9	—	—	4	14	2	3
Phenethicillin	9	20	—	—	6	9	3	3
Propicillin	11	6	—	—	5	11	2	2
Phenbenicillin	11	15	—	—	4	5	3	3
6-Aminopenicillanic acid	1	1	—	—	1	2	3	1
Methicillin	—	—	—	—	—	—	—	—
Oxacillin	8	7	—	—	—	—	—	—
BRL 1577	—	—	—	—	—	—	—	—
Cloxacillin	—	—	—	—	—	—	—	—

— Rates too slow to obtain accurate results. Results with *E. coli* 214 *O* are not shown since the activity of whole cell preparations was too low to measure precisely.

known penicillinases of *Staph. aureus* and *Bacillus* spp. are few[4–6]. With the advent of α-aminobenzylpenicillin ('Penbritin', ampicillin), which is relatively active against Gram-negative organisms[7], interest has been aroused in the penicillinases produced by such organisms. In a clinical trial[8] all penicillinase-producing coliform organisms were found to be resistant to ampicillin. The penicillinases from several strains isolated in this clinical trial have been found to differ in many important respects from others previously described. Some of these results have already been published[9–12], and it is intended here to present a summary of the enzymatic differences found between penicillinases produced by Gram-positive organisms and those obtained from eight coliform organisms.

The organisms were screened for penicillinase production first by the filter membrane method[13] and after tests for penicillin amidase[14] had proved negative, the enzymes were confirmed as β-lactamases by chromatographic identification of penicilloic acid[13,15] in suspensions of the various organisms incubated with penicillin *G*.

Location of penicillinase. Cell-free preparations of penicillinases were made as previously described[11], by disrupting organisms in an extrusion press and centrifuging at 3,000*g* for 20 min to remove whole cells and large debris. In all cases the enzymatic activity remained in the supernatant fraction after subsequent centrifugation of such disrupted preparations at 20,000*g* for 50 min, suggesting that the enzyme is in the soluble cytoplasm, in contrast to previous reports[16–19] concerning Gram-positive organisms, which indicate that penicillinases from these sources are associated with the cell wall fraction.

Effect of pH on enzymatic activity. Penicillinases obtained from a number of sources show an optimum activity at about pH 7 (ref. 20) for benzylpenicillin and this was the case for all the penicillinases obtained from Gram-negative organisms except for the enzyme produced by *Aerobacter cloacae* 53 (Table 1). This latter enzyme, however, is very unusual as it has been shown to possess essential sulphydryl groups[10]. The penicillinase of this organism was examined at pH 6·0, its optimum, and at pH 7·4—the value commonly used in the study of penicillinases.

Specificity patterns. Suspensions of whole or disrupted cells in M/40 phosphate-buffered saline or M/40 phosphate buffer at pH 7·4 (except where indicated) were incubated with a final concentration of 2 mg/ml. of various penicillins; samples were removed at intervals and assayed for penicillin by the hydroxylamine method as described by Knox and Smith[11]. Table 1 shows the results obtained with disrupted suspensions of the coliform organisms, together with the absolute rates of hydrolysis of benzylpenicillin, apparent activation energies and pH optima. Comparative figures are given for the penicillinases of *Staph. aureus E3* (ref. 21), *B. cereus NRRL* 569 (ref. 9) and *B. licheniformis*[12]. It is apparent that the penicillinases produced by Gram-negative organisms not only have specificity patterns different from those of penicillinases of Gram-positive organisms, but also that each species appears to have a specificity pattern peculiar to itself. For example, the *E. coli* strains hydrolyse only benzylpenicillin and phenoxymethylpenicillin appreciably; the *A. aerogenes* strains hydrolyse 6-aminopenicillanic acid rapidly; and *A. cloacae* hydrolyses 5-methyl-3-phenyl-4-isoxazolylpenicillin rapidly.

Permeability barrier. As reported previously[9,13] with two strains of coliform organisms, disruption of the cells resulted in a considerably increased penicillinase activity against certain substrates. Other strains have now been investigated and the same phenomenon has been found in every case. This suggests the presence of a permeability barrier between the enzyme and its substrate, presumably located in the cell membrane. The increases observed in penicillinase activity against various substrates on disruption of cell suspensions are shown in Table 2. In most cases it seems that the permeability barrier is selective, for example, with organisms 53, 415 and 418 6-aminopenicillanic acid is destroyed at a similar rate by disrupted or by intact cells; whereas with *A. cloacae* 53 at *p*H 6·0 disrupted cells destroyed benzylpenicillin 25 times as rapidly as intact cells. The presence of a permeability barrier in Gram-positive organisms has been shown either not to exist[2,22] or to be doubtful[9].

Antibacterial activity of penicillins. The eleven penicillins were tested by the tube dilution technique for their activity against all the coliform strains, *Staph. aureus E3* and *B. cereus NRRL* 569, using about 10^4-cells as inoculum. All these strains were resistant to at least 50 μg/ml. of all the penicillins tested, except the *E3* strain, which was sensitive to 1 μg/ml. of the isoxazolyl penicillins and 4 μg/ml. of methicillin, and strain 214*O*, which was sensitive to 10 μg/ml. of ampicillin. Furthermore, the most active penicillin against any strain was not necessarily that which most readily penetrates the permeability barrier, nor the most resistant to hydrolysis. However, with organism 418 a correlation between antibacterial activity and ease of penetration does exist[12]. A correlation between the inducing ability and antibacterial activity of penicillins has been suggested for certain Gram-positive bacteria[23,24].

Apparent activation energies. We have previously reported[11] on the difference in the apparent activation energies of penicillinases produced by different groups of organisms. It was shown that, based on the mean number of substrate molecules activated by each type of penicillinase, those from Gram-negative sources were 318 times more effective as catalytic agents than those from Gram-positive sources. The fact that the apparent activation energies of penicillinases from coliform organisms are so low suggests, on the hypothesis of Abraham and Newton[25], that the turn-over numbers of these penicillinases are even higher than those reported for the *B. cereus* and *B. subtilis* enzymes[26,27].

Inducibility. Penicillinases from the Gram-positive organisms have been repeatedly shown to be inducible, and relative inducing abilities have been studied in detail[21,23,28] for *Staph. aureus*. However, in no case has induction been demonstrated with any of the eight coliform strains, despite repeated attempts. In some cases[9,12] increased enzymatic activity after overnight incubation in high concentrations of various penicillins was observed, but only in intact cell preparations. It was later shown that this increased activity was due, not to an increased amount of enzyme production, but to damage to the permeability barrier. Similar findings have been made with the other Gram-negative bacteria reported here.

Absolute activity. The amount of penicillinase in Gram-negative bacteria varies considerably, whereas penicillinase-producing Gram-positive bacteria possess large amounts of enzyme only when induced. (Penicillinase-constitutive mutants of *B. cereus* and *Staph. aureus* have been obtained only as a result of special laboratory treatment[29]). The Gram-negative bacteria contain, generally speaking, low amounts of penicillinase except for *A. cloacae* 53 and *A. aerogenes* 366, which contain, respectively, almost as much as, or more, penicillinase than the highly induced *Staph. aureus E3*.

Discussion. The lack of precise enzymological investigations on penicillinases from different sources has led to the tacit assumption that they have the same properties and characteristics. The first clear indication that this was not the case came with the finding[1] that penicillinases from *B. cereus NRRL* 569 and *B. subtilis* 749 differed immunologically. The quantitative differences between penicillinases of different Gram-positive origin, although slight, appear to be quite distinct, for example: (*a*) the specificity patterns of penicillinases from *Staph. aureus*, *B. cereus* and *B. licheniformis*, although very similar in most respects[9,12,21] differ in regard to phenoxybenzyl penicillin, which is hydrolysed much more rapidly by staphylococcal penicillinase than by the others[9]; (*b*) the susceptibilities of the enzymes to inhibition by methicillin and the isoxazolyl penicillins differ[12,24]: staphylococcal penicillinase was not inhibited in conditions under which other penicillinases showed up to 80 per cent inhibition and it has been shown[30] that staphylococcal penicillinase requires a period of preincubation with the inhibitor before inhibition can be demonstrated. Despite these differences within this group of penicillinases, it is apparent that these enzymes show broadly similar properties and characteristics and form a homogeneous group within themselves.

However, major qualitative differences occur between penicillinases of Gram-positive origin and those derived from the eight Gram-negative organisms. As described here, the features which identify the penicillinases of the Gram-negative organisms are their specificity patterns, lack of inducibility, possession of a marked differential permeability barrier, difference in cellular location and significantly lower apparent activation energies[11]. The fact that there also exist quantitative differences, more marked than in the Gram-positive series, between the properties of penicillinases isolated from different Gram-negative sources[9,10,12] gives rise to the interesting speculation that each individual species of penicillinase-producing Gram-negative bacteria may produce a 'species-specific' penicillinase, the enzymological and physico-chemical properties of which cannot be predicted solely on the basis of comparison with such properties of other penicillinases from a different species.

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ENZYMATIC DEBRANCHING OF GLYCOGEN

A New Pathway in Rabbit Muscle for the Enzymatic Debranching of Glycogen

THE breakdown of glycogen by rabbit-muscle phosphorylase is far from complete, owing to the inability of this exo-enzyme to sever or by-pass the α-1,6-branch linkages, and action normally ceases when less than 40 per cent of the glycogen has been converted into α-D-glucose-1-phosphate. There remains a macromolecular limit dextrin (φ-dextrin). Further phosphorolytic breakdown is possible only after hydrolysis of the branch linkages. Some 10 years ago G. T. Cori and Larner[1] reported the existence of a rabbit-muscle enzyme, amylo-1,6-glucosidase, which attacked φ-dextrin with the liberation of glucose and allowed further phosphorolysis to occur. The enzyme did not attack native glycogen[1,2]. They thought the glucose was derived from 1,6-bonded side-chains which had been attenuated to a single glucose unit by phosphorylase. The correctness of this hypothesis and of the postulated mode of release of glucose was, however, questioned by Walker and Whelan[3], who could detect no single 1,6-bonded glucose side-chains in amylopectin or glycogen φ-dextrins. The side-chains were four glucose units long and they suggested that a transferase enzyme was required to expose the single glucose residues for hydrolysis, the other three glucose units being transferred singly or severally to other parts of the molecule[3]. A transferase having some of the properties of the postulated enzyme was reported recently in rabbit muscle by Brown and Illingworth[4], and Verhue and Hers[5] found evidence of the co-existence of single-unit and four-unit side-chains in φ-dextrin. The debranching process in plants operates by a different mechanism[6]. Shortening of the chains by phosphorylase is not a prerequisite. The responsible catalyst, *R*-enzyme, hydrolyses the 1,6-linkages in the intact polysaccharide, setting free the member chains. During this action the iodine stain increases. This is ascribed[6] to the ability of the unbranched chains to complex with iodine more efficiently than chains combined in the dendritic macromolecule.

We now report a debranching process operated by a rabbit-muscle enzyme preparation that does not require prior phosphorolysis of the glycogen. A highly concentrated amylo-1,6-glucosidase preparation, kindly given to us by Prof. E. Bueding, was incubated with glycogen and amylopectin and a slow but eventually very pronounced increase in intensity of iodine stain occurred (Fig. 1). During the incubation with amylopectin, polysaccharide precipitated from solution, staining blue-purple with iodine, in contrast to the original red stain. These changes were accompanied by increases in the extent of β-amylolysis. Amylopectin, amylopectin β-limit dextrin and rabbit-liver glycogen, having initial degrees of β-amylolysis of 49, 0 and 44 per cent respectively, yielded 77, 54 and 72 per cent maltose with β-amylase after 24-h treatment with the glucosidase preparation. The iodine stain of amylopectin did not increase when ammonium molybdate was added to the digest. Molybdate also inhibits plant *R*-enzyme[7].

The effect now reported has previously been unnoticed probably because amylase frequently contaminates 1,6-glucosidase preparations[1,2] and causes the iodine stains of the polysaccharides to decrease. Indeed, of ten glucosidase preparations given to us by Dr. Bueding, only four were sufficiently free from amylase for increase in staining to occur.

In respect of increase in iodine stain, increase in degree of β-amylolysis and molybdate inhibition, the muscle-glucosidase preparation behaves very much like *R*-enzyme[6]. (There is the important difference that plant

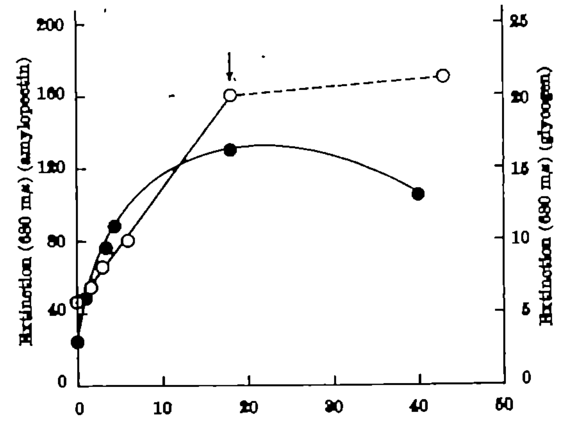

Fig. 1. Action of rabbit-muscle glucosidase on amylopectin (○) and rabbit-liver glycogen (●) at pH 7·2 and 30°. The amylopectin was waxy-maize starch. At the point indicated by an arrow precipitation of polysaccharide was noted and the iodine-stained solution at this time and at 43 h contained suspended polysaccharide. The extinction coefficient was therefore lower than if the polysaccharide had been dissolved

R-enzyme does not debranch glycogen of normal unit chain-length (about 12)[8]. Only glycogen of abnormally high chain-length, comparable with that of amylopectin (20 or more), is attacked by plant *R*-enzyme.[9]) It is, however, possible that the debranching process is mediated by the same combined actions of a transglucosylase and amylo-1,6-glucosidase which we have already postulated occurs with φ-dextrin[3]. It is a logical extension of our earlier hypothesis that the native polysaccharides should also be debranched by these two enzymes. The transferase action that exposes single glucose residues for hydrolysis by glucosidase will lengthen some of the polymer chains and increase the intensity of the iodine stain, and the removal of the 1,6-linked glucose residues by glucosidase will increase the degree of β-amylolysis. The limit of transferase–glucosidase action on glycogen would be reached by its conversion into glucose and the linear polysaccharide amylose. Indeed, the precipitation of polysaccharide from amylopectin during the action of the muscle enzyme preparation suggests the formation of amylose, with its pronounced tendency to retrograde from solution. When plant *R*-enzyme acted on amylopectin the total increase in intensity of iodine stain was only half that observed with the muscle system, and the polysaccharide did not precipitate[8,9]. This comparison strengthens the view that the muscle preparation causes chain-lengthening rather than simple debranching. Further support for the transferase–glucosidase system comes from a paper-chromatographic examination of polysaccharides treated with muscle system, but conclusive proof must wait on the further purification of the enzymes involved, particularly with respect to their freedom from amylase.

The observation of an increase in iodine stain when the glucosidase preparation acts on amylopectin and glycogen recalls the recent report by C. F. Cori *et al.*[10] that such increases occur when crystalline muscle phosphorylase is incubated with these substrates in presence of a trace of inorganic phosphate or α-glucose-1-phosphate. This was ascribed[10] to a phosphorolytic disproportionation of the member chains, not to a debranching action. Our enzyme preparations are free from phosphorylase *a*, since the iodine stain still increases in presence of an excess of phosphate sufficient to permit the full degree of phosphorolysis, and consequent diminution of iodine stain, if active phosphorylase were present.

We were informed by Dr. D. H. Brown that he and his colleagues have made similar observations of increase in iodine stain on incubation of glycogen with muscle glucosidase and their results are published in the following communication.

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Combined Action of Oligo-1,4→1,4-glucantransferase and Amylo-1,6-glucosidase in Debranching Glycogen

THE development of a procedure for the purification of rabbit muscle amylo-1,6-glucosidase to a specific activity of approximately 80,000 U./mg protein[1] facilitated a study of the action of this enzyme preparation on various polysaccharides. The fact that the specific activity was 10–15 times that of earlier preparations[2] was reflected in the rapidity with which very small amounts of this protein acted together with phosphorylase *a* to catalyse the total degradation of glycogen. Thus, 1·0 µg of such a glucosidase preparation and 7·0 µg of phosphorylase *a* caused 100 per cent degradation of 1·5 mg of glycogen in *p*H 6·7 phosphate buffer at 30° within 1 h. As expected, small amounts of the glucosidase preparation also acted directly and rapidly on a limit dextrin of glycogen prepared by prior phosphorylase treatment to yield free glucose (Fig. 1). Since several other enzymes are known to form glucose from polysaccharides, the various glucosidase fractions obtained during purification were assayed for their ability to form glucose from maltose and from glycogen. It was found that the highly purified glucosidase never formed any glucose from maltose; however, an action on glycogen was always demonstrable. Table 1 shows a comparison of the extent of glucose formation by a small amount of a glucosidase preparation with a specific activity of 42,000 U./mg protein acting on various polysaccharides. In view of the fact that the glycogen sample (M. L.) had been isolated from the liver of a patient with Type III glycogen storage disease (absence of amylo-1,6-glucosidase) and was known to have short outer chains (17 per cent degraded by phosphorylase), it was not surprising that it yielded considerably more glucose than the normal human glycogen sample. The possibility was considered that the glucose arising from the latter glycogen sample as well as the much smaller quantity from amylopectin might have had its origin in some exposed glucose stubs as branch points on the outer chains of the native polysaccharides. A similar explanation was offered by Larner who found the liberation of 0·3 per cent glucose by 200 units of glucosidase acting on glycogen for up to 2 h (ref. 3). It was not possible to decide this point with certainty from these findings alone. However, when an attempt was made to measure the total amount of glucose which could be removed from the human pectoralis glycogen by exhaustive treatment with large amounts of a glucosidase preparation, the results of Fig. 2 were obtained. Here, it can be seen that glucose is produced in rather large amount and without any apparent endpoint either in time or with increasing enzyme concentration. Glucose formation was so extensive that an explanation in terms of preformed, exposed glucose branch points was extremely unlikely.

Table 1. ACTION OF A PROTEIN FRACTION CONTAINING AMYLO-1,6-GLUCOSIDASE ON SOME POLYSACCHARIDES

Polysaccharide	Glucose formed in 5 h (per cent)
Glycogen, human pectoralis muscle	0·41
Glycogen, human liver (M.L.)	1·65
Corn amylopectin	0·17

Protein concentration, 4·6 µg/ml. corresponding to 193 units. Polysaccharide, 6 mg/ml. Glycylglycine buffer, 0·003 M, *p*H 6·8, 30°.

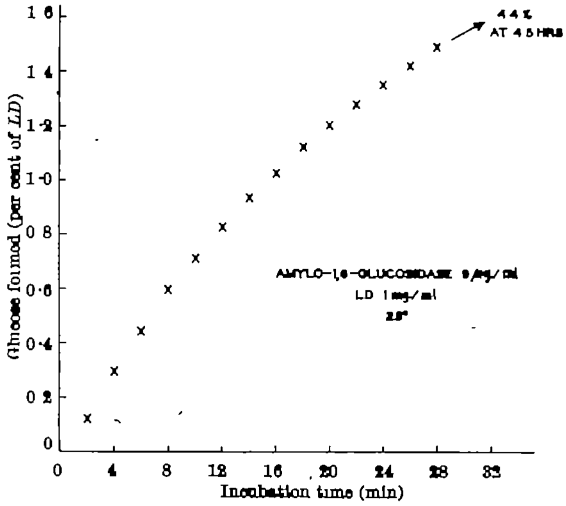

Fig. 1. Action of amylo-1,6-glucosidase on a phosphorylase limit dextrin. The reaction mixture (in a Beckman spectrophotometer cuvette) contained limit dextrin as indicated, 50 µmoles *tris* buffer, 3 µmoles mercaptoethanol, 0·5 µmole ethylenediamine tetraacetic acid, 10 µmoles $MgCl_2$, 2 µmoles ATP, 0·4 µmole NADP, yeast hexokinase and glucose-6-phosphate dehydrogenase (Boehringer) in excess, and amylo-1,6-glucosidase (sp. act., 42,000 U./mg protein) *p*H 7·2, 1·0 ml. The formation of glucose was followed by the increase in absorption at 340 mµ

The reaction of glucosidase preparations with glycogen was found to be accompanied also by large increases in iodine colour, often after a lag relative to glucose formation (Fig. 3). This finding served to show that α-amylase

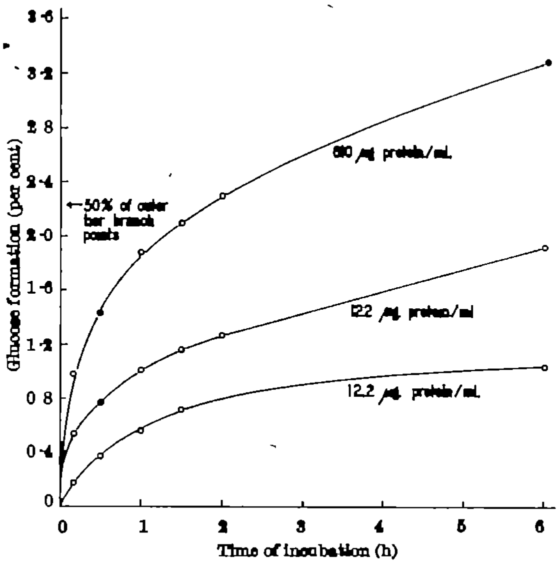

Fig. 2. Formation of glucose from glycogen as a function of time at various concentrations of a protein fraction containing amylo-1,6-glucosidase (sp. act., 41,000 U./mg protein). The reaction mixtures (0·50 ml.) contained 1·8 mg glycogen, 10 µmoles glycylglycine, and enzyme as indicated, *p*H 6·6. Aliquots (0·05 ml.) were removed at intervals and assayed micro-enzymatically for glucose

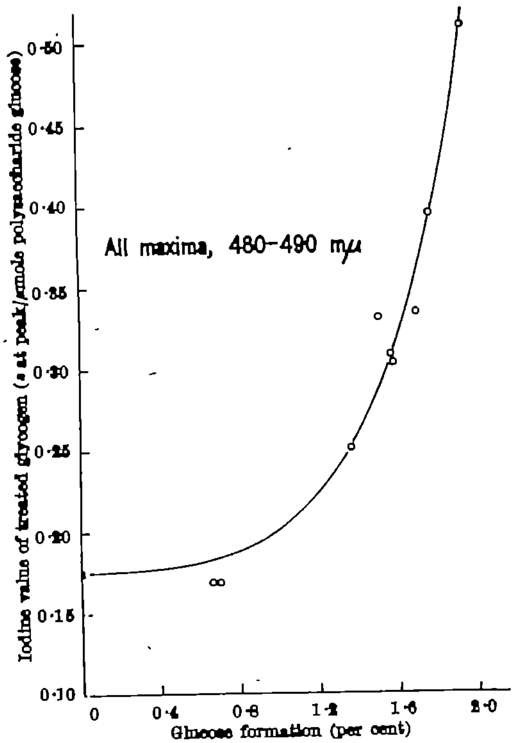

Fig. 3. Change in chromogenicity of the iodine complex of glycogen as a result of pretreatment with various amylo-1,6-glucosidase fractions. The extent of enzyme action is expressed in terms of the glucose formed. Aliquots containing about 0·2 mg of glycogen were diluted to 0·9 ml. with water and 0·1 ml. of 0·2 per cent iodine–0·4 per cent potassium iodide was added with thorough mixing. In each case extinction values were determined between 450 mμ and 550 mμ in the Beckman *DU* spectrophotometer

was not a contaminant of any of the highly purified fractions and so could not have been responsible for any part of the observed formation of glucose. Absence of action of the enzyme system on maltose showed that there was no detectable contamination by the glucose-forming enzyme studied by Stetten[4] and found to be a transglucosylase.

Since Illingworth *et al.* reported[5] that phosphorylase itself in the presence of traces of inorganic phosphate or glucose-1-phosphate can catalyse a rapid redistribution of some of the outer glucose units of glycogen and amylopectin so that the iodine colour of the polysaccharide shows a large increase, it was important to establish that the highly purified amylo-1,6-glucosidase preparations were free of phosphorylase. By direct analysis phosphorylase *a* was found to constitute less than 0·1 per cent of the total protein of a preparation in which the specific activity of amylo-1,6-glucosidase was 80,000 u./mg. In addition to using this preparation containing such an extremely small amount of phosphorylase *a*, the test solution of glycogen was electrodialysed to remove any trace of phosphate ion. These precautions when taken were without effect on the capacity of the glucosidase preparation to increase the iodine colour of glycogen. Hence, it must be concluded that this reaction is separate from the increase of similar magnitude which phosphorylase brings about. The latter will be reported in detail in a subsequent communication.

Evidence that the action of the glucosidase preparation on glycogen was actually accompanied by a partial debranching of the molecule was obtained by structure determinations of the polysaccharide remaining after measured amounts of free glucose had been produced. In six such experiments the quantity of glucosidase and the time of its action were varied in order to produce up to 1·6 per cent glucose formation. After boiling the solutions to stop enzyme action, phosphorylase *a* free of amylo-1,6-glucosidase was added together with excess inorganic phosphate to one aliquot and degradation of the outer chains of the glycogen was measured at various times by micro-enzymatic determination of glucose-1-phosphate. The final degradation observed is plotted in Fig. 4*a* against the amount of glucose which had been produced by prior glucosidase action. The results clearly show that the molecule has longer outer chains as a result of its pretreatment by the glucosidase preparation. The increase in iodine colour shown in Fig. 3 is another expression of this fact.

Another aliquot of the glycogen solution after glucosidase treatment was subjected to total enzymatic degradation by the combined action in phosphate buffer of phosphorylase and added amylo-1,6-glucosidase. The amount of free glucose formed thereby was calculated from the total present after degradation (determined micro-enzymatically) and that present at zero time due to the prior glucosidase action. In this way the end-group percentage of the treated glycogen was found, and the results are plotted in Fig. 4*b* against the measured prior glucose formation. In general, it is seen that a reduction in the number of branch points accompanies glucosidase action. The precision of these results is less than the measurement of outer chain-length by phosphorylase (Fig. 4*a*).

In order to investigate whether any part of the glucose formed by the glucosidase preparations had its origin in 1,4-linked units, a phosphorylase limit dextrin of the human pectoralis glycogen was enlarged with ^{14}C-glucose-1-phosphate by the synthetic action of phosphorylase. The extent of enlargement was 28·6 per cent, corresponding to the average addition of 2·4 ^{14}C-glucose units to each outer chain. The specific activity of the added glucose residues was 2,120 c.p.m. per μmole. This radioactive glycogen was treated for 23 h at 30° with 2·6 μg

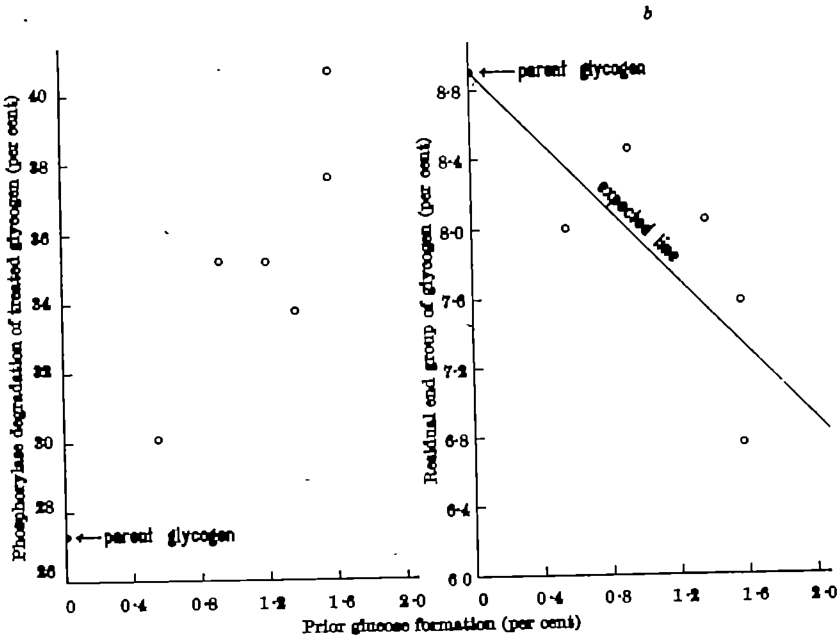

Fig. 4. *a*, Determination of the outer chain-length of various glycogen samples as a function of the extent of preceding action by amylo-1,6-glucosidase fractions; *b*, determination of the end-group percentage of various glycogen samples as a function of the extent of preceding action by amylo-1,6-glucosidase fractions

of glucosidase (sp. act., 70,000 U./mg), and in another experiment, with 28·5 μg of glucosidase (sp. act., 6,300 U./mg). In these two experiments the units of glucosidase activity added were the same, although the enzymes differed greatly in their purity. It is interesting that the amount of glucose produced during incubation was nearly the same in each experiment, 1·57 and 1·77 per cent. A measured amount of carrier glucose was added, the glycogen was precipitated by alcohol, and buffer salts removed by passage over a short column of a mixed bed resin ('Amberlite *MB*-3'). Recovery of glucose was complete. The solutions were subjected to paper chromatography (Whatman No. 1) in a butanol–pyridine–water solvent (3 : 2 : 1·5) and the glucose zones eluted (90 per cent recovery). The glucose was analysed microenzymatically and its radioactivity measured in a low background counter (Nuclear). From the known dilution with carrier it was possible to calculate that the glucose formed by glucosidase action in 23 h of incubation had a specific activity in one experiment of 27 c.p.m. per μmole and in the other of 33 c.p.m. per μmole. Thus, it could have contained no more than 1·5 per cent of molecules derived from 1,4-linkages. An enzyme with glucamylase action on the outer chains of glycogen such as that found in rat muscle by Torres and Olavarria[6] seems to play no part in the phenomenon studied here.

An explanation for the changes in the structure of glycogen (and amylopectin) which highly purified amylo-1,6-glucosidase preparations are able to bring about became available after definitive experiments were devised to show that the enzyme preparation in fact contains an oligo-1,4 → 1,4-glucantransferase which acts to transfer maltotriosyl and maltosyl (but not glucosyl) residues in α-1,4-linkage from donor to acceptor chains[7]. Possibly longer chains of glucose units may also be transferred. Walker and Whelan had suggested earlier[8] that a transferase with some of the properties of the enzyme described by Brown and Illingworth[7] would be found to play a part in glycogen metabolism. From a study of the structure of a phosphorylase limit dextrin they concluded that movement of glucose residues from outer side-chains to other parts of the molecule is necessary before action of amylo-1,6-glucosidase on a limit dextrin is possible.

In the reactions described here, glycogen gradually becomes debranched as amylo-1,6-glucosidase removes the α-1,6-linked glucose residue whenever such a unit becomes exposed by prior action of the oligotransferase. The longer outer chains of the partially debranched glycogen are, of course, more chromogenic when tested with iodine. It is not excluded that a small part of the glucose liberated has its origin in glucose stubs which are already exposed in the native polysaccharides as they are isolated. It should be emphasized, as shown in Fig. 2, that the debranching of glycogen by the non-phosphorolytic pathway described herein is slow and requires rather large amounts of the highly purified glucosidase in which oligo-1,4 → 1,4-glucantransferase is a minor contaminant. A determination of the speed at which the debranching action would proceed if the latter enzyme could be added in excess awaits the success of experiments to purify the transferase itself.

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CULTURE OF STRAIN *L* CELLS IN SUSPENSION: REPLACEMENT OF POLYMER BY TRACES OF TRYPSIN IN A DEFINED MEDIUM

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METHODS for the culture of tissue cells *en masse* have been studied in a number of laboratories[1-5] with the view of opening up to the student of tissue cells the valuable cytostat and washed suspension techniques familiar to the microbiologist. All the methods described so far, however, fall short of their microbiological counterparts in that they require media containing serum protein or some other polymer, such as methyl cellulose, to support normal rates of dispersed cell growth[6,7]; and it has often been reported that the cells nevertheless tend to clump and undergo necrosis in the clumps[1,4,8-10]. We have recently had some success in overcoming these technical difficulties, and as our findings appear to be both simple and useful, we are reporting them briefly here in a preliminary form.

We have used the defined medium *MB*752/1 described by Waymouth[11], modified[12] by the addition of 0·0032 mM ferrous sulphate, 0·25 mM α-ketoglutarate, by the increase of glutamine to 3·4 mM, and by maintaining an atmosphere of 5 per cent (v/v) carbon dioxide in air in all culture vessels. It was confirmed that although strain *L* mouse cells grow well (mean generation time about 48 h) from mechanically dispersed ('scraped') roller tube inocula in unshaken 'Pyrex' vessels, identical cultures decline, or grow very slowly when gently reciprocated or stirred. Attention was thus directed to the mechanism by which movement inhibits the growth of cultures in defined polymer-free medium.

The effects of different speeds of simple harmonic reciprocation (stroke-length 4 cm) were examined in 50-ml. conical flasks, at 37°, beginning with 4 ml. scraped cell suspensions ranging in population from 0·2 to 1·0 × 10^6 cells per ml. At reciprocation rates below 30 cycles/min, the cells adhered to the bottom of the flask in a uniform monolayer, and growth was not significantly different from stationary control cultures. Rates of 60–80 cycles/min caused many of the cells to aggregate at a 'high water mark' on the sides of the flask. This aggregation could not be prevented by siliconizing the glass, or by the addition of 0·1 per cent methyl cellulose to the medium. Microscopical examination after 24 h reciprocation at 60–80 cycles/min showed that the cells in the interior of the aggregates were undergoing necrosis, but cells free in suspension were apparently healthy, although the latter were occasionally seen to be associated with folded membranous debris, containing nuclei. Cell counts made after dispersing the clumps with 0·25 per cent crude trypsin indicated that the live cell population remained almost stationary over a few days. Higher reciprocation rates resulted in the formation of increased amounts of membranous debris, and the cell number declined rapidly. At 150 cycles/min, for example, the total cell count was reduced to less than 30 per cent of the initial count after only 15 min.

These, and related experiments, suggested two main mechanisms of inhibition of growth: (*a*) aggregation, causing locally unfavourable nutritional and physical conditions; (*b*) cell disruption, due to cell-to-cell, cell-to-interface, and cell-to-vessel adhesion, followed by extension of the adhering cell surfaces, forming membranous debris which later autolyses slowly. We therefore sought to minimize the lethal effects of the motion of the medium by reducing the adhesive properties of the cell surfaces.

It was observed that cells which had been treated briefly with trypsin were much less readily destroyed by high rates of reciprocation. Scraped cell suspensions were exposed to 10 µg crystalline trypsin per ml. for 15 min at 37°, washed, re-suspended in fresh medium and reciprocated at 130 cycles/min for 15 min. Cell counts were made before and after reciprocation at 130 cycles/min, and at daily intervals for the next four days, during which a shaking rate of 65 cycles/min was maintained. Total cell number is recorded in Fig. 1, curve *A*, for the culture after trypsinization, and curve *B* for a corresponding control culture, not trypsinized. It is significant that cell aggregates appeared in the trypsin-treated culture only after the third day.

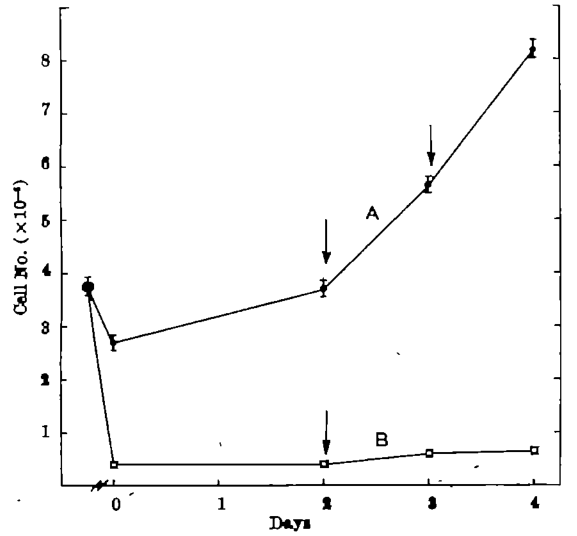

Fig. 1. Total cell number each day (with standard deviation) in culture of trypsinized cells (curve *A*) and in control culture (curve *B*), after reciprocating at 130 cycles/min for 15 min, and afterwards at 65 cycles/min. Fresh medium (2·0 ml.) was added to the starting volume of 4·0 ml at the points indicated by the arrows

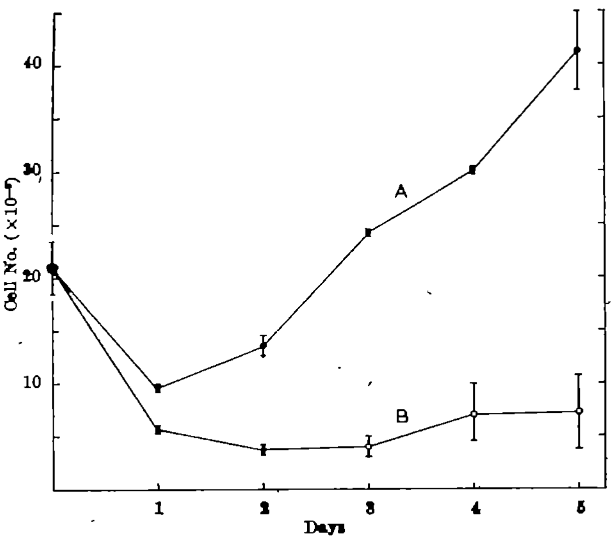

Fig. 2. Total cell number each day in trypsin-treated culture (curve *A*) and in control culture (curve *B*) reciprocated at 65 cycles/min. One-fourth volume of fresh medium and 0·2 ml. 0·005 per cent trypsin were added daily to the starting volume of 50 ml.

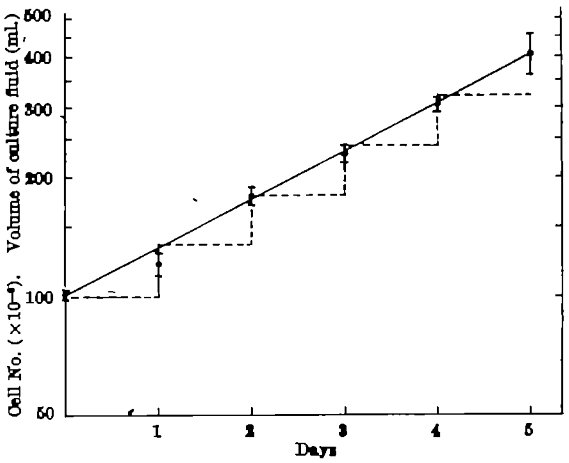

Fig. 3. Cell growth in spinner flask (200 r.p.m.) in defined medium plus trypsin. The concentration of crystalline trypsin was daily made up to 10 µg/l. of culture fluid. Volume of culture fluid (– – – –)

The excellent protective effect of trypsin in this type of experiment led us to examine the effect of maintaining low concentrations of crystalline trypsin in the culture medium at the intermediate reciprocation rate of 65 cycles/min. Fig. 2 shows the results of a typical experiment in which 0·2 ml. 0·005 per cent crystalline trypsin in basal salt solution was added to 50 ml. scraped cell suspension initially, and at each daily addition of one-fourth volume of fresh medium. The control culture (curve *B*) received an equivalent volume of basal salt solution without trypsin.

As already indicated here, reciprocating motion is especially liable to cause cell aggregation by sequestering the cells at 'high water marks'. Therefore, we have adopted the more conventional spinner culture technique, using a silicon-rubber covered follower impelled at 200 r.p.m. by a magnet attached to a clock motor. Long-necked, flat-bottomed flasks have been found ideal in shape for this method of stirring. The follower is in contact with the floor of the flask, but despite this, little evidence of cell damage is observed. The growth curve shown in Fig. 3 was obtained by the daily addition of one-third volume of fresh medium followed by 0·1 ml. 0·001 per cent crystalline trypsin in basal salt solution per 100 ml. cell suspension. Similar growth experiments have shown that crystalline trypsin does not exert an appreciable cytostatic or cytocidal effect up to a concentration of 0·2 mg/l.

A simple continuous culture system has now been established. Fresh medium containing 50 µg crystalline trypsin/l. is filtered directly into a refrigerated reservoir which is connected to the growth flask through a drawn-out capillary tube. Medium is allowed to flow in at a rate which trebles the starting volume of cell suspension (for example, 0·5 l. in a 10-l. flask) after four days, when two volumes are siphoned off. One such unit, yielding about 6×10^8 cells at each collection, has been operated for thirteen weeks. We are now developing this type of arrangement on more sophisticated cytostat principles.

The use of trypsin in such small amounts (10–50 µg/l.) is, of course, possible only in protein-free media. The trypsin appears to function by breaking down an extracellular material by which the cells adhere to surfaces and to each other, and it is a characteristic feature of suspended growth in trypsin-containing media that, as in normal cultures of micro-organisms, the cells mostly exist singly or in pairs. We are at present investigating what crystalline trypsin does to the cell surfaces, and whether other enzymes, such as neuraminidase, may also assist growth in suspension.

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HISTOGENESIS OF THE CEREBRAL CORTEX

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IT is generally accepted that stratification in the mammalian neocortex is established as a result of three cellular migrations in the wall of the telencephalon which, occurring in the rat on the 16th, 19th and 21st days of gestation, give rise respectively to layers II and III, layer IV and layers V and VI (ref. 1). A recent examination of silver and Golgi–Cox preparations showing the effects of X-irradiation on the developing nervous system has suggested, however, that the manner in which cortical lamination arises may be more complex than has been inferred from the Nissl-stained material previously examined.

In the developing nervous system the elements which are particularly susceptible to the effects of ionizing radiations are mitotic and differentiating neuroblasts[2] and it would accordingly be expected that X-irradiation during the period of general cortical differentiation would destroy the germinal and mantle layers but would leave cells in the marginal layer largely unaffected. This prediction has, in fact, been borne out by examination of the effects of X-irradiation (200 r.) given to the fœtal rat on either the 17th or 19th day of gestation. In this species 12, 24, 36, 48 h and, in some cases, 9 days after irradiation both the mantle and germinal layers are extensively and seemingly irreparably damaged while the majority of cells lying in the marginal layer near the pial surface are apparently normal (Fig. 1*A* and *B*). These cortical remnants are found to give rise later in life (30 days of age *post partum*) to a cerebral cortex composed of three layers only (Fig. 1*C* and *D*), tentatively identified on the basis of histological appearances as layers I, V and VI. However, the cortex of rats in which X-irradiation has been delayed until the 21st and 22nd days of gestation (and in which destruction of the mantle layer is less severe) includes, in addition, a thin band of disorganized cells lying superficially and deemed to represent layers II, III and IV (ref. 3). On the basis of this interpretation it would seem that, in spite of the total destruction of those elements classically destined to give rise to the infra-granular layers, the development of these layers is considerably less impaired than is that of the granular and supragranular. One possible explanation of this phenomenon is that the pyramidal cells which develop in the cortex of X-irradiated animals may, at least in part, be hyperplastic pyramids derived from layers III and IV which have differentiated abnormally in an atypical environment. The findings could equally be explained, however, in terms of a pattern of cellular migration similar to that seen in the developing cerebellar cortex[4]. Thus some of the first cells which

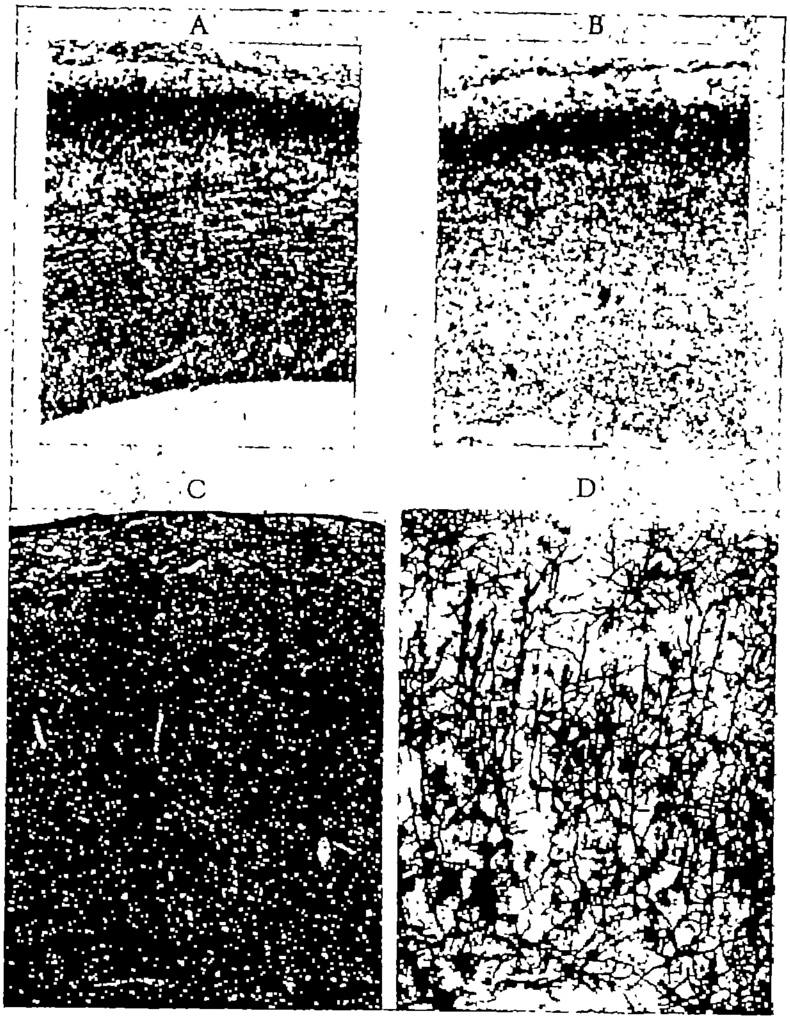

Fig. 1. Cerebral cortex of rat

A, normal, nineteenth day of gestation (methylene blue, × *c.* 83); *B*, *C* and *D*, X-irradiated (200 r.) on nineteenth day of gestation; *B*, 36 h after irradiation (methylene blue, × *c.* 83); *C*, 33 days after irradiation (silver impregnation, × *c.* 60); *D*, as *C* (Golgi–Cox, × *c.* 60) migrate into the marginal layer to occupy a position juxtaposed to the zonal layer may be destined to form layers V and VI, the granular and supragranular layers arising as a result of subsequent migrations which are vulnerable to X-irradiation between the 17th and 19th day of gestation. This schema implies that the normal six-layered cortex is established by an inward migration of cells, ultimately constituting the developing layers V and VI, intermingled with an outward migration of cells destined to form layers II, III and IV.

With this possibility in mind, an attempt has been made to identify the large pyramidal cells in the cortex of the X-irradiated animals at 30 days *post partum* by both qualitative and quantitative examination of silver and Golgi–Cox preparations. The shape of some cells in the irradiated cortex is abnormal (Fig. 1C); but if these are excluded from quantitative analyses the results indicate that morphological characteristics such as the cross-sectional area of the perikarya, the number and length of basal dendrites and the number and position of branching points along the length of these dendrites closely resemble those of the normal pyramidal cells of layer V*b* and bear little resemblance to data relating to the pyramids of layers III and IV in the normal animal. The view that the pyramidal cells present in the X-irradiated cortex are the counterparts of those of the normal lamina ganglionaris receives further support from examination of the developing three-layered archipallium in which the effects of irradiation are found to follow a course similar to that seen in the neocortex. On the 17th and 19th days of gestation the subicular area is not present, and the cells lying superficially in both the neocortex and hippocampus form, in coronal sections, a continuous band around the medial aspect of the lateral ventricle; yet Ammon's pyramids, regarded as homologous with the pyramidal cells of layer V*b* (ref. 5), can be seen in the hippocampus of the irradiated rat aged 30 days and, although disorganized and hypoplastic, form a zone of cells which, in the absence of a corpus callosum, is continuous with that occupied by the neocortical pyramids.

These findings, which imply that cells destined to form the phylogenetically older infra-granular layers appear before those giving rise to the more recently acquired superficial laminae, would seem to suggest a more compatible relationship between ontogeny and phylogeny than that indicated by the earlier interpretations. It is still not possible, however, to infer from such indirect evidence as is so far available that events occurring in the irradiated cortex also take place during the normal course of development, and studies, now in progress, of the behaviour of tritiated neuroblasts may yield more direct information concerning cellular movements during cortical differentiation.

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RAPID CHANGES IN TRANSPIRATION IN PLANTS

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It has been repeatedly shown that excision of a leaf from a plant causes a rapid change in the transpiration rate from the leaf. The change may be an increase or a decrease[1]. It has also been found that an addition of an osmotically active substance to the root medium or an increase in the salt content of a nutrient solution surrounding the root system causes an immediate increase in the transpiration rate[2,3] and in the former case also in the stomatal width as measured with a porometer[4]. The addition of various chemicals such as ethyl alcohol, diethyl ether and some respiration inhibitors also causes a rapid increase in transpiration[3].

The increase obtained after an excision of a leaf in air (the Ivanoff effect) was interpreted by Ivanoff[5] as caused by a sudden release of the water stress in the conducting elements, which means that it is caused by an increase in the water supply to the leaf. This interpretation was adopted by workers using the corona hygrometer[1] and was strongly supported by the experiments of Allerup and Nielsen[6]. The increase in transpiration obtained after addition of mannitol to the root medium was interpreted by me to be caused by an increase in the water permeability of the roots[3]. Thus, also in this case the rush of water might be an Ivanoff effect caused by a release of the water stress in the plant. The mechanism suggested can be characterized as hydroactively induced.

The alternative explanation was first suggested by Darwin and Pertz[7]. They assume that the stomatal opening is caused by a loss in turgor of the epidermal cells surrounding the stomata, mainly the companion cells. This would mean that the opening is caused by a decrease in the water supply to the leaf instead of the increase assumed by Ivanoff.

An inherent requirement of the hypothesis is that the epidermis cells dry out faster than the guard cells. The movement can be characterized as mechanically induced. Later on the guard cells lose water too and a hydroactive closure of the stomata occurs. This explanation was the only one which was referred to by Slatyer[8]. During investigations of the water relations of various plants in the laboratory as well as under field conditions several results have been obtained which are in favour of the theory of Ivanoff.

During work on the transpiration of forest trees under field conditions, a series of excision experiments on leaves and shoots of various species was performed. Rapid changes normally followed the excision. However, this immediate effect was sometimes a decrease and sometimes an increase, and in some cases no immediate change at all was found. Aspen mostly gave very considerable increases, while all conifers tested (pine, spruce, larch) gave decreases. Birch behaved as an intermediate, as this tree was found to be able to give increases, decreases or no changes at all. I assume that the direction of the change is determined by the amount of water available in the leaf together with the intensity of the transpiration.

Extremely high increases were obtained in the following way in the laboratory. Excised leaves were placed with their petioles in water and were allowed to increase their turgidity in darkness. Then they were illuminated, and when the transpiration had attained a constant value they were taken from the water. Even in these circumstances coniferous twigs gave no increases. I assume that the lack of Ivanoff effect is due to the fact that the water transport in the coniferous stem takes place via tracheids and not in vessels and thus the water column is less movable. Wood of conifers has a very low water conductivity[9].

Aspen leaves were found to give extremely high Ivanoff effects. Fig. 1 shows an experiment with an aspen leaf

Table 1 Relative Turgidity (RT) of Leaf and Petiole of Aspen Leaves before and during the Increase in Transpiration caused by Lifting the Leaf out of Water

(The determinations were performed by M. and P. Jarvis at this Institute.)

Sample		RT	
T_1	Leaves	99 5	99 3
		100 0	
		98 5	
	Petioles	97·0	97·2
		97·3	
T_2	Leaves	97·3	97·2
		96 7	
		97 7	
	Petioles	94 0	93 7
		93·4	

where the petiole was taken from and brought in contact with the water repeatedly. It is apparent that the Ivanoff effect is greater and starts earlier at the higher light intensity. Furthermore, the increase caused by the removal of water commences faster than the decrease caused by the restoration of water. This result can be explained on the basis of the Ivanoff theory, while no mechanical effects from the surrounding cells can be traced. The changes in relative turgidity of leaf and petiole were determined. Detached leaves were put in water and were illuminated for 15 min Then samples (T_1) were taken. Then the petioles were lifted and left in air for 6 min and new samples were taken (T_2). It was found (Table 1) that: (1) the relative turgidity (RT) decreased more rapidly in the petioles than in the leaves; (2) the RT was lower in the second sample where the stomata, according to the transpiration record, were more open.

Wheat plants were placed as is shown in Fig. 2. The transpiration from the leaves enclosed in the cuvette (c) was recorded and the leaves were illuminated until the transpiration had attained a constant value. Then sodium chloride was added to the vessel A to give a concentration of 1 M. This treatment can only cause an increase in the water transport to the immersed leaf which may in turn bring about a decrease in water supply to the transpiring leaves but cannot possibly cause an increase. The effect on the transpiration was a decrease (Fig. 3, curve I). According to the mechanical theory already referred to, the decrease should be preceded by a transient increase, but no such increase was found. After the transpiration had decreased to the low value the immersed leaf was cut off; this was followed by a return to the higher transpiration rate. When it had re-attained a constant value, sodium chloride was finally added to the root medium in vessel B, which was made 1 M. The effect on transpiration is shown by curve II. The increase obtained in this case means that the salt addition to the roots has an effect peculiar to this organ. So far as I am aware nothing contradicts the assumption that an increase in permeability of the roots is elicited.

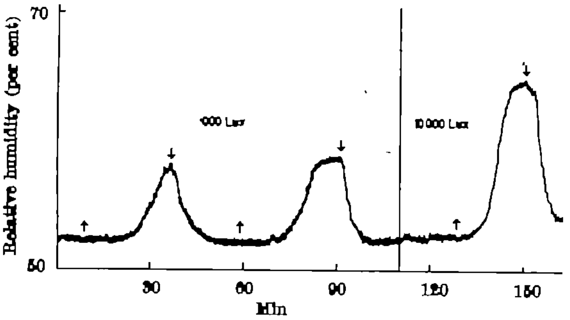

Fig. 1. Corona hygrometer record of the transpiration of a detached aspen leaf placed in water. At ↑ the water was removed, at ↓ the water was restored. Light intensities (lux)

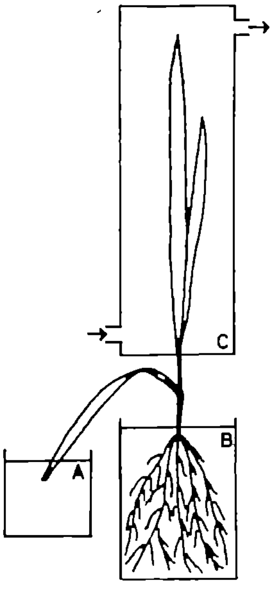

Fig. 2

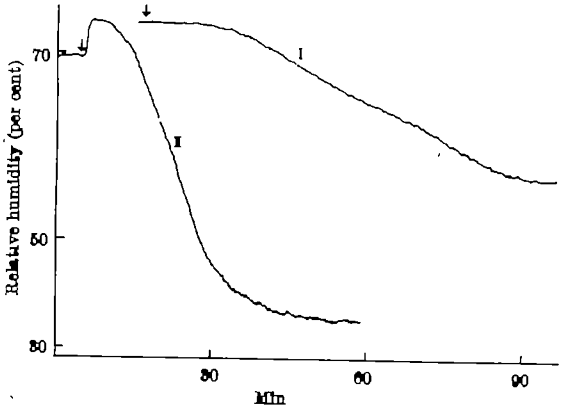

Fig. 3. Corona hygrometer record of the transpiration from wheat leaves arranged as is shown in Fig. 2. Further explanation in the text. The additions of sodium chloride are marked by an arrow

It seems to me that these experiments are interesting from another point of view. The fact that no mechanical effects could be found would imply that the water-stream from the roots to the guard cells passes the companion cells without changing the volume of these to a measurable extent. However, changes in the water supply to the leaves immediately affect the water content of the guard cells. This is possible if Strugger's[10] idea that the water transport occurs in the cell walls is accepted (see also ref. 11). A second necessary condition is that the guard cells take up water faster than the other epidermal cells, that is, a supposition which is quite opposite to that of Darwin and Pertz[7]. Experiments by Stålfelt[12] indicate that the guard cells change their water content very fast. A logical consequence of these considerations would be that hydroactive control of stomatal width is not mediated by the water-content of the leaf but by the water content of the cell walls. In this connexion the RT determinations of Table 1 are illustrative. Even if the RT decreases after the excision of the leaf which means that the leaf water deficits has increased, the stomata become more open. It seems necessary to distinguish between leaf water deficit and cell wall water deficit.

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ELECTRON-TRANSPORT SYSTEM IN PHOTOSYNTHESIS OF GREEN PLANTS ANALYSED BY SENSITIVE FLASH PHOTOMETRY

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THE observation of Emerson *et al.*[1] showed that light absorbed in the far red limit of the absorption spectra of green algae at ~700 mμ, that is, in a long wave-length chlorophyll *a*, shows a poor yield of photosynthesis but is enhanced to a much greater efficiency when it is supplemented by shorter wave-length light absorbed by chlorophyll *b* (peak at ~650 mμ). He concluded from this effect that two different photochemical reactions may be involved in photosynthesis, one sensitized by a long wave-length chlorophyll *a* and the other by chlorophyll *b*-650. Govindjee *et al.*[2] have shown that, in addition to light absorption by chlorophyll *b*-650, absorption in a chlorophyll *a* with a peak at 670 mμ can enhance the light-reaction sensitized by a long wave-length chlorophyll *a*. Correcting Emerson they concluded that two chlorophyll *a* sensitize the postulated two light-reactions. Blinks[3] and Myers and French[4] have extended the measurements of Emerson by different methods. The Emerson effect occurs also in non-green plants such as the brown, red and blue algae, etc.

Different hypotheses have been postulated to explain how a co-operation of two light-reactions may occur. Franck[5] developed a theory based on *n*–π and π–π-triplet-states. Hill *et al.*[6] proposed a hypothetical scheme, suggesting that cytochrome *b* and *f* may be involved. Brody[7] discussed the participation of monomere and dimere chlorophylls. French and Fork[8] suggested a two-pigment mechanism based on work of changes in oxygen.

Further indications for two light-reactions were obtained by other methods.

Kautsky[9] suggested from fluorescence measurements that coupling of two light-reactions occur. Allen *et al.*[10] obtained indications for two light-reactions from paramagnetic resonance signals. Losada *et al.*[11] derived a two-light-reaction mechanism from oxygen and TPN measurements in combination with poisons.

There was no experimental evidence: (1) on the chemistry of all the substances which are involved in the mechanism, except plastoquinone[12]; (2) on the nature of the light-reactions; (3) on the mechanism how the substances are correlated with each other; or (4) on the kinetics of the partial reaction itself.

Direct evidence about substances which are in action during the excitation of plants by light came from measurements of absorption changes. Duysens[13] discovered with a static method cytochrome oxidation particularly in red algae. In green plants he observed unidentified absorption changes at 420, 475 and 515 mμ. Kok[14] discovered the one-electron-oxidation of a pigment *P*700, with a redox-potential 0·43 V and with absorption changes at 703 mμ and suggests that this pigment is a chlorophyll *a*. We measured: (1) the π–π*-triplet-state in the bulk of chlorophyll *a* and chlorophyll *b* respectively (symbolized as $Chla_0$ and $Chlb_0$ (ref. 21); (2) a metastable state of a special chlorophyll *a* ($Chla_I$) which is not a π–π*-triplet-state[20]; (3) the oxidation of cytochromes (Cyt) in normal green plants[22,24,26]; (4) the reduction of plastoquinone (*Q*) (ref. 25); (5) the reduction of a substance *X*, which is a special plastoquinone[18,22,23,29] sensitized by a chlorophyll *a* ($Chla_{II}$); (6) pronounced absorption changes in photoactive chloroplasts at 433 mμ which are exactly correlated with the changes at 703 mμ and which verify the suggestion that *P*700 is a chlorophyll *a* symbolized

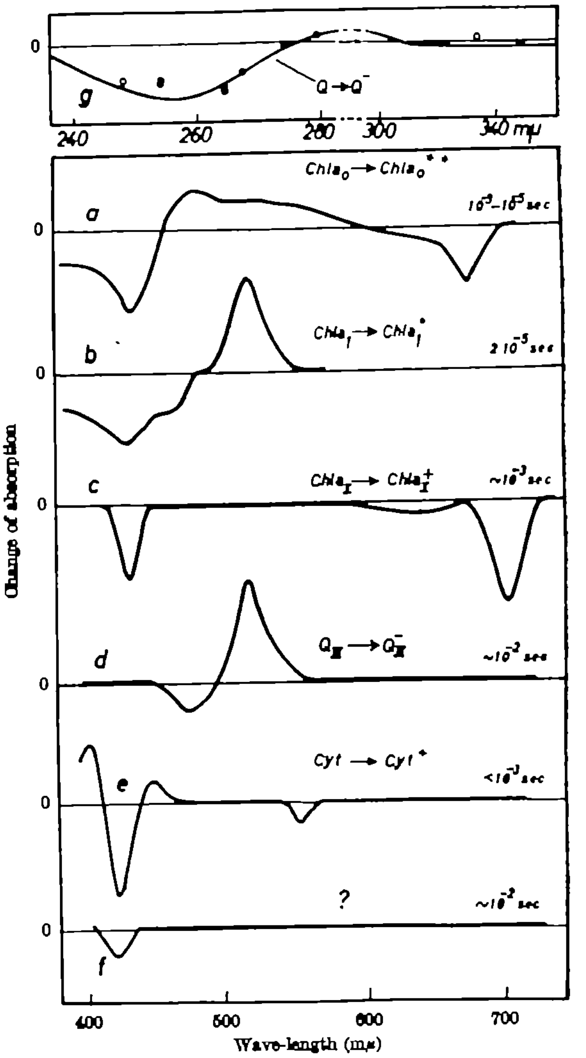

Fig. 1. Separated difference spectra caused by flashing light in chlorella and chloroplasts of spinach. (*a*) Caused by the π–π*-triplet state in the bulk of chlorophyll *a* ($Chla_0$). A corresponding spectrum was also obtained for chlorophyll *b*. The life time depends on the oxygen content and on the viscosity in the suspension (refs. 21 and 25). (*b*) Caused by a probably metastable state of a chlorophyll *a* ($Chla_I$) (refs. 20 and 28). (*c*) Caused by photo-oxidation of a chlorophyll *a* ($Chla_I$) in photoactive chloroplasts (refs. 24 and 26). (*d*) Caused by oxidation of cytochrome Cyt-*f* (refs. 22, 24 and 26). The difference spectrum can be obtained when the reduction of Cyt^+ is delayed. This can be done in different ways (ref. 26). The depicted spectrum was obtained especially by uncoupling Cyt from Q_{II} in fresh chloroplasts with added dichlorophenol indophenol. (*e*) Caused indirectly in surrounding pigments by photo-oxidation of plastoquinone (Q_{II}). Formerly Q_{II} was symbolized by *X* (refs. 17–19, 22, 23, 28 and 29). (*f*) Caused by an unknown reaction (ref. 26). (*g*) Caused directly by plastoquinones (Q_I and Q_{II}). The *in vitro* spectrum of plastoquinone is shown as a solid curve (refs. 25 and 29)

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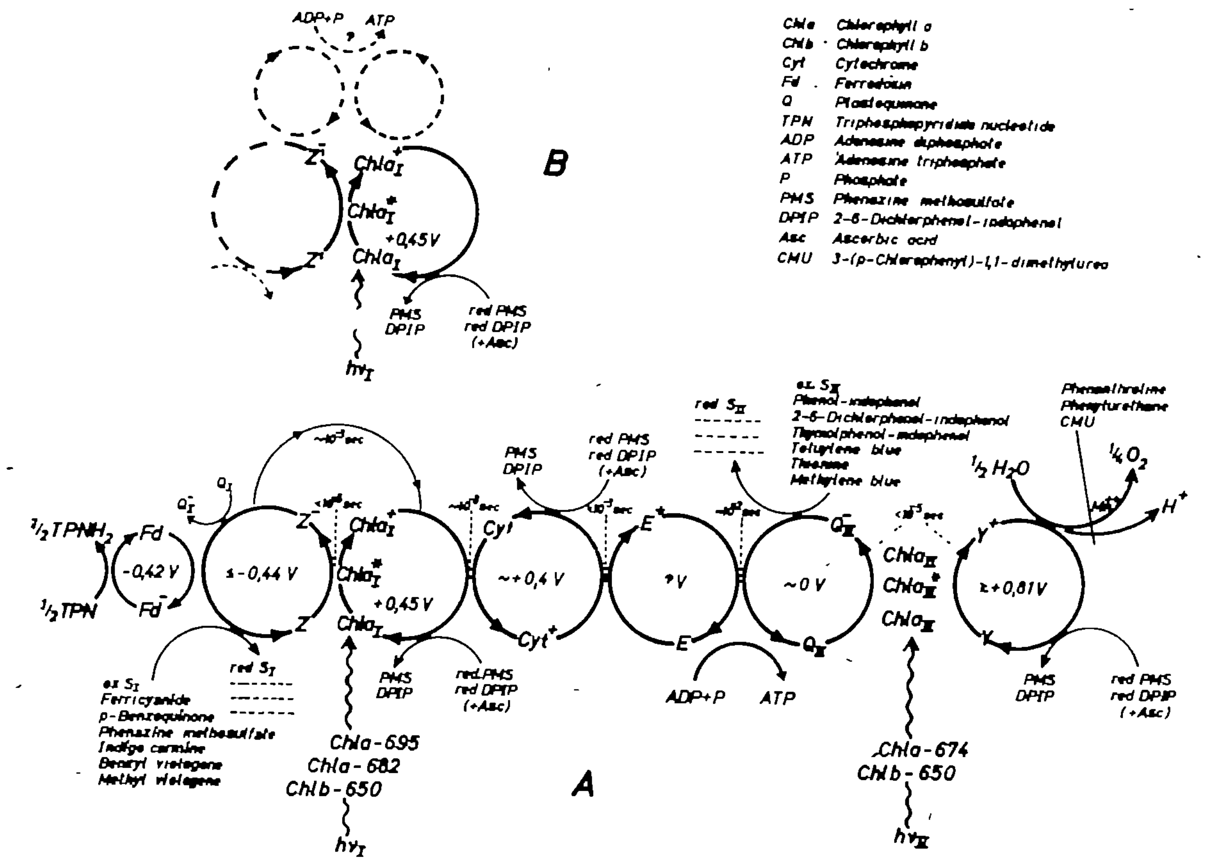

Fig. 2. Reaction scheme of the electron-transport system in photosynthesis derived from analysis of short time absorption changes measured by sensitive flash photometry

therefore here as $\mathrm{Chla}_{\mathrm{I}}$ (refs. 24 and 26); (7) the existence of $\mathrm{Chla}_{\mathrm{I}}$-oxidation in different systems *A* and *B* (ref. 26); (8) an unidentified difference spectrum with a maximum only at 420 mμ (refs. 26 and 28).

Using the two-colour effect of Emerson in combination with measurements of absorption changes, it was possible to demonstrate directly the existence of two light reactions coupled in series. This was done independently by Kok[31], Duysens[32] and Witt *et al.*[33]. Kok[31] showed that 700-light causes an oxidation of *P* 700, and shorter wave-length its reduction by an unknown product. Duysens[32] demonstrated in red algae that red light causes cytochrome oxidation and shorter wave-length its reduction again by an unknown product. We demonstrated in green plants that 700 mμ-light causes cytochrome oxidation while shorter wave-length causes the reduction of a substance *X* (identified as plastoquinone) which reduces the oxidized cytochrome[33,34].

Our measurements of absorption changes were done with the method of very sensitive flash photometry[15]. Photosynthesis was excited by single as well as periodically weak flashes (~ 1 W sec) with a duration variable between 3×10^{-6} sec and 1 sec. The detectors could measure transmission changes of 5×10^{-3} within 3×10^{-6} sec. In this way 7 different types of absorption changes between 10^{-5} sec and 1 sec could be analysed[24]. From the difference spectra depicted in Fig. 1 the participation of the afore-mentioned substances was derived. Besides this analysis, the changes in absorption have been investigated since 1954 also in relation to numerous physical and chemical parameters[15-30]. In this way numerous results on the properties of the substances, its kinetics and its relation to each other were obtained. With this information a rather detailed reaction scheme of photosynthesis was developed, including a two-light-reaction mechanism[22-24]. In this article an extended scheme is presented in which newer results have also been considered (Fig. 2).

It can be seen from scheme (*A*) that TPN or substances such as ox.S_{I} are reduced by transfer of electrons originating from water through at least eight reaction cycles. The electron-transfer between $\mathrm{Chla}_{\mathrm{I}}$ and *Z*, and the transfer between *Y* and Q_{II} (both endergonic in the dark, energy gaps ≥ 0·85 eV, ≳ 20 kcal) are made possible by two light absorptions $h\nu_{\mathrm{I}}$ and $h\nu_{\mathrm{II}}$. A simplified energy diagram of Fig. 2 is shown in Fig. 3.

In light-reaction $h\nu_{\mathrm{I}}$, $\mathrm{Chla}_{\mathrm{I}}$ is oxidized, giving up its electron in the excited state $\mathrm{Chla}_{\mathrm{I}}{}^{*}$ to the substance *Z*. $\mathrm{Chla}_{\mathrm{I}}$-oxidation is accompanied by pronounced absorption decreases at 433 mμ and 703 mμ (refs. 33, 24 and 26) which are characteristic for light reaction $h\nu_{\mathrm{I}}$ (Fig. 1c). Decreases of absorption changes just within the two absorption bands of chlorophyll *a* indicate that a chlorophyll *a* ($\mathrm{Chla}_{\mathrm{I}}$) is in action. The decreases indicate, furthermore, an oxidation because of a corresponding dependency on

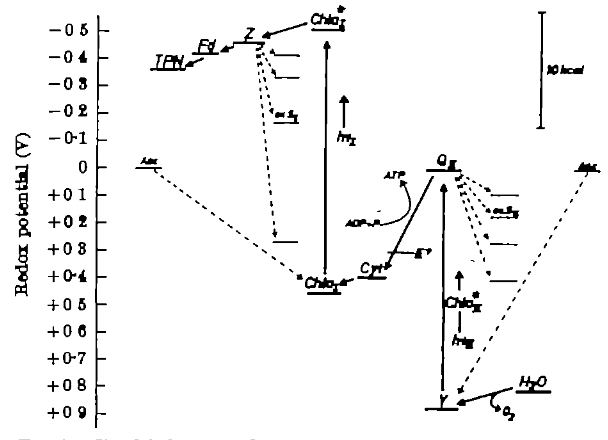

Fig. 3. Simplified energy diagram of the reaction scheme in Fig. 2

Table 1

Properties	Light-reaction $h\nu_{\text{I}}$	Light-reaction $h\nu_{\text{II}}$
Absorption	Chla$_{\text{I}}$: 433, 703 mμ	$Q_{\text{II}}(e)$: 475, 515 mμ*
Concentration	[Chla$_{\text{I}}$] ≈ 10^{-3} [chlorophyll]	[Q_{II}] ≈ 10^{-3} [chlorophyll]
Extinction	ε(703) ≈ 10^{5} M/l cm	ε(475) ≈ 10^{5} M/l cm
Redox-potential Donor	+ 0·45 V (Chla$_{\text{I}}$)	≳ + 0·81 V (Y)
Acceptor	< − 0·44 V (Z)	~ ± 0 V (Q_{II})
Supplied by	(Chlb-650) Chla-682, Chla-695	Chlb-650, Chla-675
Limit of excitation	~ 730 mμ	~ 705 mμ
Reaction constant	2 × 10^{7} L/m.sec (Chla$_{\text{I}}^{+}$ with red. PMS at pH = 8)	2·7 × 10^{6} L/m.sec (Q_{II}^{-} with DPIP at pH = 6·5)
Activation energy	4 kcal (Chla$_{\text{I}}^{+}$ with red. PMS)	7·4 kcal (Q_{II}^{-} with DPIP)
pH-range	4–11	5–8
Temperature of inactivation (5 min)	~ 65° C	~ 50° C

added ferro/ferricyanide[33]. From such measurements the redox-potential of Chla$_{\text{I}}$ could be calculated as + 0·43 V (ref. 33). Our measurements gave a slightly higher value (+ 0·45 V) (ref. 24). Different evidence that Chla$_{\text{I}}$ is oxidized in light was provided by the fact that added reduced phenazine methosulphate (red. PMS) or reduced dichlorophenol indophenol (red. DPIP) (both with ascorbic acid in excess) can be directly coupled to the light product[24,26] (reaction constant and energy of activation; Table 1). This coupling can be demonstrated by the strong acceleration (factor 100 and more) of the decay time of the absorption changes of Fig. 1c with increasing concentrations of red. PMS or red. DPIP (Fig. 4). The coupling between Chla$_{\text{I}}^{+}$ and red. PMS or red. DPIP is possible when light-reaction $h\nu_{\text{II}}$ is inactivated (by ageing chloroplasts[24] or heating to 50° C (ref. 22) or extraction of Q_{II} (ref. 22)). The production of Chla$_{\text{I}}^{+}$ within 10^{-5} sec and the trapping of Chla^{+} at − 150° C gave evidence that this oxidation is a primary act[24]. The Chla$_{\text{I}}$ reaction is stable in the pH range 4–11 and up to ~ 65° C. Z^{-} reduces substances ox.S_{I} as ferricyanide, benzoquinone, phenazine methosulphate, indigo-carmine, benzyl viologene, methyl viologene and TPN-reductase. This is demonstrated in Fig. 5 (see later). From ox.S_{I} the redox-potential of Z was estimated as ≲ − 0·44 V.

When no ox.S_{I} is added to the chloroplasts a back-flow of electrons from Z^{-} to Chla$_{\text{I}}^{+}$ occurs in ~ 10^{-3} sec (ref. 26) (Fig. 2). This is demonstrated in more detail also in Fig. 5 (see later).

A ferricyanide reactive plastoquinone Q_{I} (absorption changes at 260 mμ, Fig. 1g) is probably associated with Z (ref. 25).

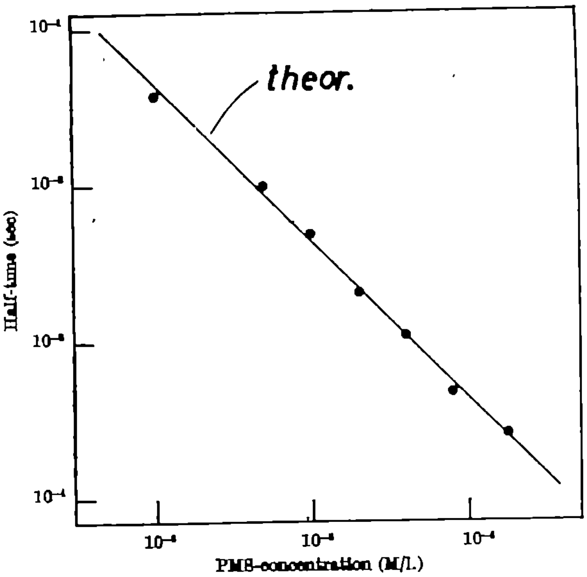

Fig. 4. Half-time of the absorption changes at 705 mμ in aged chloroplasts related to the concentration of red. PMS (with 3 × 10^{-3} M/l. ascorbic acid in excess). 20 C, pH = 8

The changes at 433 and 703 mμ show a biphasic behaviour in relation to various conditions[26]. From this the oxidation of Chla$_{\text{I}}$ in two different systems A and B could be derived. The redox-reaction in system B occurs probably only in a cyclic e-flow which may be accompanied by phosphorylation. One example for the biphasic behaviour of Chla$_{\text{I}}$ was given in ref. 26 and is also included in the discussion of Fig. 5. From this follows the ratio of Chla$_{\text{I}}$ (in A) : Chla$_{\text{I}}$ (in B) = 1.

In light-reaction $h\nu_{\text{II}}$, Chla$_{\text{II}}$ transfers in its excited state an electron from Y to plastoquinone Q_{II}: $Q_{\text{II}}\,\text{Chla}_{\text{II}}{}^{*}Y \rightarrow Q_{\text{II}}{}^{-}\text{Chla}_{\text{II}}Y^{+}$ (ref. 29). The reduction of Q_{II} (formerly symbolized with X) causes pronounced short-timed absorption changes at 475 (decrease) and 515 mμ (increase) in surrounding pigments (Fig. 1d). That these changes are caused by Q_{II} was shown by extraction experiments and redox-potential measurements[22,23,29].

Absorption changes caused directly by Q_{II} were observed at 260 mμ (Fig. 1g) (ref. 25). In the meantime, it was shown that the kinetics of the changes at 260 mμ correspond to those at 475 and 515 mμ under different conditions so far as investigated[19]. The suggestion that Q_{II} is engaged in a light-reaction follows from the very fast appearance of the changes (< 10^{-5} sec) and the temperature independence of its magnitude at all light intensities[17]. That in light a reduction of Q_{II} occurs was verified by the fact that added oxidized substances ox.S_{II} as dichlor-thymol- and phenol-indophenol, toluylene blue, thionine and methylene blue can be directly coupled to the light product[19] (reaction constant and activation energy see Table 1). This coupling can be demonstrated by the strong acceleration (factor 200 and more) of the decay time of the absorption changes at 515 mμ with increasing concentrations of ox.S_{II}. From ox.S_{II} the redox-potential of Q_{II} (formerly X) was estimated as ~ 0 V (ref. 19). Since ox.S_{II} is a Hill reagent and that with its reduction a simultaneous stoichiometric production of oxygen from water takes place, it follows that Y^{+} oxidizes directly or indirectly water[22]. Therefore, the redox-potential of Y is ≳ + 0·81 V. The Q_{II} reaction is stable in the pH range between 5 and 8 and up to + 50° C (ref. 17). The co-operation of Mn^{++} in water oxidation was shown by Kessler[34].

Action spectra[30] of the isolated light-reaction $h\nu_{\text{I}}$ and $h\nu_{\text{II}}$ respectively show that light absorbed in the bulk of the different chlorophylls is transmitted to the two reaction centres in the following way. Chla-682, Chla-695 and probably also Chlb-650 excite Chla$_{\text{I}}$ per energy-migration. Chlb-650 and Chla-674 excite Chla$_{\text{II}}$. The far red limit for $h\nu_{\text{I}}$-excitation is at ~ 730 mμ, the limit for $h\nu_{\text{II}}$-excitation at ~ 705 mμ (refs. 22 and 30). This is in good agreement with the Emerson effect. Hence, using far red light (~ 720 mμ), the light-reaction $h\nu_{\text{I}}$ is almost solely excited[22,30].

The Chla-674 is very probably the fluorescing pigment *in vivo* (fluorescence peak at ~ 685 mμ). Therefore, quenching the reaction of Q_{II} should increase the fluorescence and vice versa. A corresponding relationship was shown by the simultaneous measurements of the absorption changes of Q_{II} (at 515 mμ) and the fluorescence at different conditions[18].

The product of light-reaction $h\nu_{\text{II}}$, Chla$_{\text{I}}^{+}$, reduces via intermediates the product of light-reaction $h\nu_{\text{II}}$, Q_{II}^{-} (refs. 24 and 26). By comparing the absorption changes caused by Q_{II} with those of Chla$_{\text{I}}^{+}$ (in A) under different conditions it was possible to demonstrate the *quantitative* kinetic relation between the light-reactions $h\nu_{\text{I}}$ and $h\nu_{\text{II}}$ (ref. 26).

Intermediates between Chla$_{\text{I}}^{+}$ and Q_{II}^{-} are cytochrome Cyt and the unknown substance E. Light-induced cytochrome reactions have never been observed in normal green plants. The difference spectrum (Fig. 1e) which indicates participation of cytochrome-oxidation is only observable when the reduction of Cyt^{+} is strongly delayed or prevented (exciting only $h\nu_{\rm I}$, cooling to $-150°$ C, uncoupling from $Q_{\rm II}$ by red. DPIP, ageing, etc.)[24]. From this it was concluded that under normal conditions the reduction time of Cyt$^+$ is faster ($< 10^{-3}$ sec) than the oxidation time of Cyt ($\sim 10^{-5}$ sec). That the observed cytochrome is probably Cyt-*f* (redox-potential $+ 0{\cdot}37$ V *in vitro*[45]) is supported by the redox-potential measurement *in vivo*[23] ($\sim + 0{\cdot}4$ V). That Cyt is one reaction partner of $\mathrm{Chla_I}$ follows from three observations: (*a*) the oxidation time of Cyt corresponds (within the limits of accuracy) to the reduction time of $\mathrm{Chla_I}^+$ ($\sim 10^{-5}$ sec) (ref. 24); (*b*) both accumulate in the oxidized form when only light-reaction $h\nu_{\rm I}$ is excited (at $\sim$ 720 mμ) and both are reduced by exciting $h\nu_{\rm II}$ (at $<$ 700 mμ) (refs. 22, 24 and 26); (*c*) $\mathrm{Chla_I}^+$ and Cyt$^+$ are both trapped at $-150°$ C (ref. 24).

(Lundegårdh[35] states that he measured in *Chlorella* a "light-induced" cytochrome-*f* oxidation. Due to his measurements the lifetime of Cyt$^+$ must be of the order of 10 sec. Neither Chance *et al.*[34] nor we could confirm his results. Chance suggests that he measured an oxygenation of a previously anaerobic *Chlorella* and not a light-induced cytochrome oxidation (see special note on this point in ref. 36). Duysens[13] suggests that the absorption changes at 420 mμ may be caused by cytochrome. But the separation of the difference spectrum which belongs to 420 mμ (Fig. 1*f*) shows that the changes are not caused by cytochrome. Chance[34] suggests that the failure to detect light-induced absorption changes of cytochrome in green plants might be caused—especially in the blue—by the strong absorption of carotenoids and chlorophylls, so he used a mutant with only 1/500 the total carotenoids of normal cells and 1/10 the chlorophyll, and observed small cytochrome-*f* oxidation but only under aerobic conditions. Since our measurements were done in normal cells with high pigment concentrations and since we saw pronounced difference spectra of cytochrome (when Cyt$^+$-reduction was delayed) the pigments cannot be the reason for masking different spectra. Perhaps the abnormal conditions in the mutant delay Cyt$^+$-reduction which leads to accumulation of Cyt$^+$.)

The kinetics show that Cyt$^+$ is reduced by $Q_{\rm II}^-$ at least via E (ref. 26). E is not a cytochrome since corresponding absorption changes of 10^{-5} sec do not exist.

The energy released in the back-reaction between Cyt$^+$ and E or between E^+ and $Q_{\rm II}^-$ ($\sim 0{\cdot}4$ eV, $\sim$ 10 kcal) can be used for a phosphorylation[22,24].

From the quantitative measurements of reduced ox.$S_{\rm II}$ per flash the concentration of $Q_{\rm II}$ was estimated as $\sim 10^{-3}$ of the bulk of chlorophyll[19]. From the magnitude of the absorption changes of $\mathrm{Chla_I}$ and Cyt the same concentration also follows for these substances.

The reaction times, indicated in the scheme, follow directly from the kinetic of the absorption changes.

According to system A, eight quanta are needed for the production of one oxygen and two $\mathrm{TPNH_2}$.

The whole process can be separated into 7 partial reactions[27,28]. The flow of electrons from water to $Q_{\rm II}$ via Y and $\mathrm{Chla_{II}}$ can be blocked by addition of CMU, phenanthroline and phenylurethane (no more changes at 475 and 515 mμ, Fig. 1*d*). In this event, instead of water, at least added red. DPIP and red. PMS (with ascorbic in excess) can supply electrons to Y^+ (changes at 475 and 515 mμ reappear in full[24]). $Q_{\rm II}\mathrm{Chla_{II}}Y$ can be inactivated by ageing[24], heating up to 50° C (ref. 22) or extraction of $Q_{\rm II}$ by petroleum ether[33] (no more changes at 475 and 515 mμ, Fig. 1*d*). In this case added red. PMS or red. DPIP can provide Cyt$^+$ with electrons[24,26] (Fig. 2). With concentrations of PMS (or DPIP) $\gtrsim 10^{-5}$ M/l. (*p*H = 8, ascorbic acid in excess) Cyt is by-passed[26] (no more changes of cytochrome, Fig. 1*e*), and red. PMS reacts directly with $\mathrm{Chla_I}^+$ (only changes at 433 and 703 mμ, Fig. 1*c*). This is indicated by the linear decrease of the half-time of $\mathrm{Chla_I}^+$ with increasing concentrations of red. PMS. In these circumstances, light reaction $h\nu_{\rm I}$ is isolated[24]. (This reaction (ox.$S_{\rm I} \leftarrow Z \overset{h\nu_{\rm I}}{\leftarrow} \mathrm{Chla_I} \leftarrow$ red. PMS) must be responsible for the system that operated when Vernon, Kamen and Zaugg[46] used aged chloroplasts with red. substances which are capable of reducing TPN in the light.

At 20° C the reaction of Z–$\mathrm{Chla_{II}}$ can be suppressed by permanent oxidation of $\mathrm{Chla_I}$ with ferricyanide ($> 10^{-3}$ M/l.) (no more changes at 433 and 703 mμ, (Fig. 1*c*). In this case addition of ox.$S_{\rm II}$ (for example, DPIP) can directly accept the electron from $Q_{\rm II}^-$ (only changes at 475 and 515 mμ, Fig. 1*d*). In these circumstances, light reaction $h\nu_{\rm II}$ is isolated[27,28]. This reaction:

$$\mathrm{ox.}S_{\rm II} \leftarrow Q_{\rm II}\,\overset{\leftarrow h\nu_{\rm II} -}{\mathrm{Chla_{II}}}\,Y \leftarrow \mathrm{H_2O}$$

must be responsible for the system which was described by Losada *et al.*[10] (oxygen-producing chloroplasts with ferricyanide and DPIP). In contrast with their interpretation we conclude from our optical measurements that the essential function of ferricyanide is the chemical oxidation of the $\mathrm{Chla_I}$ in A and B.

When the chlorophylls in the bulk are separated or both light-reactions are inactivated by heating to 65° C, the migration of energy to the reaction centres $\mathrm{Chla_I}$ and $\mathrm{Chla_{II}}$ is interrupted. In this case the light energy should stay in the π–π^*-triplet states of the molecules in the bulk (Fig. 1*a*). This has been directly proved[31].

The chlorophyll *a* reaction causing the very fast changes[29] (difference spectrum in Fig. 1*b*) is as yet not included in the scheme. It might be that this reaction is in relation with the reaction of $\mathrm{Chla_{II}}$.

Since Hill[38] made the discovery that isolated chloroplasts can oxidize water and can reduce added substances during illumination, extensive work was done on the reducing and also oxidizing capacity of illuminated chloroplasts. For the highest reduction power, for example, a potential of $-0{\cdot}44$ V was estimated[39]. With the upset of the reaction scheme it now becomes clear that at least 8 sites are possible for a reduction or oxidation reaction and the question arises at what site the different substances are reduced or oxidized respectively. On this point we already gave detailed information (Fig. 2) which was derived from the behaviour of the different types of absorption changes.

On the reaction capacity of Z, we have published only a short note[24]. There are two ways of measuring which substances react with Z^-, although Z is not accompanied by absorption changes in the visible range (Fig. 5, top).

(1) By exciting photosynthesis with flashing light at t_1 the $\mathrm{Chla_I}$ (A) and $\mathrm{Chla_I}$ (B) at level α are immediately oxidized to level δ. Afterwards, $\mathrm{Chla_I}^+$ (A) is reduced by Cyt in $\sim 10^{-5}$ sec and $\mathrm{Chla_I}^+$ (B) via the cyclic way also in $\sim 10^{-5}$ sec back to level α. The corresponding absorption changes are shown in Fig. 5 (middle, first picture). Exciting only light-reaction $h\nu_{\rm I}$ with soft permanent far red background light ($\sim$720 mμ) at t_2, $\mathrm{Chla_I}$ (A) as well as Cyt and E should accumulate in its oxidized form (level β) since no $Q_{\rm II}^-$'s are produced for its reduction. ($\mathrm{Chla_I}^+$ (B) cannot accumulate because of the cyclic *e*-flow in B.) A supplementary flash with $h\nu_{\rm I}$ and $h\nu_{\rm II}$ lights ($<$ 700 mμ) at t_3 oxidizes $\mathrm{Chla_I}$ (B) and the level δ is reached. Simultaneously the same flash produces $Q_{\rm II}^-$. Afterwards, $\mathrm{Chla_I}^+$ (B) is reduced in $\sim 10^{-5}$ sec via the cyclic way. Because $\mathrm{Chla_I}$ (A), Cyt and E are oxidized the $\mathrm{Chla_I}^+$ (A) is reduced only so fast as $Q_{\rm II}^-$ can react with E^+, that is, in $\sim 10^{-2}$ sec. So the time course depicted in Fig. 5*b* should be characteristic for $\mathrm{Chla_I}^+$ accumulation in system A. Nevertheless the experiment in Fig. 5 (middle. second picture), shows no accumulation. The reason may be that $\mathrm{Chla_I}^+$ (A) can also be reduced by a cyclic back flow of electrons from Z^- to $\mathrm{Chla_I}^+$ (A). If this is so. trapping of the electrons of Z^- should prevent the back flow. Indeed, with addition of oxidized indigo-carmine absorption, changes occur which are characteristic

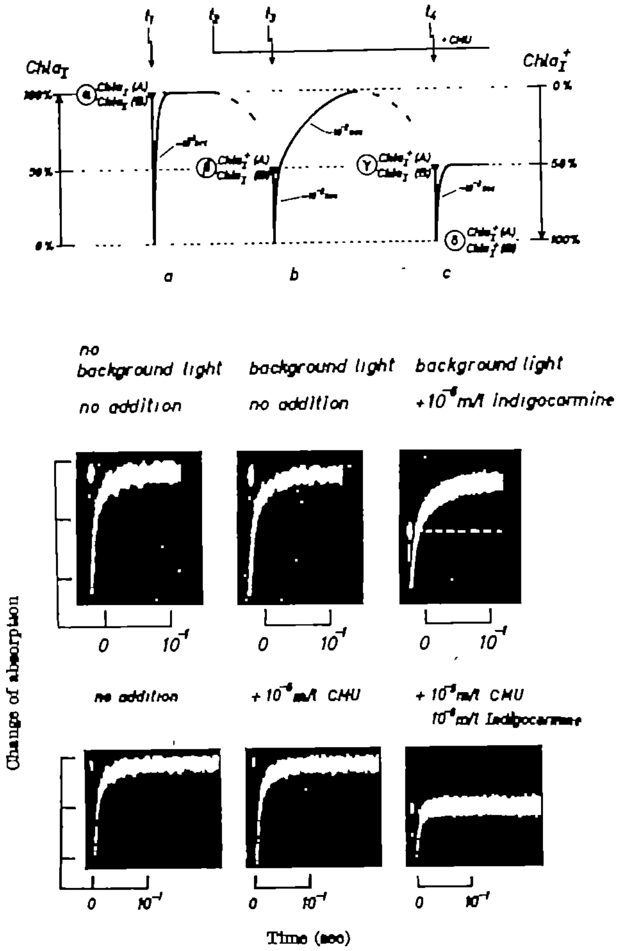

Fig. 5. Top: Scheme of the expected kinetics of Chla under different conditions. Middle and bottom. Absorption changes of Chla at 703 mμ in chloroplasts (spinach) caused by blue flashing light under different conditions. 20 C, *p*H = 7. The flash was turned on at $t = 0$. Flash duration = 10^{-5} sec. For details see text

for accumulation of Chla$_{I}^{+}$ (*A*) (Fig. 5, middle, third picture).

(2) Another consequence from the scheme is the following. With addition of CMU the electron inflow from water is totally blocked. Then Chla$_{I}^{+}$ (*A*) should be permanently oxidized at level γ. A flash can only oxidize Chla$_{I}$ (*B*) to level δ which is afterwards reduced via the cyclic way in $\sim 10^{-2}$ sec to level γ. So disappearance of the absorption changes which are caused by Chla$_{I}$ (*A*), that means half reduction of the magnitude of the changes at 433 and 703 mμ (Fig. 5c), is characteristic for the blocking of system *A* by CMU. Nevertheless, the experiment in Fig. 5 (bottom, second picture) shows that with CMU the changes are not influenced at all. Again, the cyclic back-flow may be the reason. Indeed, by trapping the electrons from Z^- by oxidized indigo-carmine the changes caused by Chla$_{I}$ (*A*) disappear, indicating that system *A* is now totally blocked (Fig. 5, bottom, third picture).

By these two tests it is possible to decide directly which oxidized substances ox.S_I can react with Z^-. So far as investigated, ferricyanide, benzoquinone, phenazine methosulphate, indigo-carmine, benzyl viologene and methyl viologene react with Z^-. Henriques[40], in our laboratory, tested that not TPN but TPN-reductase[41] traps also the electrons from Z^- in the same way as indigo-carmine, etc. The reaction of ox.S_I with Z^- is specific in so far as all substances surnamed under ox.S_I cannot react with Q_{II}^-. This was shown by the observation that ox.S_I does not influence at all the absorption changes of Q_{II}^-. From these results the following conclusions can be drawn:

(1) Because methyl viologene can be reduced by Z^- the redox potential of Z^-/Z is ≤ -0.44 V. Then the redox potential of the excited Chla$_{I}^{*}$ must be ≤ -0.44 V.

(2) Because TPN-reductase is reduced by Z^-, TPN-reductase must contain a redox system. This is a confirmation of the result of Arnon *et al.*[42], who have recently shown in a different way that TPN-reductase contains a redox system, ferredoxin. Ferredoxin was discovered by Mortenson *et al.*[43]. The difference spectrum not identified in Fig. 1*f* may be caused by ferredoxin.

(3) Ferredoxin is not identical with the electron acceptor of Chla$_{I}$ (*A*), since also without ferredoxin the oxidation of Chla$_{I}$ occurs (furthermore, absorption changes at 433 and 703 mμ, Fig. 1c).

Ferredoxin reduces directly or indirectly TPN, probably with the help of the enzyme component of TPN-reductase. With TPNH$_2$ and with ATP carbon dioxide can be reduced to sugar via the Calvin cycle[44].

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LETTERS TO THE EDITOR

GEOPHYSICS

Growth of the Earth's Core

To his recent communication[1], proposing that the cause of convection in the mantle is chemical, Dr. Stacey added a note in proof attacking the idea used by me[2,3] of a gradual growth of the Earth's core to give a tentative explanation of continental drift. He makes the objection that the settling of the Earth's core from an undifferentiated Earth, formed by the accretion of iron and silicate bodies like the meteorites, would release an amount of energy greater by an order of magnitude than that which would have been conducted through the Earth's surface since 3,000 million years ago, when I suggest separation began, supposing the geothermal gradient had always its present value. I do not think Stacey's objection is valid. I have a detailed paper in preparation giving an account of the thermal history of the Earth, assuming that convection in the mantle is possible: naturally this differs radically from the thermal history of the Earth previously presented by those[4] who have only assumed conduction in the mantle.

It is possible here only to make the following points:

(1) If the Earth was formed by the accretion process, and if the heat generated at the surface of the developing planet by impacts is radiated off into space, then the initial temperature of the Earth would have varied from 0° C at the surface to about 800° C at the centre, owing to the adiabatic gradient. It is possible, if the planet accumulated sufficiently fast, for some of this energy to be trapped within the Earth and it is of course possible to suppose that the short-lived radioactive isotopes, for example, aluminium-26, might also contribute initial heat, but again this assumes a very rapid collection of matter. There seems no strong reason to postulate much initial heat.

(2) If the radioactivity of the silicates composing the Earth's mantle is that of chondritic meteorites, which now generate $1{\cdot}6\times10^{-7}$ joules/g/year, and the radioactivity of the iron core is negligible as is the case for iron meteorites, the temperature of the Earth would have risen by only 1,800° C in the last 4,500 m.y., even if no heat escapes. Also H. C. Urey (private communication) believes that this is probably an over-estimate, as the chondritic meteorites may be over-rich in potassium. One may therefore deduce that on this theory there has had to be another source of heat in the Earth's interior, for the lowest estimate ever made of the temperature at the core mantle interface is in excess of 3,000° C and probably is much greater.

The gravitational energy released in the process of forming the dense core may reasonably be identified as the likely source. Urey[6] calculates the heat generated as being between $1{\cdot}67 \times 10^{31}$ joules and $2{\cdot}19 \times 10^{31}$ joules assuming that no changes in temperature have occurred. About a tenth of this heat has been lost through the surface; perhaps a twentieth has gone to melt the core, supposing the latent heat is 100 calories/g (mass of core is 2×10^{27} g). Also, the increase in gravitational energy in the expansion of the Earth consequent on the Earth's temperature rising through, say, 4,000° C is considerable. Assuming the volume coefficient of expansion to be 3×10^{-5}, an increase in volume of 12 per cent is to be expected. This requires the gravitational energy to increase by 2 per cent. As the total gravitational energy of the Earth is about 2×10^{32} joules, half the energy released by separation of the iron core has been accounted for.

The remaining energy, between 7 × and 12×10^{30} joules, is available for heating up the Earth to its present temperatures. The mass of the Earth is 6×10^{27} g, so that the increase in temperature lies between 1,000° C and 2,000° C, of the order of that required.

The fallacy in Dr. Stacey's argument lies in the fact that it is tempting to believe that a system of convection currents will carry away exactly the heat generated, but the proviso must be added that the heat sink is adequate. But it is quite clear, from the present-day values, that the heat conducted through the crust is insufficient, and if the Earth's initial temperature were low it could scarcely be otherwise. The convection currents will, of course, distribute the heat generated uniformly through the 'fluid' part of the mantle and the general temperature of this will rise. The thermal gradient through the crust will therefore increase with time.

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Photometric Observations from the Southern Hemisphere of 5577*A* Emission from the Aurora on October 28, 1961

IN a recent communication[1] Silverman reports unusual fluctuations in 5577*A* airglow emission intensity on October 28–29, 1961, at Sacramento Peak (geomagnetic latitude 41·6° N.). Silverman gives a curve, consisting of readings taken at 10-min intervals for the period 0200–1100 h U.T. on October 29, 1961. The peak intensity for that night is almost 450 and the minimum about 50, the units being unstated. It is of interest to compare these results with some recorded around 1200 U.T. October 28, from a similar experiment at Lauder, New Zealand (170° E., 45° S. geographic; 50° S. geomagnetic).

The Lauder photometer points continuously to the zenith and views an angle of 0·5°. The instrument was designed to search for rapid fluctuations in the airglow and uses a photon pulse-counting technique; the wavelength is selected with a dielectric filter having a half-width of 70*A*. It came into operation early in September 1961 and recorded 5577*A* emission until December 1961—the same period as that reported by Silverman.

On the night of October 28, 1961, a bright aurora was observed at Lauder. This aurora, which consisted of an active rayed arc, was the brightest observed here during the whole of 1961. The sky was clear in the zenith, and during the period 1135–1235 U.T. the curve reproduced in Fig. 1 was recorded by the photometer. Until 1141 the recorded intensity was at the normal airglow-level, although the aurora in the south had been bright for an hour or more. The rays rose higher in the sky until at 1144 they reached the zenith and the photometer went full scale with the recorder at normal sensitivity. (At 1147 the recorder sensitivity was reduced by one half, at which setting it remained for the rest of the night.) The rays stayed in the zenith for only 3 or 4 min and then retreated to the south. By 1230 the auroral display had quietened to no more than a glow. The moon rose at 1208, and after 1235 the photometer record was contaminated by moonlit cloud. For the period 0900–1200 *Kp* was 5, rising to 6 for the period 1200–1500 U.T.

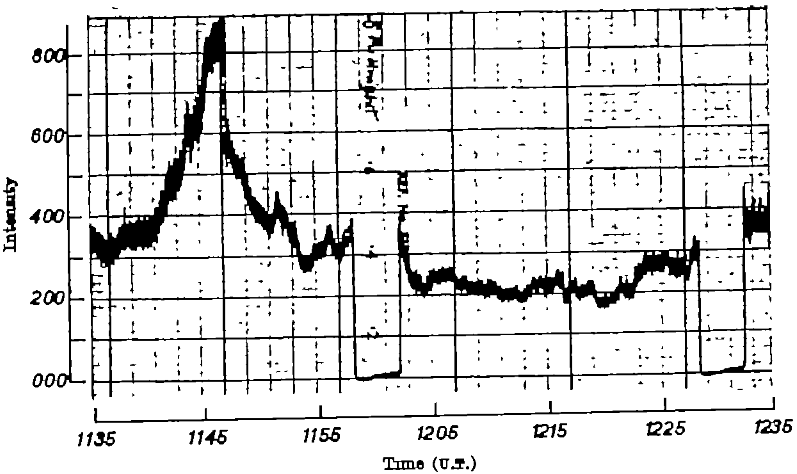

Fig. 1. Photometer trace for the period 1135–1235 U.T. of 5577 Å emission observed from Lauder. The 4-min decreases centred at 1200 and 1230 are due to the shutter closing for a reading of the photomultiplier dark current. The intensity scale is in rayleighs for the record until 1147 and should be doubled for the record after this time; contamination by moonlight becomes serious from approximately 1220

Silverman's record covers the period 0200–1100 U.T. on October 29. During this period K_P was 6, 5, 6 and 4. He does not state whether an aurora was visible, but elsewhere Gartlein[2] reports that a "large aurora" which began early in the morning of October 28 and continued until daylight was observed in North America. On the following night, which is covered by Silverman's record, glows were reported.

An all-sky camera is run in conjunction with the Lauder photometer. The photograph for the period 1132–1137 U.T. is reproduced in Fig. 2. During the nights when the photometer was operating, seven auroræ bright enough to be visible to the naked eye were recorded by the all-sky camera; but the photometer recorded a definite increase associated with an aurora only on October 28. This is evidence that this night was indeed unusually active.

Silverman recorded an intensity variation on October 29 of two to one in a period less than 36 sec. As mentioned earlier, the Lauder photometer was specifically designed to look for very rapid variations in airglow. Due to the short time-constant of about 1/100 sec, the photometer registers statistical variations of about 15 per cent: I have been unable to detect any short-period fluctuations in airglow during the period September–December 1961. If the short-term fluctuations do exist their amplitude must be less than 15 per cent of the mean level. The only major fluctuation observed during more than three months of operation was on October 28, when a visible aurora was in the field of the instrument.

The last 2 h of the Sacramento Peak record corresponds to the first 2 h of the night's run at Lauder for October 29; but the sky at Lauder was almost completely overcast for this night, so no comparison can be made.

The evidence shows that on October 28, 1961, commencing about 1100 U.T., considerable auroral activity was observed in both hemispheres. It appears that the large fluctuations recorded at Sacramento Peak were a manifestation of this widespread auroral activity rather than simple airglow variations. As Silverman makes no mention of an aurora being visible it is concluded that the activity at Sacramento Peak was sub-visual. It would be interesting to know the nature of the Sacramento Peak record for the last hour of the previous night, when the aurora at Lauder was most intense.

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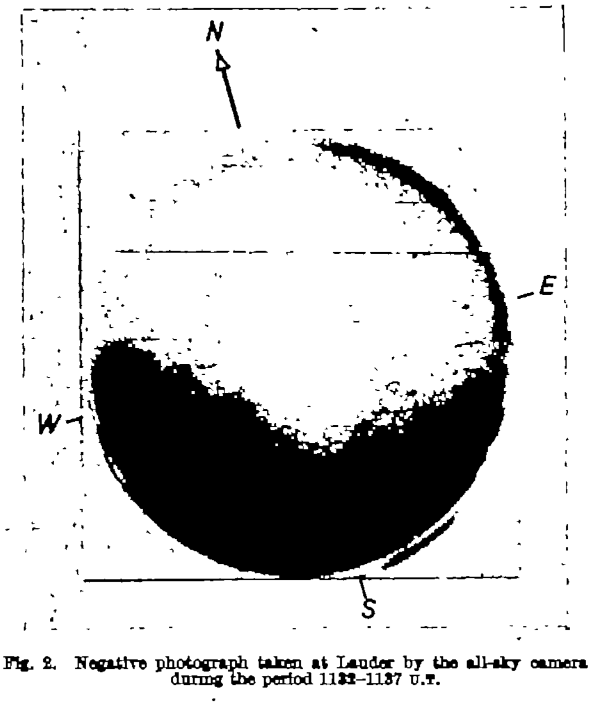

Fig. 2. Negative photograph taken at Lauder by the all-sky camera during the period 1132–1137 U.T.

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PHYSICS

Neutron Numbers 98, 108 and 116

In a discussion of neutron capture processes and theories of element origin, Malkiél[1] has suggested that the neutron numbers 98, 108 and 116 may be favoured. Malkiél mentions that mass data also appear to support the "special character" of $N = 116$. It may be recalled that these numbers are not predicted as significant by the shell model of the nucleus.

The possibility that $N = 98$ and $N = 116$ might be significant had in fact already been briefly noted by me[2] in a systematic study of the relative abundances (per cent proportions) of the isotopes of the elements. It is the purpose here to consider further the probability that these neutron numbers are favoured using as evidence a systematic study of the principal (most abundant) isotopes of the elements. It is assumed that the principal isotopes are particularly significant.

Fig. 1 is an N–Z, Z diagram of the principal isotopes of the stable elements plus those of uranium and thorium. The diagram is based on my work elsewhere (Fig. 19, ref. 2; and Figs. 3 and 4, ref. 3). A few points have been placed in brackets. These are not the true principal isotopes but are not regarded as seriously exceptional as their proportions are very slightly less than those of the principal isotopes[3]. On the basis of diagrams similar to Fig. 1 and other evidence, I have proposed[3] a 3-fold division of nuclei:

(1) N (and Z) < 20 (${}^4_2$He region)
(2) $N > 20 < 50$ (transition region)
(3) $N > 50$ (${}^6_2$He region)

and have suggested the possibility that ^{4}He ($2p + 2n$) and ^{6}He ($2p + 4n$) be considered as cluster components of nuclei. Let us now consider the region $N > 50 < 82$. ^{6}He

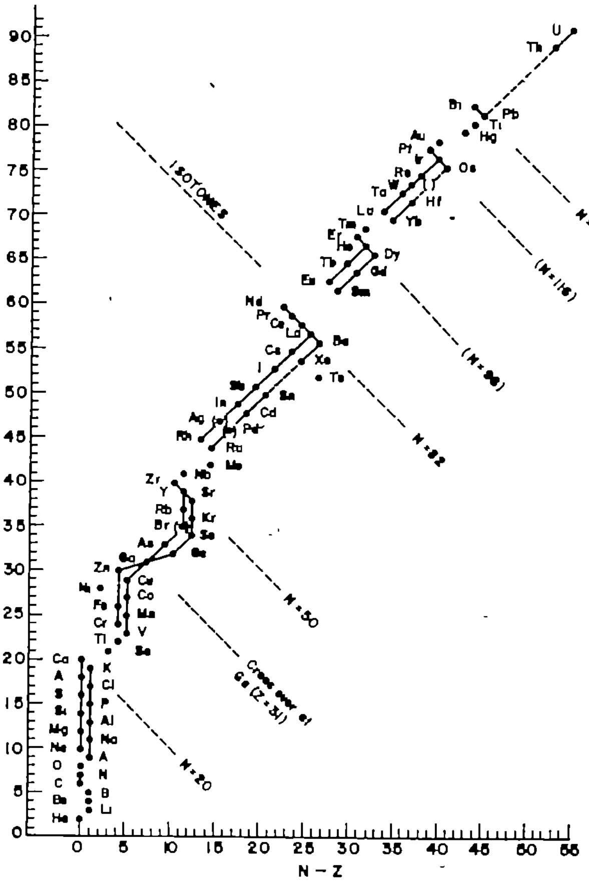

Fig. 1. $N-Z$, Z diagram for the principal isotopes of the elements; adapted from ref. 3. ○, Even Z; ●, odd Z

sequences are apparent and terminate at highly favoured $N = 82$. Such sequences are apparent also in the region $N > 82$, but these sequences are displaced with respect to those for $N < 82$. Similar discontinuities and displacements, but on a smaller scale, occur at $N = 98$ and $N = 116$; these numbers have been placed in parentheses in Fig. 1.

The well-developed discontinuities and displacements at $N = 82$ and the related discontinuity-developments at $N = Z = 20$ and $N = 50$ indicate that the systematics of stable isotopes clearly reflect the effects of the well-known highly favoured neutron numbers 20, 50 and 82. Accordingly, if such principal isotope systematics are sensitive in this respect, it is not unreasonable to assume that the discontinuity-displacements at $N = 98$ and $N = 116$ indicate that these neutron numbers are also favoured, corresponding perhaps to sub-shells or to cluster groups.

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Period and Amplitude in Atmospheric Gravity Waves from Nuclear Explosions

Fig. 1 is a log-log plot of the peak-to-peak pressure Δp of the first full cycle of the waves against the period τ measured from the first to the second overpressure peak. The points shown refer to waves with a well-defined first cycle and are taken from microbarographic records, made in Stockholm[1], of nuclear explosions in the vicinity of Novaya Zemlya. The instrument response is essentially flat for lengths of periods between 0·5 and 300 sec. All points correspond to explosions seismically recorded by Båth[2] in Uppsala during 1961 and 1962. The site of the explosions from Stockholm are in the range of 1,900–2,300 km.

The straight line in Fig. 1 approximates the apparent interdependence of pressure and period as:

$$\Delta p = 3{\cdot}5 \times 10^{-3} \times \tau^2$$

when Δp is in dyne cm^{-2} and τ in sec.

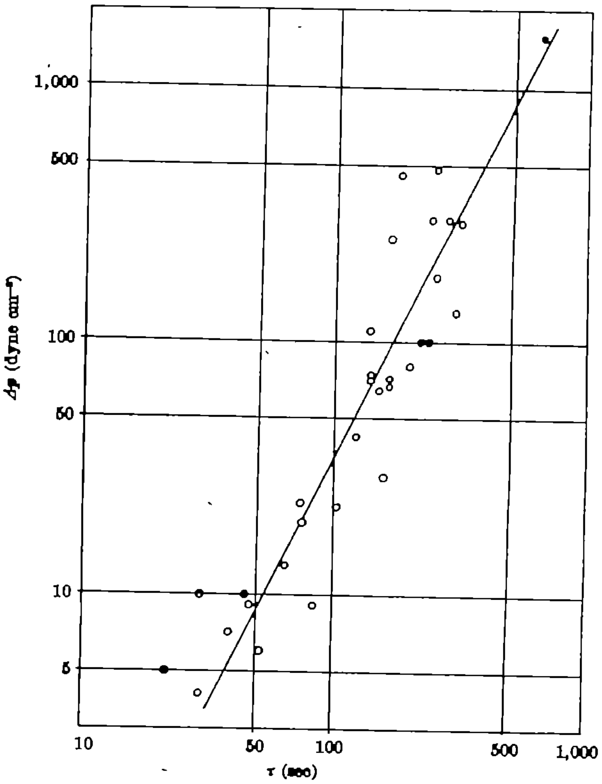

Fig. 1. Pressure and period of atmospheric gravity waves from nuclear explosions

Donn and Ewing[3] have mentioned that they could not find any significant correlation between explosion yield and record appearance in records obtained before 1961: they attributed this to the then inadequate instrumentation. In view of the proportionality of Δp to yield W used in the theoretical investigations of atmospheric gravity waves by Scorer[4], by Hunt, Palmer and Penney[5], and by Weston[6], our results indicate a proportionality between W and τ^2.

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Connexion between Local and Global Physics

If relativistic quantum mechanics can be founded on the 'principle of triality' as I have claimed elsewhere[1,2] then this ought to be reflected in the global structure of physics. Locally, triality means the invariance of a form trilinear in phase functions and two kinds of wave functions. Globally the latter are replaced by the expectation values of phase functions and globally defined fields. So the question becomes, what kind of triality is possible in the relativistic phase space E_8, considered as a quasi-classical object?

It was shown by Cartan and Schouten[3] that the elliptic space S_7 admits a triality which is unique. That is, in addition to possessing two kinds of absolute (Clifford) parallelism[4], these together with the points of the space form a triality. Here I show that this observation throws entirely new light on possible cosmological models.

All models so far considered[5] have the structure of a group space $H_n = G/K$. That is, a group G, connecting the observations of different observers, and a maximally compact subgroup K of symmetry for each observer. If $G = D_2$ the Lorentz group, and $K = B_1$ the rotation group of 3-space, then the model is determined up to an arbitrary gauge function which determines the equation of motion of a test particle. This can be chosen to give either general relativity (geodesics) or kinematic relativity (velocity–distance relation). As is well known, neither of these is satisfactory owing to the impossibility of reconciling the dynamic and kinematic requirements.

The reason for the impasse is two-fold. First, the group of dynamical transformations is potentially greater than kinematics and is only reduced to the latter by the assumption (E) that it is a group of extended point transformations; that is, momentum $p^\alpha = m_0\, dx^\alpha/ds$ with proper mass m_0 an absolute constant.

In a sense this assumption is already dropped in the steady-state theory in which proper mass is not conserved, but the only formulation of this theory[6] does not differ essentially from the introduction of a 'cosmological term' into the energy-momentum tensor, an admittedly *ad hoc* procedure. A satisfactory theory would have to relate such a term to locally observed fields as indeed others[7,8] have recognized.

The second restrictive assumption (H) lies in the fact that for a group space H_n, the number of parameters $r = n$ the number of dimensions. But the essential property of the H_n, namely, the existence of absolute parallelism, is according to the Cartan–Schouten result also possessed by just one other space, S_7. The group $G = B_3$ here acts in such a way that $r = 3n$.

Consider an affine space V_8 in the phase variables $x^\alpha = (p,x)$ with linear connexion:

$$\delta v^\alpha = dv^\alpha + \Gamma^\alpha_{\beta\gamma}\, dx^\gamma \qquad (1)$$

and of constant curvature:

$$R_{\alpha\beta} = \lambda g_{\alpha\beta} \qquad (2)$$

If the connexion is split:

$$\overset{\pm}{\Gamma}{}^\alpha_{\beta\gamma} = \overset{0}{\Gamma}{}^\alpha_{\beta\gamma} \pm S^\alpha_{\beta\gamma} \qquad (3)$$

into a metric and a totally antisymmetric part, then the pair of parallelisms given by equation (3) is absolute if:

$$S^\alpha_{\beta\gamma} S^\delta_{\alpha\epsilon} S^\beta_{\delta\eta} = \pm R_{\gamma\delta} S^\delta_{\epsilon\eta} \qquad (4)$$

The positive sign corresponds to assumption (H), while the negative, only possible for $n = 7$ in homogeneous co-ordinates with:

$$g_{\alpha\beta}\, x^\alpha x^\beta = M^2 \qquad (5)$$

gives triality between the connexions (3) and the points of S_7.

In this case $G = B_3$ is the holonomy group of V_8 which leaves invariant the Riemannian curvature:

$$R = R_{\alpha\beta\gamma\delta}\, v_1^\alpha v_2^\beta v_1^\gamma v_2^\delta \qquad (6)$$

associated with the directions v_1, v_2. Since:

$$S^\delta_{\gamma\alpha} S^\gamma_{\delta\beta} = \lambda g_{\alpha\beta} \qquad (7)$$

the structure of V_8 is entirely determined by the trivector field $S_{\alpha\beta\gamma} = g_{\alpha\delta} S^\delta_{\beta\gamma}$ depending on 21 parameters.

To see the physical significance of the foregoing, consider the two possible interpretations of S_3 in existing theory; namely, as on Einstein static[4] space S_3^4 in four homogeneous co-ordinates x^i (excluding time) or the velocity space S_3^4 of special relativity in the four-velocity co-ordinates $v^\alpha = dx^\alpha/ds$. Writing y as a quaternion representing either co-ordinates, we have[4]:

$$\frac{dy}{d\tau} = PyQ = A\,B\,y\bar{B} \qquad (8)$$

as an equation of motion due to a bivector field the polar and axial parts of which are the quaternions ABB, $AB\bar{B}$. Thus, for S_3, this is the electro magnetic field $F = (E, H)$, where (8) is the equation of ponderomotive force (without radiation terms):

$$\frac{dv}{ds} = e/m\,(v_4 E + v \times H) \qquad (9)$$

Thus, F is an acceleration-creating field. Analogously one might expect a velocity-creating field G in S_3^4, but because the norms $N(y) = N(A) = N(B) = 1$ this cannot be proper mass-creating. For that one has to go to S_7, where the weaker condition (5) allows mass creation off the light cone in time-like regions.

In S_7, (8) still holds with $z = (y,y')$ being interpreted as an octonion with the additional hypercomplex unit e, and non-associative multiplication:

$$(A + A'e)(B + B'e) = AB - \bar{B}'A' + (B'A + A'\bar{B})e \qquad (10)$$

If, now, G is regarded as weakly coupled to its sources with relative strength N^{-1}, (10) shows that cross-terms will appear with relative strength $N^{-\frac{1}{2}}$ which can be interpreted as nett charge creation. We thus have a type of model, which is not static, in which stationarity is maintained by electromagnetic forces.

Equation (8), and by triality its cyclic permutations, also hold in the local quantum theory with the skew fields P, Q, replaced by 8-component spinors. (This analogy for S_3 has been known for some time[9].) As noted previously[1], the group here is F_4, which, since it is of rank one higher than B_3, must describe an additional invariant, namely, a scale factor given by a short range field. Thus it seems likely that the number N will be given by the representation theory[10] of F_4.

Note added in proof. The same geometrical interpretation[11] of the sources of a skew field can be given to G as for F; the essential point is that congruences of parallels in elliptic geometry are not simply connected.

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METALLURGY

Electrolytic Detachment of Anodic Oxide Films on Aluminium Alloys

THE common technique for removing anodic oxide film from aluminium alloys is by dissolution of the metal substrate or attack along the metal–oxide interface using a bromine–methanol solution[1,2], or a mercuric chloride solution[3]. Both these procedures are slower, offer more risk of contamination of the oxide and yield smaller pieces of intact film than the electrolytic detachment technique developed by Lacombe and Beaujard[4].

In spite of its advantages the electrolytic detachment technique has not been widely used. This is due in part to its use of a potentially explosive mixture of perchloric acid and acetic anhydride, the necessity for filing panel edges before treatment, and a treatment period of up to 5 min. The substitution of a solution with lower perchloric acid concentration and with methanol replacing acetic anhydride has been suggested[5] to reduce the explosive hazard.

A further examination of the electrolytic detachment technique has shown that unsealed sulphuric acid anodic films can be detached in pieces up to 4 in. × 2·25 in. in size by anodic treatment for periods of less than 5 sec. Filing of the edges is not required. The solution used requires only normal care in preparation and use to avoid any hazard. The air-dried anodized panel is immersed in a perchloric acid (70 per cent)–methanol (abs.) solution with a composition of 1 : 5 parts by volume held at 21° C. The panel is made anodic by sudden application of voltage (50–65 V) for a period of from 1–4 sec during which the current rises steadily to a density of 6–12 amp/in.^{2}. The cathode is an aluminium sheet bent to fit around the panel. After the current flow is discontinued, the panel is immediately removed and rinsed for 1 min in warm water, then rinsed in cold water for 30 min.

In many cases full detachment of the film from the five surfaces of the panel occurred during the electrolytic treatment. In other cases, the film had to be removed by gentle insertion of a thin blade between the film and the metal or by tapping the air-dried film to shake off smaller portions of the film.

This new technique has been used successfully to remove oxide films from a wide range of commercial aluminium alloys. The optimum voltage required and the time needed for the current density to rise to the most effective value varied slightly with alloy. Films ranging from 7 to 60μ have been readily removed. Thinner films were more difficult to retain in large pieces than the thicker coatings. Oxide films prepared in electrolytes other than sulphuric acid and including sulphosalicylic acid–sulphuric acid type solution were detached but with somewhat less success. Cathodic treatment[6] has been tried but proved much less effective.

The colour and opacity of the detached films varied greatly with the condition of the metal surface, alloy, film thickness and electrolyte. Films from superpurity sheet were colourless and transparent, those from aluminium–5 silicon alloy were dark grey and opaque while films from certain alloys were tinted due to the effects of specific alloying constituents.

The two surfaces of the detached films differed slightly in that the electrolyte side surface took up colour from dye solution in normal quick fashion, while the metal side surface became coloured at a much lower rate. However, the colour produced by prolonged dyeing did not differ on the two sides. Agreement between the coating weight per unit area measured on the detached film and that obtained by chemical dissolution of the oxide on the metal was excellent, well within the possible experimental error. Most of the films after electrolytic detachment curled slightly, producing a concave effect on the metal side. This appeared due to the film having been formed under compression in the anodizing procedure, and to differences in the degree of compression at the two surfaces.

Anodic oxide films detached by the bromine–methanol method were compared with coatings removed by the electrolytic technique. The former film took up no colour on the metal side surface in sharp contrast with the normal colouring that ensued on its other surface. The mechanism of the detachment of the oxide films by this electrolytic technique is not clear but is believed due to the sudden evolution of large amounts of oxygen (possibly contaminated to a slight degree with reaction products of the perchloric acid and methanol). The accompanying pronounced local electrical heating effect is also a factor. The average current density is 1,000 amp or more per square foot while the actual value in the pores of the anodic film will be very much greater. Gas evolution occurs probably at the base of the pores adjacent to the barrier layer. This contributes to the penetration of the barrier film, which permits electrolyte flow to the metal substrate which becomes polished. The film ruptures laterally, either before or after penetration, at the metal-barrier layer interface or close to it.

Sealing of the anodic film, even for a very short period, interferes with its removal by the electrolytic technique described. However, sealed films have been removed with partial success in a solution with the acid reduced to 4·5 per cent, methanol to 77·5 per cent and water added to 18 per cent. Pieces detached were much smaller than detached unsealed films. It was necessary to file the edges of the panel before treatment and to slightly increase the voltage and the time of treatment.

I thank W. N. Stassen for help with the experimental work.

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Delayed Failure in a Hydrogenated Face-centred Cubic Alloy of Nickel

RECENTLY[1] it has been suggested that interstitial hydrogen in a face-centred cubic lattice will produce embrittlement and cause delayed failure under the appropriate conditions. Basically, the required conditions are that sufficient flow must occur to produce dislocation pile-ups and that the interstitial, under the influence of stress, must diffuse to the region of the piled-up array. In both the body-centred cubic and face-centred cubic lattices, dislocations have been observed to pile-up at an obstacle[2,3].

It has been postulated that stress-induced diffusion of hydrogen to the region of the dislocation array will result in the formation of a crack when a critical concentration has accumulated. The leading dislocations in the array will then coalesce to form a crack which propagates almost instantaneously and eventually is stopped by plastic flow; whereupon this process is repeated. Since the segregation of the solute is a time-dependent diffusion-controlled process, crack propagation is expected to be discontinuous in the appropriate temperature range. Presumably, substitutional atoms could produce a similar effect but the behaviour is particularly apparent with interstitials.

The delayed failure of the face-centred cubic structure which has generally been believed to be resistant to hydrogen embrittlement was examined in a high-strength alloy with the nominal composition, 66 nickel–30 copper–3 aluminium. Fig. 1 illustrates the results of static fatigue tests at various temperatures for the hydrogenated 'monel' alloy. Delayed failure was observed in the range 350°–500° F over a relatively wide range of stress (~80,000 lb./in.^{2}). The curve is typical of those observed for hydrogen in steel[1]. There is an upper limit of stress, a range of delayed failure, and a lower critical stress. Because of the lower mobility of hydrogen in the face-centred cubic lattice[4] the delayed failure phenomenon was observed at temperatures well above ambient. The creep characteristics at these temperatures rule out the association of this behaviour with conventional creep damage often observed in this type alloy.

Resistance measurements (Fig. 2) delineated the crack kinetics. There did not appear to be an initial incubation time. The absence of an induction period prior to crack initiation is attributed to a non-uniform distribution of hydrogen. The way in which the cracks grow, a series of initiations with virtually instantaneous propagation, is

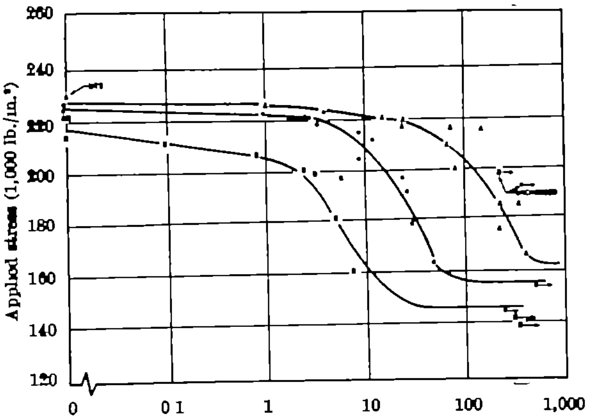

Fig. 1. Static fatigue curves for hydrogenated 66 nickel–30 copper–3 aluminium alloy. Sharp notch specimens. 185,000 lb./in.² strength-level. Test temperatures. △, 350° F; ○, 400° F; ■, 500° F

analogous to that observed with brittle delayed failure in steel[6]. It follows that the geometric details of crystal structure cannot be significant elements in a basic mechanism.

The typical strain rate sensitivity associated with hydrogen embrittlement was also obtained for this alloy. At room temperature and to 400° F or above, a pronounced decrease in ductility was observed as the strain rate was decreased. The ductility of the uncharged material was virtually unaffected by the large change in strain rate.

X-ray diffraction studies as well as mechanical tests were conducted in order to explore more fully the behaviour of hydrogen in this 'monel' alloy. The introduction of hydrogen into this alloy did not result in the formation of a second phase such as a hydride. Large increases in lattice parameter were observed in the hydrogenated material, illustrating that interstitial hydrogen caused a dilation of the lattice. This observation strongly suggests the concept of a hydrogen-dislocation interaction in face-centred cubic materials and lends further support to the same concept previously applied to body-centred cubic materials where the diffraction data are somewhat less conclusive.

Ageing at 212° F demonstrated the reversibility of this process. The lattice parameter was restored to the uncharged value when hydrogen was removed from solid solution. There was some line broadening which did not completely disappear. This phenomenon, which did not apparently influence the mechanical behaviour, was tentatively attributed to non-uniform plastic deformation resulting from hydrogenation.

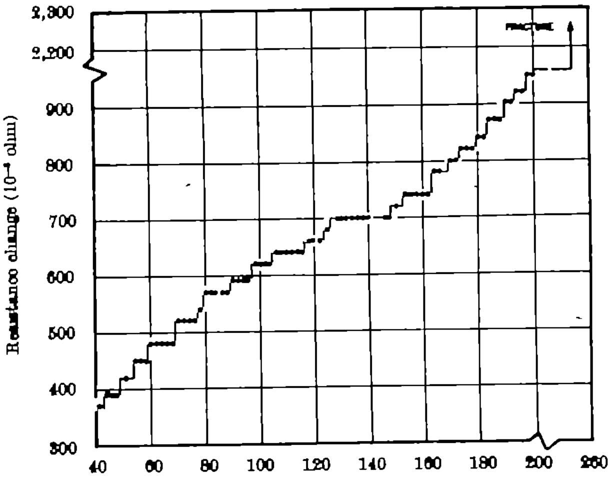

Fig. 2. Resistance increase as a function of time for hydrogenated 66 nickel–30 copper–3 aluminium in the stress-range of delayed failure. Test temperature: 400° F; applied stress, 221,000 lb./in.²

The influence of hydrogen on the stacking fault energy of face-centred cubic alloys has considerable interest in its relation to the basic mechanism of embrittlement. Interstitial hydrogen is expected to decrease the energy of a stacking fault[4]. The expanded fault implied by this change in energy can result in embrittlement by affecting the deformation processes and/or the cohesive strength. The investigation now in progress will attempt to provide evidence for this behaviour.

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BIOPHYSICS

Effect of Radio-frequency Fields on the Zeta-potential of a Colloidal Suspension

Reports from this laboratory[1–4], afterwards confirmed elsewhere[5], have shown non-thermal effects of radio-frequency on living organisms and non-living material. To preclude heat in these experiments, the radio-frequency was pulsed with pulse widths in the μsec range, and the duty cycle usually was less than 1 per cent. In these conditions any rise in temperature could be controlled so as to be virtually undetectable.

The manifold non-thermal biological changes were puzzling because the energy input on an $h\nu$ basis, at these frequencies (1–100 Mc/s), are orders of magnitude below that necessary to break the weakest chemical bond.

Because of the complexity of the biological materials and the difficulty of quantifying changes, it was decided to investigate a simpler system. For this purpose polystyrene latex was used. This material, obtained from Dr. John Vanderhof at Dow Chemical Corporation, is monodisperse and of known particle size. If these particles are placed in a radio-frequency field, the well-known pearl chain formation[6–8] may be observed under the microscope. However, as was pointed out previously[8], there appears to be a frequency optimum for alignment. This should not be the case if the dielectric constant of the particles and the medium does not vary with frequency over the range explored.

Since these particles have a high zeta-potential (about 50 mV in distilled water) which is responsible for the potential energy of repulsion between them, it was suspected that during pearl chain formation the zeta-potential might in some way be reduced. If this were the case, significantly greater reduction should occur at the frequency of optimal alignment.

Initially radio-frequency was applied at right angles across a micro-electrophoresis cell, and it was shown that the zeta-potential decreased as a function of applied radio-frequency voltage. However, the indication of a permanent change on the colloid induced by the radio-frequency led us to abandon this approach and undertake the following.

A suspension of polystyrene particles (1·305μ diam.) was dialysed against 10^{14} volumes of distilled water and

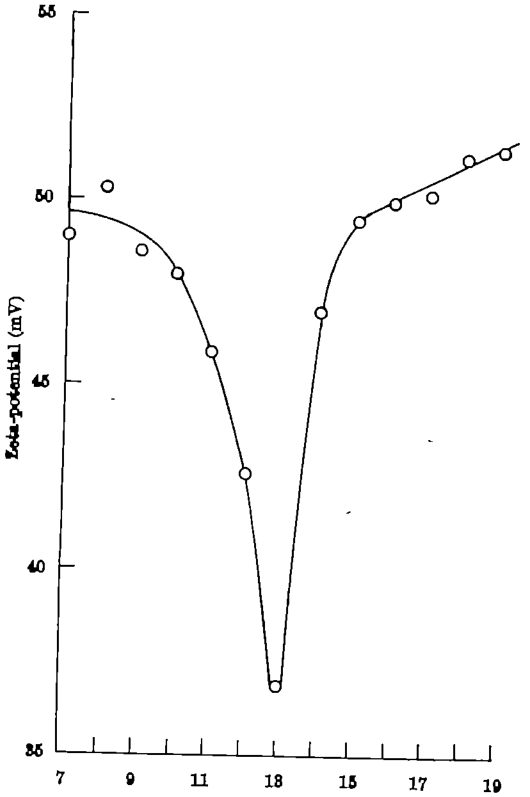

Fig. 1. Frequency dependency of change in zeta-potential. Control value of zeta-potential = 51·2 mV; applied voltage, 500 V, peak to peak/cm; pulse width, 10 μsec; pulse repetition frequency, 500; time in radio-frequency field, 1 min

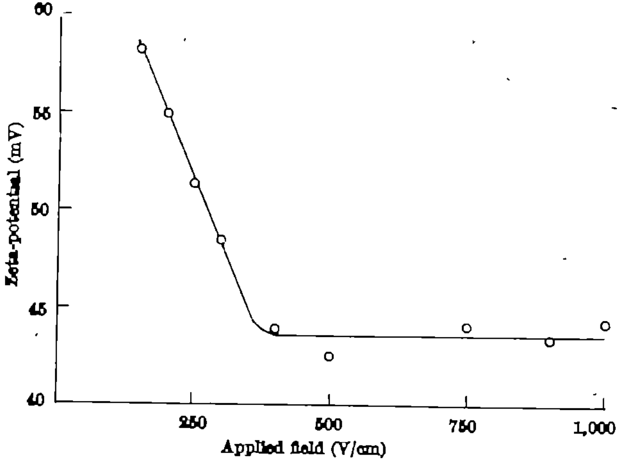

Fig. 2. Voltage dependency of change in zeta-potential at 13 Mc/s. Control = 58·7 mV; pulse width, 10 μsec; pulse repetition frequency, 500; time in radio-frequency field, 1 min

diluted with more distilled water to 10^7 particles/ml. The suspension was placed in a rectangular 2 × 2 × 5-cm cuvette. Two of the vertical sides were of sheet platinum serving as electrodes, and the other sides and base were made of polystyrene sheet.

A generator capable of delivering pulsed radio-frequency, variable over the range of 5–50 Mc/s, was connected to the platinum plates. The radio-frequency was applied in 10 μsec pulses at 500 pulses/sec for 1 min. The suspension was then removed and the zeta-potential measured in a microelectrophoresis apparatus 10 min later.

Fig. 1 illustrates the effect of varying frequency at a constant voltage on zeta-potential.

It can be seen that the maximum reduction in zeta-potential occurs at 13 Mc/s—the frequency at which optimal pearl chain formation occurs with 1·305μ spheres.

Fig. 2 shows the change of zeta-potential at 13 Mc/s as a function of applied voltage.

Such a decrease of zeta-potential might be explained by: (1) adsorption on the surface of either ions or molecules; (2) an increase in ionic strength of the medium; (3) the removal of surface charge groupings (in this case either $—COO^-$ or $—SO_3^-$); or (4) the re-association of dissociated surface charge groups. It is difficult to visualize how applied radio-frequency fields could influence any of the foregoing, especially on a permanent basis.

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Phase Shifts in the Human Retina

Brindley[1] has shown that in the human eye a part only of the attenuation responsible for flicker fusion phenomenon is localized in the receptor cells.

When previously De Lange's[2] experimental attenuation characteristics were fitted to a simple diffusion model[3] of retinal transfer characteristics the quantum efficiency of the process came out too low[3,4]. At that time, no experiments capable of yielding phase-shift characteristics for cross-checking could be[4] or had been taken. To understand why the diffusion model as applied was unable to produce an acceptable value of the quantum efficiency only a guess could be offered[4], which is now largely borne out by Brindley's[1] work.

The model[3,4] essentially yields expressions for the amplitude- and phase-shift characteristics, for the responses to a pulse and to a step as well as for the quantum efficiency, containing two parameters, namely, the time constant τ for the diffusion inside the receptor cell of photo-chemical decomposition products and the decay constant ρ, describing (pseudo-) monomolecular fading away of these products. The ranges in which on the ground of physiological data these parameters can be expected to fall have been discussed earlier[4].

From the original fit[3,4] to De Lange's[2] data the results $\tau = 0·4$ sec and $\rho = 25$ sec^{-1} were obtained, which in themselves seemed reasonable enough. The quantum efficiency Q for the diffusion process alone—equalling $(\cosh \sqrt{2\rho\tau})^{-1}$ and so being strongly dependent on parameter values—came out at 0·025, which is perhaps some 10 times too low.

Now that Brindley[1] has obtained phase-shift data on the receptor process it might be of some interest to compare these with the predictions of the diffusion model[3,4]. Unfortunately, phase-shifts below 40 c/s are not reported. The probable course of the phase-shift curve from 0 to 40 c/s must be discussed first.

Since the transit time of the system for optical stimulation is finite and for electrical stimulation essentially zero, the curve has to indicate zero phase-shift at zero frequency, if it is agreed that it is necessary to have the electrical pulse coinciding with the top of the optically excited wave at a certain site—and from there onwards—in order to obtain maximum flicker. In passing, it should be noted, however, that in view of the form of De Lange's[2] curves at elevated brightnesses it might be more advantageous for flicker perception in the region 1–6 c/s to

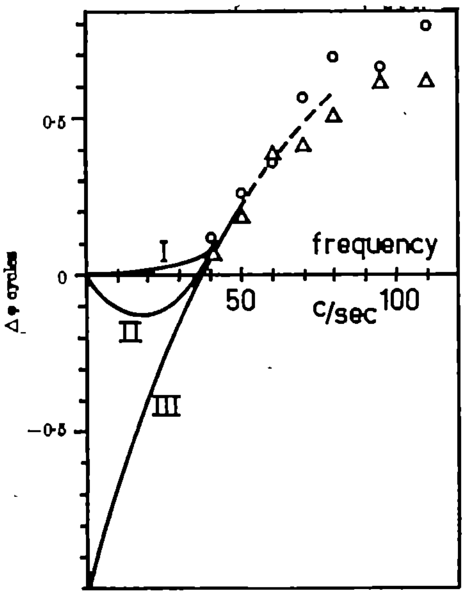

Fig. 1. Plot of Brindley's phase-shift data. ○, Cornea anode; △, cornea cathode

double the frequency by having the electrical pulses arrive at this site exactly in between optically activated wave tops; since such a mechanism could scarcely affect phase-shifts at frequencies of more than 40 c/sec it need not be considered in extrapolating Brindley's data.

A course like curve I in Fig. 1 would appear rather unnatural; such a sharp bending of a phase characteristic around 35 c/s would certainly involve a sharp top in the amplitude characteristic, which is not to be found in De Lange's[2] experimental results. Again, a course like curve II (Fig. 1) involving negative phase angles (light pulse after current pulse) would appear to have no rational meaning. I therefore believe that a reasonable extrapolation should follow a course like curve III (Fig. 1), involving a translation of the origin over one full cycle, such that Brindley's data at 40 c/s are to be read 1·12 and 1·07 cycles respectively.

In general, this full cycle can easily be overlooked in an experiment of this kind; the difficulty of the shortness of transit times encountered by Brindley[1] is thus avoided.

In Fig. 2, some of the phase-shift curves as predicted by the diffusion model[3,4] have been plotted; as shown, Brindley's data when corrected can be fitted to all curves for reasonable quantum efficiencies Q by choosing $\tau = 0{\cdot}19 \pm 0{\cdot}03$ sec, which is about half my former[3,4] value. The curves are too insensitive against Q to permit an appraisal of it on these grounds.

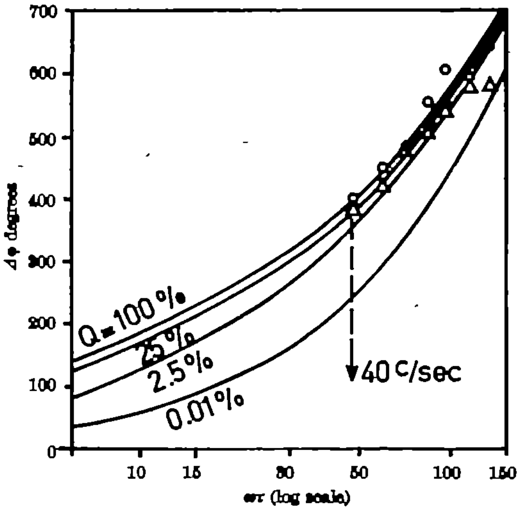

Fig. 2. Brindley's experimental points, corrected as discussed in text, can be fitted to phase-shift curves calculated for the diffusion model by choosing $\tau = 0{\cdot}19$ sec. The quantum efficiency Q cannot be appraised on these grounds

A new comparison of De Lange's[2] attenuation curves with replotted, calculated[3,4] amplitude characteristics (Fig. 3) taking $\tau = 0{\cdot}19$ sec and normalizing the experimental points on the reasonable attenuation value of 1·2–1·3 at 1·5 c/s will allow any quantum efficiency up to some 20 per cent. The existence of Brindley's[1] nervous attenuating mechanism is now clearly shown by the vertical deviation between experimental and calculated curves.

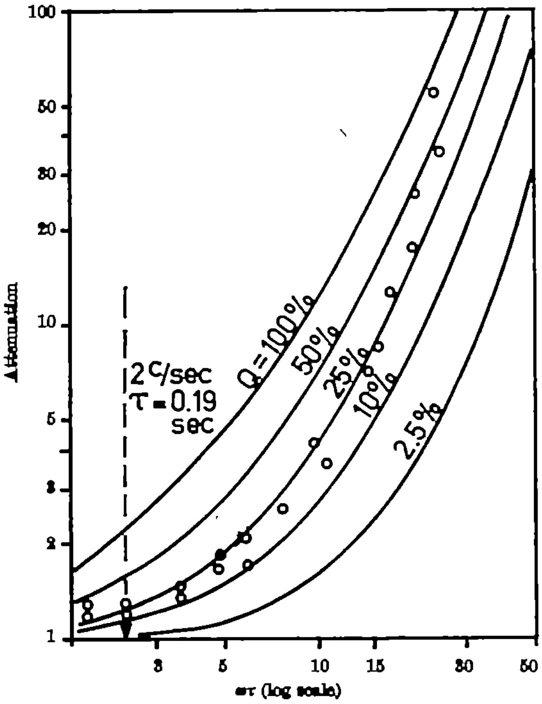

Fig. 3. Amplitude characteristics for the diffusion model for several values of the quantum efficiency Q have been plotted; if the value $\tau = 0{\cdot}19$ sec, as determined for Brindley's observer in Fig. 2, is assumed valid for De Lange's observers, that author's measurements will lie as shown. The existence of an extra nervous attenuating mechanism is apparent; Q could have any value up to some 20 per cent

Of course, the numerical values obtained for parameters τ and Q should be considered tentative for three main reasons, namely, that Brindley's[1] measurements are made with pulsed, in contradistinction to modulated, lights and currents at very elevated brightness, that De Lange's[2] measurements used here apply to one particular test field configuration (2° ⊖) and brightness (0·375 troland) only and that the model of one-dimensional diffusion used[3,4] is certainly much simplified as compared with physiological configurations. Moreover, the complete comparison given here depends on the reasonability of the addition of a full cycle to Brindley's data, which should be checked experimentally, and on the validity of the same value of τ for Brindley's and De Lange's observers.

It would appear, however, that models based on diffusion and recombination of photochemical decomposition products inside the receptor cells of the human retina can easily comprise a large body of experimental data by reasonable adjustment of few parameters.

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CHEMISTRY

Use of Xenon Adsorption for Measurements of Surface Area

MEASUREMENTS of surface area with xenon adsorption have not been used extensively despite the obvious advantage that very low dead space corrections are obtained at liquid nitrogen temperature. The reluctance to use xenon for surface area measurements seems to be due to the past difficulty of obtaining it in a pure state. Xenon with impurities totalling less than 100 p.p.m. by volume is now commercially available, and there should be no reason why the xenon Brunauer–Emmett–Teller (B.E.T.) method should not enjoy wider application.

In this laboratory, xenon adsorption at 77·3° K has been used for determining surface areas and roughness factors of nickel and nickel oxide-coated specimens. The geometrical areas of the specimen ranged from 20 to 100 cm^2 and roughness factors were 1·7–3·2. The areas were too small for accurate estimation by krypton adsorption, especially in view of the large dead space required by the configuration of the specimens (rectangular flat sheets). While relative values of surface area may be obtained irrespective of the cross-sectional area chosen for the adsorbate molecule, the determination of roughness factors requires an accurate estimate of the effective cross-sectional area (σ_{Xe}) of the xenon molecule. The literature, until recently, yielded no information concerning comparative monolayer volume (V_m) for xenon and other adsorbates of known molecular area.

Singleton and Halsey[1] give relative V_m values for nitrogen and xenon on carbon black, silver iodide, and anatase, but the measurements were made at different temperatures. The σ_{Xe} values at 148° K calculated from the data of Singleton and Halsey are 18·2 Å^2 for silver iodide, 20·2 Å^2 for carbon black, and 27·3 Å^2 for anatase. Anderson and Baker[2] comment on the observed xenon monolayer coverage on anatase and conclude that the packing is dependent on the arrangement of adsorption sites at the surface. They adopt a model in which the xenon atoms occupy preferred sites on the (001) plane, which is assumed to predominate because it is a natural cleavage plane. Due to the size of the xenon atom, adjacent sites cannot be occupied, and the xenon atoms form an array in which the effective coverage per atom is calculated to be 28·3 Å^2. Using this concept of crystallographical adsorption sites, Anderson and Baker compute average σ_{Xe} values of 23·0 and 19·5 Å^2 on polycrystalline tungsten and nickel surfaces, respectively. Pritchard[3] has recently compared V_m values for krypton and xenon on an evaporated copper film. The V_m value for krypton was determined by the B.E.T. method at 77° K and for xenon by a surface potential method at 90° K. The experiment led to a value for σ_{Xe} equal to 24 Å^2, taking 19·5 Å^2 for σ_{Xe}. The observed ratio of σ_{Xe}/σ_{Kr} is in nearly perfect agreement with the relative coverages calculated from the lattice constants of solid xenon and krypton.

Ponec and Knor[4] recently published comparative V_m data for krypton and xenon on evaporated nickel films. At 78° K the observed ratio of σ_{Xe}/σ_{Kr} was 0·92, showing that the molecular area was less for xenon than for krypton. The authors allude to the possibility of binding forces related to chemisorption playing a significant part in the case of xenon.

The lack of agreement in published molecular coverage data and uncertainty concerning the manner of packing in adsorbed films prompted our investigation of krypton and xenon adsorption on representative surfaces at the same temperature and over the same range of relative pressures (P/P_0). Krypton isotherm measurements have been carried out at 77·3° K on nickel powder, oxidized nickel powder, and glass wool. Specific surface areas and V_m values were obtained by the B.E.T. method. Smaller samples of the same materials were used for xenon isotherm measurements from which V_m values and σ_{Xe}/σ_{Kr} ratios were calculated.

The krypton B.E.T. method used was essentially that described first by Beebe, Beckwith and Honig[5]. Sample sizes were selected to yield total surfaces in the range from 2,000 to 4,000 cm^2. The vapour pressure of solid krypton was used for P_0 rather than the extrapolated liquid vapour pressure. (Justification for the use of the true vapour pressure can be found in ref. 6.) Specific surface areas calculated for this P_0 value are approximately 5–10 per cent lower than if the extrapolated liquid vapour pressure were used.

For the xenon adsorption measurements, an apparatus similar to that of Wooten and Brown[7] was used. Samples were used which had total surface areas in the range 200–

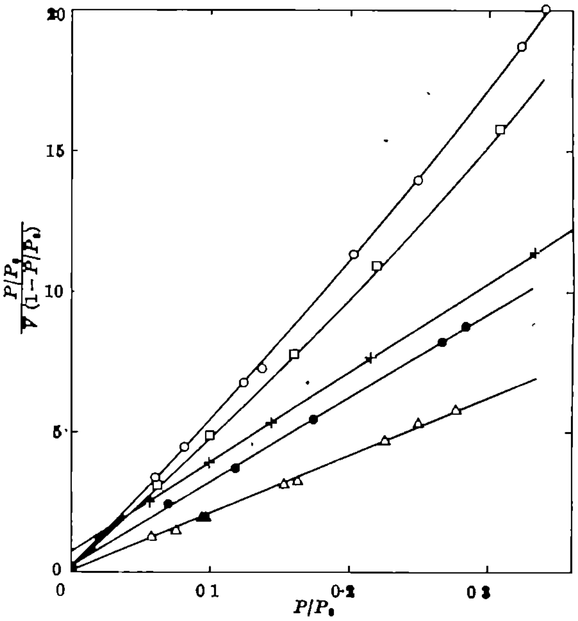

Fig. 1. Krypton B.E.T. plots. ○, Ni powder II; □, oxidized Ni powder II; +, glass wool; ●, oxidized Ni powder I; △, Ni powder I

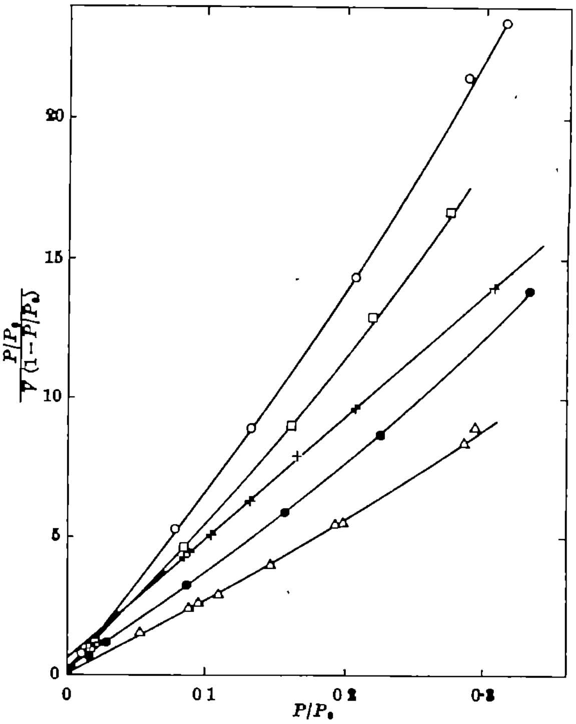

Fig. 2. Xenon B.E.T. plots. ○, Ni powder II; □, oxidized Ni powder II; +, glass wool; ●, oxidized Ni powder I; △, Ni powder I

Table 1. Relative Monolayer Volumes for Krypton and Xenon

Sample material	Specific surface area (cm²/g)	V_m, ml./g Krypton	Xenon	σ_{Xe}/σ_{Kr}	σ_{Xe} (Å²)
Ni powder I	2,530	0·0482	0·0362	1·33	25·9
Ni powder II	950	0·0182	0·0142	1·28	25·0
Glass wool	1,590	0·0304	0·0226	1·35	26·3
Ni powder I (oxidized)	1,730	0·0331	0·0262	1·26	24·6
Ni powder II (oxidized)	1,090	0·0207	0·0171	1·21	23·6

400 cm². Corrections were applied for the internal area of the glass sample holder and connecting tube, assuming a unit roughness factor for glass. Thermal transpiration corrections were applied, and P_0 was taken as the solid xenon pressure, which was calculated from the data of Podgurski and Davis[8]. Values of P_s were in the range 1·95–2·03 × 10^{-3} torr.

Monolayer volumes for krypton and xenon in ml. (S.T.P.)/g of adsorbent are given in Table 1. The V_m values are taken from the B.E.T. plots for krypton and xenon vapour shown in Figs. 1 and 2, respectively. Specific surface areas were calculated from the krypton data assuming 19·5 Å² for σ_{Kr}. The mean value of σ_{Xe}/σ_{Kr}, 1·29, is in fair agreement with the ratio found by Pritchard[3] (namely, 1·23) but does not agree with the findings of Ponec and Knor[4].

The nickel powders used were commercial materials of high, but unspecified, purity. They were used without any surface treatment and hence, even after outgassing, undoubtedly had the normal thin oxide film characteristic of an active metal. Nickel I was a 200 mesh (Tyler standard) powder containing a considerable amount of fines, while nickel II was a 150–300 mesh material. The oxidized powders were prepared by igniting nickel I and II until a black oxide film coated all particles. The gain in weight in each case was about 10 per cent of the theoretical weight gain for complete conversion to nickel oxide. Oxidation caused a slight gain in specific surface area of the Nickel II, whereas Nickel I lost area, due probably to sintering and agglomeration of the finer particles. The glass wool used was a commercial borosilicate material which had been degreased in benzene prior to outgassing in the adsorption apparatus.

All the B.E.T. plots for nickel and nickel oxide exhibit a curvature convex to the abscissa. The curvature is slight up to a relative pressure (P/P_s) equal to about 0·2, and slopes were obtained by taking the best straight line up to this value. The B.E.T. plots for both krypton and xenon on glass do not have perceptible curvature up to relative pressures of at least 0·35.

An independent measure of σ_{Xe} was made by determining xenon adsorption isotherms on samples of glass tubes and rods for which the total apparent geometrical area of the samples plus the internal area of the glass sample container could be readily measured. The roughness factor for the glass surface was assumed to be unity, in agreement with several workers who have presented experimental data to show that the roughness factor of glass and fused silica is very close to unity.

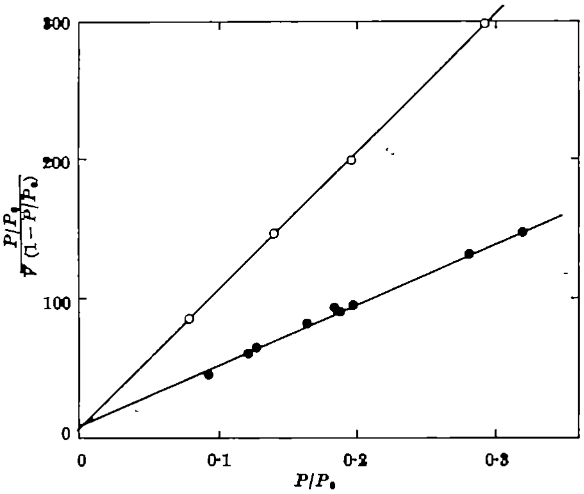

Fig. 3. Xenon B.E.T. plots on glass. ⊙, Glass tube, 72 cm² area; ●, glass rods, 166 cm² area

Table 2. Monolayer Volume and Molecular Area for Xenon on Glass Surfaces

Sample material	Geometrical area (cm²)	V_m (ml./g)	σ_{Xe} (Å²)
Glass tubing	72	0·00099	27·0
Glass rods	166	0·00226	27·1

The B.E.T. plots for the two experiments are given in Fig. 3; the corresponding computed V_m and σ_{Xe} values are shown in Table 2. Because the roughness factor of glass cannot be less than unity, the calculated σ_{Xe} values should be considered minimum values, and yet they are higher than any of the results given in Table 1. These results indicate the possibility that, with the given ratio of σ_{Xe}/σ_{Kr}, the accepted molecular area of 19·5 Å² for σ_{Kr} may be too low. Indeed, it appears from the literature that this value has been derived by averaging the various results obtained by comparison with nitrogen adsorption, and using the extrapolated liquid krypton vapour pressure data to estimate P_0. Values obtained by using the true solid vapour pressure require a higher σ_{Kr} value to bring the relative nitrogen and krypton coverages into agreement.

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A Bouncing Liquid Stream

I wish to report an unexpected effect observed when a thin stream of a solution of polyisobutylene in 'Decalin' is poured into solution in an open dish.

The solution was poured from a height of about 25 cm in a stream approximately 1 mm diameter into solution contained in an evaporating dish of diameter 10 cm. The ciné still photographs in Fig. 1 consist of every fifth frame from a 16-mm film shot at 64 frames/sec. The whole sequence shown therefore occurred in slightly less than 1 sec.

In Fig. 2 a key to the various features in the photographs is given.

As the stream enters the liquid the condition in the first frame of Fig. 1 is quickly established. A small 'heap' of liquid is formed at the point of entry. As pouring proceeds a small stream of liquid appears to emerge from the heap with a trajectory approximately parallel to the liquid surface (frame 2). The trajectory of this secondary stream gradually steepens and usually changes in azimuth (frames 3, 4, 5, 6 and 7) and at the same time the heap disappears. The secondary stream then begins to die away and disappears (frames 8, 9, 10 and 11). The heap of liquid grows again (frame 12) and the whole sequence is repeated. The secondary stream is approximately the

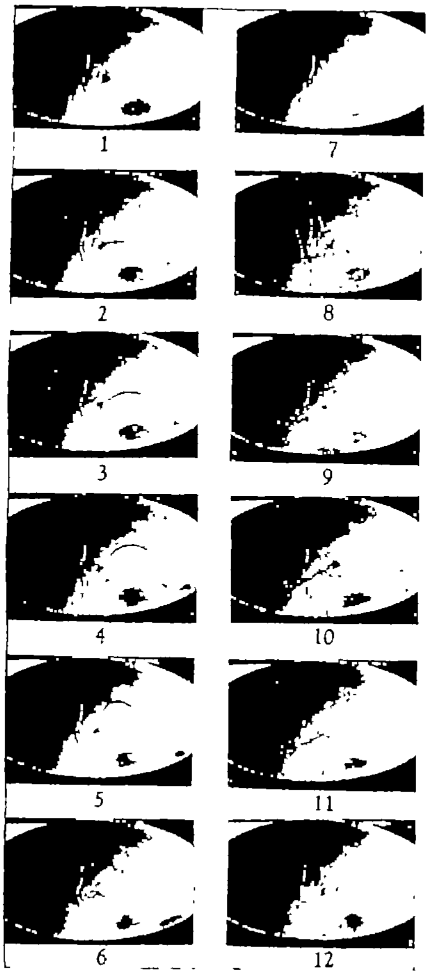

Fig. 1

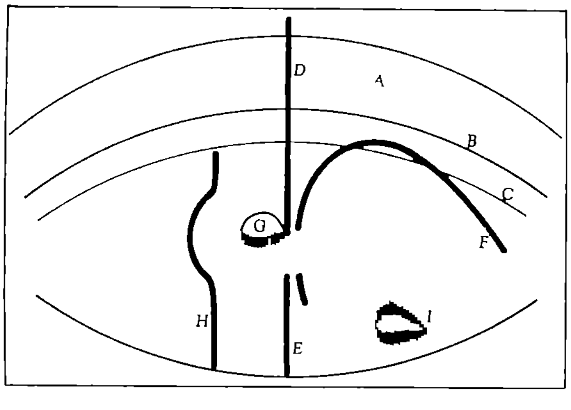

Fig. 2. *A*, wall of the evaporating dish; *B*, edge of the surface of the solution in the dish; *C*, reflexion of the rim of the dish in the liquid surface; *D*, liquid stream entering the dish; *E*, reflexion of the stream in the surface; *F*, bouncing liquid stream; *G*, 'heap' formed where the stream enters the liquid; *H*, reflexion, in the surface, of the stand used to hold the flask from which the liquid is being poured; *I*, shadow, on the bottom of the dish, of the 'heap' *G*

same diameter as the primary stream and its trajectory has a base of about 2 cm when the stream is fully developed.

I can offer no explanation for this behaviour, but it may be relevant to note that solutions of the type described are markedly non-Newtonian and visco-elastic, as shown by the work of Brodnyan, Gaskins and Philippoff[1]. It is tempting to speculate that when the 'heap' reaches a critical condition the primary stream effectively bounces off it, forming the secondary stream. The supply of liquid to the heap is thus cut off, and it therefore sinks into the main liquid surface, changing its shape and thus, perhaps, the trajectory of the secondary stream. When the heap has disappeared, 'normal' behaviour is resumed. The heap reforms and the cycle is repeated.

The phenomenon was first observed as the result of a chance observation, and no systematic investigation of the conditions necessary for its occurrence has been undertaken.

The particular solution used consisted of 5·8 g of *L*100 grade 'Vistanex' (Esso Petroleum Co., Ltd.) in 100 c.c. of 'Decalin'. The solution viscosity, measured in a concentric cylinder viscometer, was approximately 3·3 poise at a shear rate of 10 sec^{-1} and at 25° C.

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Uranium (VI) – Chromotropic Acid Chelate

DISODIUM-1:8-dihydroxy naphthalene-3:6-disulphonate (trivial name chromotropic acid–sodium salt) yields coloured lakes with various cations[1]. Investigations of chelates of chromotropic acid with iron (III)[2] and titanium (IV)[3,4] have already been made. Sommer *et al.* have extensively examined chelates of titanium with chromotropic acid and various polyphenols[5–7] and have discussed the structure of the metal chelates. The use of chromotropic acid in the colorimetric determination of uranium[8,9] has also been reported.

Here, the composition and stability of the uranyl ion–chromotropic acid chelate have been examined by spectrophotometry.

Solutions of disodium-1:8-dihydroxy naphthalene-3:6-sulphonate were prepared and standardized by determining the sulphur content. Solutions of uranyl sulphate (B.D.H. AnalaR) were prepared by dissolving the salt in double distilled water, free from carbon dioxide. All solutions used were freshly prepared to avoid the effects of hydrolysis of the metal salt.

The spectrophotometric examination was made with a Unicam *SP* 500 spectrophotometer, with matched glass cells 10 mm thick. Measurements were made at 25° C and the *p*H of the solutions adjusted to 4·5 ± 0·2. *p*H measurements were carried out with a mains-operated Leeds and Northrup *p*H indicator with a glass–calomel electrode system.

Examination by the method of Vosburgh and Cooper[10] showed that the complex species in mixtures containing varying proportions of the reactants is identical, having a λ_{max} at 435 mμ.

To examine the stoichiometry of the components the method of continuous variations was used, the total volume in each case being kept constant at 50 ml. The absorbances were measured at two different wave-lengths of 435 and 445 mμ. The plot between the differences in absorbance of the mixtures and that which would have been shown by the chelating agent alone, if no chelation occurred against the composition of the mixture, showed a composition of 1:1 for the chelate. Some typical observations are plotted in Fig. 1.

In the legend (Fig. 1) *c* represents the concentration of uranyl sulphate and *c′* the concentration of disodium-1:8-dihydroxy naphthalene-3:6-sulphonate, that is, $p = c'/c$.

The λ_{max} of 1:1 mixtures of uranyl sulphate and chromotropic acid lies at 435 mμ when the *p*H of the solutions are between 3·2 and 8·25.

The apparent stability constant of the uranyl–disodium-1:8-dihydroxy naphthalene-3:6-sulphonate, as calculated

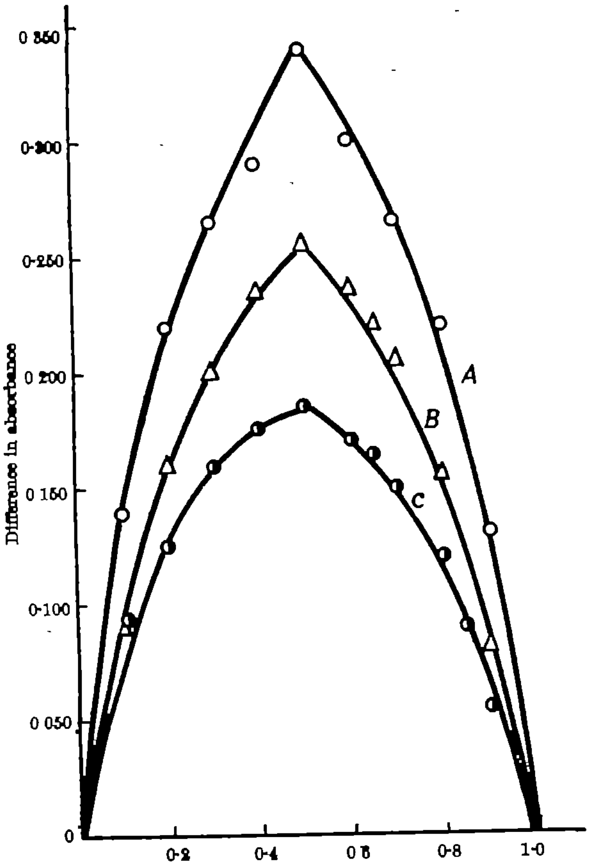

Fig. 1. Determination of the composition from absorption spectra studies at 435 mμ (equimolecular solutions). Curve *A*, $c = 2·0 \times 10^{-4}$ M, $p = 1$; curve *B*, $c = 1·33 \times 10^{-4}$ M, $p = 1$; curve *C*, $c = 1·0 \times 10^{-4}$ M, $p = 1$

from absorbance data using the method of Dey *et al.*[11], is 7·14 ± 0·11 at 25° C. The free energy of formation $\Delta F°$ of the chelate corresponds to −9·85 ± 0·15 cal. at 25° C.

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Coding of Open and Closed Carbon Chains

IN a previous communication[1], a method of coding open-ended carbon chains was suggested, which amounted, in effect, to forming the autocorrelation function of the net. This code had the advantage of being nearly unambiguous, but suffered from the drawback that, while the code of any given net could be determined straightforwardly, the net corresponding to a given code could not. A. Savitsky has suggested (private communication) that, nevertheless, this code could be useful as a check; that is, if added to the usual chemical name of a given compound, this code could serve to verify whether the topology of the compound reconstructed from the name is the correct one.

If so used as a check, complete unambiguity is of lesser importance, and a probabilistic point of view can be adopted. Thus, if a C_n net can have $N(n)$ different possible configurations, and if there are $m(n)$ ambiguities, the probability that, once having reconstructed the incorrect configuration, the code of this configuration is identical to the code of the correct configuration can be written as:

$$p = \frac{2m}{N(N-1)}$$

With this fresh point of view, the question was then examined whether, when extending the code proposed to nets containing closed chains, the appreciable number of ambiguities encountered would still leave p tolerably small, and whether p would tend to decrease for increasing n.

The postulate was made, when making this extension, that rings of 5, 6 and 7 carbon atoms only would enter into consideration. The code was formed, in line with the procedure followed for open chains, by counting the number of pairs of carbon atoms at minimum distances 3, 4, etc.

Table 1

n	N	m	p	m_1	p_1
7	15	0	0	0	0
8	34	4	0·71%	0	0
9	85	13	0·36%	4	0·14%
10	220	50	0·21%	24	0·24%

The findings are summarized in Table 1 in which the number of ambiguities were counted as 1, 3, 6, etc., for the double, triple, quadruple, etc., occurrence of the same code. The conclusion, implicit in the fourth column, is that p exhibits a satisfactory tendency to decrease with increasing n.

When extended to closed chains, the location code, formed by counting, for any one carbon atom, the number of other carbon atoms at distances 1, 2, etc., appears to be less satisfactory for increasing numbers of atoms. The probability of determining an erroneous position having the same location code as the correct one, $p_1 = \frac{2m_1}{N\,n(n-1)}$ where m_1 designates the number of location ambiguities (not counting those due to the molecule symmetry) is indicated in the last column.

I thank Prof. A. I. M. Keulemans, who directed my attention to this problem, and Prof. C. Koningsberger for a discussion of the most reasonable postulates to be made for closed chains.

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Existence of Two Soil Species in Detergency Investigations

ALTHOUGH a considerable amount of research has been devoted to the examination of detergency, little work has been done on rates of soil removal. Durham[1] has commented on the need for kinetic studies in detergency research. We have examined the kinetics of detergency in a model circulation cleaning system. In this model system a detergent solution (0·03 M sodium hydroxide) is pumped from a 50-gallon tank through a short length of 1½-in. diam. pipe in a closed system. Test strips of stainless steel 3·6 cm × 32 cm are soiled on one side with a very thin layer of pure synthetic tristearin labelled with carbon-14. The test strip is placed in the pipe, the detergent solution is pumped past the strip for a fixed time under closely controlled conditions after which the pipe is drained and the strip is removed. The amount of tristearin taken off the strip is found by measuring the radioactivity before and after each treatment. By means of repeated cleaning treatments on one strip, a cleaning curve is obtained.

A typical cleaning curve is shown in Fig. 1 where log tristearin (measured as counts per 10 min) is plotted against the number of 10-sec washings. Because of the contribution of a time-independent mechanism to the total cleaning[2], the abscissa units are number of washings rather than the time of washing. Cleaning curves obtained over a wide range of experimental conditions are of the same general shape as Fig. 1.

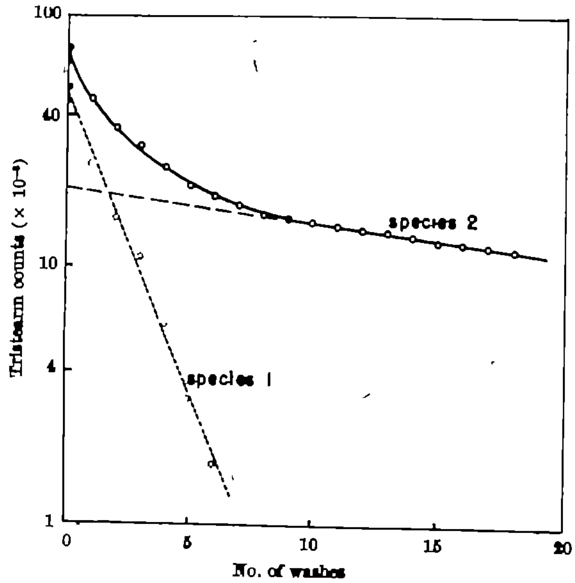

Fig. 1

The shape of the cleaning curve is similar to that obtained for the sum of two independent first-order processes occurring at different rates, for example, the over-all decay of a mixture of two radioisotopes, one with a much longer half life than the other. When the linear portion of the cleaning curve is extrapolated back to zero washes and the extrapolated line subtracted from the curve, the result is a second straight line with a much steeper slope. The line marked species 1 in Fig. 1 is obtained in this way.

This result could be explained by assuming that two species of tristearin are present, species 1 and species 2, each of which is removed from the surface independently and simultaneously by a first-order process, species 1 being removed much faster than species 2. Using kinetic theory:

$$\frac{-\mathrm{d}(A)}{\mathrm{d}t} = k_1(A), \text{ and } \frac{-\mathrm{d}(B)}{\mathrm{d}t} = k_2(B)$$

where (A) and (B) are the amounts of species 1 and 2 respectively at any given time, k_1 and k_2 are the rate constants and $k_1 > k_2$, t is the number of washes (constant time per wash).

Re-arranging and integrating gives:

$$\ln(A) = \ln(A_0) - k_1 t, \text{ and } \ln(B) = \ln(B_0) - k_2 t$$

where (A_0) and (B_0) are the amounts of species 1 and 2 at zero time. The total amount of tristearin present at a given time $= (A) + (B)$.

The experimental cleaning curve is described with a high degree of precision by the sum of the values of (A) and (B) for each value of t. From Fig. 1, for example, A_0, B_0, k_1, k_2 can be evaluated, giving the equations:

$$\ln(A) = 10{\cdot}88 - 0{\cdot}574t \text{ and, } \ln(B) = 9{\cdot}99 - 0{\cdot}0337t$$

It should be pointed out that the close agreement between the experimental cleaning curve and the sum of two independent simultaneous first-order processes is entirely empirical. There is no theory in existence at this time that would predict a relationship of this kind.

We have established that the existence of two soil species does not arise from impurity or polymorphism in the tristearin; that species 2 is not a mono-molecular layer; and that redeposition of the removed soil is highly unlikely. The proportion of species 2 is dependent on the thickness and age of the soil film, temperature of washing, and possibly other parameters that are unknown at this time, but it is not affected by the flow rate of the detergent solution. The effect of temperature, flow rate, and time of washing treatments on both rate constants k_1 and k_2 has been measured by the method described above.

Examination of the literature shows that the cleaning curves obtained by other workers using a wide variety of soils, surfaces, detergents and cleaning conditions are similar to Fig. 1 and could probably be reduced to the sum of two independent first-order reactions occurring simultaneously; a species 1 being removed at a fast rate and a species 2 being removed at a slow rate. We believe that this phenomenon is not restricted to thin films of tristearin on stainless steel but that two distinct soil species are present in most, if not all, thin soil films present on most surfaces. The nature of the forces that cause species 2 to be so resistant to removal from a surface can only be surmised at this time.

The existence of two soil species in variable proportions probably accounts for much of the variability that is encountered in detergency testing and research. Other problems of cleaning, such as the greying of textiles after repeated washings, might be explained by the gradual accumulation of the resistant species 2 soil.

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RADIOCHEMISTRY

Viscosity of Single-stranded Deoxyribonucleic Acid after X-irradiation in the Native State

Several authors[1] have discussed the ionizing radiation which causes single-stranded as well as double-stranded breaks in DNA. The single-stranded breaks seem to occur more frequently. Butler's[2] observation that the loss in molecular weight due to radiation is more intensive after denaturation of DNA by heating confirms this idea. The work of Doty *et al.*[3] provides a method for separating the two DNA strands without further degradation of the polynucleotide chain. I used this method to study the radiosensitivity of the single strand DNA.

Calf thymus DNA was prepared according to the method of Kay *et al.*[4]. The intrinsic viscosity $[\eta]$ was determined at 21° C in 0·01 M sodium chloride using a four-bulb multigradient viscosimeter of the Ubbelohde type to allow an empirical extrapolation to zero gradient. Denaturation was completed within 10 min at 90° C followed by rapid chilling. The viscosity of native thymus DNA was 38·0 dl./g; the viscosity of the single strand DNA, 20·5. The DNA was irradiated in the native state (0·05 per cent in 0·01 M sodium chloride) and then denatured within 30 min. Fig. 1 shows the viscosity of the native DNA and that of the single strand one after irradiation as percentages of the control. The irradiation data are: 150-kV X-rays, 20 m.amp, filter : 2·0 mm aluminium, *FD*, 10 cm,

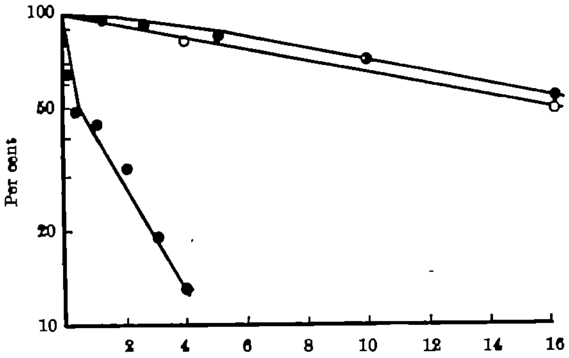

Fig. 1. Radiation effect on DNA as percentage of the control. Open circles, viscosity of native DNA; closed circles, viscosity of the single-stranded DNA; half-filled circles, breaking of the hydrogen bonds. 0·05 per cent DNA in 0·01 M sodium chloride

dose rate : 3,150 r./min). We also determined the amount of open hydrogen bonds in the native irradiated DNA according to a method described in an earlier article[5].

The viscosity of the native DNA and the hydrogen bonds between the two strands have nearly the same radiosensitivity; the D_{37} is about 30,000 r. After separation of the strands, however, the viscosity drops to 50 per cent after 500 r. The dose effect curve seems to have two components: one with D_{37} of 700 r., the other with D_{37} of 2,500 r. The two components of this curve may be discussed in a similar way as Latarjet *et al.*[6] did when considering the inactivation of the transforming factor in the DNA, where the D_{37}-values of the two components also have a ratio of 1 : 4 (1,000 and 4,000 kr.).

The breaks of the single strands are permanent in native DNA over a longer period. If the DNA is denatured only 24 h after irradiation of 500 r., the viscosity of the single strands decreases to 40 per cent of the control instead of 50 per cent. This means that there is a small 'after effect' of the irradiation.

If DNA is first denatured and then irradiated, the loss of viscosity is very small. After 1,000 r., no change of the viscosity was observed. The same effect has been described by Marmur *et al.*[7] after irradiation of DNA with ultra-violet light. The high sensitivity of the single nucleotide chain in native DNA might be explained by the fact that the target volume in the stiff double helix is larger than the target in the coiled single-stranded DNA.

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RADIATION CHEMISTRY

Possible Radiation Damage Effects in Barium Chromate Crystals

BARIUM chromate obtained by the method elaborated in our laboratory[1–3] forms crystallites the shape of which depends on the kind of nucleus on which the crystallite has been formed. Under special conditions, for example, by fractional precipitation using the same method or by precipitation from dilute solutions, reagent grade potassium chromate produces sediments of prismatic-shaped barium chromate crystallites having a rhombic cross-section.

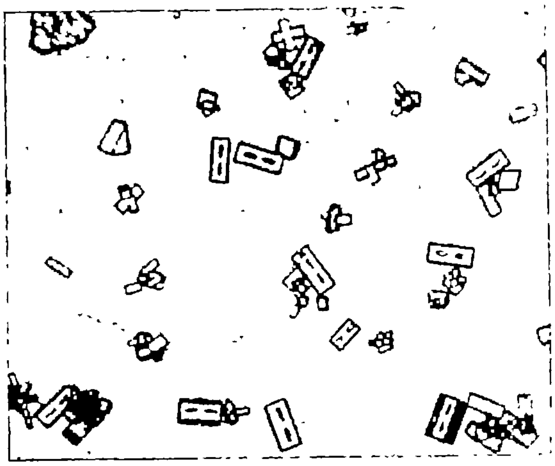

Fig. 1. × 400

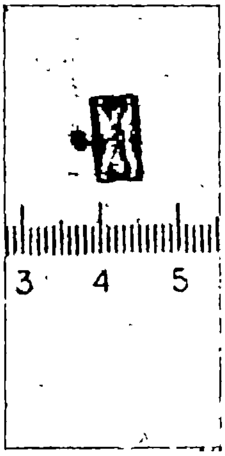

Fig. 2. × 400

Very often it has been observed that two elliptical 'gas bubbles' inside the prisms were formed. Crystallites of smaller size show two 'funnels' on the ends, instead of the 'bubbles', while others show only two or four dark points (Figs. 1 and 2). A similar feature was observed by us earlier, while precipitating barium chromate from solutions containing uranium and radium[4]. Observations described elsewhere[1,3] show that crystallites are formed on nuclei consisting of foreign substances. It is supposed that, in the case recorded here, barium chromate crystallizes on isomorphous radium chromate nuclei, traces of radium coming from uranium and thorium always present in chromite ores.

The radioactive decay of radium causes strong radiation damage effects similar to those described, for example, by Yoshida[5] and Vineyard[6]. The great energy of the decay as also the concentration of the source—that is, the crystal nucleus—produces effects sufficiently strong as to be visible by simple microscopical observation. Also it has been shown by us (by slightly levering the tube of the microscope) that the crystalline substance in the vicinity of the 'bubble' is of greater optical density and acts as a regular optical lens. These observations fit very well to the features of vacancies and interstitial formation as postulated for radioactivity effects in crystal lattices. The 'funnels' already mentioned have been observed on very small objects 5–7μ long. This fact seems to be in accordance with a similar effect described for uranium metal by Rogers and Adams[7].

In some experiments crystallites showing radiation damage effects have been obtained, where no further changes could be detected. In other cases crystallites of exactly the same shape showed no effects of this type. Differences, as such, can be ascribed to the different sizes of radium chromate nuclei, or to different radium isotopes present, that is, radium-224 ($t_{\frac{1}{2}}$ = 3·64 d.), radium-226 ($t_{\frac{1}{2}}$ = 1620 y.) and radium-228 ($t_{\frac{1}{2}}$ = 6·7 y.). The strongest damage is expected to be caused by radium-224. Many other observations made are in good accordance with the interpretation of facts presented here.

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CERAMICS

A Simple Flame Fusion Apparatus

THE need for large single crystals of refractory oxides has been ever increasing in recent years. In many instances further technological advances are being hampered by the lack of materials of suitable purity and perfection. One of the major sources of contamination of materials prepared at high temperature is the container in which the material is heated. The flame fusion technique is one high-temperature crystal growth method which overcomes the container problem as the material under investigation acts as its own support. A rather complete review of the advantages, limitations, and operation of flame fusion furnaces is given by Popov[1] in a book edited by Shubnikov and Sheftal.

This communication describes a simple portable flame fusion apparatus which can be constructed in most laboratories with readily available materials.

The apparatus consists of three major components: (1) the feed hopper and burner assembly; (2) lowering mechanism; (3) the gas system. The usual feed hopper assembly consists of an outer and inner hopper. The inner hopper is fitted with a fine mesh sieve which is vibrated by a tapper mechanism for control of feed flow. A new design of a one-hopper feed assembly has been incorporated into a flame fusion apparatus. In this version continuous and uninterrupted feed rates are accomplished[2]. The feed hopper is designed such that commercially available burner assemblies can be used and interchanged with ease.

The pedestal on which the crystal is grown must be lowered at a rate equal to the growth rate of the crystal. In addition the rate of lowering must be variable to coincide with slow or rapid crystal growth. It is also very important that the lowering mechanism transmit as little vibration as possible to the growing crystal. A compact and versatile lowering mechanism was designed to eliminate the difficulties usually encountered. A variable-speed d.c. motor and a gear train system permit the growing boule to be lowered in the continuous range from zero to 4 in. per h. The lowering mechanism is equipped with an independently variable rotation mechanism which can rotate from a fraction to 100 r.p.m. by suitable choice of gears and motors. The housing for the lowering mechanism is shock mounted and can be positioned by means of a two-dimensional slide rest. A schematic diagram of the lowering and rotation mechanism is shown in Fig. 1.

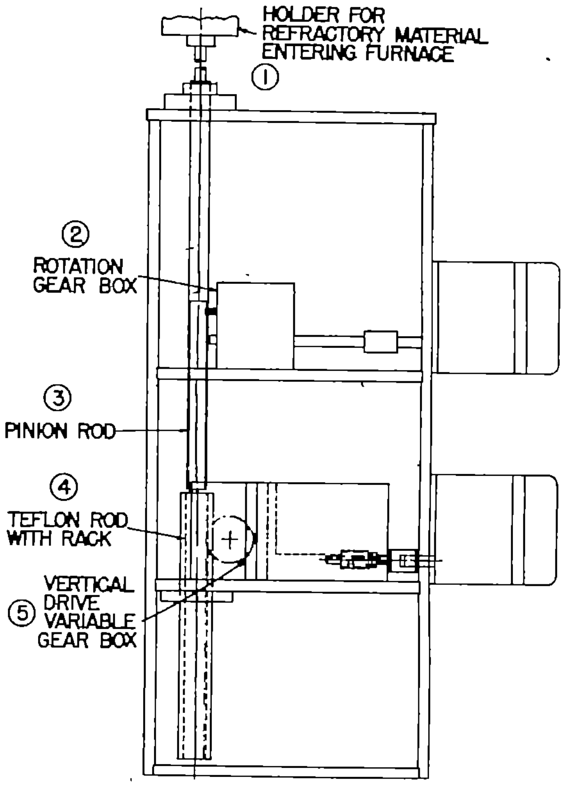
HOLDER FOR REFRACTORY MATERIAL ENTERING FURNACE
①
②
ROTATION GEAR BOX
③
PINION ROD
④
TEFLON ROD WITH RACK
⑤
VERTICAL DRIVE VARIABLE GEAR BOX

Fig. 1. Lowering mechanism

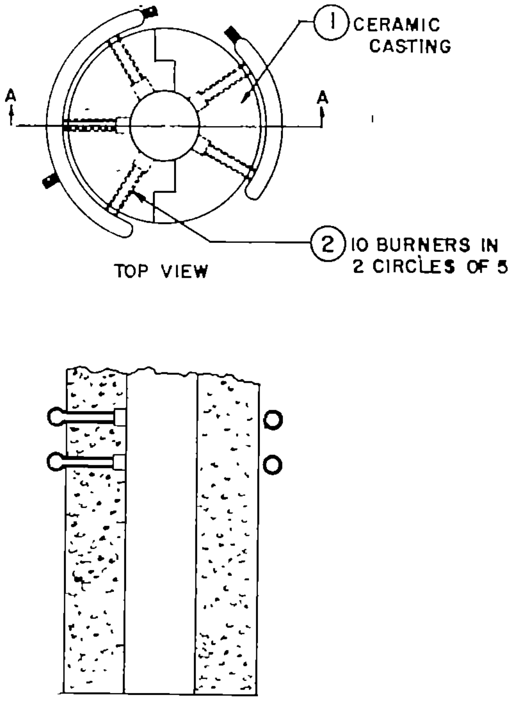

Fig. 2. Annealing furnace

A refractory furnace muffle with tongue and groove joints was cast from 33-*HD* alundum (Norton Refractories, Worcester, Mass.) to contain the heat source. An adaptation of this muffle has been designed as an annealing furnace to reduce the strains in growing boules by *in situ* annealing. This furnace consists of two rows of five burners each using oxy-hydrogen gas. The burners are positioned and the manifold cast in a refractory muffle as shown in Fig. 2.

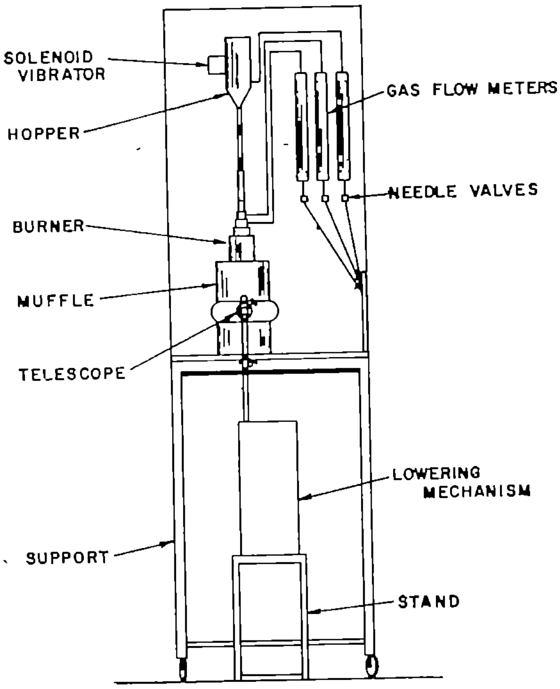

Fig. 3. Schematic drawing of apparatus

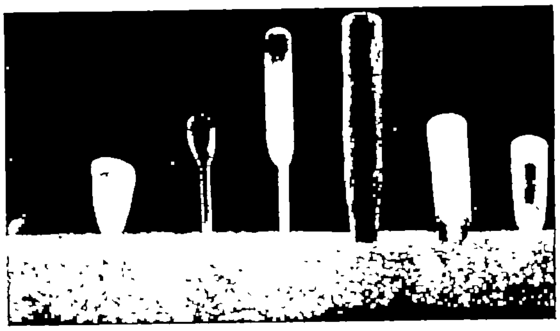

Fig 4. Photograph of typical boules

The entire apparatus is mounted on a portable frame which can be relocated with ease. The location of the flow meters, precision needle valves, feed assembly, burner, furnace muffle, telemicroscope and lowering mechanism are shown schematically in Fig. 3. The support frame is provided with casters and jack screws for purposes of relocation and levelling, respectively.

The apparatus has been used for preparing numerous crystals of refractory oxides including transition metal and rare earth oxide doped titanium dioxide, aluminium oxide and spinels.

Suitable titanium dioxide feed powder was prepared by calcination of the oxalate[3]. The transition metal impurities were added in the form of sulphates prior to calcination.

Cerium, chromium, iridium, iron, manganese, nickel, niobium, praseodymium, tantalum, vanadium, and yttrium in weight percentages of from 0·001 to 1 were successfully incorporated in titanium dioxide. Magnetic resonance investigations of some of these crystals are reported elsewhere[4].

Aluminium ammonium alum was calcined at 1,050° C to prepare a suitable aluminium oxide feed material. Reagent grade sulphate was added prior to calcination in the preparation of the spinels and doped spinels. Single crystals of $MgAl_2O_4$-Al_2O_3 : Cr^{3+} grown with this apparatus have been investigated[5].

A photograph of typical boules is shown in Fig. 4.

I thank Mr. Maxwell Hopkins for help in designing the lowering mechanism, and Messrs. E. Aleshin, R. F. Bates and R. Arlett, who assisted in the growth of some of the crystals.

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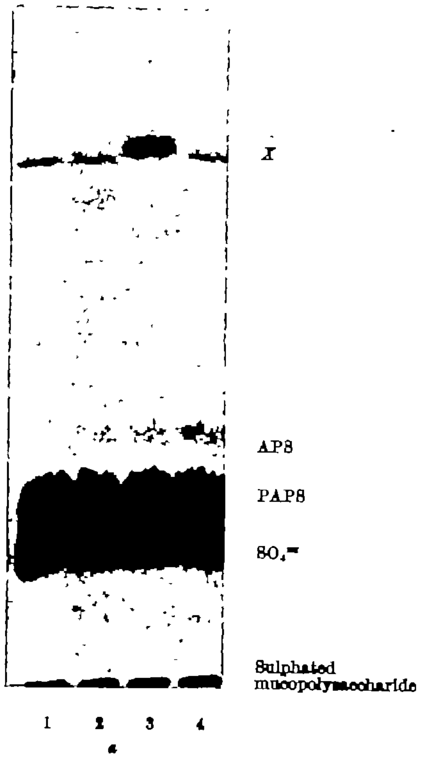
BIOCHEMISTRY

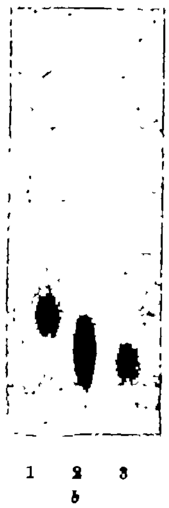
Presence of Œstrogen Sulphokinase in Chick Embryonic Cartilage

Enzymes capable of conjugating steroids to form the sulphate esters have been found only in the liver[1,2]. Although unfractionated liver preparations are able to sulphate a wide range of steroids, Nose and Lipmann[3] were able to separate an enzyme fraction which sulphated œstrone from one which sulphated dehydroisoandrosterone. A partial separation of the activities towards œstrone and *p*-nitrophenol was obtained. Results indicated that several rather specific steroid sulphokinases exist. Phenol sulphokinase, on the other hand, is relatively unspecific and has been found in such tissues as liver, kidney, intestinal mucosa and granulation tissue[4]. In the work reported here, extracts of chick embryonic cartilage were found capable of sulphating œstrogenic steroids but not a variety of other steroids tested.

During a study of the $[^{35}S]$ sulphate metabolism of cartilage derived from chick embryos, calf embryos and a human chondrosarcoma, extracts of each of these tissues activated the $[^{35}S]$ sulphate to adenosine-3′-phosphate 5′-sulphatophosphate and transferred the sulphate group to exogenous phenol or *p*-nitrophenol. This was shown by comparison with authentic material in a number of solvent systems. When incubated with steroids and chromatographed, the chick cartilage extract alone was capable of forming new $[^{35}S]$ sulphate ester zones in certain cases. Of the steroids tested, œstrone, α-œstradiol and œstriol were sulphated, but pregnan-3α,20α-diol, dehydroisoandrosterone, androsterone, testosterone and cortisone were not affected (Fig. 1). Active enzyme was found in the condyles of the tibias and femurs and also in the sternum of 15-day-old chick embryos. Extracts of condyle and costal cartilage of 12-week-old calf embryos and the cartilage matrix of a human chondrosarcoma arising in the sternum were inactive.

The zone *X*, present in the control of Fig. 1*a*, was found to be formed by a wide range of tissue extracts on incubation with $[^{35}S]$ sulphate. Its properties will be described in greater detail elsewhere[5]. An increase in the level of radioactivity was found in this zone in chromatograms of incubations containing œstrone and α-œstradiol. Œstriol gave a new zone with a lower R_F. All the other steroids gave autoradiographic patterns identical to the control. The radioactive material in the *X* zones, from chromatograms of incubations containing œstrone and α-œstradiol, were eluted and re-chromatographed in solvent 3 of Schneider and Lewbart[6]. This demonstrated that a new radioactive species had been formed in the presence of the steroids (Fig. 1*b*). Corresponding eluates of *X* zones, from incubations with mammalian cartilage extracts in the presence and absence of œstrone, are shown in Fig. 1*c* for comparison. Radioactivity in the œstrone sulphate position is absent in this case. Proof that the radioactive component, formed in the presence of œstrone, was the sulphate ester was obtained by two-dimensional co-chromatography with authentic material. The autoradiograph and the chemical stain, as revealed by spraying with Zimmerman reagent, were exactly superimposable. When incubation mixtures containing œstriol were subjected to two-dimensional chromatography in the system described by Bostrom[7] for studying $[^{35}S]$ sulphate metabolites in urine, the results shown in Fig. 2 were obtained. An additional $[^{35}S]$ zone is obtained in the presence of œstriol and is in the 'steroid sulphate' region of the chromatogram. In addition, it is in the exact position for œstriol sulphate, relative to œstrone sulphate, as depicted by Bostrom in work with $[^{35}S]$ steroid sulphates formed by rat liver[7].





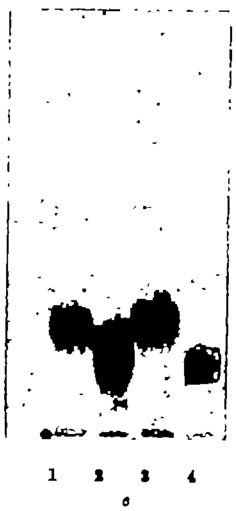


Fig. 1a. Autoradiograph of chromatogram of incubation mixture in isobutyric acid, 0·5 N ammonium hydroxide (5 : 3). The mixture contained ATP, 5 μmoles; $MgCl_2$, 6 μmoles; *tris* buffer, pH 8·2, 60 μmoles; carrier-free $[^{35}S]$ sulphate, 25 μc. and high-speed chick embryonic cartilage extract, 0·2 ml in a total volume of 0·7 ml.[11]. Steroid, 0·5 μmole was added as an alcoholic solution to the empty tube and evaporated prior to addition of the other components. 1. Control. 2. Addition of œstriol. 3. Addition of œstrone or α-œstradiol. 4. Addition of other steroids mentioned in text

Fig. 1b. Autoradiograph of eluates of *X* zones from chromatograms shown in Fig. 1a. Chromatography carried out in ethyl acetate, *n*-butanol, conc. ammonia, water (180 : 20 : 20 : 180) (ref. 6). 1. Control. 2. Incubations containing œstrone. 3. Incubations containing α-œstradiol

Fig. 1c. As for Fig. 1b. 1. *X* zone from control using chick cartilage extract. 2. *X* zone from chick cartilage and œstrone. 3. *X* zone from chondrosarcoma or calf embryonic cartilage extracts and œstrone. 4. Authentic œstrone sulphate

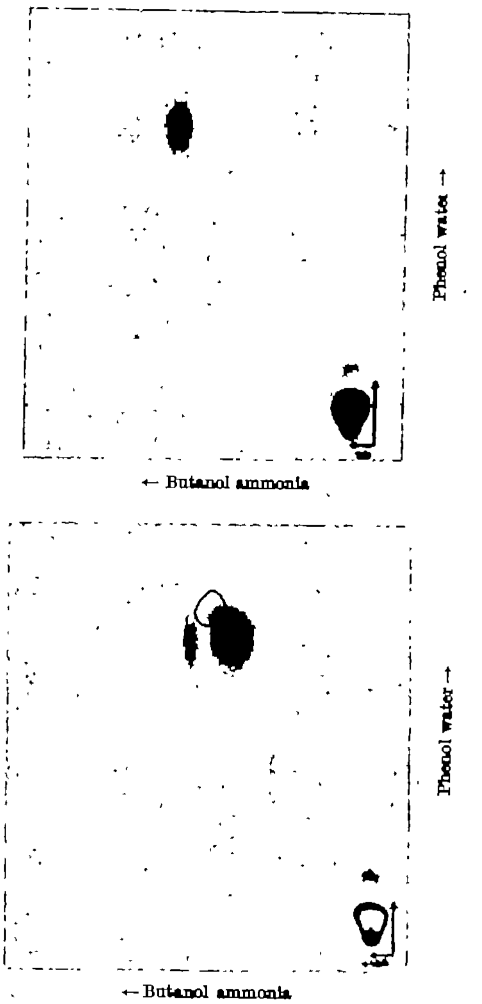


Fig. 2. Autoradiographs of two-dimensional chromatograms of incubation mixtures given in Fig. 1a. Top: control. Bottom: incubation containing œstriol. The circle outlines the position of authentic œstrone sulphate

In mammalian cartilage extracts, the ability to sulphate simple phenols and the inability to sulphate phenolic steroids very probably indicate that two separate enzymes are responsible for sulphating the phenolic group of these two classes of compounds[8]. The presence in the chick cartilage of an enzyme system capable of sulphating steroids is the first instance of such activity in a tissue other than liver. This unique behaviour of chick cartilage in its effect on the phenolic steroids could mean that the phenol sulphokinase of this species is capable of conjugating a wider spectrum of phenols than is the case with the mammalian enzyme. Alternatively, a specific phenolic steroid sulphokinase may be present. Although conjugation is generally believed to 'detoxify' or result in loss of the physiological activity of steroids, œstrone sulphate has been reported to be biologically active[9]. While this has been quoted as evidence for steroid sulphatase activity *in vivo*[10], Roy is of the opinion that this view is premature and there is nothing against the idea that such steroid sulphates might be active *per se*[4]. Some debate would then surround the proposal that œstrogen sulphokinase in chick cartilage plays the part of controlling the level of free physiologically active steroid by converting it into a water soluble form.

I thank K. N. Wynne for helpful discussion.

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Separation of Polypeptides bound by Albumin in Human Plasma

THE role of plasma albumin in maintaining oncotic pressure is generally known, but its significance as a potent binder of other substances is little appreciated. In addition to binding and transporting materials such as steroids, bilirubin, fatty acids, hormones, and dyes[1], albumin may similarly distribute many biologically important polypeptides. Therefore, the hypoalbuminæmia of certain disease states, including uræmia, hepatic failure, and chronic infection, may be associated with diminished transport of polypeptides to their sites of action and detoxification.

Groups of polypeptides bound to plasma albumin have not been demonstrated previously. This communication describes the separation of such a group.

Blood from normal subjects and uræmic patients was collected, under ice, in plastic bags containing the anti-coagulant, disodium ethylenediaminetetraacetate. It was centrifuged immediately at 4° C. The plasma thus separated, in amounts of 130–200 ml, was dialysed against a volatile buffer, triethylamine/acetic acid (TEA), at starting *p*H 9·0–9·3 at 4° C for 16 h to remove all unbound dialysable substances including electrolytes. Albumin, more than 95 per cent pure and unchanged electrophoretically, was separated by continuous-flow paper electrophoresis at 580–620 V (ref. 2). Less than 5 per cent α-1 globulin was also present.

The albumin was then redialysed against the TEA buffer for 24 h and the dialysate concentrated under vacuum, lyophilized, and saved for later chromatography. This specimen served as the chromatography control for materials liberated by acidification of the albumin. The albumin was then dialysed against 1 N acetic acid for two 24-h periods and the dialysates again concentrated under vacuum, lyophilized, and saved for chromatography.

By Folin-Lowry protein test[3] and ultra-violet absorption at 280 mμ, differences were found between the acetic acid dialysates and control specimens. In successive 24-h dialyses, progressively smaller amounts of peptide material were liberated. By these tests, acetic acid dialysates contained up to 10 per cent protein material per gram of albumin whereas the control samples contained a negligible quantity.

Ascending chromatography of these samples was performed with a butanol/acetic acid/water solvent in proportions of 4 : 1 : 5; Whatman number 3 paper; a 16-h time period; and amidoschwarz, Reindel-Hoppe protein[4], and ninhydrin stains. The acetic acid dialysates differed significantly from control specimens. Better separation of the constituents was achieved using high-voltage electrophoresis for 3 h at 3,000 V, using a 3 per cent ammonium carbonate buffer (Fig. 1). Since these substances are dialysable, give a protein reaction, absorb ultra-violet light at wave-length 280 mμ, stain with amidoschwarz, and on hydrolysis show an amino-acid pattern, it is concluded that they are polypeptides.

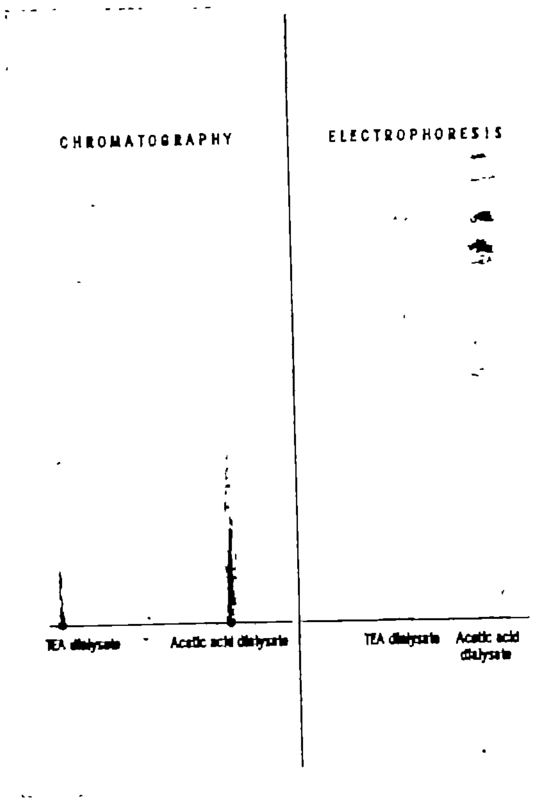

Fig. 1 Chromatographically, a significant difference is noted between substances liberated by acetic acid treatment and that obtained by dialysis against the basic TEA buffer. Better definition is achieved when the acetic acid dialysate is subjected to high-voltage electrophoresis. On this retouched figure from a normal subject, staining of the specimens is with amidoschwarz. Grossly, the patterns obtained from normals and uræmics appeared similar

Treatment with acetic acid at *p*H 2–3 converts albumin from its usual configuration to its expanded form[5], and binding sites are exposed and disrupted. The release of bound peptides by exposure to low *p*H is therefore not unexpected. It is necessary, however, to exclude the possibility that the peptides detected after acid treatment arose from proteolytic degradation of albumin itself. The amino-acid composition of the dialysable material is quite distinct from the overall amino-acid composition of albumin; but this does not disprove selective rupture of labile bonds. Analysis of the sedimentation diagrams of the albumin, however, gave no evidence of heterogeneity of the protein; that is, no dimers or fragments were detectable either before or after dialysis against acetic acid. The sedimentation coefficient of the albumin was 2 per cent lower (4·0 svedbergs) after treatment with acid, but this difference is not necessarily significant since the variation is within the range of experimental accuracy. The diffusion coefficient, derived from the sedimentation schlieren pattern[6], was not changed significantly. The molecular weight of albumin before and after acid treatment was approximately 67,000 Electrophoretic diagrams of the albumin before and after acid treatment also gave no evidence of molecular heterogeneity. There is, therefore, no reason for believing that hydrolytic or proteolytic degradation of the albumin molecules occurs. Finally, failure of serial microbiological studies to reveal bacterial growth excluded this possible cause of albumin breakdown.

The biological significance of these bound polypeptides remains undefined. Work now in progress on normal and abnormal subjects aims at comparing polypeptide patterns by 'fingerprint technique', and to determine their amino-acid composition.

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Glenn D. Lubash
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Acid Phosphatase and Catheptic Activity in Rheumatoid Synovial Tissue

Acid phosphatase and cathepsin are two of the hydrolytic enzymes shown by de Duve[1] to be present in the lysosomal fraction of liver, and similar particles have been shown in many other tissues[2]. Increases in lysosomal enzymes have been shown to be present in cases of rapid tissue breakdown[3,4]. The synovial tissue in a rheumatoid arthritic patient may proliferate rapidly, forming many villi, and can extend as the so-called pannus tissue growing over the articular cartilage. When this pannus tissue forms, erosion of the cartilage takes place. An increase in enzymes of the lysosomal type might therefore be expected in rheumatoid tissue.

Rheumatoid synovia were obtained from patients undergoing synovectomy of the knee. For comparison 'normal' synovia were obtained from patients undergoing menisectomy. Each synovium was separated from the fatty underlayer of tissue and frozen over solid carbon dioxide. While frozen, the tissue was chopped finely and weighed. A representative sample was used after appropriate treatment[5] for deoxyribonucleic acid phosphorus[6] (DNAP) and ribonucleic acid phosphorus[6] (RNAP) determination and the remainder homogenized in ice-cold water. The homogenate was spun at 4° C for 15 min at 12,000 r.p.m. The nitrogen content of the residue and acid phosphatase and catheptic activities of the supernatant were determined[7-9].

The choice of a basis for comparing enzyme activities in proliferating heterogeneous tissue such as synovium is difficult. Consequently, results are expressed on a DNAP, RNAP and nitrogen basis as well as wet weight. The DNAP/g wet weight in rheumatoid synovia is 284·5 ± 14·67 g while that for normal is 65·85 ± 6·81 g; the RNAP-level is 184·7 ± 11·24 g and 79·07 ± 7·10 g respectively. Thus in the proliferating tissue the ratio of nuclear material to cytoplasm is doubled.

Table 1

Catheptic activity at *p*H 3·6 (10^{-4} m.equiv. tyrosine released in 20 min ± *S.E.*) of synovial tissue
Substrate haemoglobin

	per g wet weight	per mg N	per μg RNAP	per μg DNAP
Normal (6)	21·45 ± 6·43	1·36 ± 0·35	0·26 ± 0·070	0·34 ± 0·11
Rheumatoid (8)	84·29 ± 11·31	6·57 ± 0·93	0 47 ± 0·070	0·31 ± 0·03

Acid phosphatase activity at *p*H 5 (μg phosphorus released in 20 min ± *S.E.*) of synovial tissue
Substrate β glycerophosphate

	per g wet weight	per mg N	per μg RNAP	per μg DNAP
Normal (6)	67·73 ± 12·23	5·82 ± 2·31	0·92 ± 0·22	1·13 ± 0·28
Rheumatoid (8)	647·32 ± 57·54	50·50 ± 5·30	3·70 ± 0·54	2·34 ± 0·31

No. of tissues in brackets.

Increases in the concentration of both enzymes were found irrespective of the basis for comparison except in the case of cathepsin/μg DNAP (Table 1).

Significant increases in two hydrolytic enzymes which could cause the breakdown of tissues if released in an active form have thus been demonstrated in rheumatoid synovia. No attempt was made to differentiate between bound and free enzyme activity as synovial tissue is difficult to homogenize and the subcellular particles are damaged. If these enzymes are normally enclosed in lysosomal particles in synovial tissue a high free activity might be expected in rheumatoid tissue as the lactic acid production is high[10] and low *p*H values cause rupture of lysosomal walls[2]. An added factor may be that of increased local joint temperature during the inflammatory process[11], as both lysosomal membranes[12] and collagen[4] are known to be affected by increased temperature.

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Optimal Conditions and Comparative Effectiveness of Dimethyl Sulphoxide and Polyvinylpyrrolidone in Preservation of Bone Marrow

The growing interest in dimethyl sulphoxide (DMSO) as an effective additive in low-temperature preservation of bone marrow prompted us to gather more information on the optimal conditions of its protective action. Several factors were examined: the concentration of the protective additive in the freezing medium, the length of the equilibration period of the cells with the additive at 0° C and at room temperature, and the toxicity of DMSO to bone marrow. A series of parallel experiments with polyvinylpyrrolidone (PVP) was conducted to compare its effects with those of DMSO in the preservation of bone marrow.

Bone marrow cells were obtained from the femurs of rats of an inbred Long-Evans strain 6–8 months old. The marrow was homogenized by aspiration with a syringe, strained, and the cells suspended in Hanks's tissue culture medium. After dividing the suspension into a number of equal parts, each part was centrifuged and resuspended in 1 ml. of the freezing medium consisting of various concentrations of DMSO in Hanks's solution, and of 10 per cent (w/v) PVP in Hanks's solution. The preparations were either frozen immediately or kept for various lengths of time at 0° C or at room temperature and then frozen. The freezing was done in accordance with our previous procedure[1], the cooling rate being 1° C/min and ice formation initiated at $-5°$ C by seeding. After the temperature reached $-25°$ C the preparations were transferred to an alcohol bath at $-79°$ C for 30 min. They were rapidly thawed by immersion into a water bath at 37° C. Immediately afterwards the preparations were washed by diluting with 4 ml. of Hanks's solution and centrifuging for 5 min at 900 r.p.m. The cells were resuspended in Hanks's culturing medium containing 10 per cent PVP and 33 per cent rat serum. The culturing and the assessment of cell viability by phase microscopy was carried out in accordance with our previous technique[1].

When various concentrations of DMSO were examined in the preservation of bone marrow, it was found that 10 per cent (v/v) offered the optimum protection, the cell survival being over 50 per cent. The viability declines sharply with slightly lower (7·5 per cent) or higher (12·5 per cent) concentrations (Fig. 1). Much lower viability was obtained with both 5 and 15 per cent.

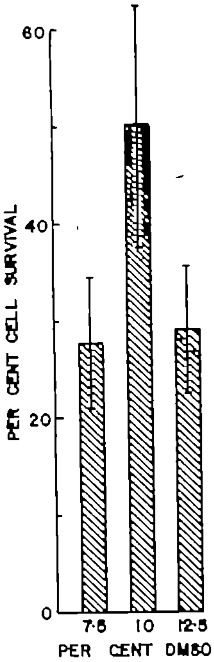

Fig 1. Effect of various concentrations of dimethyl sulphoxide on cell survival after freezing, vertical lines representing standard errors

To determine the optimal equilibration period of the cells with the additive, the cells were suspended in 10 per cent DMSO and either frozen immediately or after 10-, 30-, or 60-min exposure both at 0° C and at room temperature (Fig. 2). The exposure for 10 min at 0° C prior to freezing allows sufficient permeation of the additive into the cells to yield the optimal viability. Longer exposure lowers the cell viability; exposing the cells at room temperature decreases viability more than that at 0° C.

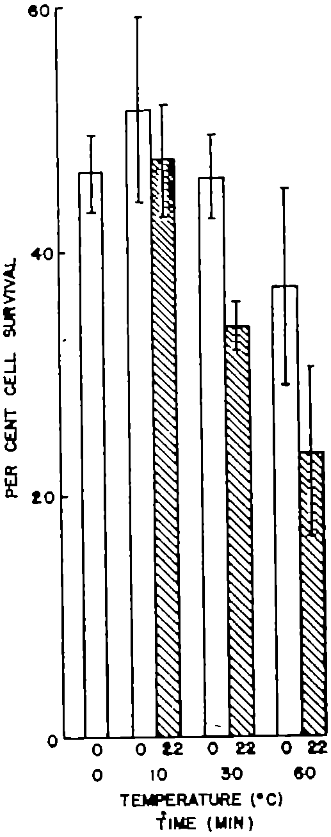

Fig. 2. Optimal exposure time of bone marrow cells to dimethyl sulphoxide (10 per cent) prior to freezing at 0° C and 22° C, vertical lines representing standard errors

The toxicity of DMSO was studied by addition of 0·1, 0·5, 1·0, 3·0, and 5·0 per cent (v/v) DMSO into the culturing medium in which the cells were then incubated for 24 h. We found a progressive decrease in the number of viable cells with increasing concentration of the compound. The bar graph in Fig. 3 shows that 3·0 per cent is much more toxic than 0·1 per cent DMSO, the viability being, respectively, 3 and 44 per cent. No cells survived a 24-h period of incubation with a 5 per cent concentration of this compound. When cells were subjected to a short period (10 min) of exposure to 10 per cent DMSO at 0° C and

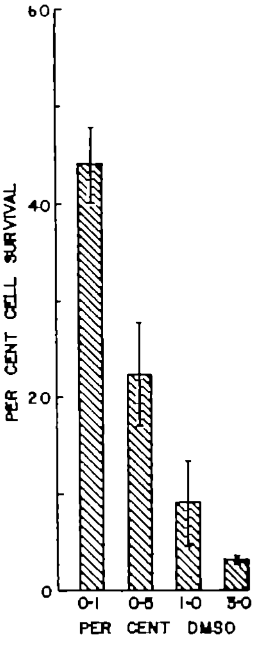

Fig. 3. Toxic effect of various concentrations of dimethyl sulphoxide in bone marrow culture after 24 h of incubation, vertical lines representing standard errors

then washed and cultured, only 70 per cent of the cells remained viable.

In discussing the results of this work, the average cell recovery value of 50 per cent obtained here with DMSO under optimal conditions and based on our method of assaying by tissue culturing and phase microscopy[1] is in close agreement with the results reported by Ashwood-Smith[2], according to which, bone marrow retained 50 per cent ability to synthesize proteins and lipids after being preserved with DMSO.

Our study indicates the considerable toxicity of DMSO to bone marrow cells. When the optimum equilibration time of 10 min (Fig. 2) was studied for its toxic effect, this short period of contact with DMSO reduced the viability of unfrozen cells by 30 per cent when compared with untreated cells. The decline of viability after freezing from the optimal value of 50 per cent when equilibration periods longer than 10 min are used is apparently due to the toxic effect of DMSO. The toxicity of this compound must be responsible for the sharp decrease of cell viability after preservation with DMSO in concentrations higher than 10 per cent (Fig. 1). This toxicity necessitates either the prompt removal of DMSO from the preparation after thawing, or dilution, or immediate administration to the recipient, which results in a rapid dilution of DMSO to a non-toxic concentration.

In comparing DMSO to PVP as a preservative, we found no sharp decrease in viability with an increase of PVP concentration from 10 to 20 per cent (w/v). The broader optimal range is perhaps indicative of the non-toxicity of PVP; the optimum concentration of PVP, however, is 10 per cent. An equilibration period with PVP is unnecessary since it is an extracellular preservative, as was shown in our previous communication[3]. This is substantiated in this investigation by the fact that there is no difference in viability whether the preparations treated with PVP were frozen immediately or after several hours of exposure. The prolonged exposure to PVP has no toxic effects such as those found with DMSO. When 10 per cent PVP is present in the tissue culturing medium there is no detectable decrease in cell viability after 24 h. Since PVP is non-toxic, there is no need to remove it from the preparation after thawing.

In conclusion, regarding the optimum recovery of cells after freezing with both preservatives, although DMSO offers, on the average, 50 per cent survival as compared with 30 per cent attained with PVP, there is a much larger variability range in the protective action of DMSO than that of PVP. We feel that, because of the many precautions which must be exercised with DMSO that are not necessary with PVP, the latter additive is simpler to handle and the results with it are more predictable.

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Immunoelectrophoretic Characterization of Proteolytic Enzymes

THE detection of proteolytic activity after electrophoresis in agar gel has been described previously[1,2]. It has been shown with a number of hydrolytic enzymes[3] that the precipitation lines formed by the antigen–antibody reaction after immunoelectrophoresis in agar gel exhibit enzymatic activity. This enzymatic activity can be demonstrated qualitatively after washing and drying the slides in the usual way by utilizing conventional histochemical techniques.

The analysis of proteolytic activity by the usual staining procedures frequently reveals the substrate as well as the enzyme since both are usually proteins or protein-like substances.

The method reported here is based on the principle that the proteolytic enzyme located in a specific antigen–antibody arc will degrade an appropriate substrate on a second slide when the latter is superimposed so that the two agar surfaces are contiguous. After incubation the indicator slide is removed and stained in the usual way. The area of enzymatic activity is revealed as a clear zone against a dark background. Thus, precise correlation of a specific antigen–antibody arc with its enzymatic activity is achieved.

Using a crude mixture of human urinary macromolecular components and serum antibodies produced by rabbits against these components, a wide variety of precipitation lines was demonstrable when the modified microscopic slide method of Scheidegger[4] was used (Fig. 1, slide *A*). After 3 days of washing in 0·9 per cent sodium chloride to remove excess proteins, the slide was immersed in glycine–hydrochloric acid buffer *p*H 2·0 for 15 min. At the same time, a saline agar slide was kept for 30 min in a solution containing 1 per cent hæmoglobin in 0·1 M glycine–hydrochloric acid buffer *p*H 2·0 (Fig. 1, slide *B*).

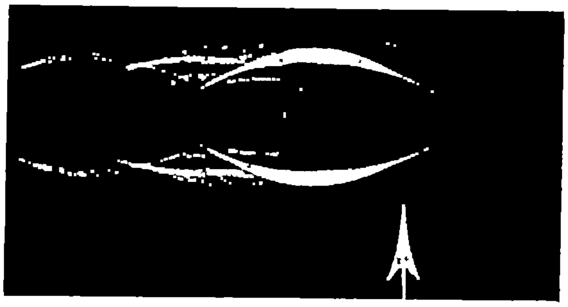

Fig. 1. Slide *A*—Agar immunoelectrophoresis after two days development. Veronal buffer $\mu = 0{\cdot}05$, *p*H 8·2, 7 V/cm, 1 h. Origin: Crude mixture of human urinary macromolecules. Trench: Rabbit serum antibodies against these macromolecules. Arc with arrow corresponds to arc of slide *B* exhibiting proteolytic activity

Slide *B* (Fig. 1) was then placed carefully on to slide *A*, agar-to-agar, so that a uniform and tight contact between the surfaces of the agar layers was obtained. The slide 'sandwich' was incubated in a moist chamber for 1 h. The slides were then separated and fixed for 2 h in 50 per cent ethanol containing 5 per cent acetic acid. Finally the plates were stained with amido black.

The result is shown in Fig. 2, slide *B*. The transparent arc indicating digestion of the hæmoglobin corresponds, when matched with slide *A*, to the location and shape of the arc marked with an arrow in Fig. 1.

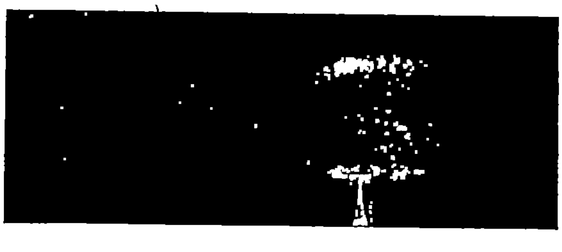

Fig. 2. Mirror image of slide *B* stained with amido black as explained in text after incubation. Demonstration of proteolytic activity by transparent area against evenly dark background

The same slide *A* can be re-used by repeating this 'sandwich' technique. Work is in progress to apply this method to the demonstration of other enzymes.

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PHYSIOLOGY

Activity of Fusimotor Fibres Innervating Muscle Spindles in the Intercostal Muscles of the Cat

IT has been shown that thoracic motoneurones are monosynaptically excited by the lowest threshold afferents in the intercostal nerves[1]. These afferents are assumed to arise from primary endings of the muscle spindles present in cat intercostal[2] muscles. The intercostal muscle spindles are shown here to be regulated through their fusimotor fibres by a central control closely resembling that which governs the activity of alpha respiratory motoneurones.

The sensory and motor innervation of the external (inspiratory) and internal (expiratory) intercostal muscles is through separate nerves which provide fine filaments (0·1–0·2 mm diam.) to their respective muscles[1]. These filaments contain large and small-diameter myelinated fibres (Fig. 1*A*). The filament in Fig. 1*B* is from an intercostal nerve de-afferented by dorsal root section distal to the dorsal root ganglion; both large and small diameter motor fibres are present. In the main intercostal nerve from which the filament in *B* arises, there were two groups of fibres ranging from 2–7μ and 10–18μ in diameter, with peaks at 5μ and 13μ respectively. As for limb muscle nerves[3], this bi-modal distribution is related to the presence of muscle spindles.

Cats were lightly anæsthetized with sodium pentobarbital or urethane and chloralose and recording was made of the efferent discharges present in more than 60 isolated filaments that innervated either the external ('inspiratory nerve filament') or internal ('expiratory nerve filament') intercostal muscles (*Th* 4–*Th* 11). Recordings from expiratory nerve filaments are shown in Fig. 2*A*, *B*, and *C*, and from an inspiratory filament in *D*. Spikes of two distinct sizes are present, and the frequency and pattern of these two types of discharge showed characteristic differences. In the expiratory filaments, the small spikes fired either periodically during the expiratory pause (*A*) or continuously throughout the entire respiratory cycle (*B* and *C*). In this latter case the frequency immediately increased at the onset of the expiratory pause as seen in *B*, where a single small spike fired at approximately 6 imp./sec in mid-inspiration and accelerated to 25–30 imp./sec towards the end of expiration, and in *D* where the frequency of the small spike approximately doubled from 50–100 imp./sec; progressive recruitment of small spikes throughout the expiratory pause is also common. Almost invariably, the onset of large spike activity occurred much later in the expiratory pause, and the maximum discharge frequency ranging from 8–12 imp./sec, as in *A*, *B* and *C*, was usually much lower than that of the small spikes. Small and large spikes are also present in inspiratory nerve filaments, but in contrast their periodic activation or their periodic acceleration in frequency, as seen in Fig. 2*D*, always occurred in inspiration, the onset of small spike activity usually preceding that of the large spikes. Although other patterns of activity were seen in quiet respiration, due principally to different levels of anæsthesia, the expiratory and inspiratory phasing of the activity in filaments innervating the internal and external intercostal muscles respectively was an invariable finding.

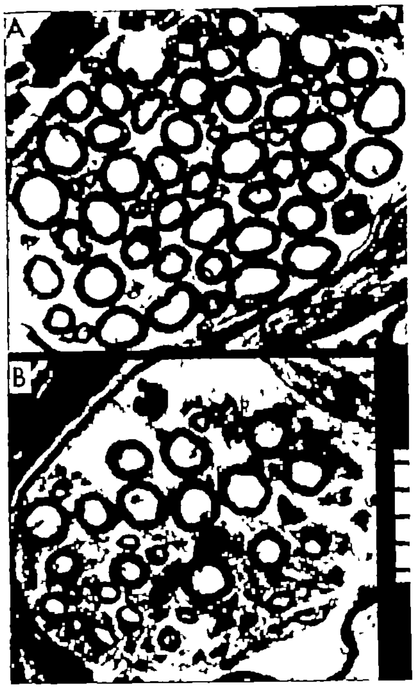

Fig. 1. *A*, Transverse section of normal 'expiratory nerve filament' (*Th*. 8) stained by the Flemming–Wolter method, as described by Williams and Wendell-Smith (ref. 7); *B*, 'expiratory nerve filament' (*Th*. 8) de-afferented by section of the dorsal roots distal to the ganglion 34 days previously, same animal as in *A*, contralateral nerve (scale 10μ)

As the recordings were made from whole, naturally occurring nerve filaments, it is assumed, following Hunt[4], that the direct relation between spike height and fibre diameter[5] is preserved and that the small and large spikes arise in fibres belonging to the groups of small and large

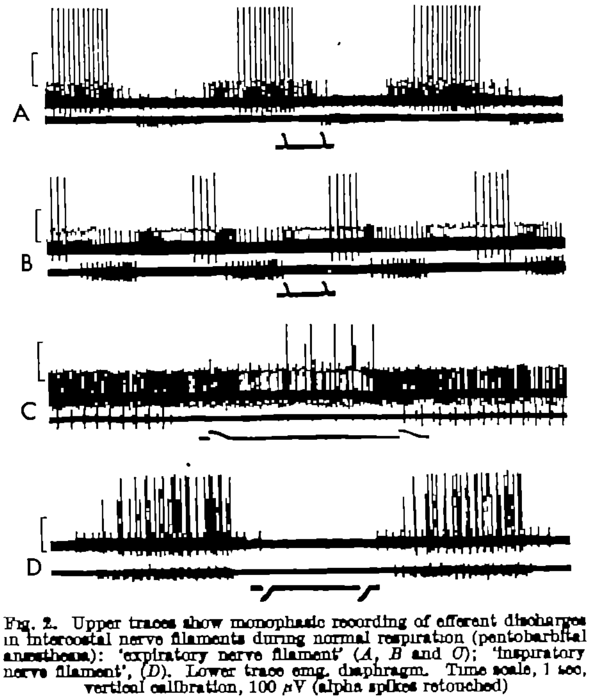

Fig. 2. Upper traces show monophasic recording of efferent discharges in intercostal nerve filaments during normal respiration (pentobarbital anæsthesia): 'expiratory nerve filament' (*A*, *B* and *C*); 'inspiratory nerve filament', (*D*). Lower trace emg. diaphragm. Time scale, 1 sec, vertical calibration, 100 μV (alpha spikes retouched)

diameter fibres demonstrated histologically. In other experiments it was shown electromyographically that the activity of motor units correlated only with the activity of the large spikes in the nerve recordings, hence it is concluded that the small spikes must arise in fusimotor fibres. Other significant findings were: (1) Inhalation of carbon dioxide enhances and hyperventilation abolishes the periodic discharge of alpha and fusimotor fibres, although during artificial respiration the large spikes of the expiratory filaments may be activated with each inflation of the lungs due presumably to a proprioceptive reflex activated by stretch. (2) The activity of both alpha and fusimotor fibres in inspiratory and expiratory nerve filaments respectively is augmented during the 'inspiratory' and 'expiratory' activating reflexes of Hering and Breuer. (3) The periodic activity of fusimotor fibres is not dependent on thoracic movements since it persists in the 'Flaxedil'-paralysed preparation not being respired artificially, and also after dorsal root section. Dorsal root section does, however, abolish the activity of some large spikes, even in quiet respiration.

Recently, Critchlow and Euler[6] obtained indirect evidence of a tonic and periodic modulation of intercostal fusimotor fibre activity which they attributed to a spinal reflex initiated by movements of the thoracic wall. This is not the conclusion reached here, where direct recording of inspiratory and expiratory intercostal fusimotor fibre activity has shown it to be controlled by a central mechanism closely resembling that regulating alpha respiratory motoneurones.

The significance of this central control is seen as follows. Failure of the extrafusal muscle fibres to shorten at the same rate as the intrafusal fibres, due to resistance to air flow to and from the lungs, would lead to an increased discharge from the muscle spindles with the consequence that the monosynaptic pathway previously described[1] would exercise an increased excitatory drive on the intercostal motoneurones. There would be summation with the 'central respiratory drive potential'[1] and so an increase in the discharge frequency of active motoneurones and a recruitment of others into activity. Thus, these results throw great emphasis on the importance of fusimotor fibre activity in the regulation of normal and forced respiratory movements.

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Reduction by Chlorpromazine of Ulcers due to Acute Starvation in Mice

EVIDENCE has accumulated indicating that starvation induces acute ulceration in the gastric glandular mucosa of mice[1] but not in rats[2,3], and that chlorpromazine inhibits ulceration in the Shay rat[4]. Also, recent evidence indicates that chlorpromazine reduces gastric acid and/or pepsin secretion in a number of circumstances in man and in dogs[5-8]. The investigation recorded here was undertaken to establish whether the tranquillizer, chlorpromazine, could prevent or decrease the incidence of acute gastric ulceration in mice as caused by starvation. In addition, a survey of the incidence of gastric glandular ulcers in normal, well-fed mice of both sexes was undertaken to establish this parameter, and to serve as a basis for comparison of the starvation procedures.

A total of 400 adult Swiss mice were utilized in the experiments. In the initial experiment 50 male and 50 female, well-fed, normal mice were killed by chloroform, and the gastric glandular mucosa of each mouse was immediately examined grossly for the presence of hæmorrhagic ulcers of any severity. In every subsequent starvation experiment approximately 50 mice of the same sex were put together in a large cage (24 in. × 14 in. × 14 in.) or were housed in groups of 10 mice per small cage (10 in. × 7·5 in. × 6 in.). The fasted mice were not fed during the three-day duration of each experiment, but water was available *ad libitum*. During this period the experimental mice were injected subcutaneously in the inguinal region twice a day (12-h intervals) with chlorpromazine ('Thorazine'), and control mice were injected similarly with water. All mice were individually weighed prior to each injection, and experimental mice received 10 mg chlorpromazine per kilogram body-weight (approximately 0·1 c.c.) for the initial injection, and 5 mg/kg for each of the remaining five injections. At the end of the third day the mice were killed with chloroform and autopsied immediately. Because of the minute size of the mouse stomach attempts were not made to quantitate grossly the severity of ulceration; stomachs were simply categorized as ulcerated or normal. It was found during an experiment with grouped male mice that the pugnacity and cannibalism characteristic of this sex during fasting resulted in very high mortality rate (65 per cent). Thus, data on fasting male mice were assumed invalid and incomplete, and are not included here.

Table 1. CHLORPROMAZINE REDUCTION OF ACUTE STARVATION ULCERS IN MICE

Treatment	No. of animals	Mean weight at autopsy (g)	No. of animals with ulcers	Percentage of animals with ulcers
Normal males	50	29·4	2	4*
Normal females	50	29·4	0	0
Starved females water-control (subcutaneous—twice daily)	92	19·4	27	29·4
Starved females, chlorpromazine (10 mg/kg, initial subcutaneous injection, 5 mg/kg, subsequent subcutaneous twice-daily injections)	87	20·0	15	17·2†

* Denotes a value significantly higher than female value ($P < 0.05$).

† Denotes a value significantly lower than control value ($P < 0.01$)

The results of the experiments are illustrated in Table 1. It was observed that two male mice in the presumably normal population of 50 male and 50 female mice possessed gastric glandular ulcers. Statistical evaluation (χ^2 method) indicates a significant difference ($P < 0.05$) between sexes in this regard, but the overall rare occurrence (2 per cent) of ulcers in normal laboratory mice concurs with reports of other normal laboratory species[9]. It was most notable that a very significant ($P < 0.01$) reduction of gastric glandular ulcers occurred in mice treated with chlorpromazine.

Each dose-level (10 mg/kg and 5 mg/kg) induced lethargy, decreased motor activity, and a pronounced decrease in postural muscle tone in all treated animals within 30 min after administration. Although the mechanism by which starvation induces ulcerogenesis in mice is not known, it has been reported that the blood level of glucose in dogs decreases during fasting[10]. If this phenomenon is consistent in mice, it may be speculated that gastric secretion is stimulated by the stress effect of hypoglycæmia as it is in man[8]. Unfortunately, the validity of recent reports in regard to alterations in intra-gastric *p*H in fasted mice is abated by complications of intragastric hæmorrhage, which would tend to elevate the *p*H of gastric contents[1]. In the present investigation, it is plausible that chlorpromazine reduced the incidence of ulceration by means of its well-established inhibition of gastric acid secretion. Indeed, other investigators have demonstrated that chlorpromazine inhibits the late phase of gastric secretion stimulated by the stress effect of insulin hypoglycaemia[6]. Furthermore, if starvation ulcers in mice are induced by a hypoglycaemia-hypersecretion effect, chlorpromazine would perhaps additionally negate the cause (hypoglycaemia) of hypersecretion, since it has been demonstrated that chlorpromazine induces hyperglycaemia in mice[11].

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Stimulation of Bone Resorption by Parathyroid Hormone in Tissue Culture

GAILLARD[1] has demonstrated resorption in cultures of embryonic bone when parathyroid hormone is added to the medium. We have used this response to assay parathyroid activity by measuring the release of calcium from bone during cultivation.

Albino rats 15–16 days pregnant (dated from time of sperm-positive vaginal smears) were injected with 200–400 μc. of calcium-45 as calcium chloride. Three days later the 18–19-day-old embryos were removed, the radius and ulna were dissected out, their cartilaginous ends cut off, and the shaft washed in Eagle's medium in Hanks base[2] at pH 7. These bones were then cultivated in small embryological watch-glasses containing 0·5 ml. of medium. They were placed on pieces of 'Millipore' filter supported at the surface of the medium by a metal screen. The medium was a mixture of 50 per cent human or rat serum and 50 per cent Eagle's maintenance medium in a modified salt solution. The initial calcium concentration was 1·8–2·2 m.moles/l., inorganic phosphorus concentration 1·3–2·5 m.moles/l., and sodium bicarbonate 25 m.moles/l. The cultures were incubated for 72 h at 37° C under an atmosphere of 5 per cent carbon dioxide, 20 per cent oxygen, and 75 per cent nitrogen. To measure the response to parathyroid hormone, 0·005 ml. of varying dilutions of a single lot of commercial parathyroid extract containing 0·2 per cent phenol ('PTE', Eli Lilly and Co.) was added to 0·5 ml. of medium bathing 1 bone rudiment. The opposite untreated limb served as a control for each treated limb; the medium contained 0·005 ml. of the 'PTE' diluent (0·2 per cent phenol in 0·9 per cent sodium chloride solution). In separate control assays the bones were treated with diluent or with inactive parathyroid extracts and compared with untreated bones. To assess the role of physicochemical exchange of calcium between bone and medium 'PTE'-treated and untreated bones were cultivated after the cells had been killed by heating to 47°–48° C for 1 h.

At the end of incubation the amount of calcium-45 released from the bone to the medium was measured in a liquid scintillation counter[3] and the stable calcium concentration in the medium determined by micro-titration[4]. The ratio of calcium in media from the treated bone to that in the untreated paired bone culture was used to permit comparison of experiments in which different amounts of calcium-45 were initially present in embryonic bone and in which there were small differences in the initial calcium concentrations in the medium. In each assay experiment the response of 3 or 4 pairs of bones of similar size were tested for a given treatment.

Pairs of control bones, one of which was treated with an inactive parathyroid extract or with phenol diluent, showed similar concentrations of stable calcium in the medium and released the same amount of calcium-45 during cultivation. The final calcium concentration was close to the initial value of 1·8–2·2 m.moles/l. (Table 1).

Table 1. EFFECT OF 'PTE' ON MEDIUM CALCIUM CONCENTRATION AND CALCIUM-45 RELEASE FROM EMBRYONIC BONE

Figures are mean and standard error of the ratio of treated versus untreated paired cultures

Treatment	No. of pairs	Treated/untreated Calcium concentration	Calcium-45 release
Control	30	1·0 ± 0·01	1·00 ± 0·01
'PTE' 0·01 unit/ml.	25	1·02 ± 0·02	1·12 ± 0·02*
'PTE' 0·1 unit/ml.	28	1·04 ± 0·02	1·66 ± 0·12*
'PTE' 1 unit/ml.	24	1·14 ± 0·02*	2·36 ± 0·16*

* Significantly different from control and from other doses of 'PTE', $P < 0·01$.

Twelve pairs of killed bones were compared with surviving control bones from the same litter. The dead bone usually took up some calcium from the medium so that the final calcium concentration in the medium was 11 per cent lower than for controls. Calcium-45 release was only 39 ± 4 per cent of that for living control bones. This finding suggests that considerable resorption and remodelling occurred in the control cultures and is consistent with the histological appearance of abundant osteoblasts plus a few osteoclasts in these bones. Adding 'PTE' to the medium had no effect on calcium-45 release or stable calcium equilibrium in cultures of killed bone. When 'PTE' was added to living bone cultures, release of calcium-

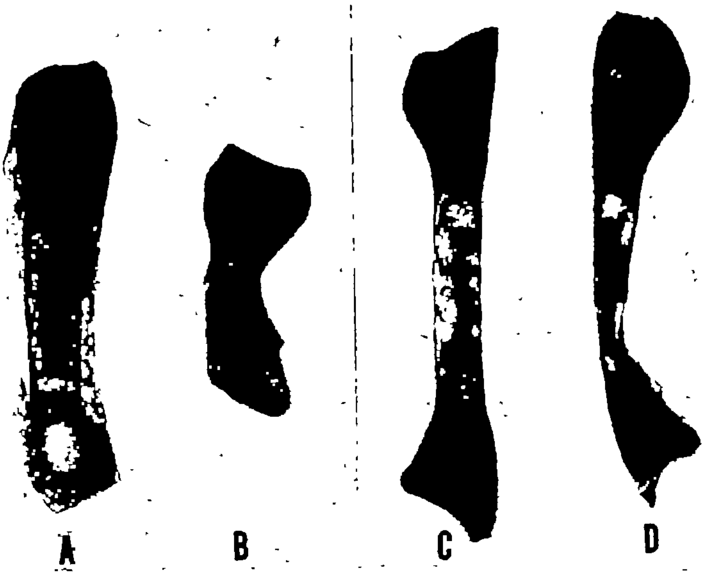

Fig. 1. Paired radii from two 18-day rat embryos after 3 days cultivation. *A* and *C*, untreated controls; *B*, effect of 1·0 unit 'PTE'/ml.; *D*, effect of 0·1 unit 'PTE'/ml. Cartilage has not been cut off prior to culture to show its relative preservation. (Azan stain, × *c*. 33)

45 to the medium was increased roughly in proportion to the dose administered. A dose of 1·0 unit/ml. caused almost complete resorption of bone matrix (Fig. 1) and increased calcium-45 release 2–3-fold while 0·1 unit/ml. usually caused less resorption. The histological changes resembled those described by Gaillard[1]. There was a marked increase in osteoclasts, some fibroblastic proliferation, and a disappearance of osteoblasts. The increase in calcium-45 release with 0·01 unit/ml. of 'PTE' was significant but small, and in 2 of 7 assays the value was within the control range. Histologically, cultures treated with 0·01 unit/ml. of 'PTE' resembled controls. Although the rise in stable calcium concentration in the medium paralleled calcium-45 release, the change was smaller and not significant except with 1·0 unit/ml. of 'PTE', presumably because of the relatively small amount of stable calcium available for release from bone compared with the amount present in the medium. Since calcium-45 was initially present only in the bones its release provided a more direct and sensitive measure of resorption.

A number of factors which affect the response to 'PTE' have been identified. A low initial concentration of calcium in the medium itself caused disappearance of matrix and proliferation of osteoclasts but addition of 'PTE' caused further resorption. If medium phosphate was increased above 3 m.moles/l., or if *p*H was raised above 7·5 by adding sodium bicarbonate, the 'PTE' effect on resorption was inhibited. Ordinarily, medium *p*H was between 6·8 and 7·4 at the end of cultivation. At the lowest *p*H values higher stable calcium concentrations were found in the control medium, but a further increase in calcium concentration was found in 'PTE'-treated cultures. This 'PTE' effect was not due to a greater acidification of the medium since there was no *p*H difference between medium from paired treated and untreated bones. The size and calcification of the explants were critical. Some 18-day embryo bones were too small and had not incorporated sufficient calcium-45 for assay. The bones of large 19-day embryos were less sensitive to 'PTE', perhaps because the bones did not survive well in culture.

These experiments confirm Gaillard's observation that parathyroid hormone stimulates bone resorption *in vitro* and show that this resorption can be measured as the release of labelled calcium from embryonic bone rudiments into the medium. The results are consistent with there being a direct effect of 'PTE' on calcium transport. The response is related to the dose of parathyroid extract.

This work was begun while I was a Special Research Fellow of the U.S. Public Health Service at the Strangeways Research Laboratory, Cambridge, England. I thank Dr. Honor B. Fell, director of the Laboratory, for her encouragement and advice.

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PHARMACOLOGY

Nature of Acetylcholine-like Activity released from Brain *in vivo*

ACETYLCHOLINE-LIKE activity appears in saline solution applied to the surface of the brain by means of a cup, when cholinesterase is inhibited locally[1]. When the cup is placed on the somatosensory cortex, the activity in the solution increases if the contralateral paw is electrically stimulated at a low frequency[2]. Similar acetylcholine-like activity can be recovered from the cortex and the caudate nucleus using Gaddum's push-pull cannula[3]. Recently evidence was brought forward by Hosein *et al.*[4] that the acetylcholine-like activity of brain homogenates treated with trichloroacetic acid was largely due to coenzyme-*A* esters of γ-butyrobetaine, crotonbetaine and carnitine and to propionyl and butyryl choline. In view of this, it was of interest to see whether evidence could be found for the release of these esters from brain *in vivo*.

The area of maximum evoked potentials in the somatosensory cortex following the stimulation of the contralateral fore-limb of a cat anaesthetized with 0·6 ml/kg 'Dial' and injected with 2 mg/kg atropine sulphate was localized by means of a wick electrode. Gaddum's push-pull cannula[3] was introduced at this location approximately 2 mm deep and Locke's solution was perfused through it at a rate of 0·25 ml./10 min. After perfusing the cortex for 20 min with Locke's solution containing 5 × 10^{-4} (v/v) diisopropylphosphofluoridate, perfusate was collected for 2 h during which the contralateral fore-limb was stimulated continuously with pulses of 1 msec duration at a rate of 1/sec. With the 3·0 ml. perfusate collected, 2 + 2 point assays were performed on a longitudinal strip of leech muscle suspended in a microbath[6,7] and on the blood-pressure of the eviscerated cat sensitized by 0·1 mg/kg eserine[8]. Similar assays were performed with a sample of γ-butyrobetaine ethyl ester, kindly supplied by Dr. Hosein.

Table 1. ACTIVITY OF BRAIN PERFUSATE AND OF γ-BUTYROBETAINE ETHYL ESTER IN TERMS OF NANOGRAM OF ACETYLCHOLINE CHLORIDE. 5 PER CENT FIDUCIAL LIMITS IN PARENTHESES

	Cat's blood pressure	Leech muscle	Ratio: Cat's blood pressure/Leech muscle
1 ml. perfusate	23·0 (19·3–29·8)	18·0 (15·0–22·5)	1·26
1 μg γ-butyrobetaine ethyl ester	14·0 (11·5–17·1)	0·48 (0·38–0·61)	29·2

As shown in Table 1, the activity of the perfusate in terms of acetylcholine was quite similar on the two preparations, while γ-butyrobetaine ethyl ester was about 30 times weaker on the leech muscle than on the blood-pressure.

Secondly, 0·15 ml. of a perfusate obtained from the somatosensory cortex as described here and estimated to contain 50 ng/ml. acetylcholine equivalent was applied to a 15-cm wide Whatman No. 1 filter paper and developed in water-saturated *n*-butanol for 15 h by descending chromatography. Similarly, 0·15 ml. of acetylcholine (50 ng/ml.) and γ-butyrobetaine ethyl ester (0·1 mg/ml.), both in Locke's solution, were applied and developed. After drying, strips were eluted with 20 ml. methanol, the methanol evaporated under nitrogen and the residue dissolved in 0·25 ml. of diluted Locke's solution and assayed on the leech muscle.

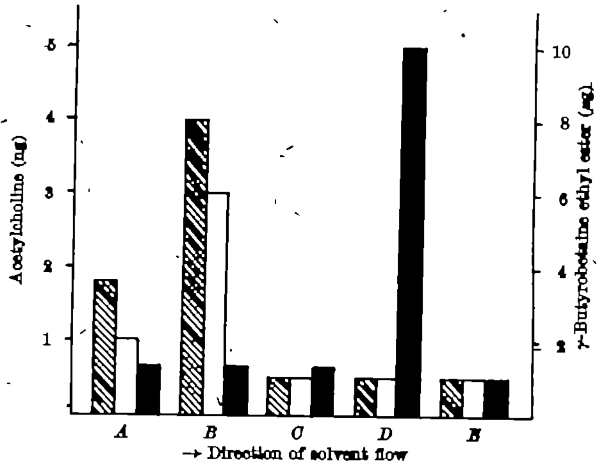

Fig. 1. Hatched, acetylcholine; white, brain perfusate; black, γ-butyrobetaine ethyl ester. *A*, R_F −0·05 – +0·05; *B*, R_F 0·05–0·15; *C*, R_F 0·15–0·30; *D*, R_F 0·30–0·50; *E*, R_F 0·50–0·70. Acetylcholine and brain perfusate assayed against acetylcholine chloride as shown on the left, γ-butyrobetaine ethyl ester assayed against itself as shown on the right

Results in Fig. 1 show that most of the activity of the perfusate could be recovered at R_F 0·05–0·15, where also acetylcholine was found, while γ-butyrobetaine migrated to R_F 0·30–0·50. These findings do not provide any evidence for the release of betaine esters from the brain *in vivo* but rather show that acetylcholine or a very similar ester is the transmitter at the cholinergic synapses in the cortex of the brain.

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Dose-response Relations of Amphetamine–Barbiturate Mixtures

MIXTURES of amphetamine and barbiturates are fairly widely used in psychiatry for treating some kinds of neurotic patients. Relatively little seems to be known about the effects of such mixtures from laboratory experiments on animals or man. On most kinds of response the two types of drug separately have opposite effects, since barbiturates are general depressants of the central nervous system and the amphetamines are general stimulants. In small doses, however, barbiturates can produce stimulant effects on animal activity[1–4], as measured, for example, by 'activity cages'.

In previous experiments[5] it was found that very small doses of amphetamine sulphate (0·75 mg/kg) and amylobarbitone sodium (15 mg/kg) which individually had little effect could, when given in combination with each other, markedly increase the spontaneous activity of rats in an unfamiliar environment, even though most of the animals were ataxic and moved clumsily. The dose ratio (1 : 20) was different from those used therapeutically since it involved much more barbiturate. For example, 'Drinamyl' (Smith, Kline and French) contains dexamphetamine sulphate and amylobarbitone sodium in a ratio of 1 : 6·5; on the assumption that dexamphetamine sulphate may be about 1½ times as potent as amphetamine sulphate[6], the ratio in these experiments was therefore of the order of 1 : 30.

In the first series of the experiments recorded here, amphetamine sulphate and amylobarbitone sodium in this same ratio were studied separately and in combination over a range of doses in order to determine whether, at its peak dose, a mixture of them did in fact produce greater stimulant effects on the spontaneous activity of rats than could be obtained with either drug on its own. In order to study the relation between spontaneous activity and ataxia, records of the rats' footprints were obtained[7,8] during a standard run and their spacing was analysed. A second set of experiments was then carried out which was similar in all respects except that dexamphetamine sulphate and amylobarbitone sodium were used in the 'Drinamyl' ratio of 1 : 6·5.

The apparatus used to study spontaneous activity was a symmetrical Y-shaped runway similar to that previously described[8]. A trial consisted of placing a rat in the centre of the Y and leaving it in the apparatus for 5 min; the number of times it entered the arms with all four feet was the measure of activity. The rats were 329 Lister hooded females aged 115–140 days which had been reared under standard conditions. In the first series, 10 rats, randomly selected, were used at each dose and 16 rats were saline controls; in the second series the numbers were 8 and 15 respectively. The drugs were dissolved in saline so that the volume injected, subcutaneously, was 2 c.c./kg; 35 min after the injection each rat was given a single trial in the runway, and immediately afterwards the record of footprints was made.

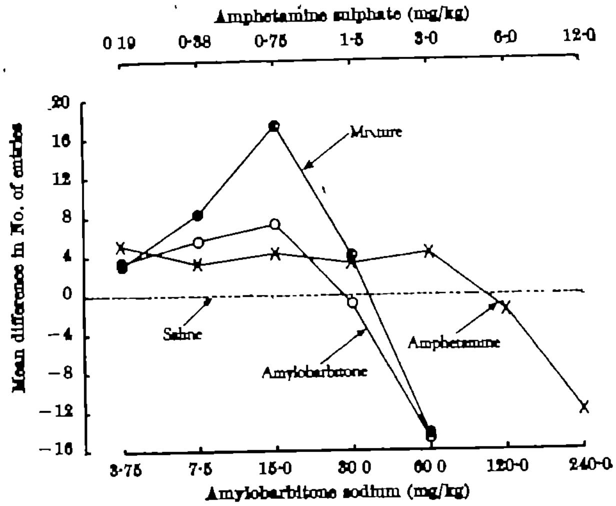

Fig. 1. Results of first series of experiments: activity of rats influenced by amphetamine sulphate and amylobarbitone sodium, given separately and in combination, over a range of doses. The figure shows the number of entries into the arms of a Y-shaped runway during 5 min, expressed as mean differences from the activity of the saline control group the mean number of entries of which was 14·7, *S.D.* 5·8. Each point represents the mean results for a different group of ten rats. The ratio between the two drugs was kept constant at 1 : 20

Fig. 1 shows the results of the first series of experiments. At its peak the activity with the drug mixture was indeed found to be greater than activity with any of the doses of the separate drugs, and it was more than twice that which was found with saline ($t = 5·6$, df 51, $P < 0·001$). Amylobarbitone alone produced a curve which was similar to but much flatter than the mixture curve, with a peak which was observed to be directly below that of the mixture curve. Amphetamine by itself produced in the first five doses used slightly more activity than saline, and the overall mean difference (4·0 entries) is statistically significant at the 5 per cent level. In the highest doses all three substances, including amphetamine, inhibited activity almost completely.

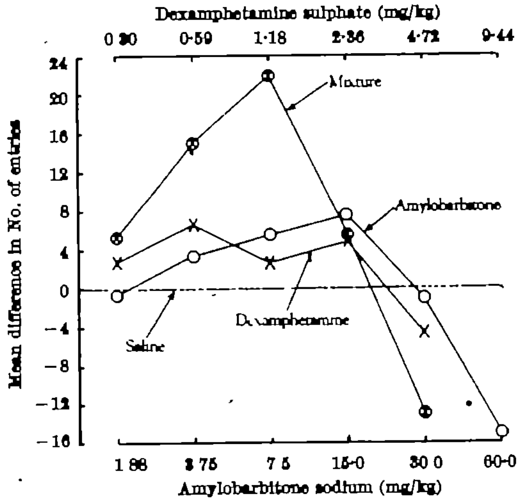

Fig. 2. Results of second series of experiments. Conditions were similar to the first series, except that dexamphetamine sulphate and amylobarbitone sodium were used, in the ratio of 1 : 6·5 ('Drinamyl' ratio). There were eight rats per group

Fig. 2 shows the results of the second series of experiments where proportionately about 6 times more amphetamine was used. It can be seen that the shape of the mixture curve was similar to that in the first series; but the peak of activity occurred at half the previous dose of amylobarbitone, that is, at 7·5 mg/kg, combined with 1·18 mg/kg dexamphetamine, which corresponds to more than twice the dose of amphetamine sulphate at the peak dose in the first series of experiments. The difference between the peak mixture activity and activity with saline was again significant at the 0·1 per cent level. As before, the largest doses of all three substances inhibited activity almost completely.

Various aspects of the footprint records were examined, and it was found that the regularity of the spacing of the prints was a useful measure of ataxia[8]: the spacing was regular in animals given saline and became irregular in animals given amphetamine–barbiturate mixtures, and the irregularity became greater the larger the dose. In the first series of experiments the smallest dose at which significant ataxia was detected was the peak activity dose ($P < 0{\cdot}001$), but in the second series ataxia was only detected at double the peak dose ($P < 0{\cdot}001$). Hence, in the first series, where proportionately much more barbiturate was used, the peak mixture dose markedly increased the rats' spontaneous activity even though it also made them ataxic, while in the second series, where the 'Drinamyl' ratio was used, the peak mixture dose produced a similar increase in activity but no ataxia.

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HÆMATOLOGY

Hæmoglobins of *Galago crassicaudatus*

Galago crassicaudatus is a nocturnal, arboreal prosimian Primate of relatively wide distribution in Africa. Recently we have examined hæmoglobin from 19 individuals of this species. All the animals came from the Sokoke forest, two to three miles west of the town of Gedi in south-eastern Kenya. The pelage colours of the 19 animals, all from the same population, spanned the characteristic shades and markings of the subspecies *G. c. agisymbanus*, *G. c. lasiotis*, *G. c. panganiensis*, *G. c. kikuyuensis*, *G. c. crassicaudatus* and *G. c. argentatus*[1]. The last-named variety, however, has characters other than the superficial ones of pelage markings and colour which make a distinction between it and the other five useful for the present. Our long-standing suspicion that the sub-species, other than the *argentatus*, are merely normal colour variations within *G. crassicaudatus* seems to be confirmed.

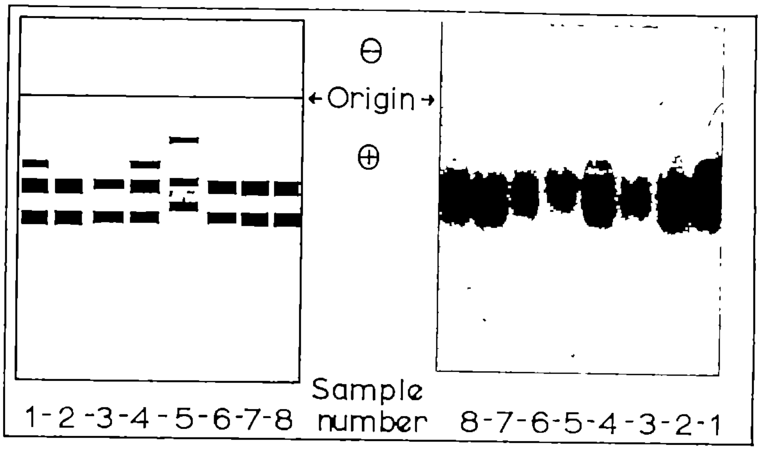

Fig. 1. A typical starch gel of galago hæmoglobins. The right-hand part is a photograph of the half of the gel stained with benzidine. The left-hand part is a diagram of the other half of the same gel after it was stained with amido-black. All samples except sample No. 5 are from galagos. Sample No. 5 shows hæmoglobins A_2, S and A, in order of increasing anodic mobility, from a human AS heterozygote; the dotted area in the diagram indicates the position of human hæmoglobin F in this system

Blood was drawn from the femoral veins of the tranquillized galagos into 7 ml. sterile vacuum tubes containing 0·7 ml. anticoagulant (citric acid, sodium citrate, and dextrose). Cells and plasma were separated by centrifugation and hæmolysates prepared in the usual manner[2].

Electrophoresis of hæmoglobin was in vertical starch gels made with *tris* borate buffer, pH 8·6 (0·027 M *tris*-(hydroxymethyl)aminomethane plus 0·020 M boric acid). Buffer in the electrode vessels was 0·27 M *tris* and 0·20 M boric acid. Electrophoresis was carried out for 7 h at approximately 10 V/cm at room temperature. At the end of a run, gels were chilled for 0·5 h at 10° C and then sliced. One half was stained with amido black and the other with a benzidine reagent.

Hæmolysates were tested for alkali-resistant hæmoglobin by the denaturation technique of Singer *et al.*[3].

Fig. 1 shows a typical gel. The half of the gel which was stained with benzidine was photographed. The other half was diagrammed after being stained with amido black. There are two hæmoglobin patterns to be seen in the galago samples on this gel. One has three distinct bands, the other has two. Ten samples had two bands and nine had three. The smeared band which has moved farthest anodally is, we believe, analogous to human A_2 or 'senescent' hæmoglobin. The slowest, or most cathodal, band moves farther, or faster, than the A_2 human band. As can be seen in Fig. 1, the fastest galago hæmoglobin has moved farther than human A, and the band with intermediate mobility has moved slightly farther than human S, but not as far as human F. The presence of minor hæmoglobin components in these galago samples, analogous to those in human hæmolysates, is not clearly demonstrated on these gels. This point awaits analysis of the hæmolysates by ion-exchange chromatography[4].

The results of alkali denaturation tests show an average value, for 19 samples, of 27·7 per cent alkali-resistant hæmoglobin. The individual values ranged from 19·2 to 36·3 per cent. In a previous communication[5] we reported slightly higher average values (36 per cent) in a group of 23 *G. crassicaudatus*.

There is no obvious association, in the present group of 19 samples, between higher or lower percentages of alkali-resistant pigments and the number of bands found on the starch gels. Nor is there any relationship between pelage colour of the animal and number of bands found after electrophoresis. We have not yet identified the alkali-resistant pigment with any of the components discovered on electrophoresis of the samples. There may be striking structural similarities between human alkali-resistant fœtal hæmoglobin and galago hæmoglobin.

This work was supported by grant *RG* 6053 of the U.S. Public Health Service and was carried out during the tenure of a National Science Foundation senior postdoctoral fellowship held by one of us (J. B.-J.). The facilities of the Wellcome Trust Laboratory at the Medical Research Laboratory, Nairobi, Kenya, were generously made available by Dr. H. Foy and Dr. A. Linsell. The co-operation and assistance of Mr. R. Caldecott and Mr. R. Clift is gratefully acknowledged.

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Effect on Antibody Production of Injection of an α_2-Protein Fraction

While investigating the effect of large doses of fractions of blood on the survival of rat skin homografts, it was found that intraperitoneal injections of an α_2-protein fraction from human, rat, rabbit, or bovine sera could prolong the survival of homografts in outbred albino rats. At the time of writing, 4 of 20 of these grafts have survived for eight and 4 of a further 20 for six months and are still apparently healthy. This fraction was prepared from whole serum by a modification of the chromatographic method of Steinbuch and Quentin[1] on DEAE cellulose. Of Steinbuch and Quentin's three fractions only that eluted with 0·5 molar *p*H 5 acetate buffer was effective. Kamrin[2] had previously produced a marked prolongation of survival of rat skin homografts by injecting a series of plasma fractions, of which the most potent contained the highest concentration of α_2-protein. It seems, therefore, that the fractions used by Kamrin and the fraction I have used probably contain a common active principle.

The work with the 0·5 molar eluate has also shown that this fraction inhibits the production of antibodies to other antigens.

The immune elimination technique of Talmage *et al.*[3] was used as an index of antibody production. 10 mg of human albumen labelled with iodine-131 was injected into six rabbits each of which had received 100 mg of the eluate intraperitoneally 30 min after being given the antigen and again three days later. There was no evidence of immune elimination in five of the six rabbits, but in the remaining rabbit immune elimination was observed on the eighth day. Nine rabbits injected only with the labelled human albumen all showed immune elimination between the eighth and eleventh days ($0{\cdot}01 > P > 0{\cdot}001$).

In rabbits injected intravenously with 1·0 ml. of packed, washed human red cells hæmagglutinating antibody developed in each of nine control animals, but it could be detected in only two of ten animals treated by intraperitoneal injection of 100 mg of the eluate given 2 h before injection of the red cells.

The timing of the injection of the protein fraction was found to be important. Suppression of immune elimination of iodine-131-labelled human albumen was observed in all animals receiving the fraction 1 or 2 h before administration of the antigen. Normal immune elimination was observed in all animals receiving the fraction 4 h after the antigen. The production of hæmagglutinating antibody was unaffected if the fraction was injected later than 15 min before the antigen, and was most effective if given 2 h before.

It therefore seems likely that the protein fraction affects the early stages of the immune response.

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PATHOLOGY

Platelet Clumping *in vivo*

Wharton-Jones[1] and Bizzozero[2] showed that, when an artery was injured, white masses would build up at the site of injury and embolize. Honour and Ross-Russell[3] examined this response to injury in considerable detail in arteries on the surface of the rabbit brain. They showed that the white bodies were composed of closely packed but morphologically discrete platelets, and they were unable to influence the rate of formation of the white bodies or their size by heparin, anti-histamines, or 5-hydroxytryptamine (5-HT) antagonists. The demonstration by Gaarder *et al.*[4] that adenosine diphosphate (ADP) was capable of producing clumping of human platelets *in vitro*, led Mitchell and Sharp[5] to investigate related substances, together with other substances normally carried by the platelet. They confirmed that ADP clumped citrated human platelets at a concentration of 0·04 μg/ml. and that adenosine triphosphate (ATP) was much less active, a concentration of 1·5 μg/ml. being required. They also found that human platelets were clumped by 5-HT, and by noradrenaline at a concentration of 0·06 μg/ml. Rabbit platelets were clumped by ADP at a concentration of 0·05 μg/ml., were much more sensitive to ATP than human platelets, 0·22 μg/ml. sufficing to produce clumping, were insensitive to noradrenaline, and showed a variable response to 5-HT.

These observations led us to examine the effect of ADP, ATP and 5-HT on injured brain vessels in the rabbit.

(1) *The major injury.* The cerebral cortical arteries of anæsthetized rabbits were exposed and observed microscopically using the techniques described by Honour and Ross-Russell[3]. An artery 100–200μ in diameter was then pinched firmly with needle-pointed ophthalmic forceps, producing slight external bleeding, and transient vaso-constriction. When this subsided a steady succession of white bodies would form at the site of injury and embolize. The application of ADP, ATP and 5-HT solutions to the external surface of such a site did not modify the size or rate of production of the white bodies which were forming spontaneously.

(2) *The minor injury.* If, however, a cortical artery was gripped gently with the forceps, no external bleeding occurred and apart from transient vaso-constriction the site looked normal. No white bodies formed spontaneously, but the application to such a site of ADP, ATP or 5-HT dissolved in 0·9 per cent saline produced white bodies, which continued to build up and embolize so long as the active agent was applied, production stopping as soon as the application was discontinued. The lowest concentration of these agents which would produce white bodies when applied to the outside of a minimally injured rabbit brain artery was determined, each substance being tested on 8 rabbits. The results were:

Substance	Range of lowest effective conc. (μg/ml.)	Mean (μg/ml.)
ADP	0·25–16·0	3·0
ATP	0·25–16·0	5·3
5-HT	0·125–32·0	4·8

Application of the test solutions to uninjured cortical arteries did not produce white bodies.

(3) *Effect of 5-HT antagonists.* Minor injuries were inflicted on cortical arteries in two rabbits and their

responsiveness to externally applied ADP, ATP and 5-HT was tested. The rabbits were then given 15 mg and 60 mg respectively of the 5-HT antagonist *Ro*-3-0837 intravenously (4·4 and 19·5 mg/kg; the material was supplied by Roche Products). After the administration of the 5-HT antagonist, externally applied 5-HT in any concentration was not capable of producing white bodies, whereas ADP and ATP applied to the same site produced white bodies at concentrations identical to those found before the 5-HT antagonist was given.

Application of the antagonist locally in low concentrations also abolished the 5-HT response; dripping *Ro*-3-0837, 25 µg/ml., on to a minor injury site rendered it unresponsive to 5-HT, but did not modify the response to ADP and ATP. Parenteral and local application of 5-HT antagonists to animals with major injuries, where a steady stream of white bodies was forming spontaneously, did not modify the rate of production or size of the white bodies, suggesting that the site of major injury is producing its white bodies by a mechanism which does not involve 5-HT induced clumping.

(4) *Effect of enzyme inhibitors*. The substances tested were dissolved in 0·9 per cent saline, and applied for 10 min to the external surface of an artery with a major injury which was producing white bodies spontaneously. The substance was then washed off with saline and the effect on white-body production was observed.

(*a*) *Sodium fluoro-acetate*, 1 mg/ml. For the first 15 min after the beginning of the application the white bodies continued to appear, but they then diminished in size, fragmented during formation, and production stopped altogether at 20 min. No further white bodies were produced during a 2-h observation period.

(*b*) *Sodium mono-iodoacetate*, 750 µg/ml. 20 min after beginning the application the white bodies became smaller, small masses of platelets could be seen streaming off their surface as they were building up, and at 30 min, no further white bodies developed. The site remained quiescent for some 30 min, when tiny white bodies began to form and embolize, gradually getting bigger, until at about 90 min the injury site was behaving in the same way as in the control observation period.

(*c*) *Sodium azide*, 10 mg/ml. White body production stopped at 5 min and did not begin again in a 2-h observation period.

(*d*) *Potassium cyanide*, 25–1,000 µg/ml., and

(*e*) *2:4 dinitrophenol*, 12·5–125 µg./ml., showed no effect on white-body formation.

The external application of ADP, ATP and 5-HT to a major injury site the spontaneous white-body production of which had been stopped with fluoroacetate, or iodoacetate, produced white bodies as long as their application was continued; an application rate of 0·1 to 0·2 µg/min of ADP produced a succession of white bodies similar in size and timing to those occurring before the enzyme poison had been applied.

These observations show that agents which will produce clumping of citrated rabbit platelets *in vitro*, such as ADP, ATP and 5-HT, can also produce platelet masses in injured brain arteries. Our investigations of the effect of the various enzyme poisons suggest that the phenomenon first observed by Wharton-Jones[1] of white-body production in injured arteries may be related to the release from the damaged tissues in the artery wall of substances similar in properties to ADP, which instruct the passing platelets to clump together.

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Auto-Immune Selection of Carcinoma Cells in Man

IN 1954 it was suggested by Green[1] that normal cell contain an antigen which is absent from tumour cells and that, in the presence of an 'auto-immune' reactio in which carcinogenic substances behave as haptenes the tumour cells have a selective survival advantage Loss of tissue-specific antigen has been demonstrated i the cells of experimental tumours of hamster kidney[2, and rat liver[2,3], and spontaneous tumours of human skin and gastro-intestinal tract[4] by the use of heterologou antibodies. Recently it has been shown that some cell of most tumours of the human thyroid appear to be defi cient in a thyroid-specific auto-antigen[5] which is presen in the epithelial cells of normal and hyperplastic thyroids primary explants of thyroid cells in tissue culture wer used and the antigenicity of the cells was demonstrate by their susceptibility to the cytotoxic effects of a natur ally occurring thyroid-specific auto-antibody[6] frequentl found in the serum of patients with chronic thyroiditis.

If the kind of antigenic modification of tumour cell described here selectively favours the growth or sprea of tumours and is not merely a reflexion of the reduce specialized function common in neoplastic cells[7], ther should be an increased tendency to formation of tumours i diseases associated with auto-immunization. This is th case in two (and possibly all) of the three common disease in man in which auto-antibodies are known to be forme specifically against epithelial cells. Auto-antibodies agains cytoplasmic particles of thyroid epithelial cells are com mon in the serum of patients with chronic thyroiditis[8,9 a condition in which the incidence of thyroid carcinom is significantly increased[10–12]. Due to the clinical latenc of chronic thyroiditis it is unknown if this conditio precedes the development of carcinoma. Auto-antibodie against colonic epithelial cells[13] have been demonstrate in ulcerative colitis, and this disease definitely precede and markedly predisposes to carcinoma of colon[14].

It has recently been shown that patients with Addi sonian pernicious anæmia have auto-antibodies whic react with the parietal cells of the body and fundus of th stomach[15,16]: it is probable[17,18] but not certain[19] tha gastric carcinoma develops more frequently than would b expected in patients suffering from this disease. (There i no evidence that the carcinoma of the stomach in pernici ous anæmia arises from the cells against which the anti bodies apparently develop.)

These findings suggest that auto-antigen loss an immunological selection occur in certain tumours of th thyroid, the colon and possibly the stomach in man.

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IMMUNOLOGY

Non-immune Gel Precipitin Tests with an Enzyme Antigen

The appearance of non-immune precipitin bands in gel diffusion tests may result from manipulation of the physical environment while the immuno-diffusion test is in progress[1]. Interactions of serum with granulocytes[2] and tissue extracts[3], or reaction of albumin with hæmoglobin[4] though not antigen–antibody reactions also produce precipitates in agar. When one of the reactants can express a second biological function other than its antigenicity under the conditions of the gel test, then such non-specific precipitin lines are likely to occur. Our experience with bovine carboxypeptidase *A* and its rabbit antiserum ultimately revealed two independent artefacts of a type we had not seen referred to elsewhere.

Recrystallized carboxypeptidase *A* of commercial origin known to behave as a single molecular species though displaying limited tryptic and chymotryptic activities was the immunizing antigen. Rabbits were immunized with a Freund's adjuvant-antigen emulsion[5]. The antigen used in the gel tests had both tryptic and chymotryptic activities removed by diisopropylfluorophosphate (DFP) treatment. The gel tests were performed in a 0·5 per cent sodium chloride, 0·5 per cent phenolized agar (1 per cent) dispensed in 5-ml. volumes on to 49 mm² cover-glass plates and incubated at 37° C for 24 h. These slides were very convenient since on completion of the tests the agar films could be dried, stained and mounted between two additional cover glasses in an aluminium frame for viewing. The wells for antigen and antiserum were cut with a cork borer of 6 mm inside diameter attached to a vacuum line and placed 6 mm apart. All were charged with approximately 0·2 ml. of reactant with the antigens at a concentration of 1 mg/ml.

When the rabbit antiserum was tested against the carboxypeptidase antigen two bands appeared (Fig. 1). The heavier band was nearer the well containing antiserum and a lighter, less discernible band was nearer the well of antigen. Neither of these appeared with normal sera. It was suspected one of these might be due to the trypsin or chymotrypsin contaminants, which though enzymatically inactive because of the treatment with DFP were still functional antigenically. Accordingly, both were analysed in the gel test, but only chymotrypsin produced a precipitate with the immune serum. This band was quite diffuse, physically unlike either of the carboxypeptidase bands and did not merge with either of them (Fig. 1). It stained readily with sudan black B but not bromphenol blue, suggesting a lipid origin. It was also formed with normal rabbit, human, horse and guinea pig sera. The antiserum would not provoke positive passive cutaneous anaphylactic reactions[6] in guinea pigs with chymotrypsin as the antigen nor agglutinate chymotrypsin coated latex particles although both these tests were positive with carboxypeptidase *A*. Therefore, we believe the chymotrypsin band is spurious and arises from enzymatic liberation of lipids from lipoproteins.

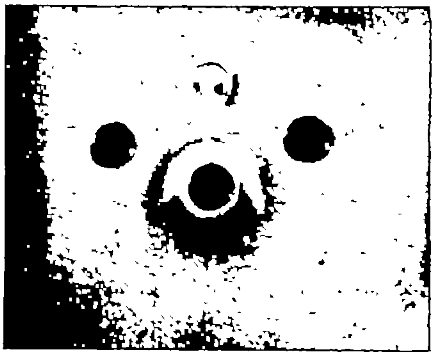

Fig. 1. Reactions of anticarboxypeptidase (lower well) with heated carboxypeptidase, carboxypeptidase and chymotrypsin (left, upper and right wells respectively)

However, the nature of the extra carboxypeptidase band remained unidentified. Believing it might arise from the natural heat instability of the enzyme, an enzyme solution was held at 37° C and aliquots removed periodically over a 5-h period. Each sample was assayed in the gel test and for enzyme content by hydrolysis of carbobenzoxyglycyl-L-phenylalanine[7]. After 5 h, only 10 per cent of the original enzyme activity remained and the previously heavier band in the gel test became even more intense. We consider this band to represent a degradation product of the enzyme which remained antigenic and stimulated formation of a second antibody.

Spurious precipitin bands may thus arise from the enzymatic activity of an antigen functioning only as an enzyme or, conversely, the antigenic activity of an enzyme functioning only as an antigen. Consequently, immunodiffusion investigations of cell extracts, culture filtrates or other enzyme-containing solutions may be anticipated to produce precipitates due to their enzyme content alone and these precipitates must be distinguished from true antigen–antibody reactions. Instability of labile antigens such as enzymes may require enzyme assays simultaneously with serological procedures to ensure that the serological test is dependent on the enzyme's antigenic function and not to some degraded form of the enzyme.

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Cheek Pouch of the Syrian Hamster and Immunity to Heterotransplantation of a Murine Leukæmia

The consistent failure of *AK*–4 leukæmia of the *AKR* mouse to grow in the cheek pouches of normal or cortisone-treated hamsters is in contrast to the routine heterotransplantability of certain other murine leukæmias[1,2]. Other investigations in these laboratories have demonstrated the accelerated rejection of second-set *AK*-4 heterografts in the cheek pouch, suggesting that the primary inability of the neoplasm to grow in hamster pouches is attributable to an immune response on the part of the host[3]. Since a method for successfully transplanting *AK*–4 in lethally irradiated hamsters has been devised[4], in which cell suspensions of the leukæmia are introduced intravenously by orbital puncture, the hamster's acquisition of immunity to the neoplasm may be examined not only with the leukæmia undergoing rejection[3], but also with the leukæmia actively growing.

Unpublished studies subsequent to the original report[4] revealed that a predictable transplantability of *AK*–4 may be achieved by a total-body dose of 1,500 r. and an intravenous inoculum of 5·0 × 10⁷ leukæmic cells within 6 h after irradiation: 100 per cent of recipients evidenced transplantability of the tumour as indicated by unusually uniform implantation of the inoculum in the regional draining (cervical) lymph nodes, sternal bone marrow, and

Table 1. IMMUNITY TO INTRAVENOUS HETEROTRANSPLANTATION OF *AK*–4 CELLS IN LETHALLY IRRADIATED HAMSTERS FOLLOWING EXPOSURE OF THE RECIPIENTS TO MURINE LEUKÆMIAS BY VARIOUS ROUTES*

Pretreating tumour	Route	Sensitizing† inoculum	30-day survival	Generalization: Liver	Generalization: Kidney	Generalization: Spleen	Generalization: Bone marrow	Total white count—5 days
AK–4(*AKR*)	i.v.	$5\cdot0 \times 10^7$	1/5	1/4	1/4	0/4	0/4	(4) $5\cdot0 \times 10^3$
	c.p.	$1\cdot0 \times 10^7$	5/13	0/8	0/8	0/8	0/8	(3) $3\cdot9 \times 10^3$
	s.c.	$1\cdot0 \times 10^3$	0/10	0/10	0/10	0/10	0/10	
	i.p.	$5\cdot0 \times 10^6$	1/10	0/10	0/10	0/3	0/10	
L–1210 (*DBA*/2)	c.p.	$1\cdot0 \times 10^7$	0/2	0/2	0/2	0/2	0/2	(5) $4\cdot2 \times 10^3$‡
	i.v.	$5\cdot0 \times 10^7$	0/5	0/3	0/3	0/4	0/5	(2) $1\cdot8 \times 10^3$
P–1534 (*DBA*/2)	i.v.	$2\cdot0 \times 10^7$	0/2	0/2	0/2	0/2	0/2	(2) $5\cdot9 \times 10^3$
*E*9514*A* (*C3H*)	i.v.	$5\cdot0 \times 10^7$	0/5	0/4	0/3	0/3	0/5	(2) $5\cdot4 \times 10^3$
Totals			7/52	1/43	1/42	0/36	0/46	
Control			0/5	5/5	5/5	5/5	5/5	(5) $6\cdot1 \times 10^4$
			0/5	5/5	3/5	5/5	5/5	(5) $1\cdot1 \times 10^4$
			0/5	5/5	5/5	5/5	5/5	(3) $1\cdot5 \times 10^5$
			0/4	3/3	2/3	2/2	4/4	
			0/5	5/5	3/4	3/4	5/5	(3) $9\cdot1 \times 10^4$
Totals			0/24	23/23	19/22	20/21	24/24	

* Experimentals and controls challenged with $5\cdot0 \times 10^7$ *AK*–4 cells i.v. at 1,500 r.
† Two inoculations, 2–3 weeks apart.
‡ Six-day blood count. Numbers in parentheses are number of animals on which average total white count is based.

spleen, with dissemination of leukæmic cells into the liver, kidney, and blood of the recipient prior to death at 5–7 days. The total white count at 5–6 days was of the order of magnitude of 10^4–10^5 nucleated cells per mm^3 venous blood, as opposed to about 10^3 cells per mm^3 in uninoculated, irradiated controls.

Transplantability of the neoplasm having been achieved under these special conditions, the specific objects of the present experiments were to determine: (*a*) whether prior exposure of the host to the prospective challenging cells would prevent implantation and dissemination of a challenging dose of intravenously administered *AK*–4 cells following irradiation; (*b*) what effect the route of administration (including the cheek pouch) of the sensitizing inoculum would have; (*c*) whether prior exposure of hamsters to leukæmic cells of other mouse strains would produce immunity to *AK*–4 challenge.

Within 6 h prior to challenge with $5\cdot0 \times 10^7$ *AK*–4 cells, previously sensitized experimentals and normal controls, anæsthetized by intraperitoneal 'Nembutal', were irradiated together by Siemens technique *A*: a clinical X-ray unit with the factors 250 kVp, 15 m.amp, additional filtration 0·5 mm. copper, 1·0 mm. aluminium, half value layer copper 1·75, dose rate in air 95 r./min, target distance 15 cm, 340° arc.

As in previous experiments[4], *AK*–4 cells for the challenging inocula were obtained from the spleens and lymph nodes of carrier lines of *AKR*/*Jax* mice: the leukæmic tissues, forced through a stainless steel screen (1/32 in. opening), were suspended in physiological saline or Eagle's basal medium, either alone or supplemented with calf serum[5]. Cell concentrations of the resultant suspensions were determined by the usual hæmocytometric methods for white cells, and final concentrations were adjusted to $1\cdot0 \times 10^8$ cells per ml. A dose of 0·5 ml., containing $5\cdot0 \times 10^7$ cells, was injected into each of the irradiated hamsters by orbital puncture, using 1 ml. tuberculin syringes equipped with 24 or 27 gauge needles.

The sensitizing inocula were similarly prepared from the spleens and lymph nodes of *AKR* mice bearing *AK*–4, or from solid subcutaneous growths of *P*–1534, *L*–1210, and *E*9514*A*, maintained by serial transfer in *DBA*/2 and *C3H* mice respectively. Suspensions of $1\cdot0 \times 10^8$ cells were further diluted to obtain sensitizing inocula of the desired concentration (Table 1). Intravenous sensitization was accomplished by orbital puncture, cheek pouch sensitization by methods previously reported for the implantation of cell culture suspensions into the cheek pouch[6]. Two sensitizing inocula were administered 2–3 weeks apart, and the animals were given one to two month's rest before irradiation and challenge.

The total white count of the blood of 5–6 day survivors of irradiation and challenge was determined by the usual methods; occasional transplants of leukotic whole blood of controls to mice resulted in leukæmia, confirming the morphological diagnosis. At death, subjects were autopsied, and representative tissues were fixed in 10 per cent formalin, and stained with hæmatoxylin and eosin for microscope evaluation. An attempt was made to examine the sternal bone marrow, liver, kidney, and spleen of every animal, but each group was finally evaluated on the basis of the proportion of recovered organs definitely judged to be infiltrated with leukæmic cells.

The results, summarized in Table 1, show that hamsters exposed to two inocula of any of the leukæmias so examined, administered by any of the routes used herein, failed to present evidence of leukæmic repopulation of the bone marrow, or leukæmic infiltration of the viscera. Control hamsters exhibited uniform infiltration of the bone marrow, liver, kidney, and spleen, with total white counts of $1\cdot1 \times 10^4$ to $1\cdot5 \times 10^5$ nucleated cells per mm^3 blood at 5–6 days, as compared to counts of $3\cdot9$–$5\cdot9 \times 10^3$ in sensitized hamsters.

The failure of the sensitizing inocula of *P*–1534, *L*–1210, and *E*9514*A* (neoplasms transplantable in the cheek pouch) to implant and disseminate when introduced centrally is corroborative evidence of special transplantation properties of the cheek pouch—what Billingham *et al.*[7] have termed the 'immunological privilege' of the cheek pouch. The characteristic failure of *AK*–4 cheek pouch transplants to grow, however, and the occasional failure of, for example, *L*–1210 (two instances of which are incorporated in the present experimental series) indicate that cheek pouch privilege is not universal. Further, since the failure of these neoplasms to grow as cheek pouch transplants resulted in immunization of the hosts against intravenous transplants of *AK*–4, it may be inferred that graft antigen was capable of leaving the cheek pouch, in contrast to the reported inability of skin isoantigens to do so[7]. Thus, at least some instances of cheek pouch transplantation failure may have an immunological basis, despite the pouch's immunological privilege.

The strong immunity to *AK*–4 challenge resulting from the rejection of murine leukæmic cells which characteristically do not grow in the cheek pouch (*AK*–4) or from the rejection of murine leukæmic cells transplantable in the pouch but which fail to grow when inoculated into non-privileged sites (*P*–1534, *L*–1210, *E*9514*A*) is revealed by these experiments to be species-specific rather than individual-specific, and is not abrogated by a total-body dose of 1,500 r., although, as the controls clearly show, that dose of irradiation is sufficient to permit first-set grafts of the neoplasm to implant, grow, and disseminate. The transplantation immunity induced under the stated conditions was of at least 1–2 months duration, but present experiments indicate that the regression of one cheek pouch implant of a murine leukæmia may result in immunity, detectable by these methods, as early as 17 days, or as late as 10 months, following sensitization.

A curious and unforeseen result of the present experiments was the slight but definite radiation-protective effect specific to the prior exposure of *AK*–4 challenged hamsters to *AK*–4 cells. Seven of 38 hamsters pre-treated with and challenged with *AK*–4 cells survived the lethal effects of exposure to 1,500 r., as opposed to none of 14 hamsters pre-treated with other strains of leukæmic cells, and none of 24 unsensitized, irradiated control hamsters. This association between radiation protection and heterotransplantation immunity is being further examined.

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Repression of Delayed Hypersensitivity to Conjugated Serum Albumin during Immune Paralysis induced in Guinea Pig by Heterologous Proteins

IT has been recently reported from this laboratory[1,2] that administration of large doses of bovine serum albumin (B.S.A.) to guinea pigs suppresses the antibody response to an immunizing dose of unrelated heterologous proteins. This effect can only be demonstrated when the immunization is performed several days after the commencement of the paralysing treatment. Moreover, in a previous investigation[3], it has been shown that similar treatment with other serum proteins is also capable of producing an inhibition of the delayed form of hypersensitivity.

In this communication, additional results dealing with the patterns of inhibition of delayed type of hypersensitivity in animals receiving large amounts of heterologous proteins are presented.

In all experiments, adult guinea pigs (350–450 g) of the *K* strain were used. Immune paralysis was induced by administering different doses of purified heterologous proteins: bovine serum albumin (B.S.A.); human serum albumin (H.S.A.); bovine γ-globulins (B.G.G.) and human γ-globulins (H.G.G.). The proteins were injected either intravenously or/and locally in the foot pads.

The delayed type of hypersensitivity was induced by injection of picrylated guinea pig serum albumin prepared according to the technique described by Benacerraf and Gell[4]: 0·1 ml. of mixture of equal parts of a saline solution containing 250 μg/ml. of picrylated guinea pig serum albumin and complete Freund's adjuvant was injected in each foot pad. The induction of the immune paralysis preceded by several days the sensitization with picrylated guinea pig serum albumin and was then continued for 4–7 days (Tables 1 and 2).

The degree of sensitization is determined by the size of the skin reactions elicited by intradermal injections of increasing concentrations of picrylated guinea pig serum in 0·1 ml saline, and was evaluated by the mean diameter of the skin reactions measured 24 h after challenge.

From the figures given in Table 1, it is evident that, by using the aforementioned method of sensitization, a strong degree of hypersensitivity could be regularly obtained in the control groups of animals, and that the size of the skin reactions is, to a certain extent, a function of the antigen concentration.

The cutaneous reactions, determined at the different time intervals after sensitization, were of the delayed type: they began to appear 6–8 h following the injection of antigen and reached their maximal intensity about 24 h later. In any event, no initial œdema or hæmorrhagic spots, characteristic of the Arthus type of hypersensitivity, were observed.

Intravenous injections of B.S.A. do not seem to affect the intensity of the reactivity to the picrylated guinea pig serum albumin (Exp. 1); although this treatment was previously found to be effective in suppressing the humoral antibody production and the anaphylactic type of hypersensitivity to ovalbumin and rabbit γ-globulins[3]. Treatment with H.S.A. by both intravenous and local routes exerts a definite inhibition of the delayed hypersensitivity provided that it has been started 10 days prior to sensitization (Exp. 2). When the paralysing pretreatment time is reduced to 3 days, the inhibitory effect disappears.

γ-Globulins, whether of bovine or human origin, were, on the whole, much more efficient than albumins in their inhibitory effect. This is evidenced by the fact that γ-globulins, injected in smaller amounts and for shorter periods before sensitization, produced regularly the same effect as H.S.A.: a more or less complete repression of the cutaneous reactions (Exps. 3 and 4). Human γ-globulins are also effective when injected separately by either the intravenous or local route (Exps. 5 and 6).

The results shown in Table 1 emphasize two important conditions for obtaining the inhibition of the delayed hypersensitivity: (1) induction of immune paralysis should in general precede sensitization by several days; (2) the injection of the paralysing antigen by the local route appears to be of a decisive importance.

Table 1. EFFECT OF PARALYSING TREATMENT WITH DIFFERENT SERUM PROTEINS ON DELAYED TYPE HYPERSENSITIVITY TO PICRYLATED GUINEA PIG SERUM ALBUMIN (PIC.GP.S.A.)

No. of experiment	Treatment with the paralysing antigen					Mean size (mm) of skin reactions elicited by the indicated concentration of sensitizing antigen (Pic.Gp.S.A.) μg/0·1 ml.			No. of animals
	Serum protein	Route of injection and dose		Duration (days)					
		I.V. mg/24 h	Local* mg/48 h	Preceding sensitization	Following sensitization	0·6	2·5	10	
	0					6 ± 3·6	11 ± 2·3	17·9 ± 3·3	50
1	B.S.A.	1,000	—	15	4	5·5	11·3	16	7
2	H.S.A.	600	140	3	7	7·7	13·5	19	3
		600	140	10	7	—	±	8·7	5
3	B.G.G.	400	65	6	7	—	—	10	6
4	H.G.G.	300	50	6	7	—	—	10	10
5	H.G.G.	300	—	0	7	—	5·9	10·7	6
6	H.G.G.	—	120†	3	7	—	±	7	4

* Injections were given subcutaneously in each foot pad preceding sensitization. The figures in the table indicate the total amount injected in the foot pads.
† This dose was repeated every 24 h.

Table 2. DURATION OF THE INHIBITORY EFFECT PRODUCED BY A PARALYSING TREATMENT WITH HGG* ON DELAYED TYPE HYPERSENSITIVITY TO PICRYLATED GUINEA PIG SERUM ALBUMIN (PIC.GP.S.A.)

Days following sensitization	Total No. of animals per experiment	Mean size (mm) of skin reactions elicited by the indicated concentration of sensitizing antigen (Pic.Gp.S.A.) µg/0·1 ml.		
		0·6	2·5	10
7	9 Control	5·5	14·4	20
	4 Treated	—	—	—
14	7 Control	14·5	17·5	26
	4 Treated	—	—	13·5
21	2 Control	12·2	12·5	18·5
	2 Treated	—	3·5	8
60	7 Control	7·4	7·6	19·6
	8 Treated	6	6·6	16·3

* 300 mg/24 h intravenously and 40 mg/24 h locally.

The results shown in Table 2 provide information concerning the duration of the inhibition of the delayed hypersensitivity produced by immune paralysis. In these series of experiments, the treatment with H.G.G. was initiated 7 days before sensitization and continued for an additional 7 days with daily injections. Both paralysing and sensitizing injections were given only in the two hind foot pads. Under these conditions a complete suppression of the delayed hypersensitivity was observed in all the treated animals when tested with picrylated guinea pig serum albumin on the seventh day following sensitization. However, 14 days after sensitization, the recurrence of a certain degree of hypersensitivity, as evidenced by cutaneous reactions, to the highest doses of antigen was already noticed and this phenomenon became more pronounced 21 days later. After two months, the reactions in the γ-globulin-treated animals were of the same intensity as in the controls. From the results reported here it can be inferred that the inhibition of the delayed hypersensitivity, induced by immune paralysis, is a transient phenomenon. It persists as long as the paralysing treatment is continued and then gradually subsides with the discontinuance of treatment.

Our results indicate that repeated injections of high doses of heterologous proteins, a condition which is known to induce immune paralysis, can also abolish the delayed type of hypersensitivity to haptene-conjugated proteins in guinea pigs.

In this respect γ-globulins were more efficient than serum albumins. Complete suppression of the delayed hypersensitivity was only obtained when the paralysing treatment was initiated several days previous to the sensitization and when both systemic and local routes of administration were used. These observations suggest that the immunologically competent cells which are mainly located in the regional lymph nodes have to be reached previous to sensitization by adequate concentrations of the paralysing antigens. The fact that the inhibition of the delayed hypersensitivity subsides rapidly after cessation of the paralysing treatment (whereas the immune tolerance to paralysing agent will persist for several months) may be related to the local persistence of the sensitizing antigen incorporated in Freund's adjuvant. On the other hand, the concentration of the paralysing antigen in the lymph node cells decreases rapidly after the end of the treatment. These findings suggest the possibility of a quantitative relationship between the concentration of the paralysing antigen in the lymph node cells and the degree of their unresponsiveness to a sensitizing agent.

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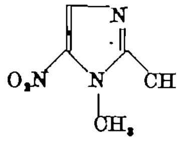
BIOLOGY

Dimetridazole: Absorption, Excretion and Metabolism in Turkeys

HISTOMONIASIS in turkeys (turkey blackhead) is caused by the protozoan flagellate *Histomonas meleagridis* and it can be controlled effectively with dimetridazole (1,2-dimethyl-5-nitroimidazole) given orally[1,2].



Since dimetridazole is given in the food continuously to prevent histomoniasis and at higher levels for shorter periods in the food or in drinking water to treat outbreaks of the disease, it is important to ascertain the metabolic fate of dimetridazole in the turkey and also to ensure that no significant amounts of the drug or its metabolites accumulate in the tissues to become constituents of a potential human food-stuff.

Absorption and excretion of dimetridazole. Single doses of dimetridazole (100 to 300 mg/kg as an aqueous solution of the dihydrogen phosphate) were administered orally to turkeys which had been deprived of food for the previous 24 h. Faeces and urine were thereafter collected for 3 successive days and the samples were examined by polarography and colorimetry. Results obtained by the two techniques were in excellent agreement and showed that 64 per cent of the dose was excreted within three days. These findings were supported by the use of radioactive dimetridazole which was labelled with carbon-14 in the 2–C and 2–CH_3 positions. (We thank Monsieur Roy and Monsieur Colson of the Département Nucléaire of the Société Rhône-Poulenc for supplying us with the labelled dimetridazole used in these experiments.) Turkeys which had received a single oral dose of dimetridazole (32 mg/kg) containing 60–100 µc. of radioactivity excreted 90 per cent of the dose in urine, faeces and expired air within three days, and 80 per cent was recovered within two days.

Polarography measured all 5-nitroimidazoles present, that is, dimetridazole and any metabolites which retained the nitro group, and it was sensitive to 0·05 to 0·1 µg/g depending on the sample size[3]. The colorimetric method, which was based on the reduction of the 5-nitro group to an amino compound which could be diazotized and then coupled with *N*(1-naphthyl)-ethylenediamine[4], detected dimetridazole, metabolites containing the nitroimidazole moiety and any 5-amino derivatives. Its sensitivity was 0·5 µg/g. The radiochemical method in which urine, faeces and expired air ($^{14}CO_2$ trapped as $Ba^{14}CO_3$) were counted with a thin end-window Geiger–Müller tube measured not only dimetridazole but also all possible metabolites in which either or both the carbon-14 atoms were retained, including carbon dioxide. This method was sensitive to about 0·03 µg/g.

Tissue residues of dimetridazole. Tissue residues of dimetridazole and its metabolites were studied polarographically and radiochemically. Initially, turkeys were given free access for six days to drinking water which contained dimetridazole (0·05 per cent) and they each ingested about 200 mg of the drug per day. The birds were killed 0, 1 or 2 days after the medicated water had been withdrawn, and samples of serum, liver, kidney, heart, muscle and skin were removed immediately for polarographic assay. No detectable residues (less than 0·1 µg/g) of dimetridazole or its metabolites were found in the serum or tissues of birds killed two days after medication had been stopped. These results were later confirmed by administering a single oral dose (32 mg/kg) of dimetridazole labelled with carbon-14 to turkeys which were killed three days later when samples were removed as before for polarographic and radiochemical examination. The concentration of dimetridazole and its metabolites found in these tissues by both methods was < 0.05 μg/g and < 0.03 μg/g respectively.

Metabolism of dimetridazole. Dimetridazole and its metabolites present in urine were separated by paper chromatography with several solvent systems. Six metabolites (I, II, IV, V, VI, and VII) were observed when the developed chromatograms were examined in ultra-violet light or were sprayed with appropriate reagents. In addition, a seventh metabolite (III) was detected by chromatography and autoradiography of urine from a bird dosed with ^{14}C-labelled dimetridazole. Metabolites IV, V, VI, and VII have been positively identified by comparing their R_F values in several solvent systems and their colour reactions with those of synthetic reference compounds. The major metabolite, metabolite V (44·4 per cent of total excreted drug), was 1-methyl-5-nitroimidazol-2-ylmethyl hydrogen sulphate. Metabolite IV (25·8 per cent of total excreted drug) proved to be 1-methyl-5-nitroimidazole-2-carboxylic acid and metabolite VI (9·4 per cent of total excreted drug) was 2-hydroxymethyl-1-methyl-5-nitroimidazole. Unchanged dimetridazole (metabolite VII) accounted for only 3·2 per cent of the excreted material. Metabolite I was shown to be a conjugated glucuronide of a nitroimidazole, probably the 2-hydroxymethyl derivative (metabolite VI).

When dimetridazole is administered orally to turkeys nearly all the excreted drug is in a metabolized form and the basic metabolic pathway apparently involves oxidation of the 2-methyl group to 2-hydroxymethyl. This hydroxymethyl derivative then undergoes either conjugation or further oxidation. The nitro group appears to resist reduction in the body and about 90 per cent of the excreted drug consists of various nitroimidazoles.

Dimetridazole has been used: (1) at 0·0125 per cent in food continuously to prevent histomoniasis; (2) at 0·05 per cent in food for 10–14 days or in drinking water for 6 days to treat the disease. Condren *et al.*[5] administered dimetridazole in food at 0·025 and 0·05 per cent to turkeys for 24 weeks and showed polarographically that two days after withdrawal of the medicated food the tissues contain no significant residues. In the work recorded here, where turkeys had received dimetridazole (0·05 per cent) in drinking water for six days, no compounds containing a nitro group were detected polarographically in the tissues two days after medication had ceased. The absence of tissue residues, indicated by the polarographic technique, is confirmed by the radiochemical measurements with birds that had received a single oral dose of dimetridazole labelled with carbon-14. This observation is of importance because the radiochemical method will reveal all metabolites which contain carbon-14, including those not detected polarographically. The evidence obtained by Condren and ourselves indicates, therefore, that no detectable residues (< 0.02 p.p.m.) of dimetridazole or its metabolites are present in the tissues of turkeys three days after administration of the drug has ceased at prophylactic (0·0125 per cent) and therapeutic (0·05 per cent) levels.

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Experimental Induction of Ciguatera Toxicity in Fish through Diet

CIGUATERA fish poisoning is widespread in the tropical Pacific and the Caribbean, causing recurrent outbreaks of illness in persons consuming snappers, groupers, jacks, barracudas, and other species that may contain ciguatera toxin[1-3]. The existence of ciguatera toxin in a fish appears to be controlled by some factors in its environment; many suggestions have been made as to the environmental factors concerned, ranging from dumped war material[3] to "flowering of coral"[4]. The most coherent hypothesis is that of Randall[5], who compiled circumstantial evidence and suggested that the origin of the toxin is in a benthic organism, perhaps an alga, and that it is passed through the food chain in the process of normal feeding.

The following observations made during field studies and during investigations at the Hawaii Marine Laboratory in the past two years favour this hypothesis: (1) a number of obligate herbivorous fishes such as *Acanthurus triostegus* (Linnaeus) and *Ctenochaetus striatus* (Quoy and Gaimard) from toxic areas in the Line Islands are toxic; (2) these herbivorous fishes are found in the stomach contents of the large carnivores such as the snappers and groupers which are also toxic; (3) only the larger carnivores appear to be toxic in affected areas. Randall[5] suggests the last may be because most of the herbivorous fishes are high-bodied, so that only the larger carnivores are capable of readily ingesting them.

However, the crux of the food chain hypothesis rests on three presumptions: (1) that the fish can acquire the toxin in their diet; (2) that the toxin can be eaten by the fish without detrimental effects; (3) that the toxin so ingested can be stored within the body of the fish in a pharmacologically unchanged condition. If these conditions cannot be obtained, the hypothesis would have to be discarded. Until now there has been no evidence that such food transmission was indeed possible.

In the course of work on the origin of ciguatera toxin, a simple feeding experiment was carried out using non-toxic *Acanthurus xanthopterus* Cuvier and Valenciennes from Hawaii. These surgeon fish are common market fish in Hawaii and have never been known to cause ciguatera in this archipelago[6]; they have also been fed to mongooses, the test animal for ciguatera toxin[7], as part of their non-toxic maintenance diet, for more than seven years without causing any reactions.

Starting in 1958, four of these fish were maintained in two running sea-water aquaria of about 120-gallon capacity; the two fish in each tank were separated by a screen allowing the passage of water but not food. One of each pair was fed on alternate days 10 grams of the flesh of *Lutjanus bohar* (Forskål) from the Line Islands which had been proved to be moderately to highly toxic (+3 to +5 reaction—see footnote to Table 1) when fed to a mongoose at a dose equivalent to 10 per cent of the body-weight of the mongoose. The other fish in the tank was fed comparable amounts of the non-toxic tuna, *Katsuwonus pelamis* (Linnaeus).

On several occasions the circulating sea-water failed and the fish died. These specimens were frozen soon after death and were kept in a frozen state. As the Hawaii Marine Laboratory burned to the ground in December 1961, destroying the experimental facilities and preventing continuing studies, it was decided to determine the toxicity of these fish at this time.

Unfortunately in the fire were lost all records of the experiment, including the length of time each fish was kept and the amount of toxic food fed. We recall that the longest period a test specimen was fed was about 18 months; the shortest, about 6 months. However, it can be easily recalled that this feeding of toxic fish flesh did no apparent harm to the specimens of *A. xanthopterus*, which grew as well as did their tank-mates fed on a non-toxic diet.

Table 1. TOXICITY OF FOUR SPECIMENS OF *Acanthurus xanthopterus* MAINTAINED ON A DIET OF TOXIC *Lutjanus bohar* (FORSKAL) AS DETERMINED BY THE MONGOOSE FEEDING BIOASSAY

	Weight of *A. xanthopterus* (g)	Tissue fed to mongoose	Dosage in % body-weight of mongoose (approx.)	Maximal reaction* in mongoose in 48 h
(1)	256	Flesh	30	5
		Flesh	8	5
		Flesh	10	4
		Viscera	2	3
(2)	217	Flesh	23	1
		Viscera	2	2
(3)	119	Flesh	10	1
		Viscera	2	2
(4)	94·5	Flesh	5	5
		Flesh	5	2
		Flesh	2	5
		Viscera	2	5

* Mongoose reactions (see Banner *et al.*, ref. 1): 0, no reaction; 1, slight weakness and flexion of the forelimbs; 2, slight motor ataxia, more pronounced flexion of the forelimbs and weakness of the hind limbs; 3, moderate ataxia with weakness and partial paralysis of limbs and body musculature; 4, acute motor ataxia and extreme weakness, capable of only limited movement, or coma; 5, death.

As we now lack laboratory facilities, the more sensitive mouse tests[8] cannot be used in the assay of the toxicity of the test fish; the results of the mongoose feeding test are given in Table 1.

The results show conclusively that the fish did acquire toxicity. It was unfortunate that control fish were not saved, as were the test fish; however, there is no reason to suspect that the controls have developed toxicity.

The tests, then, show that the toxin causing ciguatera may be passed through the food chain, without apparent change in the toxin and without apparent harm to the carrier.

New long-term experiments are now in progress utilizing live *Lutjanus bohar* to confirm this experiment.

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Furunculosis in Salmon Kelts

DURING the spawning season of 1960–61 investigations were initiated in Ireland into the occurrence of furunculosis among spawning and spawned salmon. In that season 159 kelts were examined from four rivers; only one fish gave a positive reaction[1]. These investigations were continued during the spawning season of 1961–62 when 49 kelts were examined. All fish were dead when taken from the water. The distribution and results are given in Table 1.

Table 1

River	No. of kelts examined		Hatchery or naturally spawned	Result of test	Period
	Male	Female			
River Owenea (Co. Donegal)	25	11	All hatchery stripped	All negative	January 9—February 7, 1962
River Erne (Co. Donegal)	4	2	All naturally spawned	All negative	March 15—May 29, 1962
River Blackwater (Co. Waterford)	—	1	Hatchery stripped	Positive	December 6, 1961
River Boyne (Co. Meath)	1	—	Naturally spawned	Positive	December 3, 1961
River Lee (Co. Cork)	2	—	Hatchery stripped	Negative	January 23, 1962
River Moy (Co. Mayo)	3	—	Naturally spawned	1 positive	March 1–3, 1962
Total	35	14		46 negative 3 positive	

As can be seen, three fish (6·1 per cent) gave a positive reaction.

I thank Mr. E. Murphy, of the Veterinary College of Ireland, Ballsbridge, Dublin, who undertook bacteriological examination of these fish.

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Primary Productivity of Periphyton

THE difficult problem of assaying biomass and rates of colonization by periphyton in fresh and saline waters has been recognized for many years. The most common method used in these investigations has involved the measurement of rates of colonization by periphyton on artificial substrata. The literature on this subject has recently been reviewed by Cooke[1], Sládečková[2], and myself[3]. Very few of these investigations are concerned with measurements of photosynthetic rates of algal periphyton under natural conditions. A method for measuring productivity of periphyton *in situ* in lakes and ponds would be useful for comparative studies of the relative importance of each of the photosynthetic producer components, that is, phytoplankton, periphyton, and macrophytes.

A technique utilizing carbon-14 as a tracer was developed for assaying the *in situ* productivity of periphyton. The methods were similar to those that I developed[4] for submerged macrophytes. Transparent and opaque 'Plexiglas' chambers[4], cylindrical, open at one end, and containing an injection port, were inserted into the substratum along littoral transects perpendicular to the shoreline enclosing the algal-encrusted rocks and sediment. Each chamber was inoculated with 1 ml. of carbon-14 as sodium carbonate of known assay determined by gas-phase analysis and comparison to National Bureau of Standards samples. After incubation for a 4–6-h period the surface cm of sediment was removed and stored frozen over a strong desiccant under vacuum. Immediately prior to analysis the samples were exposed to fumes of hydrochloric acid for a 10–15-min period to remove any radiocarbon precipitated as monocarbonates. The algal periphyton, primarily epipelic and epilithic, was combusted from the sediments using a Van Slyke wet carbon combustion. The $^{14}CO_2$ was evolved at atmospheric pressure, treated[4], and flushed with pure carbon dioxide into an evacuated ionization chamber. Radioassay was accomplished directly using a Dynacon electrometer[5]. Total inorganic carbon available for photosynthesis was determined titrimetrically. In addition, a carbon-14 isotope effect of 6 per cent was used in the computations.

Estimates of littoral periphyton standing crop using pigment analyses are seriously impaired by the interference of the absorption spectra of chlorophyll degradation products. To circumvent partially this problem a modified technique of chlorophyll extraction was utilized. Replicate cores of the surface substrata were collected at intervals along the transects mentioned above. The pigments of the upper cm of the sediments were extracted in 90 per cent alkaline aqueous acetone in the

Table 1

	Annual mean productivity* (mg C/m²/day)	Total annual mean productivity† (kg C/lake/day)
Phytoplankton	249·3	101·0
Periphyton	781·5	75·5
Macrophytes	76·5 (372·3)‡	1·4 (6·6)‡
	1,057·3	177·9

* Annual mean of water column for the phytoplankton, mean of littoral one for periphyton and macrophytes.

† Total annual mean for the lake compensating for changes in the volume for phytoplankton and areal fluctuations of the littoral zone (periphyton and macrophytes).

‡ Data in parentheses are mean values for the growing season (75 days) of the macrophytes.

dark at 4° C to remove the chlorophylls and naturally occurring pigment degradation products. Similarly, replicated samples were extracted in acidic 90 per cent acetone to convert the chlorophylls to pheophytins. Extracts were analysed at 665, 645, 630, 510, and 480 mμ (Beckman *DU* spectrophotometer). Background absorption not a result of the chlorophylls between 600 and 700 mμ was usually negligible; where significant it was subtracted from the specific density readings. Chlorophyll *a* ('functional chlorophyll') of the periphyton was determined by difference using the density values for chlorophyll *a* and pheophyton *a*, accounting for shifts in the spectra from extinction coefficients for the pigments in acetone at this wave-length. The extinction coefficient of pheophyton *a* in 90 per cent acetone was estimated from known values in other solvent concentrations and therefore the values must be viewed as approximate. Corrected values were then used in the formulæ of Richards[6] with necessary volume modifications.

The primary productivity of the littoral periphyton was determined in a large, saline lake, Borax Lake, of northern California in 1961–62 (Fig. 1). Shallow closed lakes of this type are subject to considerable fluctuation in volume and the amount of littoral area available for periphytic colonization varies accordingly. Changes in volume of the lake and area of the littoral zone at each sampling interval were calculated by planimetry from morphometric measurements of the basin made at the time the lake was surveyed in December. This method provided a means of estimating the total primary productivity of the lake on a more realistic basis (Table 1). The littoral zone of Borax Lake is remarkably uniform, consisting largely of small angular pebbles heavily encrusted with periphyton in spring and summer months, and leads from a gentle slope to the ooze of the flat, deeper areas. The uniform littoral region is small compared with the total area of the basin, but it constitutes a major region of primary productivity.

Correlations were generally poor between the productivity (Fig. 1) and estimates of the mean biomass of the littoral periphyton from pigment analyses (Fig. 2). Only during periods of high productivity did similar trends appear. The lack of correlation was probably the result of numerous variables, for example, light, nutrition, etc., that affect the momentary concentrations of the pigments. Evidence is rapidly accumulating[7] that pigment estimates of producer biomass must be interpreted with considerable caution.

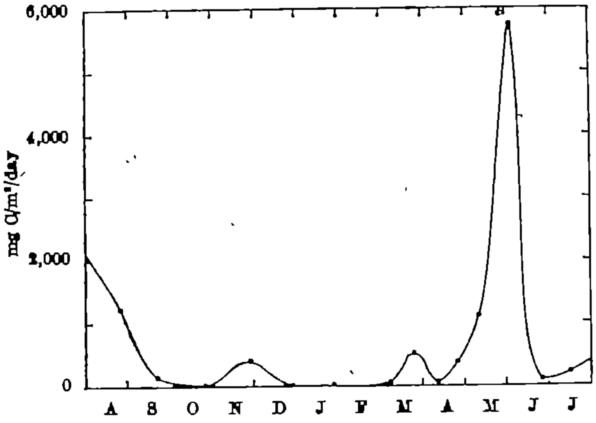

Fig. 1

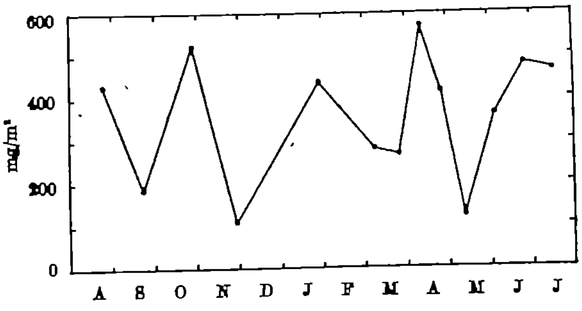

Fig. 2

Hitherto the contribution of the littoral producers to the total photosynthetic carbon fixation has generally been regarded as insignificant as compared with that of the phytoplankton. Neglect of the sessile producers is perhaps warranted in certain large, deep lakes. However, the periphyton and macrophytes constitute a major or, perhaps, the predominant source of photosynthetic energy fixation in many shallow lakes, ponds, marshes, and rivers.

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Effect of Kinetin on Powdery Mildew

IN work on wheat stem rust, Person *et al.*[1] observed that detached leaves from resistant plants which become susceptible and chlorotic when floated on water maintain their green colour and rust resistance when floated on a kinetin solution. A review of the effects of kinetin (6-furfurylaminopurine) on various plant tissues has been made by Parthier[2].

This communication deals with activity of kinetin against fungi causing powdery mildew in plants.

To screen chemicals for chemotherapeutic effect on powdery mildew, cucumber leaf disks 12 mm in diameter were floated on an aqueous solution of the compound to be tested, and inoculated by dusting conidia of *Erysiphe cichoracearum* on the upper surface. The evaluation was made after 8 days of incubation in the greenhouse at 17°–21° C. Under the conditions of the test, 10–20 p.p.m. of kinetin checked completely development of the powdery mildew, while abundant growth of the fungus was present on check disks (Fig. 1). A possibility that inhibition of this obligate parasite could be due to damage or death of the host tissues was ruled out by the fact that inoculation was again successful if it was performed two days after replacement of the kinetin solution by water. Other powdery mildews, such as *Erysiphe polygoni* on lupins, beets and peas, *E. graminis* on wheat, rye and barley, *Podosphaera leucotricha* on apple, *Sphaerotheca pannosa* on roses, and *Sphaerotheca humuli* on strawberries, were also inhibited on leaf disks by the kinetin.

For anatomical investigations of the effect of kinetin on development of powdery mildew, the cucumber leaf disks

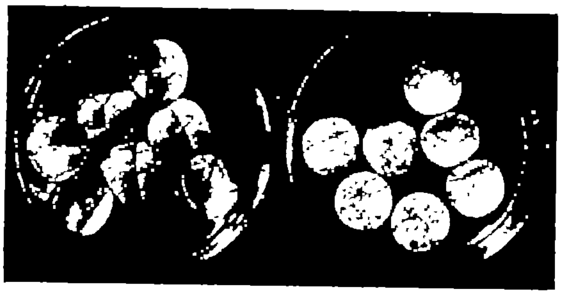

Fig. 1. Activity of kinetin on cucumber powdery mildew. Leaf disks floating on 20 p.p.m. kinetin (left, no development of powdery mildew) and water (right, covered with powdery mildew)

were floated on an aqueous solution made up to 20 p.p.m. kinetin, and inoculated by dusting with conidia of *Erysiphe cichoracearum*. After three days the inoculated epidermis was stripped, stained with cotton blue in lactophenol and subjected to microscopic examination. It appeared that neither the germination of the conidia nor the penetration of the fungus into the epidermis of the host tissue was in any way different from that in the control disks floated on water. However, on formation of the first haustorium, the development of the fungus had stopped on treated disks while the control showed an average of 7–8 haustoria per conidium during the same interval of time. These observations lead to the assumption that the action of kinetin on powdery mildew occurs inside the host epidermis. However, it is not yet clear whether kinetin acts directly on the haustorium of the fungus or via the metabolism of the host plant.

Reversal of the effect of kinetin (20 p.p.m.) on powdery mildew was in evidence when folic acid (50 p.p.m.) was added to the solution, indicating a possible relation of kinetin to the metabolism of folic acid in host or parasite. Parabenzoic acid utilized in the same manner failed to circumvent the action of kinetin on the fungus.

The attempts to control powdery mildew of cucumber systematically by application of a 300 p.p.m. kinetin solution to bare roots of cucumber plants failed. Also foliar application of kinetin by spraying a 1,000 p.p.m. solution on the leaves one and two days following the inoculation was not successful. Insufficient transport of the chemical in plant tissue may be a factor in this mode of application[2].

Kinetin appeared inactive against the other fungi which were tested. In analogous experiments with leaf disks, a solution containing 100 p.p.m. kinetin failed to reduce development of chocolate spot (*Botrytis fabae*) of dwarf French beans and 20 p.p.m. was ineffective against bean rust (*Uromyces appendiculatus*). *In vitro* the spore germination of *Ascochyta pisi*, *Botrytis fabae* and *Aspergillus niger* was not affected in a solution containing 1,000 p.p.m. kinetin, and mycelial growth of these fungi, placed on potato dextrose agar plus 100 p.p.m. kinetin, was not reduced.

The two components of kinetin, namely, adenine and furfuryl alcohol, showed no activity against powdery mildew, singly or in combination, as a 100 p.p.m. solution in the leaf disk test. However, on further testing of about 80 purines and pyrimidines, 6-azauracil appeared highly active against cucumber powdery mildew[3].

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[1] Person, C., Samborski, D. J., and Forsyth, F. R., *Nature*, 180, 1294 (1957).

[2] Parthier, B., *Pharmazie*, 15, 969 (1960).

[3] Dekker, J., *Mededelingen Landbouwhogeschool Gent*, 23, 1214 (1962)

MICROBIOLOGY

Polyimines Mutagenic for Bacteriophage *T4B*

Certain highly reactive polyimines have been suggested for use as sterilizing agents in the control of insect populations[1]. These compounds are radiomimetic, and the possibility arises that they are mutagenic. The mutagenic activities of four such compounds have been examined by testing for the induction of *r* mutants in bacteriophage *T4B*: 2,2,4,4,6,6-hexa(1-aziridinyl)-2,4,6-triphospha-1,3,5-triazine ('Apholate')[2], *tris*(1-aziridinyl) phosphine oxide (APO)[3], *tris*(1-(2-methyl) aziridinyl) phosphine oxide (MAPO)[2], and ethylene glycol bis-((1,2) propylene)-carboxamide (EGPC)[3].

The compounds were dissolved in M/15 phosphate buffer at *p*H 7 and were brought immediately to 37°. A pre-warmed aliquot of *T4B* in buffer was added, and samples were taken at intervals to be assayed for the surviving fraction of bacteriophage and the proportion of *r* mutants. Stocks of *T4B* containing a low background of *r* mutants were used, and *r* mutants were counted by visually screening plates containing about 1,000 plaques growing on *E. coli B*. Conventional bacteriophage methods were used[4].

T4B was inactivated exponentially by all four compounds. The rates of inactivation were greater at *p*H 6 than at *p*H 7, and some of the compounds themselves may be unstable in aqueous solution below *p*H 7.

Table 1

Compound	Conc. (wt/wt)	Rate k (min^{-1}) of inactivation	Mutations per lethal hit
'Apholate'	0·005	50–100	$< 0{\cdot}3 \times 10^{-4}$
MAPO	0·005	4·12	$4{\cdot}3 \times 10^{-4}$
APO	0·005	21·0	$2{\cdot}9 \times 10^{-4}$
EGPC	0·0005	154	$3{\cdot}2 \times 10^{-4}$

The results are given in Table 1. MAPO, APO and EGPC were highly mutagenic, while 'Apholate' was not detectably mutagenic in these tests. Inactivation rates obtained with 'Apholate' varied about two-fold in different experiments. Although the rates of inactivation of *T4B* by the compounds varied considerably, mutations per lethal hit were approximately constant for APO, MAPO and EGPC, suggesting that these three compounds may have similar chemical mechanisms of mutagenesis.

The high mutagenicity of APO, MAPO and EGPC indicate that their use as insect sterilizing agents should be confined to systems avoiding their free distribution in Nature, especially where transmission to human beings could occur. Although 'Apholate' was not demonstrably mutagenic in these tests, its close chemical relationship to the other compounds indicates that it might well be mutagenic under other conditions.

This work was supported by U.S. Public Health Service grant *E*3203.

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[1] For example, LaBrecque, G. C., *J. Econ. Entomol.*, 54, 684 (1961).

[2] Olin Mathieson Chemical Corp., Baltimore.

[3] Interchemical Corp., New York. These compounds are not necessarily pure.

[4] Adams, M. H., *Bacteriophages* (Interscience, New York, 1959).

Increased Survival of Ultra-violet-irradiated *Shigella sonnei* by decreasing the Surface Tension

The role of the surface tension of the suspending medium in the susceptibility of bacteria to ultra-violet irradiation, to our knowledge, has not been examined. During a course of investigations on the effect of ultra-violet light on *Shigella sonnei* we observed a very interesting relationship between the surface tension (air to liquid) of the fluid in which the organisms were irradiated and the ability to recover from the damaging effects of the irradiation. We have found that, within certain limits, a reduc-

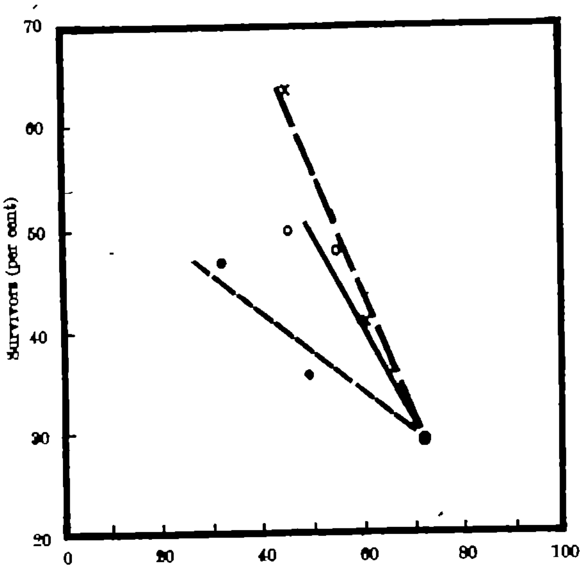

Fig. 1. Survival of ultra-violet-irradiated *S. sonnei* irradiated in buffers of different surface tension values. ×, Sodium taurocholate; O, 'Tween 20'; ●, sodium sulphosuccinate

tion of the surface tension resulted in increased survival of the irradiated cells.

S. sonnei, strain 5044–59, was obtained from Dr. W. H. Ewing, Communicable Disease Center, Atlanta, Georgia. The organisms were irradiated in buffer solutions adjusted to different surface tension values. 'Tween 20', sodium taurocholate, and sodium sulphosuccinate were the surface-tension depressants used. The agents were added to the buffer solutions and the surface tension of the solutions was measured with the Du Nuoy (Cenco) tensiometer. Readings were made at room temperature (approximately 23°–25° C); the average of three readings was recorded. The apparent surface tension which was read directly from the scale of the tensiometer in dynes/cm was corrected by dividing the values by the density of the solution being assayed and then multiplying the value by a correction factor which was dependent on the apparent surface tension reading and a ratio R/r (the radius of the platinum ring, R/the radius of the wire, r). The value of R/r was supplied by the manufacturer of the tensiometer. 24-h cells of *S. sonnei* were washed and resuspended in buffer to yield an optical density of 0·15. 5-ml. aliquots of the cell suspensions were irradiated at various surface tension values. Controls consisted of aliquots of unirradiated cells held in the buffers at the same surface tension values. A General Electric 15-W 'germicidal lamp' (*G15T8*), which emitted waves of approximately 2537 Å, was used. Under the conditions of these experiments the radiation intensity was 17·2 ergs/sec/mm². The total ultra-violet dose to which the cells were exposed was 258 ergs/mm² (15 sec); preliminary experiments have revealed that this dose resulted in a surviving fraction of approximately 30 per cent.

Fig. 1 summarizes the results. It can be seen that, in general, all three surface-tension-lowering agents increased the recovery of irradiated cells. However, sodium taurocholate was considerably more protective than sodium sulphosuccinate even though the surface tension values were similar. One possible explanation for this may be that sodium sulphosuccinate exhibited some chemical toxicity in addition to its surface tension-lowering properties. Since these experiments did not rule out the possibility that the agents examined had protective activity against ultra-violet light we increased the concentration of the agents studied to determine their protective role. Increasing the concentration of the surface-active agents did not further depress the surface tension since the maximum level of depression had already been obtained. Increased amounts of the surfactants did not increase the protection against ultra-violet light. Possibly maximal chemo-protection, if involved, had been reached.

We have demonstrated that cells irradiated in buffer with lowered surface tension survived ultra-violet irradiation better than cells irradiated at 70 dynes/cm the surface tension of the medium ordinarily used. We do not know at this stage whether the lowered surface tension reduced the absorption of ultra-violet light by the cells or if toxic substances produced by irradiation were inactivated or less stable at the lower surface tension values. Our investigations have shown that various factors affect the ultra-violet susceptibility of *S. sonnei* such as age of the organism[1] and exposure to starvation[2].

This work was supported by a research grant (*E*–3734) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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[*] U.S. Public Health Service Predoctoral Fellow (*EF*-11,372).

[1] Ramage, C. M., and Nakamura, M., *Canad. J. Microbiol.*, 8, 283 (1962).

[2] Ramage, C. M., and Nakamura, M., *Exp. Cell Res.* (in the press).

PSYCHOLOGY

Acoustic Confusions and Memory Span for Words

PREDICTION of the short-term memory span for sequences of items has been a crucial problem in testing the value of information theory as a model for remembering. A number of authors[1–3] have presented data which show that observed spans fall between predicted values and values which would obtain if span were independent of information per item. In all these cases the underlying assumption made was that if an item was forgotten the alternative items in the vocabulary were available as responses with equal probability.

I have, however, shown[4] that for the case of visually presented letter sequences this assumption is false. If an item is forgotten: (1) the alternative items are not equiprobable responses, and (2) the probability of their occurrence can be predicted by reference to a confusion matrix of listening errors when the same items are heard through white noise in a conventional intelligibility study. This result suggests, at least for verbal material, a model for predicting memory span in terms of the probability of acoustic confusion within the test vocabulary, which may be quite independent of the information per item. These contentions are supported by two experiments.

In both experiments the test material consisted of 5-word sequences which, using a memory drum, were visually presented word by word at a rate of 1 word/sec. No word was repeated in one sequence. At the end of each sequence subjects wrote down what they could recall in the correct order.

Experiment 1. Twelve naval ratings each did two conditions, half in the order (*a*), (*b*), half (*b*), (*a*). Each condition used 20 sequences. Condition (*a*): ten-word vocabulary composed of five pairs of visually different homophones, namely: *sum, some, ruff, rough, rays, raise, court, caught, past, passed*. Condition (*b*): ten-word vocabulary composed of common acoustically dissimilar words, namely: *beat, deep, fall, glue, mine, nose, play, rich, soil, town*.

Performance was scored in terms of percentage of sequences correctly reproduced. For conditions (*a*) and (*b*) the means were respectively 10·0 per cent and 58·3 per cent. No subject performed better on (*a*) than on (*b*).

Experiment 2. Eighteen different naval ratings each did two conditions, half in the order (*b*), (*c*), half (*c*), (*b*).

Each condition used 40 sequences. Condition (*b*) used the same vocabulary as in (*b*) of the first experiment. In condition (*c*) every word in the entire test (200 words) was different, and all were common four-letter nouns or verbs drawn from the *AA* and *A* lists of Thorndike and Lorge[5].

The respective mean percentage correct sequences for the (*b*) and (*c*) conditions were 68·7 per cent and 43·4 per cent. These means are significantly different ($t = 4{\cdot}26$; $P < 0{\cdot}001$).

The result of Experiment 2 is compatible with an informational model of short-term memory. But the (*b*) conditions in the two experiments are identical (apart from the number of sequences presented), and the subjects are comparable. Furthermore, the difference between the (*b*) condition scores for the two experiments is not significant (Mann–Whitney test, $P > 0{\cdot}1$). One might therefore be justified in regarding condition (*b*) as a subject matching test, and so compare the mean scores of (*a*) and (*c*). In this case a Mann–Whitney test shows the difference to be highly significant ($P < 0{\cdot}001$). This result is not compatible with an informational model.

Taken together these results provide far more support for a memory span model based on probability of acoustic confusion than for one based on information theory. I have shown that, if vocabulary size is constant, memory span is a function of probability of acoustic confusion. I have further shown that varying the vocabulary size may lead to either greater or smaller memory span. It seems *a priori* that the 200-word vocabulary of condition (*c*) has a smaller probability of acoustic confusion than the (*a*) vocabulary, and a greater one than the (*b*) vocabulary, and that these are the relationships on which the results depend.

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15 Chaucer Road, Cambridge.

[1] Pollack, I., *Amer. J. Psychol.*, 66, 421 (1953).

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[3] Crossman, E. R. F. W., in *Information Theory* (Butterworth, London, 1960).

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[5] Thorndike, E. L., and Lorge, I., *The Teacher's Handbook of 30,000 Words* (Columbia Univ., New York, 1944).

Relationship between Degree of Learning and Retention

Underwood[1] has argued that the major factor determining retention is the previous learning experience of subjects. On the basis of data from a series of earlier experiments by many different workers he suggests that recall of verbal material is a function of the number of previous lists learned. The more lists previously learned, the lower is the recall score after 24 h. Underwood claims that this is due to an increase in proactive inhibition from each list learned. In this way proactive inhibition is considered to be the main causal factor affecting recall performance.

The following two experiments have attempted to assess the importance of proactive inhibition in the learning situation Underwood discusses. The first experiment used the same design as in the work cited by Underwood. Six lists of paired associate items were learned by the anticipation method. One list was learned to the criterion of a single correct trial on each of six successive days, and each list was recalled on the following day before learning of the next list commenced.

The percentage recall for each list is shown in Fig. 1 (Exp. 1). Although percentage recall declined over successive lists, this decline was not so great as Underwood's composite curve from previous experiments would suggest. It is felt that this is because Underwood's curve combines data from paired associate and serial learning tasks; recall of serial lists is expected to be worse than recall of paired associate lists.

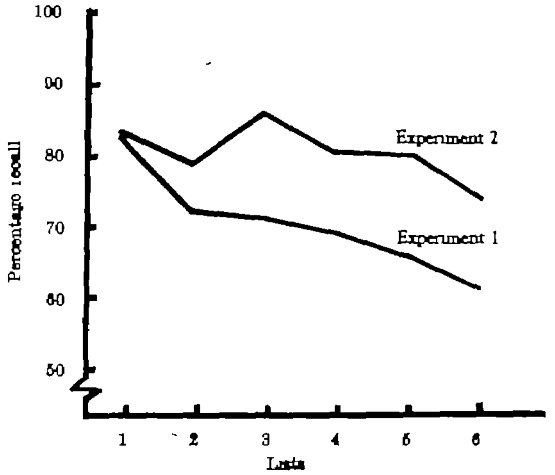

Fig. 1. Percentage recall after 24 h for six successive lists under two experimental conditions of learning

On the other hand, it was found that mean number of trials to reach the learning criterion declined steadily from 11·40 to 6·85 over the six lists. The average degree of learning of items declined in a similar fashion. Degree of learning is here operationally defined as the number of times an item is correctly anticipated during learning. The mean number of correct anticipations per item for list 1 was 5·90, and for list 6 this figure was 3·86.

The relationship between degree of learning of individual items and the probability of recall of those items was investigated for each of the six lists. It was found that probability of recall of items was directly dependent on degree of learning. It has been already reported that mean degree of learning of items in each list declined with each additional list learned. Since probability of recall is found to be dependent on degree of learning, it is felt that the observed decline in mean degree of learning provides a more economical explanation of the decline in recall scores than does an explanation invoking an additional concept, proactive inhibition.

A second experiment investigated the relationship between degree of learning and retention of items over the same six lists when trials to criterion were held constant for each list. Mean degree of learning of items was found to increase over the six lists. The recall performance is again shown in Fig. 1 (Exp. 2). It can be seen that no consistent decline in recall scores occurred in these conditions. As in the previous experiment, it was found that probability of recall was directly dependent on number of correct anticipations (that is, on degree of learning).

While the variations in degree of learning over the six lists are again taken to be the major determinant of recall score, it is however likely that an additional factor is affecting the results of the second experiment. There was a significant tendency for a given degree of learning to be associated with a lower probability of recall with each additional list learned. It is possible that this represents proactive inhibition, although other possibilities cannot be ruled out (for example, a decline in the amount of extra-experimental rehearsal of items). A similar tendency was observed in the results of the first experiment, but this was not statistically significant. If this second factor in the results of these two experiments is in fact proactive inhibition it clearly is not the main causal factor determining recall of items.

This work was supported by a grant from the Department of Scientific and Industrial Research to the Department of Psychology, University of Sheffield.

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University of Sheffield.

[1] Underwood, B. J., *Psychol. Rev.*, 64, 1 (1957).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 11

Institution of Mechanical Engineers, Industrial Administration and Engineering Production Group (at 1 Birdcage Walk, Westminster, London, S.W.1).—All-day Discussion on "Metallurgical and Engineering Problems in the Machining of Materials".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4 p.m.—Symposium on "Noise in Weaving Machinery".

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Emotional Reactions to Physical Illness".*

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. W. Sciama: "The Unity of the Universe".

Institution of Electrical Engineers, Electronics Division P.G.E8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. Lewin: "Microwave Theory or Microwave Green Fingers ?"

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1 (at 5.30 p.m.—Prof. Reinhold Baer (Frankfurt am Main): "The Reduced Rank of a Group".*

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Lords of the Forest" (Colour film).

Women's Engineering Society (at "Hope House", 46 Great Peter Street, London, S.W.1), at 7.30 p.m.—Dr. A. R. Flint: "The Channel Bridge Project".

Monday, March 11—Tuesday, March 12

Society of Chemical Industry, Chemical Engineering Group, the **Institution of Chemical Engineers, South-Eastern Branch;** and the **Institute of Petroleum** (at the Royal Commonwealth Society's Assembly Hall, Craven Street, London, W.C.2)—Symposium on "Fluidisation".

Monday, March 11—Thursday, March 14

Oil and Colour Chemists' Association (at the Royal Horticultural Society's Old and New Halls, London, S.W.1)—Fifteenth Technical Exhibition.*

Tuesday, March 12

British Computer Society (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 9.30 a.m.—All-day meeting on "Data Transmission".

University of London (at Wye College, Wye, near Ashford, Kent, at 5 p.m.—Prof Asger Klougart (Copenhagen): "Recent Advances in Danish Horticulture".*

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. Davis. "Lightning Flashovers on the British Grid".

Institution of Electrical Engineers, Science and General Division P.G.S8 (at Savoy Place, London, W.C.2.) at 5.30 p.m.—Discussion on "The Financial Background to Engineering Decisions" opened by Mr. R. E. Burnett.

University of London (at Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Prof. R. W. H. Sargent: "Chemical Engineering and Engineering Science" (Inaugural Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A Scheinin (Turku, Finland): "Vital Microscopy of the Dental Pulp".*

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. F. Alcock and Mr. W. M. Scott: "Some More Light on Diesel Combustion".

Institut Français du Royaume-Uni (at Queensberry Place, London, S.W.7), at 6.30 p.m.—Prof. Etienne Wolff "Les Agents Provocateurs des Malformations" (La Production Expérimentale de Monstruosités).

Wednesday, March 13

Society for Analytical Chemistry, Biological Methods Group (at the Friends Meeting House, Euston Road, London, N.W.1), at 10 a.m.—*Symposium* on "The Pharmacological Screening of New Drugs".

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Prof. G. Causey: "The Principles of Electron Microscopy for Histologists".*

Colour Group (Great Britain) (in the new Physics Department, Imperial College of Science and Technology, London, S.W.7), at 3 p.m.—Annual General Meeting. 3.35 p.m.—Dr. R. W. G. Hunt: "Printing Colour Negatives".

Geological Society of London (at Burlington House, Piccadilly, *London*, W.1), at 5 p.m.—Mr. R. G. Park: "The Structural History of the Lewisian Rocks of Gairloch, Wester Ross"; Dr. D. R. Bowes, Dr. A. E. Wright and Mr. R. G. Park: "Layered Intrusive Rocks in the Lewisian of the North-West Highlands of Scotland".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. O. Carter: "Pulse Techniques in Line Communications".

University of London (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. F. R. Johnson: "The Reactions of Epithelium to Injury"* (Last of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Prof. A. Rodger: "The Selection of Managers".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. S. S. Kutateladze: "Heat Transfer in Boiling".

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Mr. P. J. Geary. "Compilation of State-of-the-Art Surveys".

Institute of Metal Finishing, Organic Finishing Group (at the British Institute of Management, 80 Fetter Lane, London, E.C.4), at 6.30 p.m.—Mr. L. A. Bashford: "Chemistry of Synthetic Resins: The Whys and Wherefores".

Society of Instrument Technology, Control Technology Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. Stafford. "The Application of Computers as a Control Aid in the Development of Hovercraft".

Institute of Biology (at the Royal Entomological Society, 41 Queen's Gate, London, S.W.7), at 7.15 p.m.—Dr. J. M. Hirst: "Fungus Spore Dispersal in Plant Diseases".

Thursday, March 14

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. F. Lee, Mr. J. Scanlan and Mr. W. F. Watson: "The Cyclization of Natural Rubber".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. H. Quastler (Brookhaven): "Chemical Communication Systems in Cells".*

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. U. Grenander (Stockholm): "Probability Distributions on Algebraic Structures"* (Further lecture on March 18).

Chemical Society (at Burlington House, Piccadilly, London, W.1), at 6 p.m.—Scientific Papers.

Institution of Mechanical Engineers, Lubrication and Wear Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Elasto-Hydrodynamics".

Plastics Institute, Engineering Sub-Group (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. J. R. Martland: "Mould Design"; Mr. P. Spiro: "Electroformed Moulds"; Mr. A. S. Matthews "Special Problems Relating to Close Tolerance Injection Moulding".

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, London, W.C.2), at 7 p.m.—Mr. D. Hunter: "The Role of the Computer in Radar Systems".

Royal Institute of Chemistry, London Section (joint meeting with the **Polytechnic Chemical Society**, at the Polytechnic, Regent Street, London, W.1), at 7 p.m.—Dr. R. A. Jeffreys: "The Chemistry of Colour Photography".

Friday, March 15

Society for Water Treatment and Examination (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 9.30 a.m.—Eleventh Annual General Meeting.

Association of Applied Biologists (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.40 a.m.—Symposium on "The Effect of Environment on Crop Production".

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 2.30 p.m.—Meeting on "The Application of Inertia Navigation Systems to Air Transport".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. L. F. Boyd: "Astronomy of the Sun".

Saturday, March 16

Nutrition Society (at the Hospital for Sick Children, Great Ormond Street, *London*, W.C.1), at 10.30 a.m.—All-day Meeting on "Nutritional Problems in Infancy and Childhood".

Monday, March 18

Medical Research Council (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 4.30 p.m.—Prof. S. Spiegelman (University of Illinois). "The Use of DNA-RNA Hybridization as an Analytical Tool for the Solution of Biological Problems".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Gilbert White (Chicago University): "The Contribution of Geographical Analysis to the Planning of River Development".

Society of Chemical Industry (joint meeting of the **Pesticides, Fine Chemicals** and **Microbiology Groups**, at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Prof. B. Lythgoe and Dr. A. Rhodes: "The Chemical and Biological Properties of *Venturicidin*".

Institution of Electrical Engineers, Science and General Division P.G.S3 and **Power Division P.G. P7** (at Savoy Place, London, W.C.2), at 5.30 p.m.—*Dr. M. P. Reece*: "Circuit Interruption in Vacuum".

Institute of Metal Finishing, London Branch (at the Borough Polytechnic, Borough High Street, London, S.E.1), at 6.15 p.m.—Mr. R. J. Brown: "Electroplating and the Use of Plastics and Corrosion Resistant Materials".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Professor in Philosophy at the Memorial *University of Newfoundland*—Dean of the Faculty of Arts and Science, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, and The Secretary, Association of Universities of the British *Commonwealth* (Branch Office), Marlborough House, Pall Mall, London S.W.1 (March 15).

HEAD OF THE DEPARTMENT OF ELECTRICAL ENGINEERING, Enfield College of Technology, Enfield—Chief Education Officer (F. 3), Middlesex County Council, 10 Great George Street, London, S.W.1 (March 16).

SENIOR LECTURER/LECTURER (preferably with special interests in physiology or ecology or anatomy) IN THE DEPARTMENT OF BOTANY, University of Ghana—The Registrar, University of Ghana, P.O. Box 25, Legon, Accra; or the Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (March 18).

SECOND CHAIR IN THE DEPARTMENT OF MATHEMATICS (applicants should have special interests in the field of applied mathematics)—The Registrar, The University, Leicester (March 20).

SENIOR LECTURER or LECTURER (preferably with an interest in political philosophy) IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 22).

LECTURER IN PHYSICAL CHEMISTRY—The Registrar, Manchester College of Science and Technology, Manchester 1 (March 23).

LECTURER or ASSISTANT LECTURER (with special interests either (a) in experimental approaches to algology, or (b) in some aspect of the fine structure of plant cells) IN THE DEPARTMENT OF BOTANY—The Registrar, University College of Wales, Aberystwyth (March 23).

CHAIR OF PHYSICAL OCEANOGRAPHY—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (March 25).

MANAGER OF THE RESEARCH REACTOR "CONSORT" now under construction at Silwood Park, the Imperial College Field Station near Ascot—Dr. P. J. Grant, Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7 (March 25).

BIOCHEMIST (Scientific Officer grade) to work on plant enzyme reactions—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (March 30).

LECTURER IN PHYSICAL or INORGANIC CHEMISTRY; and a LECTURER IN THEORETICAL CHEMISTRY—The Secretary, The University, Edinburgh (April 1).

TURNER AND NEWALL RESEARCH FELLOW IN ENGINEERING, INORGANIC CHEMISTRY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 1).

TEACHING FELLOW (with an honours degree in science or equivalent qualifications) IN THE SCHOOL OF CHEMISTRY, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (April 5).

LECTURER (with postgraduate experience in research, coupled with a keen interest in teaching) IN PHYSICS at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, April 6).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOW IN CHEMISTRY, PHYSICS, ENGINEERING (including Chemical Engineering), METALLURGY, PHARMACOLOGY or related subjects—The Registrar, The University, Sheffield (April 8).

PROFESSOR IN PHILOSOPHY; and a SENIOR LECTURER/LECTURER IN PHILOSOPHY in the Faculty of Arts at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

READER IN THEORETICAL PHYSICS—The Secretary of Faculties, University Registry, Oxford (April 15).

SENIOR LECTURER or LECTURER IN ENGINEERING MATHEMATICS in the Faculty of Engineering and Architecture at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

RESEARCH ASSISTANT (organic chemist or biochemist), to undertake work on polysaccharides of intervertebral discs and connective tissues—The Secretary, Royal Infirmary, Bradford, 9, and the Registrar, Dept. (E/20), Bradford Institute of Technology, Bradford, 7.

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER, for research on marine plankton, hydrography and fisheries—The Officer-in-Charge, Oceanographic Laboratory, Scottish Marine Biological Association, Craighall Road, Edinburgh, 6.

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with an honours degree in mathematics and preferably an interest in biology) IN THE STATISTICS SECTION, for duties which include consultation and research on design and analysis of experiments, and co-operation with the research staff of the Institute in the statistical aspects of the work—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 63/6.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University of Wales, Aberystwyth. Memorandum No. 4, (1961): Synopsis of Discussions held on March 8th, 1961 on the subject of Aspects of Soil Climate. Edited by James A. Taylor. Pp. vi+44. 7s. 6d. Memorandum No. 5 (1962): Synopsis of Discussions held on March 21st, 1962 on the subject of Climatic Factors and Diseases in Plants and Animals. Edited by James A. Taylor. Pp. vi+56. 7s. 6d. (Aberystwyth: University of Wales, 1961 and 1962.) [151

Transactions of the Royal Society of Edinburgh. Vol. 65, No. 6: The Petrology and Sedimentation of Upper Ordovician Rocks in the Rhinns of Galloway, South-West Scotland. By Dr. Gilbert Kelling. Pp. 107–138. 10s. 6d. Vol. 65, No. 7: On an Arctolepid Fish Gemünden. By Prof. T. S. Westoll and R. S. Miles. Pp. 139–154+2 plates. 8s. 6d. (Edinburgh: Royal Society of Edinburgh, 1962.) [151

Meteorological Office. Scientific Paper No. 17: Extremes of Wind Shear. By A. F. Crossley. Pp. ii+23. (London: H.M. Stationery Office, 1962.) 3s. net. [171

Laboratory Animals Centre. Collected Papers, Vol. 11: The Environment of Laboratory Animals—Papers read at L.A.C. Symposium, 1962, with additional papers. Pp. 72. (Carshalton: Laboratory Animals Centre, M.R.C. Laboratories, 1962.) 12s. 6d. net. [171

Imperial College of Science and Technology, (University of London). Fifty-fifth Annual Report of the Governing Body, 1961–62. Pp. iv+138. (London: Imperial College of Science and Technology, 1962.) [171

Annual Report of the National Oceanographic Council, 1 April 1961—30 March 1962. Pp. vii+45+8 plates. (Cambridge: At the University Press, 1963.) 6s. net. [171

Annual Report of the Advisory Council on Scientific Policy, 1961–1962. (Cmnd. 1920). Pp. vi+45. (London: H.M. Stationery Office, 1963.) 3s. net. [171

Animals, Vol. 1, No. 1, (8 January, 1963). Editor-in-Chief: Armand Denis. Pp. 32. (London: Knowledge Publications, Ltd., 1963.) 2s. [171

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 45: Vertical Resolution and Line Broadening. By G. D. Monteath. Pp. 23. (London: British Broadcasting Corporation, 1963.) 5s. [171

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1054, Vol. 255, (17 January 1963): A Note on the Calculation of the Matrix Elements of the Rotation Group. By A. L. Altmann and C. J. Bradley. On the Symmetries of Spherical Harmonics. By S. L. Altmann and C. J. Bradley. The Crystallographic Point Groups as Semi-Direct Products. By S. L. Altmann. Pp. 193–240. 15s. No. 1055, Vol. 255, (17 January 1963): An Investigation into the Theory of Resonance Testing. By R. E. D. Bishop and G. M. L. Gladwell. Pp. 241–280. 12s. 6d. No. 1056, Vol. 255, (17 January 1963): The Lubrication of Rollers. IV: Measurements of Friction and Effective Viscosity. By A. W. Crook. Pp. 281–312. 10s. (London: The Royal Society, 1963.) [211

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Stanford Research Institute. Minerals in the Infrared—a Critical Bibliography. By R. J. P. Lyon. Pp. xii+76. (Menlo Park, California: Stanford Research Institute, 1962.) 1 dollar. [151

Proceedings of the Rudolf Virchow Medical Society in the City of New York, Vol. 20, 1961. Edited by Hans B Bojsch, Kurt Shalshe and Manfred Weichsel. Pp. vi+198. (Basel and New York: S. Karger, 1962.) 36 Swiss francs. [151

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Annals of the New York Academy of Sciences. Vol. 93, Article 8: Strontium-90 in the Soils of the New York City Area. By J. L. Kulp, A. Kaufman, R. Slakter Hurshman and A. R. Schulert. Pp. 761–774. (New York: New York Academy of Sciences, 1962.) [171

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 88: Cambrian Stratigraphy and Trilobite Faunas of Southeastern Newfoundland. By R. D. Hutchinson. Pp. ix+156+25 plates. 3 dollars. Paper 61-31: Mount Robson (Southeast) Map-Area, Rocky Mountains of Alberta and British Columbia. By E. W. Mountjoy. Pp. iii+114. 50 cents. Paper 62-8: Gods River Map-Area, Manitoba. By R. R. Potter. Pp. iv+12. 50 cents. Paper 62-18: Pre-Mississippian Succession of Northernmost Axel Heiberg Island, District of Franklin. By P. E. Fricker and H. P. Trettin. Pp. iv+24. 50 cents. Paper 62-30: Summary of Research: Office and Laboratory, 1961. By P. Harker and S. E. Jenness. Pp. iii+28. 25 cents. (Ottawa: Queen's Printer, 1962.) [211

University of Sydney: School of Agriculture. Report No. 5: a Report and Statistical Analysis of Factors Affecting the Yields of Canning Peaches on the Yanco No. 1 Irrigation Area, New South Wales. By L. N. Balaam and J. B. Corbin. Pp. 100. (Sydney: The University School of Agriculture, 1962.) [211

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Map 1137A: Groundwater Probability—Virden (East Half), West of Principal Meridian, Manitoba. Map 41-1962: Surficial Geology—Lethbridge (East Half), West of Fourth Meridian, Alberta. (Ottawa: Director, Geological Survey of Canada, 1962.) [211

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SCIENCE AND WORLD PEACE

THE International Conferences on science and world affairs, now known as the "Pugwash Conferences", of which ten have now been held, have, as Prof. J. Rotblat justly claims in his brief history of the Conferences[*], become established as an important and effective channel of communication between scientists for the study and discussion of the most complex problems facing mankind at the present time. This standing results from the resolute efforts of a group of scientists determined to retain an independent and unbiased outlook, though Prof. Rotblat admits that gradual development, seeking, learning and blundering have also contributed. Lord Hailsham's opening address to the tenth Conference in London last September, and also the messages received from Mr. Harold Macmillan, President Kennedy, Mr. Krushchev and the Acting Secretary-General of the United Nations, Mr. U. Thant, testify to the respect which the movement already commands. Moreover, even more tangible fruit may perhaps be seen in the proposal for sealed seismic stations as a means of detecting nuclear explosions made during the final steps of the Conference itself.

The proposal, which was endorsed in Moscow a week later at the seventh General Assembly of the World Federation of Scientific Workers, and has now been adopted as part of the official Soviet proposals for controlling the cessation of nuclear tests, involves instruments for detecting seismic disturbances sealed in containers which are placed in specified positions by nationals of the country whose territory is being inspected. If inspection stations elsewhere indicate a suspicious 'event', the sealed containers from a given area around the origin of the 'event' are required to be handed over to an international authority. The problem remains of ensuring that the containers have not been tampered with and that they have actually been placed in the specified positions, but it is believed that this could be established by a skilled seismologist from an examination of the recording of background tremors.

Lord Hailsham's opening address took a much wider view, however. Challenging the validity of the assumption that existing political institutions must necessarily continue and some sort of *modus vivendi* established between existing political systems, he urged that complete disarmament required co-operation in positive measures to promote human welfare and also an examination of the need for a central political authority. This, he felt, was particularly emphasized by the nature of the nuclear weapon and by the needs of under-developed countries for aid. There was an established opportunity for international co-operation in the purely scientific field; but, as regards international aid, what was required above all was the application of known technology for the development of human happiness and culture—means which by themselves offer no political advantage to any Power Bloc over another.

What is interesting about Lord Hailsham's address is the extent to which his ideas are reflected in the statement issued after the Conference. The statement affirmed that the scientists from thirty-five countries assembled at the Conference were united by an awareness that the scientific revolution had created a radically new situation for humanity, endowing man with an unprecedented capacity for creation and destruction. Their chief concern was to prevent war and to relieve humanity of the fearful anxieties and the grave economic burdens caused by the arms race. A general war with nuclear weapons would be a disaster of unimaginable magnitude. Against global rockets with thermonuclear war-heads there is no effective defence, and in this situation the traditional view of war, or the threat of war, as an instrument of policy is obsolete. War must be eliminated from the life of mankind. Disarmament and a stable peace are essential conditions for making a new society in which poverty can be abolished and the prospect of such a world is no longer Utopian.

In the Pugwash Conferences, therefore, attention has been concentrated on the complex problems of disarmament, and discussion has led to the belief that solutions can be found for the economic problems which might be encountered while disarming, and the measures needed to ensure the confidence and security of States during the process and to keep the peace in a disarmed world. Those present reached the conclusion that in the process of general and complete disarmament the early elimination with adequate and effective verification measures, and within a short period of time, of the means of delivery of the weapons of mass destruction would constitute a satisfactory beginning when linked with substantial reductions of conventional military forces and armaments and with the solution of the problems arising from the presence of troops and bases on foreign soil.

The experience of the Conferences led them to believe that scientists have a special contribution to make to the solution of those problems. Informal discussions between scientists contribute to the understanding of different points of view; but we are in a period of very rapid change in which new problems are constantly emerging. Scientists should therefore increase their efforts to bring about disarmament and conditions of lasting peace and should invite Governments, learned societies and other institutions to support them in this task. Because increasing confidence between nations is an essential element in such progress, the Conference also considered constructive proposals for many kinds of international collaboration in science; but, the statement concluded, we are now at a stage when general statements of principles are not enough—action is needed. Some proposals to promote disarmament and human welfare need detailed study to assess their implications; others can be implemented immediately. The statement reaffirms the conviction that the goal of full disarmament and permanent peace is realistic and urgent, and part of a long struggle for the progress of mankind in which scientists have a responsible part to play; it calls on scientists everywhere in the world to join in this task.

Here the Conference is giving expression to the belief in the moral responsibility of scientists generally which the Bishop of Exeter considered more particularly in respect of nuclear scientists in a paper presented to the Conference on "Radiation and Social Ethics" at the University of Chicago on January 16. Recognition is required of the ethical dilemma which the Bishop discussed, namely, that in spite of the unpredictable results of research, the scientist must consider the probable or certain uses to which results of his successful research could be put. There is thus a certain limit of moral freedom in experiment which is probably one of the driving forces in the Pugwash movement. The Bishop pointed out, moreover, that the ethical dilemma was one which the scientist shared with the whole community of which he is a member, but he suggested that the nuclear scientist had a moral responsibility to conduct his experiments and operations so as not to endanger either the whole human race or the community adjacent to his place of work.

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[*] *Science and World Affairs*. History of the Pugwash Conferences. By Prof. J. Rotblat. Pp. 92+4 plates. (London: Dawsons of Pall Mall, 1962.) 8s. 6d. paper bound, 14s. cloth bound. (See also *Nature*, 196, 934; 1962.)

Here the argument runs close to the subject of the second part of Dupré and Lakoff's book, *Science and the Nation: Policy and Politics* (see *Nature*, 197, 629; 1962), and to the main theme of Mr. R. Gilpin's *American Scientists and Nuclear Weapons Policy* (see p. 1035 of this issue of *Nature*). This last Pugwash Conference, in fact, raises afresh fundamental issues over the participation of scientists in politics and the way in which scientists as such can contribute to or assist in the task of Government. To some extent this was obscured by the number of the practical proposals arising out of the Conference but which only makes it the more essential for a clear understanding of the nature of politics and of the contribution of science itself.

The Conference affirmed that all its activities should be directly related to the goal of full disarmament and permanent peace even when agreeing that subjects less directly relevant might appear on the Conference agenda. The work should in itself be scientific or technical or represent a problem which could be tackled by the scientific method. It should be noted, too, that the present policy is that the Conferences should be small and non-governmental and that they should not duplicate the work of other agencies. Nevertheless, the Conferences are evidently already taken seriously in Government circles, and apart from throwing up practical suggestions which Governments could adopt they undoubtedly have an educational function among scientists themselves in respect of what is involved in negotiation if not in politics and diplomacy also. Further, they encourage and bring into prominence the special claims of scientists to debate and make recommendations on world affairs—such as their technical knowledge and habits of fruitful and objective thinking, or the professional friendship and respect between scientists of the East and of the West, which facilitate frank and cool discussion of controversial matters.

Whether more effective means can be found of mobilizing scientific talent and enabling it to act to some purpose, both at the national and at the international level, in the task of Government, is the subject of urgent inquiry in Great Britain and in the United States at least. Here the Pugwash Conferences may well help. The last Conference recommended acceptance of India's invitation for a Conference on international collaboration in the developing nations and its significance for permanent peace, for which the United Nations Conference on the application of science and technology for the benefit of the less-developed areas in February at Geneva provided some pointers.

Two other proposals were made to extend the Pugwash method. Sir Julian Huxley advocated the sponsoring of a conference on education on the grounds that advances in science are changing the requirements of education, and suggested a series of East–West meetings to discuss the aims of education in a developing world. The other proposal was for a conference on the behavioural sciences with the view of considering how science could help to remove psychological barriers to disarmament and the peaceful resolution of conflict.

Although Mr. Gilpin only makes passing reference to the Pugwash movement, his book makes a most important contribution to a discussion as to the future of the Conferences and the direction which any expansion should take. The title of the book, in fact, belittles its scope, for although the main concern is with the part American scientists have played in the development of the United States policy on nuclear weapons, that is used to illustrate the much larger theme of the relations between scientists and Government. The book is really about the nature and limits of scientific and technical advice, the implications of the relations which have already been established, and, if it does not specifically discuss the nature of politics, it is just as realistic as Prof. Miller's book, *The Nature of Politics* (*Nature*, 196, 301; 1962), and is addressed more particularly to the scientist himself.

So far as the Pugwash Conferences are concerned, Mr. Gilpin notes that its significance lies in its attempt to utilize the international character of science as a force for peace and that it is the first such movement in which social scientists have actively participated. But while he notes also, and welcomes, its more modest ambitions, and the extent to which it has succeeded in avoiding exploitation by Communist propaganda, he notes also that leadership of the movement represents only one of the schools of thought among scientists on nuclear weapons. Nor does he provide much evidence to justify the belief that the international community of science as such is a force for peace and that through appeals to Russian scientists the Soviet Union can be guided towards a more rational pattern of behaviour.

Prof. Rotblat's book is too exclusively a record of the development and the official statements of the Pugwash Conferences to discuss these vital issues, nor does it enter on the issues raised by efforts of scientists to influence Governments and to educate the public on the implications of science. It is all the more valuable that at this juncture there should appear such a careful examination of the issues as Mr. Gilpin provides, for example, in his concluding chapter on the task of political leadership and in his analysis of the reasons which led to such strongly conflicting schools of opinion among scientists themselves. Both the dominant schools of opinion in the United States are fairly treated, and the analysis indicates how much more difficult it is to reach agreed opinion on the international plane and to tender technical advice which is not the opinion of only a section of the scientific community. However, a clear understanding of what is involved in the tendering of advice, and of the impracticability of a sharp separation between scientific or technical factors and political factors, is the first step towards the more effective utilization of the scientist and technologist which world order and world peace requires.

To such an understanding Mr. Gilpin's book makes an invaluable contribution. Moreover, it is not merely the negative one of pointing to past errors and the reasons for them. It shows also how the scientist, operating at the frontiers of knowledge, provides just that note of anticipation in political and social matters that is essential ■

if a wise national policy is to be formed. Likewise, it illustrates the scientist's contribution to the analysis of its problems arising out of scientific advice which any Government ought to apply to-day. While fundamentally there is little in the book on the relations between scientist and Government that Barker and Tizard and others in Great Britain have not previously underlined, there are some particular expedients adopted in the United States which might well be considered in other settings.

Fundamentally, however, in the international context such as the Pugwash Conferences, the book is a corrective to careless thinking about the relations of expert to expert and expert to Government, to the uncritical acceptance of expert advice, or to excessive optimism as to the possibilities in a scientific approach to policy-making. It drives us back once more to the fundamental importance of education at all levels and of public understanding. If it suggests that the pace of advance is likely to be slow, and to call for hard constructive thought, for sound judgment and human insight at all times, it nevertheless firmly upholds the place of the scientist as an adviser even on non-technical matters. Supporters of the Pugwash Conferences will find much in its pages for thought and caution: they will find no reason for pessimism.

THE SCIENTIST AS POLITICIAN

American Scientists and Nuclear Weapons Policy

By Robert Gilpin. Pp. vii+352. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1962.) 56*s*. net.

THIS study of the effect of scientific opinion on the function of nuclear weapons policy of the United States has a much wider interest than its title suggests. The study of the part of the scientists in the development of nuclear weapons is a major contribution to the larger theme of the place of the expert in the formation of policy generally, the mechanism of advice and the relation between science and technology and the task of Government. The particular subject of nuclear weapons is used to illustrate this general theme, and the implications of the participation of the scientist in the task of Government, the limitations, as well as the conditions, for effective contribution are clearly indicated. The essential issues are laid bare and, with its refreshing freedom from jargon, and the curse of uninterpreted abbreviations, this well-written book is a contribution to the study of the relations between science and Government that should be as welcome and acceptable in Great Britain as in the United States. Moreover, its documentation is comprehensive and balanced, dominantly, but not exclusively, American, and while some major British sources are not mentioned, the omissions are neither such as to mar the book nor to suggest more than pardonable oversight.

Mr. Gilpin begins with a glance at the questions raised by the participation of the scientist in politics in general, and especially those arising out of the formulation and tendering of scientific advice on the solution of the vast range of problems created by the advance of science. He is concerned to discern the conditions under which such advice contributes to the taking of effective decisions, and while he is particularly impressed by the uniformity and consistency of what he terms "scientific political opinion", Mr. Gilpin is particularly helpful when he analyses the political nature of the advice tendered by scientists and notes how that advice on technical matters is influenced by the scientist's non-technical assumptions about the problems at hand. Thus, it is that agreement on the scientific facts in a situation may lead different groups of scientists to tender differing and even opposed recommendations for dealing with the situation.

From the start, Mr. Gilpin emphasizes that the very nature of the scientific and technical adviser's responsibility to combine his technical expertise with his overall understanding and good sense takes him out of the realm where the scientific method and the scientist's canons of criticism are the most relevant guides for judgment into one where wisdom is the prime desideratum. In his next eight chapters he reviews the controversies that arose between scientists themselves and between scientists and Government over the development of nuclear weapons and their use, including the effect on the structure of the American administration before returning in his final chapter to discuss, in the light of all that experience, the task of political leadership and what that involves in this rapidly changing technological age. That study, in his view, demonstrates conclusively that scientists in the United States, in one particular field at least, have become full partners with politicians, administrators and officers of the Services in the formulation of policy; accordingly it is the task of political leadership to utilize more effectively and more generally the legitimate desire of scientists to contribute to the formulation of national policy.

The value of Mr. Gilpin's contribution does not lie so much in new and constructive suggestions as in pointing to weaknesses and mistakes in the past which have made the contribution of scientist and technologist relatively ineffective—weaknesses and mistakes that are to be remedied sometimes by the administrator or statesman, sometimes by the scientist himself. There are naturally suggestions that will be noted by the alert mind for examination in settings other than the American context in which they were first tried. In this concluding chapter Mr. Gilpin is strongly reminiscent of Sir Henry Tizard's Haldane Memorial Lecture in his emphasis on the importance of education—of having administrators who understand the nature and limits of science and of scientists who understand the nature of politics and the task of administration. To the view that the scientist as such should be 'on tap' and never 'on top' he returns a firm negative, with a clear warning against exaggerating the importance of ideas in the political process gives the unequivocable affirmation that scientists have a right to advise even on non-technical matters. It does not necessarily follow that because scientists are primarily technical experts they are incompetent to advise on political or strategic matters.

Where the book is specially valuable is in stimulating thought as to the nature and limitations of scientific advice, the implications for the adviser himself, as well as for the authority responsible for deciding on any particular administrative structure for advice, and the taking of decisions. This is particularly relevant at a moment when the whole structure of Britain's Government organization for civil research and development is under review and when Government departments are already considering the recommendations of the Gibb–Zuckerman report. Mr. Gilpin demonstrates the impracticability of any sharp separation of the technical and political realms in the advice tendered by scientists. It is in this context especially that the implications of his observations on asking the right questions, on the limits within which true objectivity is possible, on the interpretation of advice, on the importance of scientific controversy and an atmosphere of intellectual give-and-take deserve to be noted and pondered critically.

Mr. Gilpin's staunch advocacy of the place of the scientist and technologist in the task of Government is not over-stated. He fully recognizes the weaknesses of the scientist himself as well as of the policy-maker. The need still exists of developing means for countering the natural inclination of the scientist to advocate technical solutions to political problems and the tendency of the administrator to accept uncritically such solutions. This is in part a task of education, but it is also a task of organization—flexible and appropriate to this purpose, including the necessary professional institutions as well as Government structure. This book is admirably suited to stimulate the thought and study which must go to the discovery of ways of integrating the technical and political knowledge in policy-making which are effective in the conditions prevalent to-day. R. BRIGHTMAN

VETERINARY SCIENCE

Advances in Veterinary Science

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As in the case of previous volumes, Volume 7 of this book embraces in eight chapters a number of subjects of particular interest to veterinary scientists at this present time.

The first chapter deals with cardiac dynamics and its clinical significance. At a time when cardiac abnormalities, especially in horses and in dogs, are receiving the attention of research workers in many countries, this chapter is of particular interest and value.

The second chapter, which deals with experimental and spontaneous tumorigenesis, is of particular significance at a time when the origin of cancer is being sought and the roles of the endocrines system, viruses, parasites, food and radiation in this field are being examined. A consideration of all these factors is discussed in this important chapter.

In the realms of parasitology, current thought is largely centred on helminth immunity, a subject which is very fully dealt with in the third chapter. The widespread use of irradiated larval vaccines calls for a thorough understanding of the antibody reactions involved, and this chapter, which discusses each animal parasite in turn, covers very fully the story as it is now understood.

Of particular interest to workers in South Africa, the short but informative fourth chapter deals with enterotoxæmia-like conditions among sheep in that country.

The fifth chapter, dealing with non-specific or cross-sensitivity reactions to tuberculin in cattle, is of particular interest to scientists in all parts of the world, but more especially to those in countries where the tuberculin test is being applied as a means of eliminating or controlling bovine tuberculosis. The interpretation of the tuberculin test in cattle may be rendered complicated by the presence of the human or avian types of tubercle bacilli as well as by the presence of Johne's disease, nocardiosis, the so-called skin tuberculosis and the presence of other micro-bacteria. All the implications arising from these intercurrent infections are here discussed.

Skin diseases are dealt with in the following chapter. Of all the problems which concern the practising veterinarian, skin diseases, especially among small animals, are frequently the most baffling. The reader will find much in this chapter to enable him to crystallize his ideas on this subject and to appreciate the correlation between skin diseases in domesticated animals and those in man.

The seventh chapter is devoted to the subject of shipping fever in cattle, a condition which has been known in many parts of the world for a very long time, but about which there have been many conflicting ideas. The evidence put forward in support of a viral origin of this condition and the suggestion made that it is essentially a disease-complex rather than one of simple ætiology will attract the attention both of research workers and clinicians interested in or confronted by this problem.

The last chapter embraces the subject of mycotoxicoses. Quite recently, owners of poultry in particular have received sharp warning of the toxicity of certain mouldy feeding stuffs and it is very opportune that this subject should be so ably dealt with in this volume of *Advances in Veterinary Science*.

As in previous volumes, the author-index and subject-index leave nothing to be desired and the bibliography set out at the end of each chapter is admirably comprehensive. Chapters I, VI, and VIII, which readily lend themselves to illustration or diagram, are well supplied in that respect and their value is thereby enhanced. The authors of the several chapters are to be congratulated both collectively and individually on this production, which will be of the utmost value to its readers and which fully maintains the high standard of previous volumes.

H. BURROW

PHYSICAL CHEMISTRY IN METALLURGY

Physical Chemistry of Process Metallurgy

An International Symposium, Pittsburgh, Pennsylvania, April 27–May 1, 1959. Edited by George R. St. Pierre. Part 1: Pp. xiv + 644. 22.50 dollars. Part 2: Pp. xiv + 645–1374. 25 dollars. (Metallurgical Society Conferences, Volumes 7 and 8.) (New York and London: Interscience Publishers, 1961.)

In 1920 and again in 1949 the Faraday Society arranged symposia on the application of physical chemistry to process metallurgy. Since the latter date, there has been a considerable broadening of the field in many parts of the world, and in 1959 the American Institute of Mining, Metallurgical and Petroleum Engineers organized the conference the papers and lectures of which are contained in these volumes. How wide the interest was is shown by the fact that papers were received not only from the United States and Canada but also from Great Britain, Japan, Australia, Belgium, France and Switzerland. In any such symposium there is an inevitable variation in the quality of the contributions, but the standard here is almost invariably high, and the editors have done an excellent bit of work in their presentation. In addition to the papers themselves, the discussions add appreciably to the value of the information provided. This information is not only in the collection and re-presentation in condensed form of the authors' own work but also in not a few instances there is quite new information or new interpretations of earlier work.

Although the major emphasis is, somewhat naturally, on ferrous problems, the scope of the contributions also ranges over non-ferrous subjects varying from the vapour pressures of liquid metals and the velocity of sound in liquid lead–tin alloys to the oxygen content of copper mattes and the kinetics of the reduction of nickel sulphate in aqueous solutions. One of the outstanding features of the work discussed is the importance of the molten state, especially of slags, and the significance of the advances made to our knowledge of this field is most striking.

In spite of this, however, very much more needs to be done. As Prof. F. D. Richardson remarks in his introductory lecture to the conference, it is improbable "that in the foreseeable future we shall be able either to extrapolate our data or to calculate thermodynamic properties from first principles with the kind of accuracy that the operator of processes would like. The properties we need to know with great precision will still have to be measured. On the other hand, by means of our imperfect models and intelligent correlations, we should be able to predict accurately enough to avoid futile experiments in both works and laboratory".

The value of the symposium can scarcely be overestimated. F. C. THOMPSON

INVESTIGATION OF THE RADIO SOURCE 3C 273 BY THE METHOD OF LUNAR OCCULTATIONS

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THE observation of lunar occultations provides the most accurate method of determining the positions of the localized radio sources, being capable of yielding a positional accuracy of the order of 1 sec of arc. It has been shown by Hazard[1] that the observations also provide diameter information down to a limit of the same order. For the sources of small angular size the diameter information is obtained from the observed diffraction effects at the Moon's limb which may be considered to act as a straight diffracting edge.

The method has so far been applied only to a study of the radio source *3C* 212 the position of which was determined to an accuracy of about 3 sec of arc[1,2]. However, *3C* 212 is a source of comparatively small flux density and although the diffraction effects at the Moon's limb were clearly visible the signal-to-noise ratio was inadequate to study the pattern in detail and hence to realize the full potentialities of the method. Here we describe the observation of a series of occultations of the intense radio source *3C* 273 in which detailed diffraction effects have been recorded for the first time permitting the position to be determined to an accuracy of better than 1″ and enabling a detailed examination to be made of the brightness distribution across the source.

The observations were carried out using the 210-ft. steerable telescope at Parkes, the method of observation being to direct the telescope to the position of the source and then to record the received power with the telescope in automatic motion following the source. Three occultations of the source have been observed, on April 15, at 410 Mc/s, on August 5 at 136 Mc/s and 410 Mc/s, and on October 26 at 410 Mc/s and 1,420 Mc/s, although in October and April only the immersion and emersion respectively were visible using the Parkes instrument. The 410 Mc/s receiver was a double-sided band receiver, the two channels, each of width 10 Mc/s, being centred on 400 Mc/s and 420 Mc/s, while the 136 Mc/s and 1,420 Mc/s receivers each had a single pass band 1·5 Mc/s and 10 Mc/s wide respectively.

The record of April 15, although of interest as it represents the first observation of detailed diffraction fringes during a lunar occultation, is disturbed by a gradient in the received power and is not suitable for accurate position and diameter measurements. Therefore, attention will be confined to the occultation curves recorded in August and October and which are reproduced in Fig. 1. It is immediately obvious from these records that *3C* 273 is a double source orientated in such a way that whereas the two components passed successively behind the Moon at both immersions, they reappeared almost simultaneously. The prominent diffraction fringes show that the angular sizes of these components must be considerably smaller than 10″, which is the order of size of a Fresnel zone at the Moon's limb.

The most interesting feature of Figs. 1(*e*) and 1(*f*) is the change in the ratio of the flux densities of the two components with frequency. The ratio of the flux density of the south preceding source (component *A*) to that of the north following source (component *B*) is 1 : 0·45 at 410 Mc/s and 1 : 1·4 at 1,420 Mc/s, indicating a striking difference in the spectra of the two components. If it be assumed that the flux densities[3] of *3C* 273 at 410 Mc/s and 1,420 Mc/s are 60 and 35 Wm^{-2} $(c/s)^{-1}$ and that over this frequency-range the spectrum of each component may be represented by $S \propto f^{n}$, then the above ratios correspond to spectral indices for components *A* and *B* of − 0·9 and 0·0 respectively. The spectral index of *A* is a representative value for a Class II radio source; but the flat spectrum of *B* is most unusual, no measurements of a comparable spectrum having yet been published. If the spectral indices were assumed constant down to 136 Mc/s then at this frequency component *A* must contribute almost 90 per cent of the total emission, a conclusion which is confirmed by a comparison of the times of immersion at 136 Mc/s and 410 Mc/s on August 5.

It has been shown by Scheuer[4] that it is possible to recover the true brightness distribution across the source from the observed diffraction pattern, the resolution being subject only to limitations imposed by the receiver bandwidth and the finite signal to noise ratio and being independent of the angular scale of the diffraction pattern. However, in this preliminary investigation we have not attempted such a detailed investigation but based the analysis on the calculated curves for uniform strip sources of different widths as published by Hazard[1]. As a first step in the investigation approximate diameters were estimated from the intensity of the first diffraction lobe and the results corresponding to the three position angles defined by the occultations and indicated in Fig. 2 are given in Table 1.

As already indicated here, the 136-Mc/s measurements refer only to component *A* and hence no diameter measurements are available for *B* at this frequency. The 410-Mc/s observations of the August occultation are the most difficult to interpret owing to the components having both comparable flux density and small separation relative to the angular size of the first Fresnel zone. At immersion the widths were estimated by using a process of curve fitting to reproduce Fig. 1(*d*); at emersion (position angle 313°) the diameter of component *B* was assumed to be 3″ as indicated by the estimates at position angles 105° and 83°. The individual measurements at each frequency are reasonably consistent but there is a striking variation of the angular size of component *A* with frequency and evidence of a similar variation for component *B*. As at the time of the August occultation the angular separation of the Sun and the source was about 50° and hence coronal scattering of the type observed by Slee[5] at 85 Mc/s is not likely to be significant, this variation in size suggests that the model of two uniform strip sources is inadequate.

Therefore, a more detailed analysis was made of the intensity distributions of the lobe patterns given in Figs. 1(*c*) and 1(*f*), and it was found that in neither case can the pattern be fitted to that for a uniform strip source or a source with a gaussian brightness distribution. The 1,420-Mc/s observations of component *B* can be explained, however, by assuming that this source consists of a central bright core about 0·5″ wide contributing about 80 per cent of the total flux embedded in a halo of equivalent width of about 7″. Fig. 1(*b*), where component *A* predominates, suggests that this source has a similar structure

Table 1. EFFECTIVE WIDTH OF EQUIVALENT STRIP SOURCE
(Sec. of arc)

Frequency Mc/s	Component *A* Position angle 105°	313°	84°	Component *B* Position angle 105°	314°	83°
136	6·4	6·4	—	—	—	—
410	3·1	4·2† 2 (6)*	4·2	3·1	3·0†	2·7
1,420	—	—	2·9	—	—	2·1 0·5 (7)*

* Estimated from an analysis of the whole diffraction pattern.
† Component *B* assumed to have width of 3″.

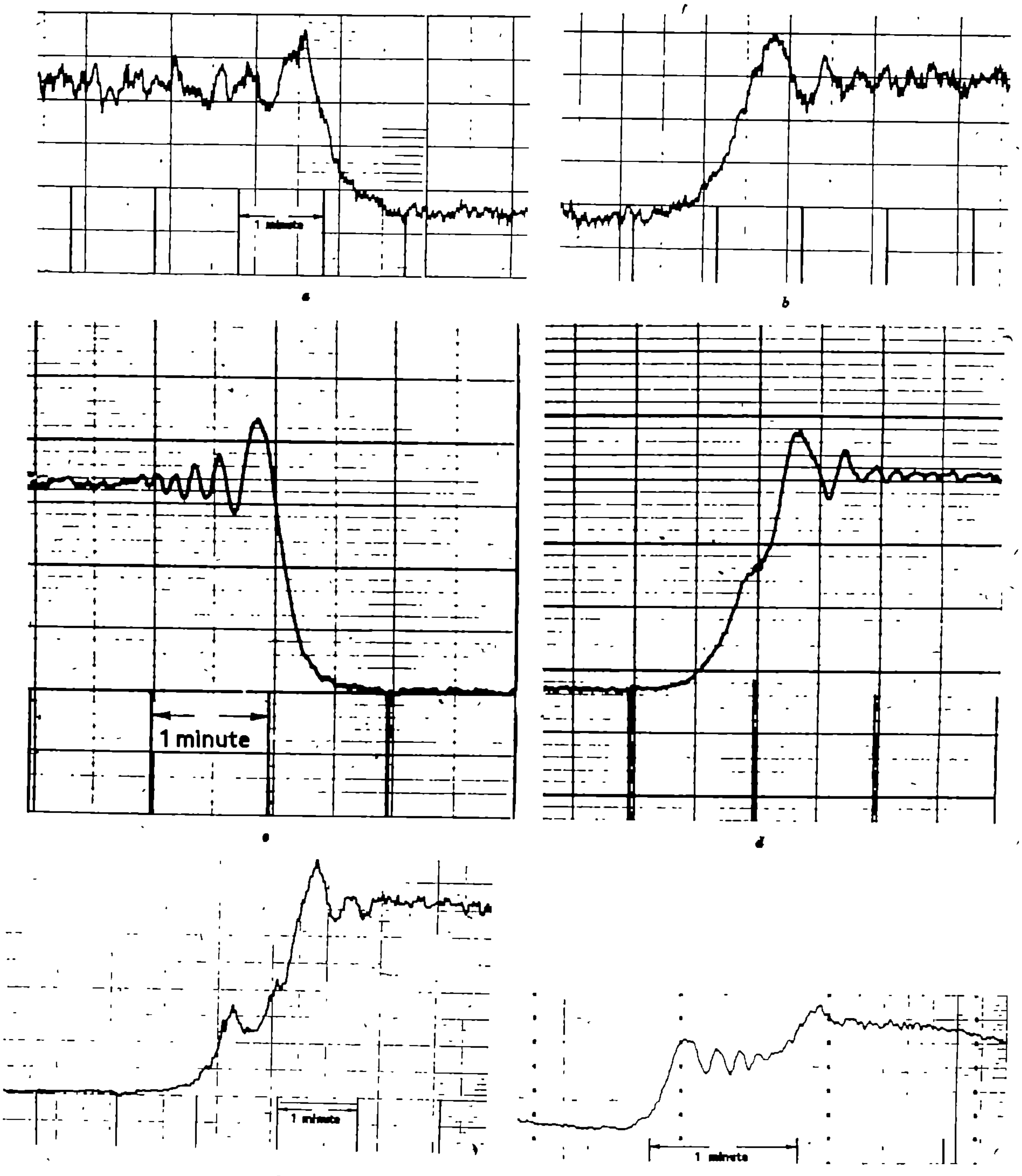

Fig. 1. Facsimiles of records showing occultations on August 5 and October 26, 1962, at different frequencies. (a) Emersion of August 5, 1962, at 136 Mc/s; (b) immersion of August 5, 1962, at 136 Mc/s; (c) emersion of August 5, 1962, at 410 Mc/s; (d) immersion of August 5, 1962, at 410 Mc/s; (e) immersion of October 26, 1962, at 410 Mc/s; (f) immersion of October 26, 1962, at 1,420 Mc/s. Abscissae, U.T.; ordinates, flux density

but with a core of effective width about 2″ at 410 Mc/s and a halo of width 6″. It therefore seems that the overall extent of both components are comparable but that the emission is more highly concentrated to the nucleus in *B* than in *A*. The close agreement between the halo size of *A* and its effective diameter at 136 Mc/s suggests that the observed variation of effective size with frequency may be due to a difference in the spectra of the halo and central regions. This would imply that the spectrum becomes steeper in the outer regions of the sources, that is, in the regions of lower emissivity. It is of interest that the integrated spectral indices of the two components show an analogous effect. Thus the spectrum of *B*, where most of the emission arises in a source about 0·5″ wide, is markedly flatter than that of *A*, where it arises in a source about 2″ wide.

The analysis is not sufficiently accurate to reach any reliable conclusions on the ellipticity of the individual components of *3C* 273, but allowing for the uncertainty in the estimated widths and position angle 314°, the 410-Mc/s observations indicate that both components may be elliptical with *A* elongated approximately along the axis joining the two components and *B* elongated perpendicular to this axis.

The position of each source was calculated from the observed times of disappearance and reappearance, which were estimated from the calculated flux density at the edge of the geometrical shadow and, where possible, from the positions of the diffraction lobes; these times are given in Table 2. In estimating the values of $T_D{}^A$ and $T_R{}^A$ from the 136-Mc/s records a small correction was applied for the effects of component *B*, this correction being estimated by comparison with the 410-Mc/s records. The corresponding times for *B* were estimated from the 410-Mc/s observations using the estimated position of component *A* and the known flux density ratio of the two components. For each component the times and associated errors given in Table 2 define three strips in each of which the source should lie; the centre lines of these strips represent the limb of the Moon at the time of observation and define in each case a triangular-shaped area. In principle, the position of the source lies in the area common to the three associated strips but it was found that for each component, and in particular for component *A*, that the size of the triangles defined by the Moon's limb was larger than would be expected from the estimated timing errors. This suggests that errors in the positions of the Moon's limb are more important than the estimated timing errors, and possibly that the effective position of the source varies slightly with frequency. The position of each source was therefore assumed to be given by the centre of the circle inscribed in the triangle defined by the Moon's limb at the relevant times. Dr. W. Nicholson of H.M. Nautical Almanac Office has kindly carried out these calculations and the estimated positions are as follows:

Component *A* (Epoch 1950)	R.A.	12h 26m 32·38s ± 0·03s
	Decl.	02° 19′ 27·8″ ± 1·5″
Component *B* (Epoch 1950)	R.A.	12h 26m 33·29s ± 0·02s
	Decl.	02° 19′ 42·0″ ± 0·5″

The average positions of the two sources given here represent the most accurate determination yet made of the position of a radio source. The quoted errors were estimated from the size of the triangles defined by the Moon's limb at the times of disappearance and reappearance, for the method is not subject to uncertainties introduced by refraction in the Earth's ionosphere or troposphere and is also free from the effects of confusion. A comparison of the times of disappearance and reappearance at different frequencies indicates that there is also no significant source of error due to refraction in either the solar corona or a possible lunar ionosphere; any refraction appears to be less than 0·3″ even at 136 Mc/s. This may be compared with the upper limit of 2″ at 237 Mc/s and 13″ at 81 Mc/s as estimated by Hazard[1] and Elsmore[6] respectively, and allows a new limit to be set to the density of the lunar ionosphere. Thus, from his observations at 81·5 Mc/s, Elsmore has set an upper limit to the electron density of 10^3 cm^{-3}; and it follows that the present measurements set a limit of about 10^2 cm^{-3}. Similarly, Buckingham[7] has estimated that at 50 Mc/s a ray passing at 50° to the Sun would be deviated by 1″ if the electron density in the solar corona at the Earth's distance from the Sun is 100 cm^{-3}. The present observations at 136 Mc/s and 410 Mc/s on August 5 indicate that at 50 Mc/s the deviation is less than 2″ at this angle, setting an upper limit to the electron density of about 200 cm^{-3}, which may be compared with an upper limit of 120 cm^{-3}, set by Blackwell and Ingham[8] from observations of the zodiacal light.

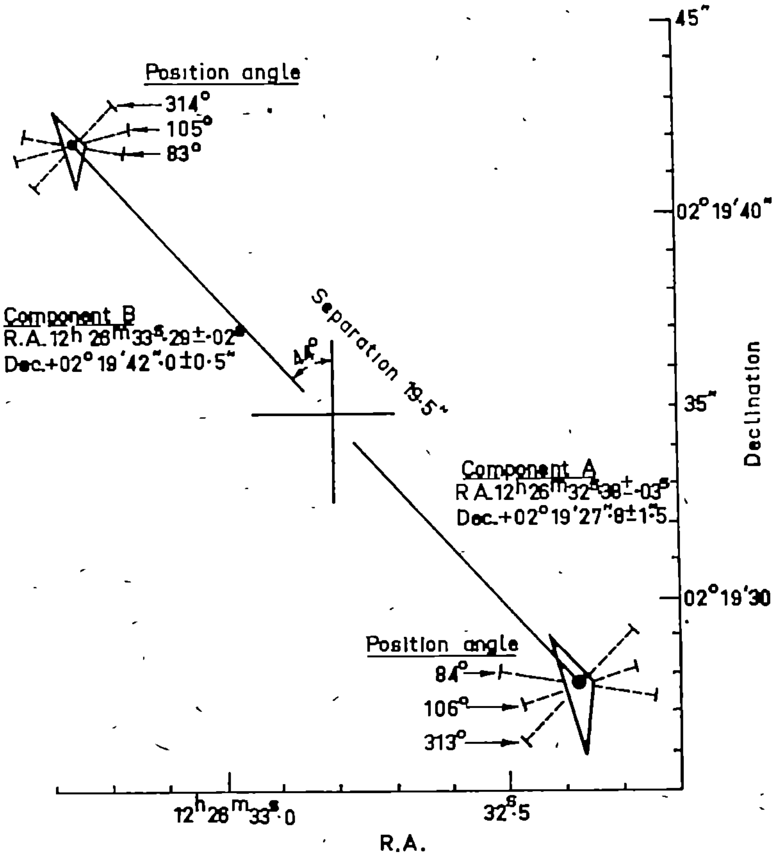

Fig. 2. Diagram of the radio source 3C 273. The sides of the full line triangles represent the positions of the limb of the Moon at the times of occultation. The broken lines represent the widths of the equivalent strip source as measured at 410 Mc/s for each of three position angles indicated

Table 2. OBSERVED OCCULTATION TIMES OF THE TWO COMPONENTS OF 3C 273

	Component *A* (U.T.)	Component *B* (U.T.)
Time of disappearance August 5, 1962	07h 46m 00s ± 1s	07h 46m 27·2s ± 0 5s
Time of reappearance August 5, 1962	09h 05m 45·5s ± 1s	09h 05m 45·7s ± 1 5s
Time of disappearance October 26, 1962	02h 55m 09·0s ± 1s	02h 56m 01·5s ± 0 4s

In a preliminary examination of a print from a 200″ plate it was noted that the position of component *B* agreed closely with that of a thirteenth magnitude star. We understand that the investigations by Drs. A. Sandage and M. Schmidt of the Mount Wilson and Palomar Observatories have revealed that this star and an associated nebulosity is very probably the source of the radio emission.

We thank Mr. J. G. Bolton for his interest in this work, and his assistance, with that of the staff at Parkes, in ensuring the success of these observations. We also thank Dr. W. Nicholson, who calculated the positions of the sources, for his valuable co-operation and interest in the occultation programme. One of us (C. H.) thanks Dr. E. G. Bowen for his invitation to continue occultation work at Parkes as a guest observer from the Narrabri Observatory of the School of Physics of the University of Sydney.

[1] Hazard, C., *Mon. Not. Roy. Astro. Soc.*, 124, 27 (1962).

[2] Hazard, C., *Nature*, 191, 58 (1961).

[3] Bolton, J. G., Gardner, F. F., and Mackey, M. B. (unpublished results).

[4] Scheuer, P. A. G., *Austral. J. Phys.*, 15, 333 (1962).

[5] Slee, O. B., *Mon. Not. Roy. Astro. Soc.*, 123, 223 (1961).

[6] Elsmore, B., *Phil. Mag.*, 2, 1040 (1957).

[7] Buckingham, M. J., *Nature*, 193, 538 (1962).

[8] Blackwell, D. E., and Ingham, M. F., *Mon. Not. Roy. Astro. Soc.*, 122, 129 (1961).

3C 273: A STAR-LIKE OBJECT WITH LARGE RED-SHIFT

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THE only objects seen on a 200-in. plate near the positions of the components of the radio source 3C 273 reported by Hazard, Mackey and Shimmins in the preceding article are a star of about thirteenth magnitude and a faint wisp or jet. The jet has a width of 1″–2″ and extends away from the star in position angle 43°. It is not visible within 11″ from the star and ends abruptly at 20″ from the star. The position of the star, kindly furnished by Dr. T. A. Matthews, is R.A. 12h 26m 33·35s ± 0·04s, Decl. +2° 19′ 42·0″ ± 0·5″ (1950), or 1″ east of component *B* of the radio source. The end of the jet is 1″ east of component *A*. The close correlation between the radio structure and the star with the jet is suggestive and intriguing.

Spectra of the star were taken with the prime-focus spectrograph at the 200-in. telescope with dispersions of 400 and 190 Å per mm. They show a number of broad emission features on a rather blue continuum. The most prominent features, which have widths around 50 Å, are, in order of strength, at 5632, 3239, 5792, 5032 Å. These and other weaker emission bands are listed in the first column of Table 1. For three faint bands with widths of 100–200 Å the total range of wave-length is indicated.

The only explanation found for the spectrum involves a considerable red-shift. A red-shift $\Delta\lambda/\lambda_0$ of 0·158 allows identification of four emission bands as Balmer lines, as indicated in Table 1. Their relative strengths are in agreement with this explanation. Other identifications based on the above red-shift involve the Mg II lines around 2798 Å, thus far only found in emission in the solar chromosphere, and a forbidden line of [O III] at 5007 Å. On this basis another [O III] line is expected at 4959 Å with a strength one-third of that of the line at 5007 Å. Its detectability in the spectrum would be marginal. A weak emission band suspected at 5705 Å, or 4927 Å reduced for red-shift, does not fit the wave-length. No explanation is offered for the three very wide emission bands.

It thus appears that six emission bands with widths around 50 Å can be explained with a red-shift of 0·158. The differences between the observed and the expected wave-lengths amount to 6 Å at the most and can be entirely understood in terms of the uncertainty of the measured wave-lengths. The present explanation is supported by observations of the infra-red spectrum communicated by Oke in a following article, and by the spectrum of another star-like object associated with the radio source 3C 48 discussed by Greenstein and Matthews in another communication.

Table 1. WAVE-LENGTHS AND IDENTIFICATIONS

λ	$\lambda/1{\cdot}158$	λ_0	
3239	2797	2798	Mg II
4595	3968	3970	Hϵ
4753	4104	4102	Hδ
5032	4345	4340	Hγ
5200–5415	4490–4675		
5632	4864	4861	Hβ
5792	5002	5007	[O III]
6005–6190	5186–5345		
6400–6510	5527–5622		

The unprecedented identification of the spectrum of an apparently stellar object in terms of a large red-shift suggests either of the two following explanations.

(1) The stellar object is a star with a large gravitational red-shift. Its radius would then be of the order of 10 km. Preliminary considerations show that it would be extremely difficult, if not impossible, to account for the occurrence of permitted lines and a forbidden line with the same red-shift, and with widths of only 1 or 2 per cent of the wave-length.

(2) The stellar object is the nuclear region of a galaxy with a cosmological red-shift of 0·158, corresponding to an apparent velocity of 47,400 km/sec. The distance would be around 500 megaparsecs, and the diameter of the nuclear region would have to be less than 1 kiloparsec. This nuclear region would be about 100 times brighter optically than the luminous galaxies which have been identified with radio sources thus far. If the optical jet and component *A* of the radio source are associated with the galaxy, they would be at a distance of 50 kiloparsecs, implying a time-scale in excess of 10^5 years. The total energy radiated in the optical range at constant luminosity would be of the order of 10^{59} ergs.

Only the detection of an irrefutable proper motion or parallax would definitively establish 3C 273 as an object within our Galaxy. At the present time, however, the explanation in terms of an extragalactic origin seems most direct and least objectionable.

I thank Dr. T. A. Matthews, who directed my attention to the radio source, and Drs. Greenstein and Oke for valuable discussions.

ABSOLUTE ENERGY DISTRIBUTION IN THE OPTICAL SPECTRUM OF 3C 273

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THE radio source 3C 273 has recently been identified with a thirteenth magnitude star-like object. The details are given by M. Schmidt in the preceding communication. Since 3C 273 is relatively bright, photoelectric spectrophotometric observations were made with the 100-in. telescope at Mount Wilson to determine the absolute distribution of energy in the optical region of the spectrum; such observations are useful for determining if synchrotron radiation is present. In the wave-length region between 3300 Å and 6000 Å measurements were made in 16 selected 50-Å bands. Continuous spectral scans with a resolution of 50 Å were also made. The measurements were placed on an absolute-energy system by also observing standard stars whose absolute energy distributions were known[1]. The accuracy of the 16 selected points is approximately 2 per cent. The strong emission features found by Schmidt were readily detected; other very faint features not apparent on Schmidt's spectra may be present.

The source 3C 273 is considerably bluer than the other known star-like objects 3C 48, 3C 196, and 3C 286 which have been studied in detail[2]. The absolute energy distribution of the apparent continuum can be accurately represented by the equation:

$$F_\nu \propto \nu^{+0{\cdot}28}$$

where F_ν is the flux per unit frequency interval and ν is the frequency. The apparent visual magnitude of 3C 273 is +12·6, which corresponds to an absolute flux at the Earth of $3{\cdot}5 \times 10^{-28}$ W m^{-2} (c/s)$^{-1}$ at 5600 Å. At radio frequencies[3] the spectral index is $-0{\cdot}25$ and the flux at 960 Mc/s is $5{\cdot}0 \times 10^{-26}$ W m^{-2} (c/s)$^{-1}$.

Between 6000 Å and 10,250 Å, eleven 120-Å bands were measured with an accuracy of 10 per cent. These measures indicate that the relatively flat energy distribution given in the equation here applies as far as 8400 Å. Beyond 8400 Å the flux may increase significantly. Between 3300 Å and 8400 Å the energy distribution cannot be represented, even approximately, by the flux from a black-body or a normal star. At least part of the optical continuum radiation must be synchrotron radiation.

During the course of the infra-red observations a strong emission feature was found near 7600 Å. Further observations with a 50-Å band-width placed the emission line at 7590 Å with a possible error of about 10 Å. The emission profile was found to be similar to that of the emission line at 5632 Å. Using this line and others in the visual spectrum Schmidt has shown that the most prominent emission features are Balmer lines and that the line at 7590 Å is *H*α. Using Schmidt's red-shift $\Delta\lambda/\lambda_0$ of 0·158, *H*α should appear at 7599 Å; this is in satisfactory agreement with observation, when it is recalled that the atmospheric *A* band absorbs strongly beyond 7594 Å. It is possible that the [*N*II] lines which have unshifted wave-lengths of 6548 Å and 6583 Å can contribute to the emission feature identified as *H*α. A large contribution, however, would shift the line significantly towards the red. The relative positions of *H*α, *H*β, *H*γ, and *H*δ cannot be produced by applying a red-shift to any other hydrogen-like ion spectrum.

Further observations, particularly in the infra-red, will be made in the near future.

[1] Oke, J. B., *Astrophys. J.*, 131, 358 (1960).

[2] Matthews, T. A., and Sandage, Allan, *Astrophys. J.* (in the press).

[3] Harris, D. E., and Roberts, J. A., *Pub. Astro. Soc. Pacific*, 72, 237 (1960).

RED-SHIFT OF THE UNUSUAL RADIO SOURCE: 3C 48

By Dr. JESSE L. GREENSTEIN

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AND

Dr. THOMAS A. MATTHEWS

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The radio source 3*C* 48 was announced to be a star[1] in our Galaxy on the basis of its extremely small radio diameter[2], stellar appearance on direct photographs and unusual spectrum. Detailed spectroscopic study at Palomar by Greenstein during the past year gave only partially successful identifications of its weak, broad emission lines; the possibility that they might be permitted transitions in high stages of ionization could not be proved or disproved. Hydrogen was absent but several approximate coincidences with He II and O VI were suggested.

The discovery by Schmidt (a preceding article) of much broader emission lines in the apparently stellar radio source, 3*C* 273, suggested a red-shift of 0·16 for 3*C* 273 if the lines were interpreted as the Balmer series. In 3*C* 48 no such series was apparent; measurable lines still do not coincide with the hydrogen series. However, the possibility of a very large red-shift, which had been considered many times, was re-explored successfully. 3*C* 48 has a spectrum containing one very strong emission feature near λ3832 which is 35 Å wide and about 10 other weaker features near 23 Å in width. The sharper lines are listed in Table 1 in order of decreasing intensity. Some broad lines or groups of lines between 50 and 100 Å width may be red-shifted hydrogen lines.

Table 1. IDENTIFICATIONS AND OBSERVED RED-SHIFTS

λ*	Wave-length λ lab.	Source	λ*/λ lab.
3832·3	2796 2803 } 2798	Mg II	1·3697:
4685·0	3426	[Ne V]	1·3676
5098	3729 3726 } 3727	[O II]	1·3679
4575	3346	[Ne V]	1·3673
5289	3868	[Ne III]	1·3671
4065·7	2975:	[Ne V]	1·3667:

The weighted mean red-shift $d\lambda/\lambda_0$ is $0{\cdot}3675 \pm 0{\cdot}0003$, an apparent velocity of +110,200 km/sec. The slightly discrepant value for λ2975 of [Ne V] is compatible with the uncertainty of ± 3 Å in the wave-length predicted by Bowen[3]. The Mg II permitted resonance doublet has a small additional displacement to longer wave-lengths, possibly caused by self-absorption in an expanding shell; it is the strongest emission line in the rocket-ultra-violet spectrum of the Sun. The forbidden lines are similar to those in other intense extragalactic radio sources.

So large a red-shift, second only to that of the intense radio source 3*C* 295, will have important implications in cosmological speculation. A very interesting alternative, that the source is a nearby ultra-dense star of radius near 10 km containing neutrons, hyperons, etc., has been explored and seems to meet insuperable objections from the spectroscopic point of view. The small volume for the shell required by the observed small gradient of the gravitational potential is incompatible with the strength of the forbidden lines.

The distance of 3*C* 48, interpreted as the central core of an explosion in a very abnormal galaxy, may be estimated as $1{\cdot}10 \times 10^{9}$ parsecs; the visual absolute magnitude is then $-24{\cdot}0$, or $-24{\cdot}5$ corrected for interstellar absorption. The minimum correction for the effect of red-shift is of the order of 2 *v*/*c* and a value between 4 and 5 times *v*/*c* is probable for a normal galaxy. The absolute visual magnitude of 3*C* 48 is then brighter than $-25{\cdot}2$ and possibly as bright as $-26{\cdot}3$, 10–30 times greater than that of the brightest giant ellipticals[4] hitherto recognized, which are near $-22{\cdot}7$ and another factor of five brighter than our own Galaxy, near $-21{\cdot}0$.

As a radio source at a distance of $1{\cdot}1 \times 10^{9}$ parsecs 3*C* 48 is not markedly different from other known strong radio sources like 3*C* 295 or Cygnus *A*. The one feature in which it does differ from most sources is in its high surface brightness. This is partially due to its extremely small radio size of $\leqslant$ 1 sec of arc[2]. The optical size is comparable, being also $\leqslant$ 1 sec of arc[5]. At the assumed distance such angular sizes indicate that both the optical and radio emission arise within a diameter of $\leqslant$ 5500 parsecs. The radio diameter might even be comparable with or less than that of 3*C* 71 (*NGC* 1068) the diameter of which is about 700 parsecs. However, 3*C* 71 has 5 orders of magnitude less radio emission.

If we determine the integrated radio emission of 3*C* 48 from the observed spectral index of the radio spectrum, and correct for the red-shift, we find that 3*C* 48 is comparable with 3*C* 295, emitting 4×10^{44} erg/sec of radio-frequency power. The cut-off frequencies were 7×10^{7} c/s and 10^{11} c/s. The lower limit is indicated by the observed radio spectrum and the upper limit is an assumed one.

The absolute magnitudes of the galaxies connected with 3*C* 295 and Cygnus *A*, corrected for interstellar absorption, are $M_v = -21{\cdot}0$ and $-21{\cdot}6$ (using a red-shift correction of 2 *v*/*c*) or $M_v = -22{\cdot}4$ and $-21{\cdot}8$ (using a correction of 5 *v/c*) respectively. Thus 3*C* 48 radiates about 50 times more powerfully in the optical region than other more normal but intense radio galaxies. In contrast, the absolute radio luminosity of 3*C* 48 is the same as that of Cygnus *A* and 3*C* 295. The unusually strong optical radiation may be synchrotron radiation as suggested (for other reasons) by Matthews and Sandage[5].

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HUMAN CANCER: MENDELIAN INHERITANCE OR VERTICAL TRANSMISSION?

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THE purpose of this article is to consider whether the familial tendency to malignant disease should be attributed to genetic inheritance or to the transmission of an oncogenic virus from either parent to offspring ('vertical' transmission[1]). It will be shown that this is one of those situations, rare in cancer research, where the available evidence is adequate to allow almost unambiguous conclusions to be drawn about an issue of fundamental importance.

Following many demonstrations of the vertical transmission of leukæmogenic viruses in mice, Gross[2] has recently stated: " . . . one could therefore regard the development of cancer, leukæmia, or that of any other [the printed form is not 'any other' but 'another': it is presumed that 'any other' is intended] malignant tumour, as the result of an activation, frequently merely accidental of an oncogenic agent, hitherto masked, and carried by the host since birth. . . . Oncogenic agents carried in descendants of certain families, in humans as well as in other species, may have a higher pathogenic potential and be more readily activated than those carried in other families. . . . Thus cancer, leukæmia, or other malignant tumours would develop more frequently in members of certain families than in others". Elsewhere it is made clear that the 'oncogenic agent' is a virus.

This authoritative and influential working hypothesis inspires much contemporary cancer research, and is supported by an enormous volume of experimental evidence obtained from chickens, mice and rats.

Nevertheless, it was claimed recently[3], in harmony with previous conclusions[4–6], that the 'inheritance factor' in human leukæmias usually takes the form of an apparent dominant 'predisposing' mutation of incomplete penetrance; furthermore, the several ætiologies of the various forms of this neoplastic disease are all polygenic in nature[3]. Haldane[7] in 1938 arrived at a similar conclusion with respect to cancer in general on the basis of Little's[8] statistical investigations of cancer inheritance. In rare families, but especially in those where the parents are consanguineous, childhood leukæmias and lymphomas can exhibit apparent recessive inheritance[9,10].

I have deduced[3,11] that malignancies in adult human beings generally develop from one or more cells containing a total of four specific (nuclear) gene mutations, one of which is often inherited, the remainder originating in one or more somatic cell lines.

The incompleteness of the penetrance of the inherited mutation arises because the probability of a somatic cell accumulating the fourth and final 'carcinogenic mutation' within the normal life-span is less than unity.

The evidence from families where the parents are consanguineous[9,10] indicates that two out of the four 'carcinogenic' mutations affect both homologous genes at a particular locus. More indirect evidence and arguments[12] are consistent with the view that the remaining two mutations affect both homologous genes at another locus. The ætiology of many childhood and adolescence malignancies[3] should involve a primarily non-genetic 'stress' factor such as (1) a hormonal stimulus; (2) a pyogenic infection (notably pneumonia[9,13]); or (3) infection, by 'horizontal' transmission, with an oncogenic virus. Direct epidemiological support for (3) has lately been obtained in the ætiology of a malignant lymphoma in children living in certain regions of Africa[14].

Familial Leukæmia and Mutation Frequency

By calculating (1) the size of (genetic) carrier subpopulations; (2) the 'penetrance' of an inherited mutation in the phenotypic form of a childhood leukæmia, the approximate frequency of childhood leukæmia in multiple sibs[9] can be accounted for[3]. Using Haldane's[15] "indirect method" for the calculation of mutation frequencies (where approximate genetic equilibrium is assumed) the derived values for the mutation frequencies of 'leukæmogenic' genes are closely comparable with known frequencies at other loci[3,11]. They lie at the upper end of the normal range[16] for dominant and sex-linked loci; but they include some effects attributable to gross chromosomal changes. Thus, trisomy for chromosome 21 (mongolism, or Down's syndrome) is associated with a very high leukæmia incidence in childhood[9,17,18], and the discovery of the *Ch*^{1} chromosome by Gunz *et al.*[19] shows that the inheritance of a chromosome 21 from which a portion of the short arm has been either deleted or translocated, predisposes to chronic lymphatic leukæmia.

There is another source of uncertainty that could introduce an overestimation of the mutation frequency. It is assumed that only two major 'leukæmogenic' loci are implicated with respect to each of the two groups of genetically related leukæmias[3]. Although immunological evidence indicates that one specific structural locus may be involved in the ætiology of many carcinomas[12], and although the phenomena of the *Ph*^{1} and *Ch*^{1} chromosomes[19,20] are consistent with specific loci being associated with leukæmogenesis, the possibility cannot be dismissed that there may be several loci of similar phenotypic expression. It is evident from the calculated mutation frequency that the number of major 'leukæmogenic' loci cannot be high, but it could be greater than two. (Other loci will undoubtedly affect the phenotypic expression of the major loci through their influence on factors such as growth, or growth-rate, but their net effect on the calculated mutation frequency should in general be small.)

The measure of agreement between established mutation frequencies and those calculated for the hypothetical major 'leukæmogenic' genes is obviously of considerable theoretical significance; it is rather unlikely that it represents a mere chance coincidence.

Direct Cytogenetic Evidence for an Inheritance Factor

With regard to the theoretical ætiology of chronic lymphatic leukæmia it was stated[3]: "Chronic lymphatic leukæmia in adults mostly arises from one or more cells containing one inherited and three somatic mutations". " . . . furthermore, a different set of common alleles may be involved in chronic lymphatic, chronic and acute myeloid, and some stem-cell leukæmias, together with certain pernicious anæmias and some cancers. . . . Given the predisposing dominant inheritance, the penetrance in the form of a specific leukæmia is typically of the order of 10 per cent for a normal life-span of 70 years".

The subsequent report of Gunz *et al.*[19] now offers considerable support for several of these predictions. Their findings show or suggest: (1) an association between a mutation of one chromosome of the pair 21 (the Ch^1 chromosome) and chronic lymphatic leukæmia in adults; (2) the inheritance of this defective chromosome yields a viable karyotype (there is as yet no indication that the Ph^1 chromosome[20] can be inherited); (3) the inherited Ch^1 chromosome is evidently a 'predisposing' factor—some adults are carriers of the defective chromosome without exhibiting any clinical signs of chronic lymphatic leukæmia; (4) if both chronic lymphatic and chronic myeloid leukæmias involve a common set of alleles, then the Ph^1 and Ch^1 chromosomes indicate that at least two loci (one on the long, and the other on the short arm of the acrocentric chromosome) are implicated in human leukæmogenesis; (5) a chromosomal deletion or translocation can again be phenotypically equivalent to a gene mutation[3]; (6) if the father of the 'C' family was a carrier, then the Ch^1 chromosome also predisposes to 'sarcoma of the mediastinum'; (7) if the sister (No. 5) of the propositus was a carrier, then the Ch^1 chromosome also predisposes to carcinoma of the uterus and to pernicious anæmia.

This cytogenetic evidence is probably relevant to rare families only. Out of eleven families Gunz *et al.*[19] have so far investigated in which there is at least one case of chronic lymphatic leukæmia, they have found only two where members exhibit the Ch^1 chromosome; it is therefore still possible that the normal mode of leukæmia and cancer 'inheritance' is neither a gross chromosomal-, nor a gene-mutation, but a vertically transmitted oncogenic virus.

To test this more general hypothesis it is necessary to examine evidence relating to large, for example, national populations.

Leukæmia Pedigree Evidence

Gross[3] points out " . . . However, transmission of certain oncogenic viruses *in utero*, or immediately after the birth of the hosts through the milk of nursing mothers, or by contact infection from parent to the new-born offspring, would also be included in the general pattern". We should expect, therefore, that transmission will occur with far greater frequency from the mother than from the father, and indeed it has been shown[21] that transmission of the Gross and Moloney viruses in mice occurs only via the mother. Videbaek[4] and Guasch[22] collected (independent) pedigrees of human "leukæmic families" from the literature and their own investigations. Videbaek's[4] collection (his Fig. 3) indicates that leukæmia was apparently 'transmitted' via the father in 16 instances and via the mother in 21. Guasch's[22] collection (his Tables 15–18) shows 18 examples of 'transmission' via the male, and 13 via the female. These small numbers therefore show equal frequencies of 'transmission' from the father and from the mother. This evidence is at variance with Gross's suggestions, but consistent with Mendelian autosomal inheritance to a remarkable (if somewhat fortuitous) degree.

A similar pattern of 'transmission' is found when all cancers are considered. Table 1 contains a summary of Little's[8] analysis of the incidence of cancer (all types) among the sons and daughters of cancerous fathers or mothers (four categories in all). The slight excess of 'transmission' from the mother is not statistically significant.

Table 1. INHERITANCE OR TRANSMISSION OF CANCER IN MAN*

Cancerous parent	Offspring investigated	No. investigated	No. cancerous	No. expected on chance basis	Ratio: No. observed / No. expected	
Father	Sons	453	14	0·85	16·5	12·6†
Father	Daughters	788	10	1·05	9·5	
Mother	Sons	565	12	1·19	10·1	14·0†
Mother	Daughters	504	27	1·59	17·1	

* Adapted from Little (ref. 8). The expected number of cases (column 5) was based on United States national statistics (1909–12). It is possible that case-finding in Little's sample (taken from the Eugenics Record Office) was better than the national average, and hence all ratios (last column) may be too high. There is no reason, however, to suspect a sex bias in the cancer statistics. Little gives no explanation of the sex-ratio for the offspring of cancerous fathers.

† Average for combined sons and daughters.

It could still be claimed that human oncogenic viruses are intimately associated with the genome, as in the phenomenon of lysogeny in bacteria. If such were the case, the 'lysogenic-type' of oncogenic virus could in principle be transmitted as readily via the sperm as the ovum. Evidently this is not the mechanism for transmitting the Gross and Moloney viruses in mice, and neither would this mechanism display normal Mendelian segregation—except by chance in rare instances. Nevertheless one may, for the sake of argument, disregard the indications of apparent dominant, and (very occasionally) recessive-inheritance, and consider instead certain broad features of cancer mortality.

Broad Aspects of Cancer Mortality

If age-specific total cancer mortality rates are plotted as a function of time, these are more or less steady in England and Wales among the lower age-groups, during the period 1915–53 [23]: a similar phenomenon is observed in the United States mortality statistics[24] over the period 1900–53. It must be admitted that it is difficult to know what significance can be attached to such statistics. They are complicated by advances in diagnosis and therapy, and the changing influence of environmental carcinogens. In the case of childhood leukæmias, other complexities have been demonstrated. Stewart and Barber[13] have directed attention to an impressive inverse correlation between mortality-rates from pneumonia and leukæmia in children during the period 1912–59. This phenomenon will be discussed at the end of this article.

Complications apart, a steady incidence-rate of pre-zygotic cancers over the years is to be expected on the basis of Mendelian inheritance. In situations of genetic equilibrium, the selective mortality among carriers before and during the reproductive phase is balanced by the introduction of new spontaneous mutations into the population. A general increase (decrease) in the mutation-rate would, of course, raise (lower) the incidence-rate of pre-zygotic malignancies until a new equilibrium would eventually be attained.

Consider in this context Gross's working hypothesis[3] of the vertical transmission of oncogenic viruses. To maintain an equilibrium incidence of cancer in the population by such a mechanism it is necessary for the virus host (mother or father) to transmit the oncogenic virus, on the average, to slightly more than half their offspring. (The selective mortality among recipients up to and during the reproductive age requires that more than half the offspring should be affected at birth—or during the period of infective contact with parents.) It is obviously impossible to achieve a stable equilibrium through vertical transmission. A slight average deviation upwards from the 'equilibrium transmission faction' would lead to an initially exponential increase in the proportion of hosts in the population until ultimately all members of the population would be affected. On the other hand, a slight departure downwards would lead to an exponential decrease in the proportion of hosts and their eventual disappearance. Any temporary equilibrium would be highly unstable.

Abandoning Gross's particular hypothesis, this leaves open the logical possibility that every individual is in fact a host to oncogenic viruses (of equal pathogenic potential from one individual to another) and that these are necessary to cancer development, but additional to all the genetic and other factors previously described[8]. On present evidence, this logical possibility is superfluous.

Evidence from Studies of Twins

On Mendelian theory, if one monozygotic twin is a genetic carrier the co-twin will also be a carrier. For the common case of apparent dominant autosomal inheritance of a single 'carcinogenic mutation', if one dizygotic twin is a carrier, the average chance of the co-twin being a carrier is 0·5. Neglecting post-zygotic cancers, the frequency of concordant cases of cancer in monozygotic twins should therefore be twice that in dizygotic twins. The phenomenon of post-zygotic cancers will, on the average, affect all non-carrier twins equally, and this will 'dilute' the ratio, to make it slightly less than 2. On Gross's vertical transmission hypothesis (*ad hoc* assumptions apart) the cancer concordance-rates should be identical in both types of twins. The results in a recent investigation[24] were combined with four previous investigations to show that the average (uncorrected) concordance-rates are 9·8 per cent for 255 monozygotic twins and 5·1 per cent for 704 dizygotic pairs; the difference is significant at the 0·01 level of confidence[25]. The nominal ratio of concordance-rates (1·9) provides further remarkable support for the hypothesis of Mendelian inheritance of 'cancer predisposition'. (It should be noted that in these studies the average penetrance of an inherited carcinogenic mutation in the phenotypic form of any cancer is $\sqrt{9{\cdot}8 \text{ per cent}} = 0{\cdot}3_1$.)

Conclusions

On the basis of all the foregoing evidence, Gross's working hypothesis of human carcinogenesis quoted at the beginning of this article is, as a general proposition, untenable. Nevertheless, the possibility cannot be excluded that it may be applicable in particular (but rare) instances. In a modified form of the vertical transmission hypothesis described here (with every individual a host to similar oncogenic viruses) the hypothesis is, on present evidence, superfluous, but not necessarily false. In this modified hypothesis, vertical transmission viruses could logically constitute familially neutral oncogenic factors additional to the genetic and other factors described here, previously[8] and in the next article[16].

Two outstanding enigmas remain.

(1) Why should the vertical transmission of oncogenic viruses be such a conspicuous feature of the ætiology of malignancies in small, short-lived animals, and so inconspicuous in man? It is suggested that the solution to this problem is an immunological one, and it will be discussed in an article to appear in the next issue of *Nature*.

(2) Although two large-scale statistical surveys[4,6,17] yield apparently unambiguous evidence of an abnormally high incidence of leukæmia in the relatives of leukæmic subjects, three other surveys[19,22,26] give no indication of familial leukæmia. Quite apart from theories of carcinogenesis, these apparent contradictions are disturbing in themselves, and the failure of three statistical investigations to reveal a familial tendency to leukæmia runs contrary to all the evidence relating to particular leukæmic families. Much of this latter evidence cannot be explained, even remotely, on a chance basis[27].

Videbaek's survey[4] was carried out in Denmark, and he found 17 confirmed cases of leukæmia in the families of 209 leukæmic patients, and only 1 in the families of 200 matched, healthy controls. Busk's[18] criticisms of this investigation have been answered by Videbaek[23]. According to the present theory, the maximum ratio of familial leukæmic incidence in the two groups should have been about 10, although the probable ratio is appreciably lower. However, even if the 'true' ratio is as low as 5, it is still consistent with Videbaek's small numbers. Steinberg[19], acting on Busk's[18] criticism, dispensed with controls, and compared the leukæmia mortality (assessed by interview and correspondence) in the families (grandparents, parents, aunts, uncles and sibs) of 249 children who had acute leukæmia with the 'expected mortality' based on 1940 and 1950 statistics. No significant difference was found (based on only 4 cases of familial leukæmia). However, the crude death-rate from leukæmia in the United States increased by more than a factor of 4 between 1921 and 1951[20], and little significance can be attached to Steinberg's conclusions regarding his statistical investigation. To minimize errors associated with cohort effects (however caused) and faulty memory recall, the use of a carefully matched control group as advocated by Videbaek[4] would appear to be indispensable to this type of investigation.

Morganti and Cresseri[26] carried out such an investigation, with controls, in Italy. In the families of 476 leukæmic probands, they found only 3 cases of leukæmia, as compared with 2 cases in the families of controls. In Guasch's[22] personal investigation in Spain, one case of leukæmia was found in the 2,290 members of the families of leukæmic patients and four in the 4,340 members of the families of controls. Both these surveys, therefore, give no indication of a familial tendency to leukæmia. However, a fascinating aspect of Morganti and Cresseri's study[26] is the demonstration of a significantly higher incidence of non-leukæmic malignancies in the families of leukæmic patients as compared with controls. The ratio they found was 1·3 (228/175 cases). Videbaek found a slightly higher ratio (1·4) and concluded "that there exists a relation between leukæmia and cancer".

In the terms of the present theory[8], a gene mutation that strongly predisposes to leukæmia development also markedly increases the susceptibility to certain other cancers. (As already mentioned, other genes will have some influence on the carcinogenic process. In mice, the agouti locus which affects growth-rate and total growth also influences the incidence of mammary tumours, the faster-growing, larger mice having, as would be expected, the higher incidence[21]. Furthermore, various hormones (especially the sex hormones) are known to modify the carcinogenic process, and it is obvious that these agents must be influenced by genes other than the major 'carcinogenic' ones.) Where the numbers of cases found were high enough to impart statistical significance, Morganti and Cresseri's data therefore indicate that the proportion of "leukæmogenic-carcinogenic genetic carriers" was higher in the families of leukæmic patients than in the families of controls. One of the principal reasons why Morganti and Cresseri[26] and Guasch[22] were unable to find statistical evidence for the genetic factor in leukæmias demonstrated by Videbaek[4] and by Stewart[6] is the low mortality from leukæmia in pre-1950 Italy and Spain, as contrasted with pre-1945 Denmark, and with England, Scotland and Wales during 1953–55. A low overall mortality rate, is, of course, equivalent to a lower expectation of mortality per individual. Hence in countries with a low overall incidence of leukæmia, the 'test' populations need to be much larger to demonstrate the existence of pre-zygotic leukæmias in the presence of a 'background' of post-zygotic leukæmias. The following discussion will amplify this point.

An interpretation of the marked differences in leukæmia mortality rates between different countries has been proposed[23]; but a brief recapitulation will be given here.

'Leukæmic mutations' in the malignant cell must abrogate the normal control of cellular differentiation and mitosis[12]. It is proposed that some degree of impairment exists in the pre-leukæmic genetic carrier and, as a consequence, the immune defence mechanism is weakened. This hypothesis readily accounts for the inverse correlation mentioned earlier between pneumonia and leukæmia mortality in children[18]; but it is proposed[32] that adult 'leukaemic genetic carriers' are likewise more prone than non-carriers to death from certain infectious and parasitic diseases. On this view, in countries where there is a high incidence of these diseases, there should be a low mortality from leukaemia and aleukaemia because of selective mortality from infectious disease among 'leukaemic genetic carriers'. The truth of this prediction is illustrated in Fig. 1. Furthermore, changes of mortality from infectious disease within a given country, over the years, should be followed by inverse changes in mortality from leukaemia. The truth of this second prediction is well known, and is illustrated in Fig. 2 with respect to statistics for England and Wales. (The mathematical form of these relationships will be exceedingly complicated.) In this context it is noteworthy that patients with diagnosed chronic lymphatic leukaemia generally exhibit hypogammaglobulinaemia[36,37] and frequent infectious complications[37].

Hence, in environments where mortality from infectious disease is high, mortality from pre-zygotic leukaemias should be low. Mortality from post-zygotic leukaemias, on the other hand, should be relatively unaffected—the immune defence system in non-carriers will not in general be impaired—consequently the ratio of pre-zygotic to post-zygotic leukaemias will be greatly diminished in countries where there is a high mortality from infectious disease. We now see why Videbaek[4] in Denmark—the country with the world's lowest recorded mortality rates from infectious disease and the world's highest mortality rates from leukaemia—was able to detect the familial tendency to leukaemia in a statistical survey, whereas in Italy and Spain, countries with a relatively high mortality from infectious disease, no such effect was detectable.

Nation-wide surveys are clearly necessary to elucidate the genetics of all forms of malignant disease through an analysis of pedigrees. It is very difficult to escape the conclusion that among the many aetiological factors in human carcinogenesis there exists a genetic basis.

Note added in proof. Dr. F. W. Gunz and his colleagues, Christchurch Hospital, New Zealand, have investigated 87 per cent of all cases of leukaemia arising in New Zealand during the period of their investigation. In a private communication to me, Dr. Gunz states: "Our figures for familial leukaemia support Videbaek, but our material is larger than his". Thus another large-scale survey in a country where there is a relatively low mortality from infectious disease (Fig. 1) confirms the existence of familial factors in leukaemogenesis.

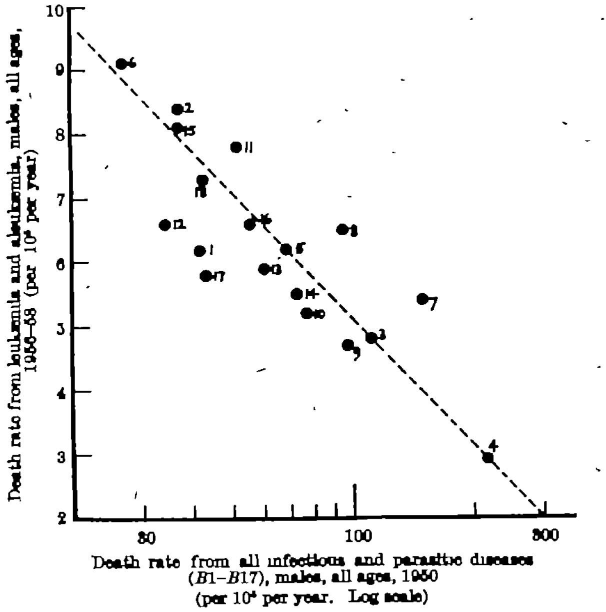

Fig. 1. Relation between gross mortality rates in males, from leukaemia and aleukaemia (ref. 33) (1956–58), and all infectious and parasitic diseases (ref. 34) (*B*1–*B*17 International Abridged List, 1948) for 1950, in different countries. (Changes in leukaemia mortality should lag behind changes in mortality from infectious diseases)

1, Canada. 2, U.S. White, 3, U.S. non-White, 4, Japan, 5, Germany, Federal Republic; 6, Denmark, 7, Finland; 8, France; 9, Ireland; 10, Italy; 11, Norway; 12, Netherlands; 13, England and Wales; 14, Scotland; 15, Sweden; 16, Switzerland; 17, Australia; 18, New Zealand

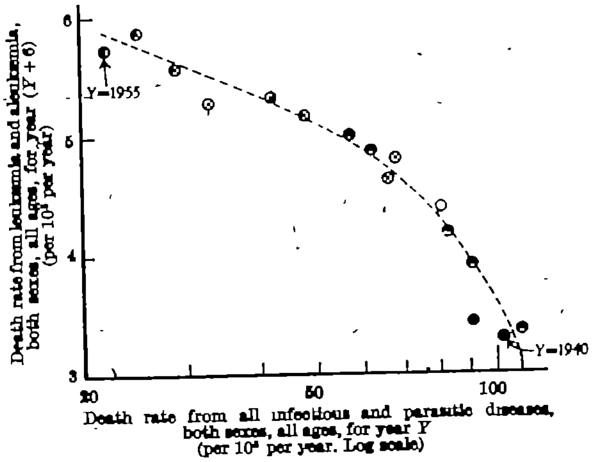

Fig. 2. Relation between mortality rates from all infectious and parasitic diseases for year *Y* (abscissa), and mortality rates from leukaemia and aleukaemia for year (*Y* + 6) (ordinate), England and Wales, *Y* = 1940–55 (refs. 34, 35)

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RELATIONS BETWEEN MOTHS AND PREDATORS

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THE visual protective adaptations of the Lepidoptera, and the selection pressures which have given rise to them, have been the subject of intensive study in the course of the past ten years. On the whole, researches in these fields follow three main lines of attack. In the first place, it is necessary to provide evidence of more than anecdotal value for the actual efficacy of a supposed adaptation; in this respect, adequate experimentation has become possible largely through the combination of satisfactory experimental design with the precise observation of the behaviour of predators which modern ethology allows. Secondly, those features of the predators' behaviour which have forced the evolution of the protective adaptation need detailed behavioural analysis. Lastly, the evolutionary possibilities of the prey–predator interaction require a genetic analysis if they are to be sufficiently understood.

The field has recently been reviewed by P. M. Sheppard[1]: few investigations other than the well-known series by Dr. H. B. D. Kettlewell on industrial melanism in *Biston* have been able to pursue all three approaches simultaneously. Nevertheless, there is an impressive body of evidence to refute the scepticism of the generation of ecologists who followed Sir E. B. Poulton, and who doubted whether even the most obvious adaptations were more than the products of morphogenetic or evolutionary accident.

It is customary to classify visual adaptations in terms of the relationship between the visual appearance of the prey and that of its environment. Species with cryptic coloration resemble some features of their normal background; aposematic species stand out from it and are distasteful; the eyespot patterns of others have been assumed to mimic the appearance of their predators' own enemies, and so forth. The behaviour patterns which disturbance elicits from such insects are rather precisely adjusted to the patterns which they bear, and the presumption that behaviour and correlated visual pattern are mutually necessary to confer protective advantage has been demonstrated experimentally for each of the three categories listed here. In addition, it has been shown that the immobility and dispersion of many cryptic insects exploits the searching behaviour and limited learning capacity of avian predators, while the detailed accuracy of their procryptic resemblances corresponds to the precision of the visual discriminations which birds can achieve under ideal conditions of presentation. Eyespot patterns have been shown to parasitize the inborn responses of passerine birds to their predators; their concealment when their possessors are at rest, and sudden exposure during protective display, are required by the rapidity with which the passerine's responsiveness to them wanes with repeated presentation. Predators have been repeatedly shown to link bright aposematic patterns with distasteful properties, and to avoid insects so coloured, often under conditions of single trial learning. There is also a suggestion in recent work that some potential predators may be equipped with built-in responses to aposematic patterns.

In the last category of analysis, Kettlewell has determined that the melanic form of *Biston betularia* is controlled by an autosomal dominant, and has traced its rapid spread through the British population over the past hundred years. In this case, selective advantage is conferred by the procrypsis of the black form of the moth when resting on tree-trunks contaminated by industrial soot. There is an intriguing possibility that the supporting gene complex of the melanic dominant has also undergone selection to enable it to confer a physiological advantage over the typical form, but the evidence is inconclusive. There are also a number of recent studies by P. M. Sheppard and C. A. Clarke of the control of genetic polymorphism in mimetic butterflies.

At the present time, then, it would seem that the advantages conferred by visual adaptations have been amply demonstrated, and the fact of their existence sufficiently explained. Can work of this kind be made to yield further results of any great importance to evolutionary theory?

In fact, the more complex selective consequences of these prey–predator interactions have been ignored; yet they present some of the most interesting evolutionary and ecological problems. They are, broadly speaking, of two main types: (1) the effects of selection by predator populations are limited or directed by concurrent interactions between the prey population and its animate or inanimate environment; (2) the special features of the relationship between prey and predator impose widespread selective effects which are not obviously related to the protective devices which are their immediate result. It will be seen that these two types of action are somewhat arbitrarily defined.

Type (1) may be illustrated by two examples, the first of which can barely be differentiated from the simple kind of direct selection usually studied. Cryptic moths have evolved colour patterns which match the backgrounds against which they usually rest. In a tropical environment which presents a florid speciation and the opportunity to compare several closely related species grouped in different habitats, such matching may be critically observed. In Panama, lymantriids, notodontids and geometrids have been seen in seasonal evergreen tropical forest at sea-level, and at altitudes of 4,000–5,000 ft. in the Province of Chiriqui. The latter forest contains a particularly high density of lichens, mosses and epiphytes on the trees; not surprisingly, the species found in it have colour patterns which seem, from the frequency with which grey and green occur in them, to be specifically adapted to the altered background. At the same time, the examination of very large assemblies of cryptic species suggests that there may be selection for interspecific divergence in procryptic forms, and the adoption of very specialized cryptic niches by individual species. It is clear that the learning capacities of predators must limit the density of individuals which can occupy any one cryptic niche.

The same two habitats provide a second example. The diurnal temperature range of the lowland habitat is only removed by some 6° C from the lowest effective working temperature of the flight muscles of Lepidoptera (35° C for sphingids[2]). Consequently, even heavy bodied saturniids and sphingids are able to make fairly efficient escape flights from rest when they are disturbed. Correlated with this ability is a poor maintenance of protective display in all but the most aposematic species. Presumably a fast escape flight is more likely to succeed than a display which may or may not be effective. In aposematic small-bodied arctiids, these transient displays are usually held for no more than a few seconds. At the higher altitudes, with diurnal temperatures which are lower by some further 8° C, the saturniids and arctiids are found to have well-defined and sustained displays. This would appear to be a genuine and selectively determined difference, for cooling the lowland forms does not notably improve their display performance. The general range of environmental temperatures appear to be critical in determining the evolutionary course taken by protective display behaviour in flying insects.

The last category is, perhaps, more speculative, and also more difficult to analyse. It is a familiar fact, for example, that aposematic insects are commonly tough and heavily sclerotized in comparison with non-aposematic forms, and it is usually argued that aposematic species have to survive sampling by inexperienced predators. In order to achieve heavy sclerotization, selection must exert rather widespread effects on the ecology of the early stages; it might be expected to impose a relatively greater food-intake, and probably a longer larval history to accommodate it. These effects do not seem to have been searched for critically, and their confirmation would be of some interest. There are, too, more complex possibilities. One of them relates to the status of a non-feeding adult insect after it has completed a reproductive role in its population. If it is a procryptic species, its continued post-reproductive survival can only prejudice the safety of its fellows, for it remains available to train one individual predator to find them. For this reason, group selection might be predicted to shorten post-reproductive survival in procryptic forms, and, in addition, to concentrate reproductive behaviour near to the beginning of adult life. Conversely, a post-reproductive aposematic individual can only benefit its still reproducing fellows, for it can train more than one predator to ignore them. Selection in this case should lengthen post-reproductive survival, and allow more latitude in time to the reproductive process. Recent evidence from hemileucine saturniids observed in seasonal tropical evergreen forest has tended to confirm these predictions, and has suggested an extension of them: while the aposematic species have behaviour which is stable in time from emergence until death, procryptic species show great behavioural instability, and become progressively more excitable as they grow older. Since they also possess 'soft' scaling, the result tends to be a considerable degree of damage to their cryptic patterns rather early in their potential life-spans, and it seems likely that mechanical damage and increased vulnerability to predation remove them from their populations even earlier than their life-spans under ideal laboratory conditions would imply[3].

Effects of this kind require the operation of group selection, and the extent to which this is possible in Nature is still controversial. Clearly, certain obvious constraints (for example, lack of mobility in the populations concerned, and the synchronous emergence of siblings) are necessary conditions which are met for tropical saturniids. Whether other families of the Lepidoptera are as liable to group selection is not known. It might serve to explain the paradox of the general fragility of so many short-lived cryptic forms. In principle, since cryptic patterns have achieved a degree of precision which has been shown to be required by the discriminatory abilities of predators, direct selection might be expected to make their structure sufficiently tough to last the course of an average life-span. If, however, a counter-selective force, generated by the predators learning abilities, tends to reduce the availability of the pattern to reinforce recognition, the outcome could conceivably be that actually found: precision of colour pattern, coupled with fragility to confer a limited availability for learning processes, particularly during the act of prey-capture.

Such complex selective effects are probably widespread, and they may prove to underlie the elaborate structuring of actual populations of closely related aposematic forms in the tropics, where instead of the simple Müllerian convergence which might be predicted the investigator more commonly finds complicated systems of partial resemblance from which the cases of classical Müllerian mimicry stand out and invite, perhaps, too easy explanation.

The original field work which formed the basis of some of this article was supported by grants from the U.S. National Science Foundation, and from the U.S. Institutes of Health (*RG*–7109). It was carried out in the Canal Zone Biological Area, U.S. Canal Zone, Panama, during 1958, 1961 and 1962. The article summarizes a lunch-hour lecture delivered at University College, London, on January 22, 1963.

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COMPUTERS AS BOTANISTS

Substance of a Friday Evening Discourse delivered at the Royal Institution on October 26

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IN any day and age some scientific opinions have been less acceptable than others. Galileo found himself in difficulties as a result of holding currently unfashionable views on the manner in which the universe conducted itself; and in Galileo's day you suffered, if suffer you did, at the hands of the Church or State. Nowadays, it has been wisely remarked, if anybody burnt a scientist at the stake it would be his fellow-scientists; for the modern counterpart of the phenomenon is that some scientific activities are professionally less respectable than others. A recent example is the melancholy argument over extra-sensory perception, where the dispute seems to have been less over whether or not it actually happens, than as to whether a reputable scientist ought to investigate it at all.

Another scientific activity which is at present in some disrepute is that which involves the extraction, from a complex mass of data, of some form of underlying pattern or general principle—an activity which occupies the time of many of those engaged in examining living organisms, whether these be plants, animals, or even that special animal, the human being. There seem to be two reasons for the present denigration of this pursuit. First, it must be remembered that all sciences start like this, by the extrication of patterns from a welter of observations. If the resulting patterns are simple and clear, we may even call them 'laws'; but this is no more than a courtesy title—no Government has ever attempted to repeal the law of gravitation, and no magistrates' court has ever fined a scientist for breaking it. Whatever they may be called, these patterns, once extracted, can be used for predicting further phenomena. Now, the predictive stage of a science is immensely powerful, immensely useful, and remarkably lucrative; and the physical sciences, which are now largely in this stage, are inclined to look down on their own humble origins. Since the biological sciences are, because of the almost inconceivable complexity of the simplest of their material, still largely in the pattern-making stage, they tend to become looked down on *en bloc*.

The second cause of this present disrepute arises, I believe, out of our lack of understanding of the logical processes involved. The extrication of patterns is an entirely human activity, and those who are most gifted in this respect are far from clear as to how they do it, and are seldom able to teach the process to others. It is at this point that the myth that pattern-making is a second-class science is vulnerable; for if we can show that the process can be reduced to a set of rigorous mathematical transformations, we can make it objective, universally available and respectable. A computer may be regarded as a model of some aspects of the human brain. Can a programme be written for a computer which will enable it, when given a set of data, to extricate its underlying pattern?

Consider the problem of establishing the pattern in an area of vegetation, where what is required is a subdivision of the area into regions as different from each other as possible. A human ecologist will do this by walking over the area, using—not always consciously—many different items of information, and drawing extensively on his past experience. If the process is to be made objective, we must control the information reaching the ecologist; he must not be allowed to see the area himself. We will instead provide him with information concerning a series of small sample areas ('quadrats') arranged in a grid over the whole area, and this information will consist solely of the presence of named plants. His task will be more difficult, but not impossible; but he may still be able to draw on his experience by using his knowledge of the proclivities—the habitat preferences—of plants with which he is familiar. The information must therefore be still further reduced: the plant species must be given code numbers or letters, and their names not divulged. Is the problem still soluble?

The work of Nordhagen[1] and Goodall[2] showed that in principle it is. Nordhagen pointed out that the existence of heterogeneities would be manifested by the tendency of the different species to occur in groups which are, or tend to be, mutually exclusive; and that the existence of some degree of association or dissociation between any pair of species would be evidence of a discontinuity in the area. Goodall pointed out that if an appropriate statistical measure of association were calculated for every pair of species, the resulting symmetrical matrix of associations would summarize the available information concerning heterogeneity. His work implied that it should be possible to establish which of the columns or rows of the matrix, that is, which of the plant species, contained the most information; and he pointed out that a division of the area into those regions containing, and those not containing, this species should delimit those regions which, so far as a single species could provide the information, were most different. Our contribution at Southampton was to suggest a means by which this species could be identified[3], and the entire process of analysis could then be transferred to a computer, and was in fact programmed for the Ferranti *Pegasus* computer.

The process was tried out on three test areas. Details of the results can be found in the literature[4,5], and for the purpose of this article the briefest summary will suffice. In the first of these areas—a strip of New Forest heathland near Beaulieu Road Station—my colleague and I hoped that the computer would extract and delimit patches of ground burnt by sparks from passing trains. It did so, stigmatizing these as of only minor importance, but first indicated that the area should be divided neatly into two. More detailed examination of the site showed that the computer had defined a soil difference—the boundary between Barton Sand and Plateau Gravel—which was far from obvious on the surface. The computer was, of course, perfectly right in assigning to this the greater importance; for it is a permanent difference, whereas the burning changes, though more spectacular, are transitory. Chastened by this experience, Dr. Lambert and I selected a very strikingly zoned area of the heath, ranging from dry heath to bog. Here we were reasonably confident that not even the most fallible botanist could err; and we were comforted when the computer produced precisely the same results as our own.

The last of the test areas was the now classic Rockland–Claxton level, in the Norfolk Broads. This had been surveyed some years earlier by Dr. Lambert with the help of a group of students, and her published maps—which were fully accepted by ecologists—related the major heterogeneities to differences in soil and grazing[6]. The computer accepted the soil, paid no attention to the grazing, and again firmly divided the area along an unexpected line—which this time was manifestly the parish boundary. Historical investigation provided the reason for this ecclesiastical interest: the 1840 tithe-maps show that by that date one of the parishes had drained its land, the other had not. The difference was still detectable in the vegetation. For good measure the computer made a further division, between different pairs of students; there had been, it appeared, some uncertainty about the identification of grasses.

All in all, the results fulfilled, and even exceeded, our expectations. In two of the cases the computer did better than the human beings, in the sense that it revealed discontinuities which had been overlooked by its human colleagues, but which on investigation proved to be of major importance. There is, then, no doubt that patterns can be extricated by computers by mathematical operations on the data; this should be a great relief to biologists, since it is to-day regarded as a mark of prestige if one's scientific activities, whatever they may look like to the casual observer, can be claimed to be, in reality, a slightly recondite branch of mathematics.

But does the fact that a computer can beat a human being at an essentially human activity carry any sinister long-term implications? In science fiction, which is the literature of extrapolation, there is to be found the recurrent theme of the omniscient computer which ultimately takes over the ordering of human life and affairs. Is this possible? I believe it is not; but I also believe that the arguments commonly advanced to refute this possibility are the wrong ones. First, it is often said that computers 'do not really think'. This, I submit, is nonsense; if computers do not think, then nor do human beings. For how do I detect the process of thinking? I present data—say, an examination paper—to a student, which he scans with a photoelectric organ we call an 'eye'; the computer scans its data with a photoelectric organ we call a 'tape-reader'. There is then a period when nothing obvious happens, though electrical changes can be demonstrated on the monitor-tube of a computer or on the corresponding device—an electroencephalogram—for the student. Lastly, information based on the data is transcribed by means of a mechanical organ called a 'hand' by the student and a 'teleprinter' by the computer. In other words, as Gilbert Ryle[7] pointed out, the only evidence we have that people think is what they do, our observations of their actions; and the actions of man and machine differ only in the appliances they use.

Secondly, it is said that computers 'only do what they are told', that they have to be programmed for every computation they undertake. But I do not believe that I was born with an innate ability to solve quadratic equations or to identify common members of the British flora; I, too, had to be programmed for these activities, but I happened to call my programmers by different names, such as 'schoolteacher', 'lecturer' or 'professor'.

Lastly, we are told that computers, unlike human beings, cannot interpret their own results. But interpretation is always of one set of information in the light of another set of information; it consists simply of finding the joint pattern in two sets of data. The mathematics of doing this is cumbersome, but well known; the computer would be perfectly willing to do the job if asked.

There are, of course, real differences. The memory store of the human brain, though slow and uncertain of access, is vastly larger than that of any computer yet built or conceived; but this is not a fundamental difference, and will become less important as computers get larger. Moreover, computers tend to think in straight lines, like the signal reaching a television tube; they do not immediately apprehend it, as the human brain can, as a two-dimensional pattern of moving images. This is merely because the human brain contains many separate thinking paths, all elaborately cross-connected; whereas even the largest computers have few distinct paths, with little cross-connexion. This is purely a matter of computer design, and could be overcome if we so desired.

Clearly, then, these well-worn arguments will provide no comfort for those who fear domination by computers. One vital distinction between computers and men, hitherto completely overlooked, does nevertheless remain. It was first pointed out by my colleague Mr. A. R. Manser, to whom I am indebted for permission to reproduce the substance of his argument in this communication. Briefly, human beings *want* to compute; and the reasons for their wanting are unrelated to the things they compute. For human beings are provided with more than brains; they have reproductive systems, digestive systems and the like. These supply them with drives, impulses, needs, wants, desires—and some of these may be satisfied by mental activity. I do not intend any reference to some sort of psychological 'compensation'; I mean only that I may undertake additional mental activity—more work—in order to obtain money to buy a refrigerator, because I enjoy food; or to buy a mink coat, to clothe someone I wish to please. Computers, being only brains, live in a cold world, free from all such desires and free, therefore, from the desire to think; and we shall be safe from their domination so long as we continue to build them solely as brains. If we go further—if we aspire to give them reproductive systems, digestive systems and all the facilities for sensuous enjoyment, then they will no longer be computers but genuine robots; and the world of Karel Čapek, Isaac Asimov and Arthur Clarke will be around us at last.

But even if we do not make this mistake, one danger remains. Computers, even in the very human field of pattern-making, are so much faster and more reliable than human beings that we may be tempted to delegate to them all our computational activities; computation would then become something that men never do. But something men never do soon becomes something men never teach; and all our knowledge of the manifold skills of computing could be lost within a few generations. So one vital question remains: has the human computer, *qua* computer, any advantages over his electronic counterpart? The answer to this question has long been current in computer circles, though I have been unable to trace its original author.

Man has indeed three advantages—advantages which he may well retain even into the far-distant future. First, given his abilities, he weighs less than any computer yet designed or even envisaged: there seems no likelihood that an electronic computer capable of so great a variety of computational facilities can be encompassed within 150 lb., even though the human computer increases somewhat in size and weight after the first fifty years' operation. Secondly, man needs far less energy; his energy requirements, being in the form of food and drink, are intensely inconvenient and clumsy, but they are collectively minute when compared with those of an electronic computer. Thirdly, and lastly, and perhaps most important of all, man is the only computer yet designed which can be produced entirely by unskilled labour.

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TEACHERS OF ENGLISH AND THE LITERACY OF SCIENTISTS

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MR. A. J. KIRKMAN has recently argued in *Nature*[1] that it was mainly up to teachers of science and technology to give the guidance and impose the standards necessary for their students to improve their written English. He gave examples of errors of grammar typical of their writings, mostly indicating rather trivial effects of carelessness: sentences incomplete or tenses changed in the middle, bad punctuation and spelling, and so on.

All the errors he mentioned were fairly easy to spot, easy to correct, and of a kind which the teacher could easily mark; but they were all irrelevant to the main problem of helping scientists to write better English and express themselves more fluently.

The damage is done in the classes called 'English', and is not the fault of science teachers who accept low standards. I can best illustrate from my own experience as a schoolboy one of the ways in which respect for English as a subject is destroyed; and I should say, lest a counter argument be sought, that there is no analogy in the lack of enthusiasm some students have for mathematics (which may, perhaps, be compared with Latin), for English is my language just as much as the English teacher's. One essay I wrote was criticized as being "too much like a *Daily Herald* leader". I was given no help in how to express views which, in those days of the 'Hunger Marchers', my generation held very strongly indeed. I was even criticized for my vehemence.

The subject set the following week was "The English Sunday". In response to my teacher, and drawing on my upbringing in a cathedral city, I concocted an inconsequential blather about cake, church bells, and chimneys, contrasting them with Continental commercialism. Without conviction about the sentiments expressed nor believing that the topic was important, I was awarded a prize for this performance in hypocrisy and, by some standards, banality.

Now science is about veracity and understanding. Scientists are not barristers, though they sometimes rather foolishly allow their emotions to get them into the position of advocate when their own ideas are attacked. To be rewarded by my teacher for my exercise without conviction had not helped me to sort out in words on paper the complicated ideas boiling up and the ideals growing inside me. It illustrates how easily teachers can kill 'English' as a subject by setting subjects in which the pupil cannot involve himself. They imagine that it is better to think up than think out ideas. They encourage padding, for it is commonly accepted that on reaching the top of the fourth page of an essay the pupil has at least done what he is allowed to call enough, whatever its quality. This takes all, yes all, the poetry from writing.

The teacher's task is to get the students to set down live theses (live, of course, to the pupil, not necessarily to the teacher), to polish until it cuts cleanly into the reader's imagination. They should invite descriptions of experiments in the science laboratory or of a football game just played, comment on an outrageous piece of current news or an imaginary case of moral injustice within the law, inquiries into the causes of accidents, advice on choosing a camera, accounts of almost anything for which the writer has some genuine feeling. Any pupil who responds will find occasion enough for drudgery when later he aspires to achievements which will require it, and he need never be assigned to it. The teacher should get the whole class to consider how to improve the effectiveness of each pupil's effort. What a change this would be from essays on characters in Shakespeare with marks for spelling, punctuation and all the other corrigenda of the grammarian.

Driven to cynicism for the fun of it, I occasionally made discoveries. On one occasion, in answer to the invitation to write on "The Best Time of Life", I opted for old age. (My views were recently endorsed in a B.B.C. sound programme on growing old.) Conviction was evolved in a mood of rebellion against the usual tedious story: the essay had many shortcomings, but apart from grammar, it was criticized for insincerity. Instead of investigating better ways of expressing my chosen thesis I was reprimanded for "trying to be clever", which convinced me that I had succeeded, wherefore I could not at the time see the shortcomings of the essay.

An interesting point about Mr. Kirkman's examples is that in each case it was immediately clear what the writer meant, though a moment's thought was necessary to spot the error for which it was quoted. The outrage against the rules of grammar evidently annoyed Mr. Kirkman who, being himself unable to understand the intended meaning, blamed the bad grammar rather than his own lack of knowledge of the subject-matter for the failure of communication. He should not suppose that the writing ought to be intelligible if the grammar is correct. Thus Carlyle writes, "The man so nourished and decorated, thenceforth named royal, does verily bear rule; and is said, and even thought to be, for example, 'prosecuting conquests in Flanders', when he lets himself like luggage be carried thither: and no light luggage; covering miles of road". Full of thought, concentrated and delightful to one who understands, but certainly obscure and of questionable grammar to one who does not. Imagine Mr. Kirkman's reaction to a laboratory experiment described in this style. Correction of the grammar in the cases quoted by him would only ensure that so far as the grammarian was concerned the words could have meaning; but grammatical correctness does not guarantee meaning, still less significance. Nonsense can be made grammatically correct; and grammatical correctness has even caused meaning to be sought in words which contained none, as in the example from ***Ezekiel*** given later in this essay.

Any ordinarily educated person can easily learn the habit of correct grammar with practice. Some errors remain only because students have not been taught how to re-read what they have written. This has nothing to do with the problem of becoming articulate on paper. In mathematics and science the pupil is encouraged to say what he needs in the least possible space. In mathematics particularly, provided the logic is correct, the briefest formulation is often the best. We have no sympathy for padding, or "leisurely periphrasis" as Mr. Kirkman approvingly calls it. We have symbols for whole sentences the intelligibility of which depends on hard-learned familiarity: it is not merely a language but a shorthand which cannot be translated into ordinary language because many other symbols and sophisticated ideas stand between it and its origins in the commonplace world. The mathematics student is therefore used to having a whole series of steps in an argument presented in half a page. Equal logical content in a political, religious, or legal argument would occupy perhaps several pages which would take an hour or more to write.

The unpractised writer has great difficulty in visualizing all the steps in a long verbal sequence as clearly and simultaneously as he can see them in a mathematical argument contained physically on one piece of paper. It takes such a long time to write out that he cannot easily keep clear in his mind where he has got to at each moment; he easily forgets the intended asides and reinforcements. It is probable that most teachers suggest the making of rough notes; but which of them dissects the pupil's final essay, reshaping it sympathetically over and over again until all unnecessary content is removed? More important —which of them does this with essays on subjects they know the pupils feel strongly about?

Experienced authors often cannot see mistakes in printers' proofs because they know too well what was intended, and it is only worth a student's while checking for grammar if some considerable time is allowed to elapse since the writing was done. In tutoring mathematics students, I have, from time to time, asked them to write essays on non-mathematical topics in order to give them practice in writing. They make plenty of trivial grammatical mistakes, but these should be tolerated with a smile: they can easily see them if I keep their work from them for a week or two. But often what was in their minds lies only between the lines and is only fully exposed by discussion of the essays. One idea is expressed two or three times while another is forgotten. Obviously the hand and the mind have not learned to move at the same speed, and the mind sees implications in the written words which are not apparent to a reader new to the matter.

English teachers have no easy task in training pupils to express their intended meaning clearly and unequivocally. Except in mathematics the meaning evoked in the reader's mind depends not only on what is written but also on the reader. The extreme example of this occurs when reviewers accuse authors of advocating points of view which they have actually expressly rejected. The reviewer is anxious to categorize the author as hastily as he can (we all do this, but are not usually in such a hurry as a reviewer) and his eyes and mind become fixed on sentences which seem to label the author with one or two '-isms' well known to the reviewer. The author has probably written the book in the first place because he finds these very '-isms' unsatisfactory; but it it is easier to read a new book and write it off as being a good or bad exposition of familiar theses than to search the depths of the writer's mind.

Some things are indescribable however good one's control of language. It is a common experience of scientists who have have been intimately concerned with a matter which has become news to see nonsense made of it by 'Our Science Correspondent' who, in the profession of journalism, has learned fluently to form sentences according to the rule of grammar which are only seen to be meaningless by those who know what he was trying to understand. Occasionally meaning gets through to those with sufficient knowledge of the topic. Thus Ezekiel, believing the Lord to be descending from heaven in a cloud to summon him, describes a thunderstorm with a tornado:

". . . Now as I beheld the living creatures, behold one wheel upon the earth beside the living creatures, for each of the four faces thereof. The appearance of the wheels and their work was like unto the colour of beryl: and they four had one likeness: and their appearance and their work was as it were a wheel within a wheel. When they went, they went upon their four sides: they turned not when they went. As for their rings, they were high and dreadful; and they four had their rings full of eyes round about . . ." and so on. This kind of writing is described by loyal theologians as "obscure", the implication being that we cannot penetrate the depth of Ezekiel's vision. One could alternatively say that the vision was very shallow: How could it be otherwise for an observer who understood neither rotation, nor condensation, nor electricity, nor wind? Moreover, how impossible for a translator who himself does not understand a tornado, nor even suspects that this was the basis of the vision, to render meaningfully into English!

One is led to suspect that a teacher of English can hope to find only the superficial deficiencies in scientific English unless he learns more about the subject-matter. Perhaps Mr. Kirkman is right after all in passing responsibility to the science teachers because most English teachers cannot bear it. But the scientist's task is so often rendered well-nigh impossible by English teachers who inoculate pupils interested in science with a distaste, or at least a disdain, for the subject known as 'English'.

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[1] Kirkman, A. J., *Nature*, 196, 807 (1962).

OBITUARIES

Dr. P. Rosbaud

With the death on January 28 of Dr. Paul Rosbaud, at the age of sixty-six, one of the most lovable personalities in the world of science has passed away. He was not an active research scientist but was engaged in scientific publication. There cannot be many contemporary authors of scientific books who have not met him some time. He was friends with many leading scientists, mainly physicists and chemists, and to some of them he was very close.

Born in Austria, he studied chemistry in Germany. Early in the 1930's he joined the big scientific publishing firm of Springer in Berlin as scientific editor, and there he began his fruitful publishing activities. Most of the important standard works in physics and chemistry published by this firm at the time must have passed through his hands, and he was undoubtedly responsible for the writing of many. One of his most fateful activities was in 1938 when, after a telephone call from Prof. O. Hahn, he decided to have a paper published in *Die Naturwissenschaften* describing Hahn's discovery of nuclear fission. Thus this important fact was made known to the world shortly before the War.

In 1933, the Nazi period began, which he loathed from the very beginning. Shortly before the Second World War he had to send his wife out of Germany—she went to England as a refugee—but Rosbaud stayed in Berlin. He did not want to take one of the few posts then available to refugees. His office in Berlin became a meeting place for dissident scientists. He was always ready to help as much as he could those in trouble. During the War he was in constant danger of his life, owing to his manifold anti-Nazi activities; but he judged the Nazi police correctly: it was more cruel than intelligent. In the middle of the War he secured the release of two French scientists from a prisoner of war camp for "the purpose of translating a scientific book into French" (after obtaining Joliot-Curie's consent that they would not be accused of collaboration after the War).

When the War ended, all the Allies knew his worth, and several Powers tried to get him. He wanted to go to England and was flown there. England became the country of his adoption and he loved it. Soon he began his publishing activities again. First, he was partner of a new scientific publishing house but later he made himself independent, working as a free lance. He was scientific adviser to many big publishing firms throughout the world, mediating between author and publisher. His contact with prospective authors was always personal and this enabled him to be uniquely up to date in the developments of science. In his small unpretentious office in London, S.W.1, with the kind landlady who was always ready with a cup of tea for the visitor, everyone must have felt at ease. His intelligence, vividness and dry humour won the heart of everyone of good will. He was tolerant of every honest opinion but very intolerant of injustice, underhandedness and so on.

In 1961 he was awarded the Tate Medal of the American Institute of Physics for his services to science.

The post-war development of science was not entirely to his liking, and he worried a lot about it. He did not like the alliance science had made with military authorities. Nor was the pretentious scale on which science was to be furthered quite to his taste, and he disliked the attitude that sponsored it. He saw the danger involved when science became too much mixed up with the interest of power or mere material advantage.

In September 1962 he was frequently seen at the Pugwash Conferences in London (in which he nominally did not participate) discussing the world's problems with the delegates—lively as ever. He fell ill in November. It turned out to be leukaemia. Up to the last he thought of his friends. He died peacefully on January 28.

W. Heitler

Prof. N. R. Sen

Nikhil Ranjan Sen, who retired in 1959 as Ghosh professor of applied mathematics at the University of Calcutta, after holding the chair for thirty-five years, died on January 13. He was honorary research professor at the Indian Association for the Cultivation of Science, Calcutta, at the time of his death.

Son of Shri Kalimohan Sen and Shrimati Bidhumukhi Devi, he was born on May 23, 1894. He was educated at Dacca Collegiate School, Rajshahi School and Rajshahi College. Later he studied in Presidency College, Calcutta, and at the University of Calcutta, and he took his M.Sc. degree in 1916, and D.Sc. in 1921. Afterwards he proceeded to Germany, where he worked with Prof. Max Von Laue. Shortly after returning to India he was appointed Ghosh professor in the University of Calcutta, where he worked ceaselessly to create a school of research in applied mathematics which in the course of years spread its influence in all parts of India.

His researches embraced several fields. He did interesting work on some intricate problems on spherical harmonics early in his career. In the early 'thirties he worked on wave mechanics, including Dirac's relativistic equations. He also worked on cosmological problems. Later he turned to relativistic effects in stellar bodies and also (a little after the advent of Bethe's law of energy generation) to the problems of stellar constitution. During the 'fifties he started working on turbulence and other problems of fluid mechanics.

To his students and colleagues Sen was a model of single-minded devotion to work, and the team of enthusiastic teachers which he built up in his department in Calcutta was inspired by his exemplary character. His weekly seminars, which attracted persons from different institutions, worked as a powerful force and pioneered a new spirit of research and study. One would also often see him on holidays holding small study groups in his house, squatting on a mat, reminiscent of an ancient Indian teacher with his disciples.

He was greatly interested in the problems of teaching mathematics at school-level, especially in relation to the teaching of higher mathematics. In the 'thirties he started a small laboratory for numerical computation for his M.Sc. students. For some years before his retirement he was working to set up a small hydrodynamic laboratory with a flume and a wind tunnel.

He was the fountain-head of inspiration of the Calcutta Mathematical Society. With the help of hard-working editorial secretaries he did his best to raise the standard of the *Bulletin* of the Society.

Though essentially of retiring temperament and averse to committee work, he responded to the demands of the country and served on various committees and advisory bodies all over India.

S. N. Bose

NEWS and VIEWS

The Royal Society of Edinburgh: New Fellows

THE following have been elected Fellows of the Royal Society of Edinburgh: Mr. A. J. Aglen, fisheries secretary, Department of Agriculture and Fisheries for Scotland; Sir William Arbuckle, secretary of the Scottish Education Department; Mr. J. W. Atwell, managing director, G. and J. Weir Holdings, Ltd., Glasgow; Prof. J. Baddiley, professor of organic chemistry, King's College, University of Durham, Newcastle upon Tyne; Dr. R. A. Beatty, senior principal scientific officer, Agricultural Research Council Unit of Animal Genetics, Edinburgh; Mr. A. J. Brook, lecturer in botany, University of Edinburgh; Prof. J. Bruce, Regius professor of clinical surgery, University of Edinburgh; Mr. J. D. S. Cameron, president of the Royal College of Physicians, Edinburgh; Mr. W. Dickson, senior science master, George Watson's Boys' College, Edinburgh; Dr. A. J. Hale, senior lecturer in pathology, St. Thomas's Hospital Medical School, London; Prof. J. Hawthorn, professor of food science, Royal College of Science and Technology, Glasgow; Dr. J. B. Helliwell, senior lecturer in mathematics, Royal College of Science and Technology, Glasgow; Dr. P. N. Hobson, head of Microbiology Department, Rowett Research Institute; Prof. A. Iggo, professor of veterinary physiology, Royal Dick Veterinary School, Edinburgh; Mr. G. S. Johnstone, principal geologist, H.M. Geological Survey (Scotland); Mr. P. P. Love, director of research and development, Glacier Metal Co., Wembley; Prof. W. T. Marshall, Regius professor of civil engineering in the University of Glasgow; Dr. D. Martin, senior lecturer in mathematics, University of Glasgow; Dr. W. G. Siller, principal scientific officer, Agricultural Research Council, Poultry Research Centre, Edinburgh; Mr. D. G. Sopwith, director of the National Engineering Laboratory (Department of Scientific and Industrial Research), East Kilbride; Mr. T. A. Stuart, principal scientific officer, Department of Agriculture and Fisheries for Scotland, Freshwater Fisheries Laboratory, Faskally, Pitlochry; Dr. R. L. M. Synge, biochemist, Rowett Research Institute, Bucksburn, Aberdeen; Dr. C. J. Taylor, acting head of the Forestry Department, University of Edinburgh; Dr. J. Tinsley, reader in soil science and head of department, University of Aberdeen; Dr. H. W. Wilson, director of the Scottish Reactor Centre.

The 1961 Corday Morgan Medal and Prize: Prof. F. Sondheimer

PROF. FRANZ SONDHEIMER, professor and head of the Organic Chemistry Department at the Weizmann Institute of Science, Rehovoth, has been awarded the Chemical Society's 1961 Corday–Morgan Medal and Prize, in consideration of his contribution to the chemistry of natural products, including his notable synthesis of the steroidal sapogenins and his studies of the synthesis and properties of unsaturated macrocyclic compounds. This award, consisting of a silver medal and a monetary prize, is made annually to the chemist of either sex and of British nationality who, in the judgment of the Council of the Chemical Society, has published during the year in question, and in the immediately preceding five years, the most meritorious contribution to experimental chemistry, and who has not, at the date of publication, attained the age of thirty-six years.

The International Commission on Radiological Protection

THE following have been elected officers and members of the International Commission on Radiological Protection for the period 1962–65: *Chairman*, Dr. E. Eric Pochin (U.K.); *Vice-Chairman*, Prof. L. Bugnard (France); *Members*, Mr. W. Binks (U.K.), Prof. Otto Hug (Germany), Dr. H. Jammet (France), Dr. Bo Lindell (Sweden), Dr. John F. Loutit (U.K.), Dr. K. Z. Morgan (United States), Dr. H. J. Muller (United States), Prof. Rolf M. Sievert (Sweden), Dr. C. Gordon Stewart (Canada), Prof. R. S. Stone (United States), Dr. Lauriston S. Taylor (United States); *Scientific Secretary*, F. D. Sowby. The Commission's secretariat has recently been transferred from Stockholm to Clifton Avenue, Sutton, Surrey, England.

The Water Resources Bill

TOWARDS the end of the report stage of the Water Resources Bill in the House of Lords, the claims of amenity were strongly pressed in discussion of an amendment moved by Lord Henley in favour of a Lakeland River Authority. The Bishop of Carlisle, Lord Chorley, Lord Conesford and Lord Hurcomb all referred to widespread concern that the amenity interests of the Lake District would not be sufficiently respected by Lancashire and Cumberland River Boards on which the dominant interest represented was that of interest as consumers. Earl Jellicoe, while unable to accept the amendment, was at pains on behalf of the Government to reassure the House that the Government was really determined that the amenity interest should be adequately protected and that this was better served by treating areas concerned as a single unit as proposed in the Bill. He further pointed out that since Clause 13 provided for the appointment of an amenity member to the Water Resources Board, it was almost certain that the Board would be alerted of all important schemes and that the amenity member of the Board would watch such schemes in the interest of amenity. Special references to natural beauty were to be found in Clause 19 dealing with minimum acceptable flaws, and there was a new sub-section in Clause 52 dealing with the transmission of applications for licences to the National Park Planning Authorities. Besides the amenity clause itself (93), there was the saving for the town and country planning legislation in Clause 63 (3) and there was a new clause recognizing the special responsibility in this field of the Nature Conservancy. Earl Jellicoe said that he himself had proposed an amendment making the Water Resources Board, in addition to the river authorities, responsible for securing economy, and he thought that all these enactments would ensure that amenity was always in mind at all stages of the river authorities' work and in the counsels of the Water Resources Board. They would ensure that the views of the local National Park Planning Authority were made known to the river authority and taken into consideration by that authority. They secured that decisions on planning permission for the construction of works were not taken by the river authorities but either by the planning board or the Minister himself, and on a controversial application the final decision would rest with the Minister. He claimed that it was impossible for any river authority to ride roughshod over local opinion in this question of natural beauty and amenity.

Working Party on Materials Science and Technology

A WORKING Party on Materials Science and Technology has been formed under the auspices of the Institution of Chemical Engineers and the inaugural meeting has been held. The interests represented included paper, concrete, rubber and plastics, in addition to metals, ceramics and glass. It was unanimously decided that every attempt should be made to co-operate with other groups concerned with materials. It is hoped that, at a later stage, conferences will be held, in conjunction with other learned and professional societies, on a series of themes, each of which involves consideration of a wide variety of materials, to stimulate the exchange of ideas and techniques between what are at present considered different disciplines. The Working Party is constituted as follows: *Chairman*, Mr. L. Holliday (Shell Chemical Co., Ltd.); *Honorary Secretary*, Dr. P. E. Evans (Manchester College of Science and Technology); *Members*, Dr. N. F. Astbury (British Ceramic Research Association), Prof. J. G. Ball (Imperial College of Science and Technology), Dr. T. H. Blakeley (The Morgan Crucible Co., Ltd.), Prof. R. W. Cahn (University College, Bangor), Dr. A. R. Collins (Cement and Concrete Research Association), Prof. A. H. Cottrell and Mr. J. A. Charles (University of Cambridge), Dr. J. I. Cox (A. Boake, Roberts and Co., Ltd.), Prof. R. W. Douglas (University of Sheffield), Dr. E. Eastwood (English Electric Co., Ltd.), Dr. G. A. R. Hartley (Courtaulds, Ltd.), Dr. J. M. Hutcheon (U.K. Atomic Energy Authority), Dr. N. P. Inglis (Imperial Metal Industries (Kynoch), Ltd.), Prof. R. Edgeworth Johnstone (University of Nottingham), Mr. A. Kennaway (B.T.R. Industries, Ltd.), Prof. A. J. Kennedy (College of Aeronautics, Cranfield), Mr. J. Luckins (British Paper and Board Industry Research Association), Mr. D. S. Mahon (Plastics Institute), Dr. J. W. Martin (University of Oxford), Prof. E. W. J. Mitchell (University of Reading), Mr. N. C. Moore (Plessey, Ltd.), Dr. P. Murray (U.K. Atomic Energy Research Establishment), Dr. J. F. Nye (University of Bristol), Mr. C. E. Phillips (National Engineering Laboratory), Dr. L. H. A. Pilkington (Pilkington Brothers, Ltd.), Dr. J. A. Pople (National Physical Laboratory), Mr. R. Llewellyn Rees (Central Electricity Generating Board), Prof. E. Rhoderick (Manchester College of Science and Technology), Mr. F. Roberts (U.K. Atomic Energy Research Establishment), Prof. T. K. Ross (Manchester College of Science and Technology), Dr. G. C. Shipp (Brunel College of Technology), Dr. E. G. Stanford (Aluminium Laboratories, Ltd.), Dr. W. F. Watson (Rubber and Plastics Research Association), and Dr. J. G. Wistreich (British Iron and Steel Research Association).

Economic Planning in France

French Economic Planning describes briefly and clearly the origin and development of economic planning in France from the First (Monnet) Plan for modernization and equipment, 1946–53, to the final approval of the Fourth Plan (Pp. 12. London: Ambassade de France, Service de Presse et d'Information, 1963). It covers economic and social development 1962–65, visualizing a 24 per cent growth in gross internal production, an increased export drive, appreciable increase in collective equipment for schools, hospitals, scientific research, town planning and country equipment and a policy of regional development. The Second Plan, 1954–57, set production and investment targets for most sectors of the national economy, particularly the processing industries, agriculture and housing. The Third Plan, 1958–61, set out to restore the balance of the economy, which had been upset by over-rapid progress under the Second Plan. The pamphlet also describes briefly the bodies responsible for planning, including the Commissariat Général du Plan and the Modernization Commissions, the procedure adopted in drawing up the Plans and the psychological and other factors which contribute to the effectiveness of the Plans.

U.S. National Institute of General Medical Sciences

The National Institute of General Medical Sciences, with Dr. Clinton C. Powell as its director, has been founded. This brings the total of the National Institutes of Health, U.S. Public Health Service, Bethesda, to nine. The new Institute was formerly the Division of General Medical Sciences of the National Institutes of Health. The Division was established in 1958 to help meet the increasing requirements of the Institutes' programmes of grants to non-federal medical schools, universities, and other medical institutions. The new Institute will be responsible for administering, fostering and co-ordinating research in the sciences basic to medicine and biology, to public health, and to certain clinical sciences and biomedical studies not within the responsibility of the other institutes.

National Science Foundation Support for Research on Information Retrieval

According to *Scientific Information Notes*, December 1962–January 1963 (No. 6, Vol. 4), an experimental study supported by the National Science Foundation showed that it is both practicable and desirable to convert the terminology used in some information systems to that being used by another and that the methodology developed can be applied to the preparation of a practical tool for the necessary conversion. Under a Foundation contract, the Datatrol Corporation, Maryland, is now developing a structured composite listing of the vocabularies used by the Atomic Energy Commission, the Armed Services Technical Information Agency and National Aeronautics and Space Administration with the view of compiling a dictionary of equivalents. Under another Foundation contract the character and degree of use of the *Chemical Engineering Thesaurus* and the index data provided for articles in *Chemical Engineering Progress*—both published by the American Institute of Chemical Engineering—are also being studied by Harner and Co., Washington, D.C., partly with the view of providing insight into the construction of thesauri and their efficiency as retrieval tools. The American Anthropological Association is conducting a rapid but thorough investigation of its publishing situation: (1) to determine the extent and nature of existing stockpiles of data and manuscripts in anthropology and the reasons why they have not been published; (2) to explore means for distributing such unpublished material; and (3) to suggest ways of integrating and organizing the flow of anthropological materials, whether published or not.

The British Library of Political and Economic Science

The annual report, 1961–62, of the British Library of Political and Economic Science records progress in the transfer of books to the Library Depository established at Egham by the University, but staffing difficulties badly handicapped the work of the cataloguing department at senior and junior levels (Pp. 15. London: British Library of Political and Economic Science, 1962). Acquisitions during the year totalled 7,353 treatises, 1,450 pamphlets, 3,551 non-serial Government publications (excluding British Parliamentary Papers and United States Public Documents) and 8,711 current serials. Bound volumes acquired totalled 11,405, 241 volumes of pamphlets bound bringing the total to 11,646, from which 252 were withdrawn or transferred to the Lending Library. Some 5,000 volumes of current acquisition were bound and 5,594 were bound, re-bound or repaired. The total of bound volumes in all libraries at July 31, 1962, was 432,208 of which 11,348 are in the Lending Library, 9,253 in study rooms, and 5,019 in the Shaw Library. Expenditure on treatises amounted to £8,135 with £1,157 on binding, on pamphlets £148, on non-serial Government publications £512, on periodicals and other serials £8,357 (including £3,498 on binding), while £5,507 was spent on current binding, and binding of arrears, repairs and rebinding. The Lending Library issued 39,314 treatises, 1,371 works were lent to other libraries, and, of 862 readers' permits (262 for overseas), 327 were in force on July 31, 1962.

Volatile Fruit Flavours

THE report of a symposium held in Berne during 1962, one of a series organized by the Scientific and Technical Commission of the International Federation of Fruit Juice Producers, summarizes the great advances made in the chemistry and technology of fruit aromas since the advent of gas–liquid chromatography (*Volatile Fruit Flavours*. Pp. 449. Zurich: Juris-Verlag, 1962. Obtainable from International Fruit Juice Union, Eschenz TG, Switzerland. 40 Sw. Fr.). The papers presented, reviews or original papers, are in English, French or German with tri-lingual summaries. Of the 38 articles, 28 deal with the chemistry of aroma components or with their commercial recovery from fruits and juices. The developments so far have already led to significant improvements in the quality of juice products; the more detailed examination of aroma components is now beginning to make possible a more quantitative assessment of quality, though sensory tests still remain the final criteria.

Investigations of Infra-red Spectra

DR. R. N. JONES, president of the Coblentz Society, has written to the Editor saying that, with the permission of the Carnegie Institution of Washington, the Coblentz Society has arranged for the re-publication of the Carnegie Institution Monograph No. 35, *Investigations of Infrared Spectra*. In this is published the original research work of Dr. W. W. Coblentz, who first established the potentialities of infra-red spectroscopy as a tool in organic chemical research, and who died in Washington last September at the age of eighty-eight. The Monograph, which was originally published in 1905, has long been out of print; it is believed that it will be of interest to many molecular spectroscopists outside the immediate circle of the Society members. A limited number of copies are still available at a price of 3.5 dollars, and can be obtained from Dr. H. B. Kessler, secretary of the Coblentz Society, the Perkin-Elmer Corporation, Norwalk, Connecticut.

The Coblentz Society was formed in 1954 with the view of bringing together physicists and chemists concerned with the development and application of infra-red spectroscopy and has its headquarters in the United States.

The Sun during the International Geophysical Year

DR. M. A. ELLISON, senior professor in the School of Cosmic Physics at the Dublin Institute for Advanced Studies and editor of Volumes 21 and 22 of the *Annals of the International Geophysical Year* (*I.G.Y. Solar Activity Maps, DI and DII*), which were reviewed on p. 1237 of the September 29, 1962, issue of *Nature*, has written to the Editor of *Nature* as follows: "In the review . . . there was no mention of the greatly reduced price available to individuals who certify that the *Maps* are for their own personal use (£18 as against £54 to Institutions). . . . Such orders must be addressed to IGY Solar Maps, Pergamon Press, Headington Hill Hall, Oxford, England".

Course in Nuclear Magnetic Resonance Techniques

A SPECIAL advanced course on "Nuclear Magnetic Resonance in Molecules of Moderate and High Molecular Weight" will be held during April 25–26. The speakers include Prof. A. R. Katritzky, and Drs. R. Freeman, D. W. Jones, J. A. S. Smith and I. M. Ward. Applications in inorganic, organic and polymer chemistry will be considered, and there will be a short session on commercial equipment. The fee for the course is 30*s*. Further details and registration forms can be obtained from the Registrar, Institute of Technology, Bradford 7.

The Night Sky in April

FULL moon occurs on April 9d. 00h. 57m. U.T. and new moon on April 23d. 20h. 29m. The following conjunctions with the Moon take place: April 3d. 05h., Mars 2° N.; April 18d. 23h., Saturn 1° N.; April 21d. 09h., Venus 3° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Jupiter on April 28d. 17h., Venus being 0·6° S. Mercury is an evening star, and is conveniently placed for observation during the last week of the month, when it sets about two hours after the Sun. Venus is a morning star, rising about an hour before the Sun. Mars sets at 4h. 10m., 3h. 20m. and 2h. 30m. on April 1, 15 and 30, respectively. It is at a distance of 101 million miles on April 15, when its stellar magnitude is +0·5 and it is in Cancer. Jupiter and Saturn are too close to the Sun for easy observation. There are no occultations of stars brighter than magnitude 6 visible at Greenwich. The Lyrid meteors are active during April 19–22, the radiant being at R.A. 18h. 04m., Dec. +33°; conditions for observation are favourable.

Announcements

PROF. BERND T. MATTHIAS, professor of physics at the University of California, San Diego, has been awarded the twenty-seventh Research Corporation Award for his outstanding contributions in the fields of superconductivity and ferroelectricity. The Award includes an honorarium of 10,000 dollars.

AN international starch conference will be held in Detmold during April 24–26. Further information can be obtained from Wolfgang Kempf, Arbeitsgemeinschaft Getreideforschung E.V., Detmold, Am Schützenberg 9.

THE 1963 Central African Scientific and Medical Congress will be held at the College of Further Education, Church Road, Lusaka, during August 26–30. Further information can be obtained from the Honorary Secretary, P.O. Box 844, Lusaka, Northern Rhodesia.

THE third European Mycological Congress will be held in Glasgow during August 31–September 7. The programme will include a symposium on "Gasteromycetes". Further information can be obtained from the Local Secretary, Third European Mycological Congress, Botany Department, the University, Glasgow, Scotland.

THE British Institution of Radio Engineers and the Institution of Electrical Engineers are organizing a joint symposium on "Automatic Control", which will be held at the University of Birmingham on March 28. Further information can be obtained from Mr. G. K. Steel, Department of Electrical Engineering, College of Advanced Technology, Gosta Green, Birmingham 4.

THE Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology will be held in London during April 16–24. Among the subjects to be discussed will be the problems in relation to: aviation; odontology; horse doping; stains by human tissues; drug addiction; psychiatry; industrial toxicology; clinical toxicology; plant and animal poisons; poisoning by therapeutic substances; questioned documents. Further information can be obtained from the Secretariat, Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology, 28 Portland Place, London, W.1.

A SYMPOSIUM on "Aspects of Chemical Change during the Processing and Storage of Foods", arranged by the Nutrition Society (Scottish Group), will be held in the Royal College of Science and Technology, Glasgow, on April 20. The programme will include: foodstuffs as unstable chemical systems; catalytic destruction of vitamin A and carotene; chemical change in fish muscle during storage; flavour changes in potatoes; the environment for chemical change in dried and frozen foods. Further information can be obtained from Dr. D. G. Armstrong, Department of Agricultural Biochemistry, King's College, Newcastle upon Tyne 1.

THE INTERNATIONAL COMMITTEE ON WEIGHTS AND MEASURES

At the October 1962 meetings in Paris of the International Committee on Weights and Measures (Bureau International des Poids et Mesures, Pavillon de Breteuil, Sèvres, France), and its specialist sub-committees, proposals were made which, when formally adopted, will result in improved or extended bases for the measurement of length and temperature. Included were 12 new secondary wave-length standards of length, and 6 proposals to modify the International Practical Temperature Scale.

It was recognized nearly a century ago, in light of increasing industrialization and the expansion of foreign trade, that some sort of international agreement was necessary in the area of physical measurements. After considerable preliminary work, both scientific and diplomatic, the Treaty of the Metre[1] was signed in 1875 by representatives of 17 nations (there are now 38 member nations). This treaty provided international agreement on the bases for the metric system, and established the International Bureau of Weights and Measures on the outskirts of Paris, France. The International Bureau, funded by assessments of the member nations, conducts research in many areas of measurement, maintains physical prototype standards, and co-operates with the national standards laboratories in promoting measurement accuracy and agreement.

Representatives of the nations adhering to the Treaty of the Metre normally meet in Paris every 6 years at what is called a General Conference on Weights and Measures. Official actions, such as the adoption of new or improved standards, are voted on by the General Conference. Details of administration, and responsibility for recommending proposed actions to the General Conference, fall in the province of the executive body or International Committee, composed of 18 individuals, each from a different nation. The International Committee, which has advisory committees in 6 areas of measurement, meets annually. The actions of the advisory committees on length and temperature at the recent meeting are outlined here.

Secondary Wave-length Standard of Length

The metre was officially defined by the 1960 General Conference[2] as equal to 1650763·73 wave-lengths in vacuum of the orange-red wave-length of krypton-86, making the wave-length of that line about 6057·8021 ... × 10^{-10} m. This action abrogated the platinum–iridium metre bar as the standard of length, and resulted in a more accurate standard of widespread availability. At the October 1962 meeting the Consultative Committee for the Definition of the Metre recommended to the International Committee 12 secondary wave-length standards of length. These are:

A	Wave-lengths in vacuum of krypton-86
	6458·0720 × 10^{-10} m
	6422·8006 ,,
	5651·1286 ,,
	4503·6162 ,,
B	Wave-lengths in vacuum of mercury-198
	5792·2683 × 10^{-10} m
	5771·1983 ,,
	5462·2705 ,,
	4359·5624 ,,
C	Wave-lengths in vacuum of cadmium-114
	6440·2480 × 10^{-10} m
	5087·2379 ,,
	4801·2521 ,,
	4679·4581 ,,

The values for krypton are within 2 × 10^{-8}, those for mercury 5 × 10^{-8}, and those for cadmium 7 × 10^{-8} of the true values, respectively. These figures represent a consensus of view of all the national laboratories. It should be noted that these values have not as yet been accepted as international secondary standards of length, but that such action is expected probably late in 1963.

The Consultative Committee, recognizing that further work remains to be accomplished in the measurement of length, is encouraging the development of secondary standards in the ultra-violet and infra-red regions, and examination of the use of masers, Zeeman filters, atomic beams and interference filters in the measurement of length.

Proposed Changes in the International Practical Temperature Scale

The International Practical Temperature Scale[3], established in 1927, is defined in terms of a series of fixed points and of interpolation formulæ for the instruments used to realize the scale. Advances in the science of temperature measurement have made it possible to consider changes to make the International Practical Temperature Scale agree more nearly with the thermodynamic temperature scale, and to cover a more extended range of temperatures.

The Advisory Committee on Thermometry of the International Committee has made 6 tentative proposals for presentation at the 1966 General Conference of Weights and Measures. These tentative proposals, which would both extend the scale and improve its accuracy, are:

(1) Incorporate the T_{58} (^{4}He) and T_{62} (^{3}He) scales into the International Practical Temperature Scale. At present, the International Practical Temperature Scale is undefined below the oxygen point (90·18° K), and the inclusion of these two scales will provide a basis on the International Practical Temperature Scale for measurements in the range 0·25°–5° K. The T_{58} (^{4}He) scale has been recognized by the International Committee on Weights and Measures since 1958, but it has not been incorporated in the International Practical Temperature Scale. The ^{3}He vapour pressure scale, developed by the Los Alamos Scientific Laboratory, was this year recommended by the International Committee of Weights and Measures for interim use with the designation T_{62} (^{3}He).

(2) Define for the practical scale the equilibrium boiling point of hydrogen to be about 20·267° K and the boiling point of oxygen to be about 90·17 °K. The current value for the boiling point of oxygen is 90·18° K. No value for the boiling point of hydrogen is given on the International Practical Temperature Scale at present. The term 'about' is included in the wording of these proposals to indicate that new results or re-evaluation of existing data may change these values between now and 1966.

(3) Incorporate a platinum resistance thermometer scale in the region between the hydrogen and oxygen points. This step would extend the scale from its present limit of the oxygen point. Platinum resistance thermometers have been used for some time in this region, and there appears to be sufficient data on which to base this extension. However, work on the proper interpolation method, including the choice of the resistance-temperature relation, is needed in order to make this extension practical.

(4) Eliminate the sulphur (444·6° C) and replace it with the zinc point (at present 419·505° C, but see proposal (5)). This action is recommended because the zinc point is more reproducible than the sulphur point.

(5) Change the values of the zinc (419·505° C), silver (960·8° C), and the gold (1063° C) points on the Inter-

national Practical Temperature Scale by about +0·07, +1·1 and +1·5 degrees, respectively.

(6) Replace the standard thermocouple on the scale with the platinum resistance thermometer. The scale is at present defined in terms of the thermocouple from the gold point down to 630·5° C, and the platinum resistance thermometer from the oxygen point to 630·5° C. As the platinum resistance thermometer is more precise than the thermocouple, this action will yield more accurate determinations of temperature, and will result (along with proposal 3) in the definition of the scale from the hydrogen point to the gold point with platinum resistance thermometers.

The U.S. National Bureau of Standards has contributed strongly in the past to the foundations of the International Practical Temperature Scale, and is now working on two major projects which may affect these tentative proposals before submission in 1966. One concerns the gap that would exist in the scale between about 5° K and the hydrogen point even if provisions (1) and (3) are adopted. The Bureau is developing the ultrasonic thermometer as an absolute device to provide a scale in this region. Preliminary results with this thermometer, which is based on the determination of the speed of sound in helium gas, indicate a precision of ±0·007 deg. at 20° K and 0·002 at 2° K (ref. 4). Also, a major programme under way in gas thermometry may result in more accurate values for certain defining points on the scale before the 1966 meeting.

New Prefixes

The International Committee also adopted two new prefixes for denoting sub-multiples of units. They are: femto, 10^{-15}, symbol 'f', and atto, 10^{-18}, symbol 'a'. The Bureau will follow this action of the Committee, which brings the list of numerical prefixes to a total of 14. The full list is given in Table 1.

Administrative

The International Committee on Weights and Measures met this year for the first time with the new director of the International Bureau of Weights and Measures, Dr. Jean Terrien. Dr. Terrien is a distinguished French metrologist who had been at the International Bureau for several years. The Committee reluctantly accepted the resignation of its secretary, Prof. G. Cassinis, rector of the Polytechnical School of Milan, who had recently become the Mayor of Milan and therefore felt compelled to relinquish international committee responsibilities. Prof. J. de Boer, director of the Theoretical Institute of the University of Amsterdam, was elected the new secretary. Other officers, elected in 1960, are chairman, Prof. Richard Vieweg, recently retired as president of the Physikalisch-Technische Bundesanstalt, Germany, and vice-chairman, Dr. L. E. Howlett, director of the Applied Physics Division of the National Research Council, Canada.

Table 1

Multiples and Sub-multiples	Prefixes	Symbols	Pronunciations
10^{12}	tera	T	tĕr′ ȧ
10^{9}	giga	G	jĭ′ gȧ*
10^{6}	mega	M	mĕg′ ȧ
10^{3}	kilo	k	kĭl′ ō
10^{2}	hecto	h	hĕk′ tō
10	deka†	da	dĕk′ ȧ
10^{-1}	deci	d	dĕs′ ĭ
10^{-2}	centi	c	sĕn′ tĭ
10^{-3}	milli	m	mĭl′ ĭ
10^{-6}	micro	μ	mī′ krō
10^{-9}	nano	n	năn′ ō*
10^{-12}	pico	p	pē′ cō*
10^{-15}	femto	f	fĕm′ tō
10^{-18}	atto	a	ăt′ tō

* These pronunciations differ from those previously given, and are the result of international discussions to promote uniformity of pronunciation in various languages.

† The official French spelling for the prefix representing the multiple ten is deca. This prefix has long been spelled deka in the United States, Great Britain, Germany, and a few other countries. The continued use of this spelling is consistent with the American use of gram for the French gramme and meter for metre. The symbol, however, should be universal and even though dk has long been used in the United States for the symbol for deka, the National Bureau of Standards will from now on adhere to the official international symbol da for the prefix representing the multiple ten and recommends that this practice be followed throughout the United States.

One of the most important actions of the 1962 meeting was the completion of negotiations with the French Government for land to house a new laboratory for the recently activated programme on standards for measuring ionizing radiations. Plans for the Laboratory were reviewed and approved and construction is expected to begin early in 1963. The new laboratory will be on land until recently part of the Parc de St. Cloud, immediately adjacent to the present building of the International Bureau of Weights and Measures.

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[2] *NBS Tech. News Bull.*, 44, 199 (Dec. 1960).

[3] Text revision of 1960. H. F. Stimson, *NBS Mon.* 37 (Supt Documents, United States Government Printing Office, Washington 25, D.C., 10 cents).

[4] *NBS Tech. News Bull.* (Jan. 1963).

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

The *Bulletin of the National Research Development Corporation* is issued twice-yearly and the twenty-first issue (October 1962) appears with a new cover and in a revised modern format. The main articles are devoted to descriptions of the "Perbury Gear" and "The Piezo-magnetic Analogue Multiplier", both of which are the subject of patents, and the development of which is being supported by the Corporation. An improved unit utilizing the Perbury gear has been designed for the transmission of an input torque of 100 ft. lb. over a ratio range of 4·7 : 1 and six of these units have been built for trials on two re-circulating power test rigs at Plint and Partners, Ltd., of Wargrave. The results so far attained indicate that the possible industrial uses of the Perbury gear cover a wide range, from machine tools to test-house equipment and vehicle transmissions. The particular advantages are simplicity, compactness, high efficiency, and the ability to work at high rotational speeds. It is known from nearly 25,000 miles running experience with the *Hillman Minx* motor-car that the transmission is quiet at all speeds and, under normal motoring conditions, gives a lower fuel consumption than a car fitted with a standard gear-box.

The piezo-magnetic analogue multiplier utilizes the expression $XY = \frac{1}{4}[(X + Y)^2 - (X - Y)^2]$ and makes use of the fact that the force in a magnetic circuit is proportional to the square of the magnetic flux. A bender type piezo-electric crystal carries two soft iron armatures at one end, one on each side of the crystal. Opposite each of the armatures, and separated from it by an air gap, is a laminated silicon iron C-core. The two C-cores carry four identical coils, one on each limb. The input quantities to be multiplied are represented by currents X and Y. On one C-core the coils are connected in the same sense and the magnetic force exerted on the armature is thus proportional to $(X + Y)^2$. On the other C-core the coils oppose and the magnetic force exerted is proportional to $(X - Y)^2$. The two forces act in opposite directions so that the voltage output from the piezo-electric crystal is therefore proportional to XY. Small multipliers measuring 1 in. diameter by $1\frac{1}{4}$ in. long have been made and with improved materials multipliers of even smaller size with greater temperature stability, sensitivity and resonant frequency can be made. The applications of multipliers up to the present time have been limited, but the trend in electronics is towards extremely complex pattern recognition systems which utilize auto-correlation and cross-correlation of wave shapes, operations which require the use of an analogue multiplier. The computational power of a multiplier compares well with that of a fast digital computer, for with a bandwidth of 5 kc/s and an accuracy of $\frac{1}{2}$ per cent it can effectively multiply two 7-bit numbers 10,000 times per sec and a network containing 100 multipliers could easily do more arithmetic per sec than the fastest digital computer yet built.

THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

THE fifty-fifth annual report of the Governing Body of the Imperial College of Science and Technology* covers the year ended July 31, 1962, in which the number of full-time students increased by 53 to 2,735, with a decrease of 4 in postgraduate students, and the academic staff increased by 25 to 444 full-time members. The report refers to the difficulty in recruiting and retaining staff of the right calibre, mainly because of the disparity between academic salaries and the inducements offered by industry. This may seriously affect the implementation of the integrated academic plan; but great concern is also expressed as to the effect of the restricted grants to be made available in the next quinquennium, as announced in June 1962. From October 1963 the undergraduate tuition fee has been increased to £85, inclusive of College and University examination and registration fees, the postgraduate fee remaining at £75 exclusive of those charges. Discussions are proceeding with the Architectural Association on the possible incorporation of the Architectural Association School within the College. The Governing Body considers that a close connexion between leading schools of engineering, particularly civil and structural engineering, and of architecture could be of great value. The £17·5 million 'ceiling' imposed on the overall cost of the Jubilee Expansion Scheme has necessitated postponing the start of two of the major buildings, with adverse effects on the Departments of Mathematics, Meteorology and Chemistry concerned.

The Rector's report notes that, during the year, students obtained 200 higher and 498 first degrees, science degrees accounting for 46 per cent of the total, and the 400 students awarded the D.I.C. included 296 technologists. Of the 653 overseas students, 406 were from the Commonwealth and four-fifths were postgraduates. Of the United Kingdom students, 27 per cent came from within 30 miles of the College. The academic staff now includes 50 professors and 73 readers, and developments in fields lying on the borderline between important disciplines include the introduction of a course on the application of engineering to medicine and biology and the institution of a chair in the history of science and technology. Library facilities were greatly improved during the year and, referring to research, Sir Patrick Linstead notes that of 37 research projects at present listed by the National Research Development Corporation, 6 originated at the College. Grants for research and income from research contracts totalled £523,000, the Department of Scientific and Industrial Research supporting 77 projects with an annual value of £235,000. Some notes on recent research are included in the Rector's report, and a list of publications during the year, arranged by departments, is appended, together with a list of special lectures.

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* Imperial College of Science and Technology (University of London). Fifty-fifth Annual Report of the Governing Body, 1961–62. Pp. iv+138. (London: Imperial College of Science and Technology, University of London, 1962.)

ENVIRONMENTAL PHYSIOLOGY AND PSYCHOLOGY IN ARID CONDITIONS

A SYMPOSIUM on "Environmental Physiology and Psychology in Arid Conditions" was held recently at the Central Drug Research Institute in Lucknow, under the auspices of Unesco and the Government of India. The arrangements were made by Dr. M. Batisse of the Department of Natural Sciences of Unesco, and locally by Dr. B. Mukerji, director of the Institute and chairman of the symposium, with Dr. J. Swarbrick of the Unesco South Asia Science Co-operation Office at Delhi. Thirteen countries were represented by approximately fifty delegates.

The scientific papers and discussion were divided into nine sections, as follows: the neurophysiology of heat exposure, psychological aspects, human performance and comfort standards, comparative heat physiology, the significance of solar radiation in heat balance, water and electrolyte problems, nutrition and heat, medical climatology, and physiological anthropology. The proceedings, which will be published by Unesco, drew strength principally from two sources: first, from a number of pre-circulated reviews of the topics chosen for discussion, and secondly, from recent fundamental and field research, particularly in Israel and the French Sahara.

Unesco will publish these reviews separately and within the next few months. They range in subject-matter from physiological adjustments to heat, to the somewhat vexed definition of tropical neurasthenia. The authors include F. A. Fuhrman (United States, effects of heat on the action of drugs), E. M. Glaser (Great Britain, circulatory adjustments to heat), A. Henschel (United States, field test methods), R. A. Kenney (Great Britain, renal adjustments), G. E. Lambert (France, thermal comfort), D. H. K. Lee (United States, arid zone physiology), W. V. Macfarlane (Australia, endocrine adjustments), J. Mayer (United States, arid zone nutrition), E. Schreider (France, physiological anthropology), and F. Sargent (United States, tropical neurasthenia). Available also to the symposium participants was a review of the works of Soviet scientists on arid zone problems (prepared by the U.S.S.R. Academy of Medical Sciences and translated from the Russian). These documents are listed here because they were the background to many of the short papers read at the symposium, but there were also unrelated communications of value, notably by O. von Euler (Sweden) on the body thermostat and its setting, and by K. Schmidt-Nielsen (United States) on comparative physiology in arid climates.

The papers relating to recent studies in Israel and the French Sahara, along with similar contributions from Indian and Australian scientists, provided most of the participants with fresh and stimulating information. The Institute of Technology at Haifa, the Negev Institute in Beersheba, the climatic unit at the Tel Hashomer hospital in Tel Aviv, and the French centre for study and information on human problems in arid zones (PROHUZA), have all contributed significantly to our knowledge of heat physiology; and Unesco has done well to foster, throughout its major project on scientific research on arid lands, studies which are carried out in the field rather than in climatic chambers in temperate countries. Developing communities in arid regions, given a new way of life and new standards of hygiene, must sooner or later face the issues of how the local climate affects health and industrial programmes; and the studies which have been made so far, principally on expatriate European and American communities, are not altogether relevant. In this respect the work of W. V. Macfarlane (Australia) on long-term heat adaptations in domestic stock animals is particularly valuable, and it is encouraging to learn that similar comparative research is carried out in Egypt and at the Central Indian Arid Zone Research Institute in Jodhpur, and that the PROHUZA team has examined the industrial-employment aptitude and long-term adaptations of nomadic tribes in the Sahara.

The visiting participants at the Lucknow symposium are indebted to Unesco and the Government of India, and to Dr. B. Mukerji for his hospitality at the Central Drug Research Institute. The published Proceedings will contain much valuable information and should stimulate continued interest in the subject in the Middle East and in South-East Asia.

C. S. LEITHEAD

CARDIOVASCULAR ANATOMY AND PATHOLOGY

A SYMPOSIUM on 'Cardiovascular Anatomy and Pathology', organized by Prof. R. G. Harrison and Prof. K. R. Hill, was held in the meeting room of the Zoological Society of London during November 28–29, 1962. This symposium, which was attended by more than 100 research workers from a wide variety of medical and veterinary departments, reflected the growing interest in the functional anatomy and pathology of the cardiovascular system.

During the first morning session, under the chairmanship of Prof. E. W. Walls, Prof. F. Navarrina Gamez (Santiago de Compostela, Spain) described researches conducted in collaboration with Prof. R. G. Harrison on the effect of cortisone on the vascularization of bone in normal and ovariectomized female rats. These researches, like others during the symposium, demonstrated the need for a detailed analysis of the functional anatomy of organs and tissues when investigating their vascularization. During the first four weeks after ovariectomy there is a reduction in the extent of vascularization of the metaphyses and vertebral bodies. This is not as extensive as in cortisone-treated rats, however, and is followed, within ten weeks, by an increased vascularization. The primary metaphyseal ischæmia can be correlated with a disorganization of cell columns in the epiphyseal cartilages of long bones, which persisted in cortisone-treated rats. These alterations in vascular pattern are accompanied by changes in the pattern and density of trabeculæ in metaphyses and vertebræ; in some animals there is an obvious fragmentation of trabeculæ in vertebral spines.

Dr. Helen N. Duke (London) summarized her investigations on the hæmodynamics of the pulmonary circulation. Factors concerned with the pressure and flow and change of blood volume in the circulation were discussed. The pressure flow is concave to the flow axis. The curve is affected by changes of left atrial pressure and by vasomotor reactions of the pulmonary blood vessels. Increase of pulmonary arterial inflow rate or left atrial pressure increases the volume of blood in the pulmonary blood vessels; some of the increased blood volume is the result of capillary dilatation. It is probable that those vessels responsible for pulmonary vascular resistance are not those responsible for changes in lung blood volume.

The functional anatomy of the vertebral venous system was described by Dr. J. D. W. Tomlinson (London). The variability of the posterior vena cava, particularly among aquatic mammals, was clearly and beautifully demonstrated. The value of the azygos venous system as a collateral circulation for drainage of structures below the level of the diaphragm was also discussed.

Dr. R. L. Holmes (Birmingham) reviewed present-day knowledge on the anatomy of the hypothalamo-hypophyseal vascular system, with particular relation to the hedgehog and ferret, and showed that there is no isolation of the vascular supply of one part of the gland from that of another or from the hypothalamus. The portal vessels are responsible for carrying the greater part of the normal blood supply to the pars distalis, and play a major part in the mediation of hypothalamic control over the pituitary.

In the afternoon session on November 28, under the chairmanship of Prof. Harrison, Mr. D. Sykes (Liverpool) reviewed some of his work on the blood supply of the human kidney. There is a segmental pattern of branching of the renal artery within the substance of the kidney which is, however, subject to some variation. A continuous arcuate artery does not exist. The venous pattern does not correspond with the arterial supply, and evidence was presented to suggest that blood vessels which penetrate the kidney capsule are carrying blood which is passing centripetally into the substance of the kidney. The longitudinal sulcus, when it exists, does not correspond with Brodel's line, which is neither linear nor avascular.

Mr. D. B. Moffat (Cardiff) summarized the research work which he had been conducting with Dr. Julia Fourman on the fine vascularization of the mammalian kidney; he demonstrated the difference to be found in various mammalian species, and clarified the nature of the vasa recta, which he found to be mostly composed of capillaries.

Prof. D. F. M. Bunce II (Des Moines, Iowa) read a paper on the architecture of the distended arterial wall, and stressed the differences in histological appearance of arteries in the collapsed and distended state, and between arteries from various sites in the body. In the teaching of students he had found the use of casts of the renal vascular tree of great value for their appreciation of the true nature of glomerular structure.

Dr. J. L. Braithwaite (Liverpool) described experiments he had been conducting with Dr. C. J. Fairhurst on collateral venous drainage of the spleen. Pursuing his original investigations on the regional distribution of the splenic vessels and their branches to the rat spleen, he had shown that the venous drainage of a region of the spleen the hilar vein of which had been occluded was effected through a plexus of newly formed veins encircling the site of ligature. Following interruption of the main splenic vein, collateral channels, consisting of preformed anastomoses (evident at 24 h) newly formed vessels (visible at 7 days) and portal-systemic anastomoses (which developed 3 weeks later), became established.

Dr. J. E. Wright (Liverpool) concluded the afternoon session by describing the effects of interruption of the vascular pedicles of the liver in the experimental animal, and compared the effects of ligation of the hepatic artery with those of interruption of the portal vein. Arterial ligation was not followed by either atrophy or necrosis, and was accompanied by the enlargement of œsophageal and omental anastomoses, and a peribiliary plexus of vessels coursing along the wall of the bile duct. Interruption of the portal vein was fatal within a few hours of operation in all cases in which the main trunk was ligated; ligation of the central lobar branch of the portal vein resulted in atrophy of the central lobe of the liver, and compensatory hypertrophy of the quadrate lobe.

During the morning session on November 29, under the chairmanship of Prof. W. I. Beveridge, Dr. R. Finlayson (London) gave a short account of the pathology and the incidence of arterial disease as it occurs in wild animals in captivity. Vascular lesions were described in fish, amphibia, reptiles, birds and mammals. Atherosclerosis is seen commonly only in some avian orders and occlusive atherothrombosis is extremely rare in animals. Age is the only factor shown to be fairly directly related to atherosclerosis, but the virtual absence of this condition in some comparatively old animals emphasizes that atheroma is not an inevitable accompaniment of senescence. The role of dietary and other factors which may play a part in the pathogenesis was discussed briefly.

Dr. B. McKinney, of Makerere College, Uganda, East Africa, gave an account of his investigations into arterial lesions discovered in free-living wild animals in East Africa. These were particularly prevalent among ruminants such as buffalo and antelopes, a surprising result in animals which fed only on herbage and the fat intake of which would be largely composed of unsaturated fatty acids. It had been suggested that ruminal digestion resulted in a high intake of acetic and butyric acids which might account for this, but the author discounted the suggestion that this factor could be of significance.

Dr. H. M. Pirie (Glasgow) gave an account of cardiovascular disease in domestic animals as seen in routine studies *post mortem*. He described a wide range of lesions from the aneurysms commonly found in horses associated with vascular invasion by nematode larvæ (*Strongylus vulgaris*) to atheroma of pigs and the atheroma-like lesions often seen in the coronary arteries of dogs.

On the experimental and physiological side, Dr. J. Poole (Oxford) described his work on experimental studies on growth of aortic tissue in baboons. He has used tissue-culture methods from which he believed that aortic endothelium was related to smooth muscle. Dr. A. F. Munro and Dr. R. S. F. Campbell (Glasgow) gave an account of their work on the respiratory metabolism of blood vessels from different species. The authors had evolved the technique of measurement of oxygen consumption by normal and diseased aortæ and had found significant impairment in the latter.

During the afternoon session, under the chairmanship of Prof. K. R. Hill, Dr. M. Crawford (London) described the epidemiology of coronary artery disease. Coronary artery disease cannot be regarded as a disease entity but is made up of two components; (1) mural atheroma; (2) occlusion of the lumen; which are obviously related, but are different from each other. Epidemiological evidence shows that occlusion of the lumen appears to be related, to physical activity and associated with certain diseases such as peptic ulcer, whereas mural atheroma appears to have no relation to these factors. In general, there appears to be an increase in prevalence of coronary occlusive disease among the populace, but not of mural atheroma.

Dr. Jack P. Strong (New Orleans) made some observations on the natural history and geographic pathology of human atherosclerosis. A geographical study of coronary artery disease has shown that white males are more severely involved than other sex or race groups. Aortic fatty streaks are practically universal after the first decade of life, but population differences in the formation of fibrous plaques seem to parallel suspected differences in the clinical manifestations of atherosclerotic heart disease.

Dr. M. I. Wright (London) discussed age changes in peripheral arteries of man. Peripheral arteries were examined from twelve sites at all ages. Two groups of change from the typical infant pattern were found: those which appeared to be associated with growth; namely, muscular elastic cushions, thickening intima, longitudinal elastin fibres, loss of continuity and swelling and beading of the internal elastic lamina, and a layer or layers somewhat resembling the internal elastica. The second group of changes includes small plaques of calcification of the internal elastica, and accumulation of excessive mucopolysaccharide in the media; these are seen best and earliest in the arteries of the leg in adult life.

Prof. S. Gero (Budapest) described his work in relation to mucopolysaccharides in the arterial wall and, in particular, the role of these compounds in the mechanism of lipid deposition. He had found that lipid could be adsorbed to mucopolysaccharide and could thereby be deposited in the arterial wall. He regarded this as one of the main mechanisms which led to the development of the atheromatous plaque.

Mr. W. J. S. Still (London) described electron microscopic studies in experimental atherosclerosis. Electron microscopic studies of dietary-induced aortic atherosclerosis in the rat and the rabbit show that lipid-bearing cells are capable of penetrating the intact endothelium. In contrast, when individual endothelial cells degenerate, lipid-bearing cells from the intima appear to extrude into the lumen. Ultra-microscopy also shows that two main cell types are present in all intimal lesions: first, foam cells, with complex lipid inclusions, and secondly, smaller cells with simple lipid inclusions. These cell types appear to be largely derived from the blood and aortic smooth muscle respectively.

R. N. Fiennes

THE EXPANDING EARTH?

By Prof. L. EGYED

Geophysical Institute, Eötvös University, Budapest

THE change of the Earth's dimensions is an old problem occurring mainly in connexion with mountain building and the moving or origin of continents. Generally, the first problem suggests contraction, while the second expansion of the Earth.

The comments made by J. G. Dennis[1] referring to a communication by C. H. Barnett[2] necessitate the summarizing of the idea of expansion. According to our knowledge Mantovani[3] was the first, at the end of the nineteenth century, to apply the idea of expansion to the explanation of the similarity of the Atlantic coast lines.

B. Lindemann[4] was the next who derived the expansion of the Earth from an excess of heat generated by radioactive elements existing in the Earth's interior. As it has been shown lately[5,6], heat is responsible for a very limited expansion only. The same idea was supported by Bogolepov[7], showing its probability on different geological features.

O. C. Hilgenberg[8] in 1933 was the first to construct a contiguous sialic surface on a sphere of a diameter about half of that of the present Earth and consisting of the present continents connected by shelves. But he had to cut and shift North America in his reconstruction considerably along a fault line. On the other hand, he suggested as the cause of expansion the physically untenable hypothesis according to which an æther-like material from space enters into the interior of the Earth, and hence, the increasing internal mass increases the Earth's volume also.

J. K. E. Halm[9] also suggested the expansion of the Earth to clear up the disruption of and drifting apart of continents. His explanation was based on astronomical considerations.

J. Keindl[10] published a hypothesis according to which the Earth possesses a central core of a very small size located in the centre of an iron core. This central core consists of a very dense material (density of some ten or hundred thousand g/cm³) similar to that of the white dwarfs (that is, corresponding to a Fermi-gas) and disintegrating in the course of time. He applied the expansion idea also to the explanation of 'continental drift'.

Pascual Jordan[11] refers to a private communication by Joel E. Fisher, in which the idea of expansion of the Earth is expressed with the aim of explaining the present distribution of continents and oceans. P. Jordan adopts this result in support of his projective theory of relativity.

The main idea of R. T. and W. J. Walker[12] essentially coincides with that of Keindl with the only difference that, instead of Fermi-gas some mysterious '*Q*-atoms' disintegrate with time and cause expansion. In the first place this idea strives to explain volcanism and mountain building.

It is curious that these authors as well as those mentioned later arrived at the idea of expansion independently, without knowledge of their predecessors. On the other hand, all these authors came upon the idea of expansion in an effort to explain the disruption and drifting apart of continents. At the same time they did not try to treat it in a quantitative way and often defied the laws of physics.

I have already pointed out how the idea of expansion is supported by analysing the interior of the Earth, contrary to the earlier authors, who derived the idea from the requirements of continental wandering. In the course of my investigations I developed a quantitative theory for the dynamism of the Earth, consistent with modern physical theories[13–19] and geological–geophysical observations. While expansion is only a special result of this theory, it also offers an explanation of the development of the Earth's surface[20], also its origin[21] and internal evolution[22]. Moreover, the amount of internal energy is estimated. In the course of my investigations it was shown in different ways and independent geological and geophysical observations that the most probable increase of the Earth's radius is 0·5–1·0 mm per annum.

As the next representative of the expansion theory W. Carey[23] may be considered. He arrived at the idea in connexion with his orocline concept. At first, his work was also influenced by the continental drift theory. The rate of expansion supposed by him, however, seems to be much higher than those values which can be considered as real on the basis of physical, geological and geophysical data.

In connexion with his investigations concerning Mach's principle, R. H. Dicke[24] also expressed the opinion that a decrease in gravity results in the expansion of the Earth. However, I think his estimation of increase in radius is probably too low.

After discovering the oceanic rifts, B. C. Heezen also came upon the idea of expansion as a possible cause of their formation[25,26].

It must also be mentioned that J. Tuzo Wilson, after his recent article[27], seems to be a representative of the expansion conception. However, in 1958 he declared himself as a supporter of contraction and an opponent of expansion. But his recent article[27] expresses no more than the validity of his tectonic concept even in the case of an expanding Earth.

Cox and Doell[28] have determined the Permian radius of the Earth based on an idea of mine[29]. It has been shown that the rate of expansion derived by Carey is too high and that the values suggested by me are between the limits of reality.

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INTERTIDAL ZONE CHANNEL DEPOSITS IN MIDDLE JURASSIC GREAT OOLITE SERIES, SOUTHERN ENGLAND

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THE Middle Jurassic Great Oolite Series of southern England consists of interbedded, shallow marine, oolitic limestone, shelly limestone, fine-grained limestone and olive silty claystone. Extensive development of oolitic limestone in the Great Oolite Series prompts one to infer depositional conditions similar to parts of the present-day Bahama Banks described by Illing[1], Newell and Rigby[2] and Newell, Purdy and Imbrie[3]. However, the Bahama Banks represent a complex of shallow marine environments which includes not only oolitic shoals[4] but also tidal creeks and mangrove swamps[5], coral reefs[3] and vast accumulations of non-oolitic lime sand and lime mud. Recognition of each of these distinct environments in sequences such as the Great Oolite Series will enable one to determine palæogeography and solve a variety of stratigraphic problems that still exist in these rocks.

A recent investigation of the Great Oolite Series in the Oxfordshire–Cotswolds Basin by me indicates that cross-stratified, shelly, oolitic limestones reported as part of a larger cycle by Arkell and Donovan[6] are characterized by

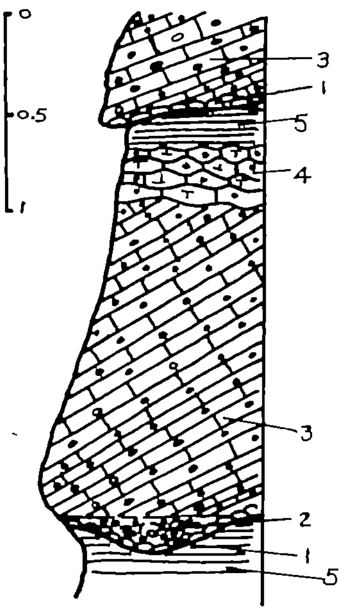

Fig. 1. Vertical sequence of limestone lithologies in channelled limestones, Great Oolite Series. (1) Basal lag concentrate of imbricate brachiopod, clam and oyster shells; (2) shelly, oolitic limestone; (3) cross-stratified, oolitic limestone; (4) wavy bedded, ripple marked, micro-cross-laminated, oolithic fine-grained limestone; (5) olive claystone. Scale in feet

a specific vertical succession of lithologies, textures and primary structures. Such a limestone sequence is also characterized by a downward-thickening, cross-sectional geometry resembling a channel. The downward thickening is at the expense of the underlying beds. Channels in the Chipping Norton Limestone, the Farmington Oolite member of the Stonesfield Slate, the Taynton Stone, the Kemble Beds and the Wychwood Beds show the following vertical sequence of lithologies from the base upward: shelly limestone (0–1 in. thick), shelly-oolitic limestone (0–1 in. thick), cross-stratified, oolitic limestone (1–4 ft. thick), wavy bedded oolitic, fine-grained limestone (2–4 in. thick) and olive claystone (0–6 in. thick) (Fig. 1). The channels average 15 ft. in width. Table 1 records the total number of cycles at the best localities exhibiting these features.

Table 1. PARTIAL LIST OF LOCALITIES SHOWING CHANNELLING PHENOMENA AND NUMBER OF CHANNELS IN GREAT OOLITE SERIES. OXFORDSHIRE–COTSWOLDS BASIN

Locality	No. of channels	Grid No.
Wychwood Beds		
Tolleys New Quarry, Bladon, Oxon	1	*SP*450150
Breakspear's Quarry, East End, Oxon	1	*SP*389153
Upper Greenhill Quarry, Gibraltar, Oxon	1	*SP*485177
Kemble Beds		
Tolley's New Quarry, Bladon, Oxon	1	*SP*450150
Breakspear's Quarry, East End, Oxon	2	*SP*389153
Crawley Road Quarry, Witney, Oxon	2	*SP*353109
Long Hanborough Station Quarry, Long Hanborough, Oxon	2	*SP*436142
Lower Greenhill Quarry, Gibraltar, Oxon	2	*SP*485177
Old Cement Works, Kirtlington, Oxon	2	*SP*495200
Holwell Downs Farm, Oxon	2	*SP*209281
Eastleach Turville, Oxon	1	*SP*195055
Leach Bridge, Glos	2	*SP*145080
¼ mile west of intersection of A433 and Ampney Crucis Road, Glos	2	*SP*059034
Railway Cut, Kemble–Tetbury Line, Glos	3	*ST*974975
Woodleaze Farm, West of Horsely, Glos	1	*ST*817979
Intersection of *B*4058 and Nymphsfield Road, Glos.	1	*ST*821984
Knockdown Quarry, Knockdown, Wilt	3	*ST*844876
Taynton Stone		
Castle Barn Quarry, Sarsden, Oxon	2	*SP*301226
Ashford Mill Railway Cut, Oxon	3	*SP*386159–*SP*389157
Taynton Quarries, Taynton, Oxon	3	*SP*234154–*SP*739156
Salperton Downs Quarry, Glos	1	*SP*071215
Roundhill Railway Cut, Glos	2	*SP*125221
Hornsleasow Quarry, Hornsleasow, Glos	1	*SP*131323
Farmington Oolite Member of the Stonesfield Slate		
Hill Barn Quarry, Glos	2	*SP*175134
Foss Quarry, Farmington, Glos	2	*SP*131160
Rte A-420, 1 mile north of North Leach, Glos	1	*SP*122163
Chipping Norton Limestone		
Highway Department, Chipping Norton, Oxon	1	*SP*320276
Cheltenham Quarries, Ltd., Swellwold Quarry, Glos	1	*SP*144260
Kewpark Quarry, Longborough, Glos	2	*SP*170283
Hornsleasow Quarry, Hornsleasow, Glos	2	*SP*131323

The basal shelly limestone consists of imbricated, convex-upward oriented shells of disarticulated clams, brachiopods and oysters. It lines the channel bottom. The shelly, oolitic limestone which overlies the imbricated shelly limestone is evenly laminated and filled with randomly oriented, disarticulated brachiopod, clam and oyster shells. This upper bed thins out to nothing as the channel margins are approached.

A thick (1–4 ft.) planar cross-stratified, oolitic and partly shelly-oolitic, limestone overlies the beds deposited on the channel floor. The oolites average 1 mm in grain size and are excellently size-sorted. Some cross-strata (about 1 in. thick) show a vertical grading from shelly limestone at the base to medium sand-sized oolitic limestone in the middle to a fine sand-sized oolitic limestone at the top. The cross-stratified limestone is overlain by thin (2–4 in.). fine-grained, poorly sorted, oolitic limestone which in some outcrops is wavy-bedded and in other outcrops is ripple marked and micro-cross-laminated. This wavy bedded, micro-cross-laminated zone is often overlain by an olive claystone, or was excavated by another limestone channel unit.

A somewhat similar cycle occurs in the Hampen Marl (Fig. 2). At the 'Old Quarries' north of Black Heath Clump, Oxfordshire (*SP*256156), the following vertical succession of lithologies was observed: slightly oolitic, quartzose limestone (1–2 ft. thick), brown siltstone (1½–3 ft. thick) and olive grey, platy claystone (6 in.–1 ft. thick). The basal sandy limestone thickens downward and is lined by imbricated, disarticulated clam and brachiopod shells mixed with clay chips. In other outcrops, the basal sandy limestone was observed to thin to nothing.

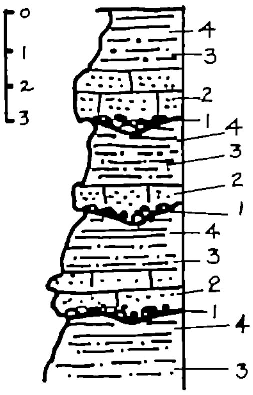

Fig. 2. Vertical sequence of lithologies in Hampen Marl. (1) Basal lag concentrate of imbricate clam and brachiopod shells and clay chips, (2) sandy limestone; (3) siltstone; (4) claystone. Scale in feet

The origin of these vertical successions can be determined by comparison with both Recent and fossil channel deposits in shallow marine environments. Van Straaten[7] has described the lithologies, textures and primary structures associated with laterally migrating creeks in modern tidal flat deposits in the Dutch Wadden Sea. He observed that during low tide, such lateral migration generated a lag concentrate of imbricated, convex-upward oriented, disarticulated clam shells. Such a lag concentrate is buried and preserved by point bar sands during lateral stream migration. The point bars are afterwards buried by inter-channel tidal flat muds. This process was described as "lateral sedimentation"[7]. A similar sequence of sediments occurs in the tidal flats of the Bay of Fundy[8], where, because of a difference in geomorphic setting[8], coarser-grained detritus is mixed in the channel-bottom lag concentrate; cross-stratified, sandy, point bars bury a channel floor lined with cobbles and clam shells. In the Bay of Fundy[9], the cross-stratified point bar deposits are overlain by ripple-marked and micro-cross-laminated, fine-grained sands.

The occurrence of channel bottoms lined with lag concentrates of imbricated, convex-upward oriented invertebrate shells appears to be a distinctive feature of meandering creeks traversing lower tidal flats at low tide. Present-day investigators[7–9], however, have confined their studies to tidal flats consisting of sand, silt and clay. No work has been undertaken on tidal flat disposition in areas of carbonate deposition. Bramkamp and Powers[10], however, have demonstrated that most limestone deposits accumulate by the same mechanical processes as silica clastic deposits. A parallel can therefore be drawn between the vertical succession of lithologies, textures and primary structures in modern tidal flat deposits and the channel deposits of the Great Oolite Series. Shell lag concentrates line channel bottoms in both modern tidal flat creeks and the limestone channels of the Great Oolite. The cross-stratified point bar sands of the Wadden Sea and the Bay of Fundy serve as modern analogues to the cross-stratified, sand-sized, oolitic limestone of the Great Oolite. The ripple-marked higher tidal flat deposits or inter-channel lower tidal flat deposits of the Wadden Sea and Bay of Fundy are analogous to the uppermost ripple-marked, wavy bedded, oolitic fine-grained limestone. I conclude from these data that the channel deposits in the Great Oolite Series were formed by the process of lateral sedimentation generated by intertidal zone creeks in an oolitic limestone-forming area.

Analogous vertical successions of lithologies, primary structures and textures have been observed by others[11–14] from channel deposits of different origin. Visher[11] has observed that modern fluvial deposits and ancient fluvial sandstone in the central and western United States are characterized by a vertical succession containing a basal clay-chip conglomerate, an overlying cross-bedded massive sandstone, an overlying ripple-stratified and thinly laminated sandstone and a capping siltstone and claystone. He infers that such features are diagnostic of meandering stream deposits where the clay-chip conglomerate represents a channel-bottom lag concentrate, the massive, cross-bedded sandstone represents rapid flow and the ripple-bedded zone and claystone represents tranquil flow. Allen[12,13] observed similar features in the Old Red Sandstone of England and drew a parallel to several meandering streams in Nigeria. Oomkens and Terwindt[14] reported such a sequence in modern estuarine sediment deposited by meandering creeks threading through tidal marshes. The only difference between the estuarine succession and the other vertical successions is the presence of a basal lag concentrate consisting of a mixture of shells and clay chips. The Hampen Marl of Oxfordshire shows such a vertical sequence of lithologies (Fig. 2) and was probably deposited by estuarine meandering creeks.

The parallelism in the vertical succession of lithologies between deposits from meandering creeks on lower tidal flats, meandering rivers on land and meandering creeks in estuaries is indicative of a common process. Van Straaten[7] has referred to the lateral shifting and re-working of sediment by meandering tidal creeks as the process of lateral sedimentation. The parallelism of the vertical succession of lithologies in tidal flat, fluvial and estuarine channel deposits suggests that Van Straaten's principle of lateral sedimentation is operative under a variety of sedimentary environments. At present, the only possible way to differentiate the three environments in which lateral sedimentation occurs is by close study of the lag concentrates lining the channel floors, as shell lag concentrates occur in tidal channels, mixed shell and clay-chip lag concentrates occur in estuarine channels and clay-chip conglomerates occur as lag concentrates in the channel floors of meadering rivers.

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RADIOACTIVITY OF BIOLOGICAL MATERIALS FROM BRAZILIAN AREAS RICH IN THORIUM COMPOUNDS

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THE areas of the world with a high background of radiation due to the presence of radioactive minerals in the soil afford great possibilities for investigations of long-term consequences to biological systems of continuous exposure for very long periods of time to relatively high doses of radiation.

There are two types of high background areas in Brazil where the thorium content of the soil is high. The first is the region of monazite sands, along the coast of the States of Rio de Janeiro and Espirito Santo, where the towns of Guarapary, Meaipe and Cumuruxatiba are situated. The average level of radiation in these towns is respectively 78·1 μr./h, 292 μc./h and 332 μc./h (ref. 1). The human population in this area is estimated at 20,000.

The second type is the region of volcanic intrusives, Morro do Ferro (Iron Hill) in Poços de Caldas, State of Minas Gerais. It is the most representative area of this type with an average level of radiation of 1,330 μr./h (ref. 1). No people are living in this area.

An extensive programme has been established by the Brazilian Atomic Energy Commission to investigate these regions. We have made some radiochemical analyses of samples brought to us by scientists working in this programme, in order to estimate the degree of contamination of their materials.

Among the thorium daughters the most important radionuclides from the physiological point of view are the isotopes of radium: radium-228 (mesothorium I) and radium-224 (thorium *X*). Being an alkaline earth, radium is a bone-seeker with a long biological half-life in animals. Radium-224 has a physical half-life of only 3·64 days and, once separated, it decays very rapidly; its daughters are all short-lived. Radium-228, however, has a physical half-life of 6·7 years and equilibrates very rapidly with actinium-228 (mesothorium II), an emitter of high-energy β- and γ-radiations. This makes radium-228 the greatest contributor, among the thorium daughters, to the internal irradiation of men and animals once they are contaminated by products of thorium decay.

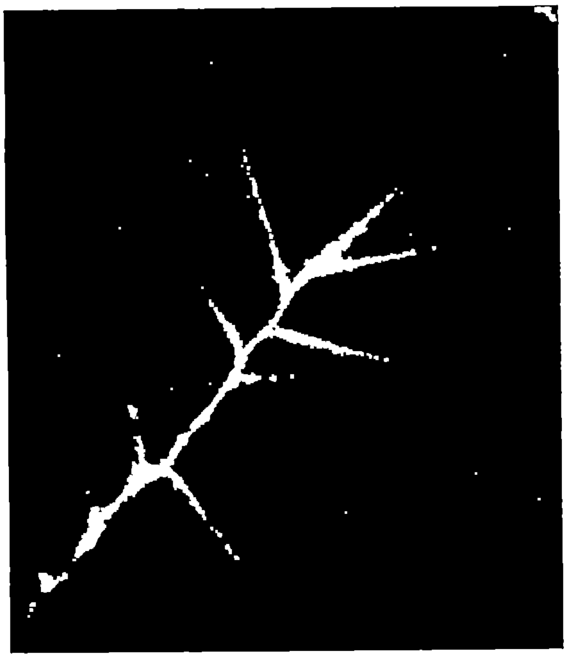

Fig. 1. Autoradiograph of leaves from a tree on Iron Hill, Minas Gerais, Brazil (exposure, 40 days)

Radiochemical separation of the radium isotopes of the thorium series, using barium and lead carriers, leads to the isolation of radium-228 and radium-224. The activity of radium-224 is insignificant after about 22 days, but the activity of radium-228 and actinium-228 in equilibrium is practically constant 22–90 days after separation[2].

The method used for separation of radium, barium and lead from calcium, iron, thorium and other interfering ions were essentially those recommended by the Health and Safety Laboratory, U.S. Atomic Energy Commission, for Sr-90 analysis of strontium-90 (ref. 3).

The separation of barium from lead was conducted by precipitation of the sulphates, followed by solution in alkaline ethylenediamine tetraacetic acid (EDTA). By acidification, only the barium sulphate reprecipitates, leaving the lead complexed in the supernatant[4].

For counting the radium-228 isolated from our samples we used a G.E.C. Geiger–Müller counter of low background (7 c.p.m.). We also counted samples of dried soil or ashed biological materials in a well scintillation counter associated with a spectrometer which was able to detect γ-radiation of 30 keV upwards. Since the isotopic composition and the degree of equilibrium among the different thorium decay products were not the same in the various samples counted, the results are not exactly comparable but give an indication of the total radioactivity present in the samples. Provided that the content in radionuclides from the uranium series is low, the results in counts per minute allow in first approximation an estimation of the number of disintegration occurring per minute, since the spectra of these samples were very similar. Of course radioactive potassium has also a contribution which is particularly important in plant ashes.

Table 1. DETERMINATIONS of RADIUM-228

Type of sample	No. of samples	d.p.m./g ash	μμc./g calcium
(a) Samples from Iron Hill			
Grass (leaves)	10	1,470–3,580	13,200–39,400
Grass (stems)	6	1,770–5,960	11,200–54,800
Bovine stools	4	425–573	13,200–24,500
Bushes (leaves and branches)	4	2,730–5,440	12,000–61,500
Moss	2	1,230–2,250	20,400–38,500
(b) Samples from Guarapary and Meaipe			
Calf bone	2	35	51
Calf teeth	2	12	18
Chick bone	2	8	11
Grass	4	160–270	680–1,340
Lobster shell	2	19	27
Bushes (flowers and leaves)	2	400	1.540
Bee wax	2	50	760
Bees	2	5	100
Bee mound	2	100	640

Table 2. GAMMA-ACTIVITIES

Type of sample	c.p.m./g ash
(a) Samples from Iron Hill	
Soil	784–3,580
Top soil (taken around the roots of grass)	1,060–5,060
Grass	1,260–6,670
Bushes	5,880
Moss	8,300
Fern	960–1,190
Bovine stools	
Mushroom (taken from the gallery of the thorium mine)	10,800
Frog (caught in the gallery of the thorium mine)	21,000
(b) Samples from Guarapary and Meaipe	
Monazite sand	57,500
Calf bone	17
Calf teeth	12
Chick bone	4
Grass (from Meaipe)	44–100
Sea shells	2–8
Lobster shell	8
Honey (*Trigona tataira*)	59–113
Bee wax (*Apis mellifera*)	20
Bees	6
Honeycomb (*Trigona tataira*)	29
Bee mound (*Trigona rufiris*)	88
Algae	616
Bushes (leaves and flowers)	468
(c) Controls.	
Potassium	92
Beach sand (Rio de Janeiro)	1
Top soil (R. J.)	8
Cow bone (bought in R. J.)	2
Honey (bought in R. J.)	15
Grass (R. J.)	57

The efficiency of our γ-radiation detector for radium-228 in equilibrium with actinium-228 was 28 per cent.

For comparison, some samples from a normal area, Rio de Janeiro (soil, sand, vegetation), or marketed in Rio de Janeiro (honey, cow bone) were counted in the same conditions, as well as potassium.

Chemical analysis of top soil from Iron Hill (carried out at the Laboratorio da Producao Mineral, Rio de Janeiro, by Dr. C. Pires Ferreira) gave contents of thorium dioxide ranging from 0·03 to 0·68 per cent in six different samples.

The great differences in the degree of contamination of biological materials from Iron Hill and from the coastal area could be due to differences in the solubility of the monazite sands and the thorium minerals occurring in the first area (thorium fluor carbonates), and also to the very irregular distribution of the monazite sands in the region.

In comparison with the external radiation-levels in the monazite sand zone, these preliminary results indicate that the internal irradiation of plants and animals due to radioactive contaminants should be relatively unimportant.

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EXCIMER FORMATION IN POLYCYCLIC HYDROCARBONS AND THEIR DERIVATIVES

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EXCIMERS are excited dimers, formed by the association of excited and unexcited molecules: they are dissociated in the ground-state[1]. In solutions of aromatic compounds the presence of fluorescent excimers is characterized by the appearance at high concentrations of a new, structureless emission band at long wave-lengths which is not accompanied by any change in the absorption spectrum. Excimer fluorescence was initially thought to be a property confined to pyrene and its derivatives[2], but recent studies by the authors[3–5] and others[6–9] have shown that it occurs in many polycyclic hydrocarbons and their derivatives. The purpose of this article is to summarize the present data, which indicate that excimer formation is the rule, rather than the exception, in this class of compounds.

The fluorescence spectra of the polycyclic hydrocarbons and derivatives listed in Table 1 have been examined in concentrated solutions at room temperature. The compounds are classified in terms of the number of benzene rings in the parent polycyclic hydrocarbon, and according to whether excimer fluorescence has been observed or not.

Anthanthrene and perylene solutions were earlier reported[9] to show new absorption bands at high concentration, which were attributed to the formation of stable dimers. Subsequent measurements on specimens of higher purity have shown the initial results to be incorrect.

Table 1. SOLUTIONS AT ROOM TEMPERATURE

No. of benzene rings	Excimer fluorescence	Ref.	No excimer fluorescence	Ref.
1	*benzene*	(6)	*p*-xylene	(6)
	toluene	(6)		
2	*naphthalene* (N)	(7)		
	1-methyl N	(8)		
	2-methyl N			
	1,6-dimethyl N			
	2,3-dimethyl N			
	2,6-dimethyl N			
3			*anthracene* (A)	
	9-methyl A	(9)	2-methyl A	
	9-ethyl A	(9)	9,10-diphenyl A	
			phenanthrene	(8)
4	*pyrene* (P)	(2)	*triphenylene*	
	1-methyl P		[*3:4-benzphenanthrene* (BP)]	
	2-methyl P		2-methyl BP	
	4-methyl P		2-isopropyl BP	
	3-cyano P	(8)		
	3-bromo P	(8)		
	P-sulphonates (aqueous solution)	(8)		
	1:2-*benzanthracene* (BA)			
	2′-methyl BA		9,10-dimethyl BA	
	3′-methyl BA			
	4′-methyl BA			
	3-methyl BA			
	4-methyl BA			
	5-methyl BA			
	6-methyl BA			
	7-methyl BA			
	8-methyl BA			
	9-methyl BA			
	10-methyl BA			
	2′,4-dimethyl BA			
	2′,6-dimethyl BA			
	cholanthrene			
	5-ethyl BA			
	5-propyl BA			
	5-amyl BA			
	5-phenyl BA			
	6-isopropyl BA			
5	*perylene*		*picene*	
	1:2-benzpyrene		*1:2,5:6-dibenzanthracene*	
	3:4-benzpyrene		*1:2:7:8-dibenzanthracene*	
	1:2:3:4-dibenzanthracene		*3:4:5:6-dibenzphenanthrene*	
6	*anthanthrene*			
	1:12-benzperylene			
	1:3:4:5-dibenzpyrene			

Anthanthrene has a typical excimer emission peaking at 594-nm wave-length. The excimer fluorescence of perylene in solution at room temperature is very weak, but the crystal fluorescence spectrum, a broad structureless band peaking at 580-nm wave-length, is clearly indicative of excimer formation.

The difference between the fluorescence spectra of a compound in dilute solution and in the crystalline phase is a further source of evidence for excimer formation, provided the crystal lattice structure is favourable for interaction between adjacent excited and unexcited molecules[9,10]. The crystal fluorescence spectra of pyrene, 5-methyl 1:2-benzanthracene, 8-methyl 1:2-benzanthracene, perylene, 3:4-benzpyrene, 1:12-benzperylene and anthanthrene[10,11] are all characteristic of excimer emission, thus confirming the solution data on these compounds (Table 1). The crystal spectra of 20-methyl cholanthrene, coronene and ovalene indicate that these compounds also form excimers.

Several of the compounds listed in Table 1 also show excimer fluorescence in the pure liquid state. These include benzene, toluene, 1-methyl naphthalene, 2-methyl naphthalene, 1,6-dimethyl naphthalene and pyrene. This method of detecting excimer formation is being currently extended to compounds of low solubility.

Studies of the rise and decay times of the monomer and excimer fluorescence of solutions[12] have shown that the excimer formation process is reversible, and that the relatively low quantum yield of excimer fluorescence in solutions of some compounds at room temperature is due to the high rate of non-radiative dissociation of the excimer. This dissociation rate decreases markedly on cooling, with a resultant increase in the excimer fluorescence yield, provided that the solvent remains sufficiently fluid at reduced temperature not to inhibit the diffusion-controlled excimer formation process. A major increase in the excimer fluorescence yield of a 5×10^{-3} M naphthalene solution in toluene has been observed on cooling from 21° to − 62° C (ref. 7). Acenaphthene in toluene solution, which has negligible excimer emission at room temperature, exhibits excimer fluorescence on cooling[13].

There are thus three distinct methods by which the data on excimer formation obtained from observations on solutions at room temperature may be supplemented or extended: from investigations of crystals, liquids and low-temperature solutions. It would therefore be premature to conclude that any compounds which do not exhibit solution excimer fluorescence at room temperature may not do so under more favourable conditions. It is of interest, however, to consider the factors which may inhibit excimer formation.

In anthracene and many of its derivatives, the dimeric interaction is so strong that the compounds form stable photo-dimers. Bonding occurs at the 9 and 10 positions in the two molecules forming the dimer, the central rings losing their aromatic character in the process[14]. It is interesting to note, however, that the formation of stable dimers in 9-methyl and 9-ethyl anthracene does not exclude the observation of excimer fluorescence at solution concentrations above 2×10^{-3} M (ref. 9).

9,10-disubstitution in anthracene prevents the formation of stable dimers, and this has been attributed to the effect of steric hindrance[9]. Similar steric hindrance is probably responsible for the inhibition of excimer formation in *p*-xylene and in the 9,10-disubstituted anthracene and 1:2-benzanthracene compounds. Except in these cases, substitution does not appear to interfere with excimer formation (Table 1).

Förster[8] has proposed certain conditions for excimer formation in solution, namely, the existence of a weak (^1L_b) lowest excited singlet state in the monomer adjacent to a strong (^1L_a) excited singlet state. Most of the compounds in Table 1 which exhibit excimer fluorescence in solution satisfy these conditions. In phenanthrene the $^1L_a - {}^1L_b$ energy separation is relatively large ($\sim$ 5,100 cm^{-1}) and probably exceeds the $^1L_a - {}^1L_a^-$ splitting, so that the $^1L_a^-$ state in the excimer lies above the ^1L_b state of the monomer. This factor has been suggested to account for the absence of excimer fluorescence in phenanthrene solutions[9]. The absorption spectra show that the $^1L_a - {}^1L_b$ energy separation is also large in triphenylene, 3:4-benzphenanthrene, 3:4:5:6-dibenzphenanthrene and picene, thus providing a similar explanation for the absence of solution excimer fluorescence. The f-values and separation of the ^1L_b and ^1L_a states in the two remaining compounds in Table 1, 1:2:5:6-dibenzanthracene and 1:2:7:8-dibenzanthracene, appear marginal for excimer formation.

The Förster conditions are not rigorous, and do not exclude the possibility, under favourable conditions, of excimer formation and fluorescence in compounds which do not satisfy them. A low excited singlet state with a strong transition moment from the ground state is required to provide the necessary interaction strength for excimer formation, but, although a lower weak singlet state is desirable to give a long life-time for the diffusion of the excited monomer molecules, it does not appear to be essential. Thus 2,5-diphenyloxazole, which has a very short monomer life-time of 2·6 nsec in xylene solution, corresponding to a strong lowest excited singlet state, nevertheless exhibits excimer fluorescence in high concentration solutions[15].

To summarize, it appears that excimer formation may be inhibited in the following circumstances: (1) when the interaction is so strong that stable dimers are formed, although excimer fluorescence may still be observed simultaneously in some compounds of this type; (2) when there is steric hindrance at the positions corresponding to the ends of the interacting dipoles; (3) when the transition from the ground state to the lowest excited singlet state of the monomer is weak, and there is no strong transition sufficiently close to provide adequate interaction for excimer formation.

We thank Prof. F. Bergel and Prof. M. S. Newman for the gift of compounds, and the British Empire Cancer Campaign for a grant in support of this work.

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PRECURSORS OF CARCINOGENIC HYDROCARBONS IN TOBACCO SMOKE

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A NUMBER of suggestions have been advanced[1] on the mode of formation of polycyclic aromatics in burning tobacco, on the tacit assumption that the thermal reactions involved are essentially unaffected by the simultaneously occurring oxidation process. In particular, tobacco paraffins[1] and cigarette paper[2] have been considered as precursors of the carcinogenic 3,4-benzopyrene (3,4-BP), since they form on pyrolysis small amounts of the latter compound. However, many organic materials have been shown[3] to yield some 3,4-BP at elevated temperature. Comparative experiments under smoking conditions have been reported[2] only in the case of cigarette paper, and it has been concluded[4] that the contribution from this source is not significant.

Terpenes have been mentioned as precursors by Wynder and Wright[1], but without giving any supporting data. In this article we wish to present evidence for the role of terpenic tobacco components in the formation of aromatics.

Earlier work[5] has led us to outline a scheme for the thermal aromatization of hydrocarbons at 550°–750° C, based on the interaction of dienic breakdown products. The reaction mechanism proposed involved mainly the intermediate formation of butadiene, followed by dimerization to 4-vinyl*cyclo*hexene and subsequent evolution of the latter into mono- and polycyclic aromatics. The polycyclic fractions formed in our experiments were not submitted to detailed analysis. Badger *et al.*[6], however, have shown recently that the pyrolysis at 700° C of both butadiene and 4-vinyl*cyclo*hexene results in considerable amounts of polycyclics and, particularly, in significant yields of 3,4-BP (0·34 and 0·48 per cent by weight of the total liquid product, respectively).

A similar pattern is emerging for the thermal reactions of isoprene. Staudinger and co-workers[7] have reported that at 700°–800° C the latter gives an aromatic tar, from which a number of polycyclic compounds were isolated. Recent experiments[8], carried out in this laboratory, showed that at 400° C and 760 mm isoprene forms a dimeric product composed of dipentene, 1-methyl-5-isopropenyl*cyclo*hexene, and a *gem*-type isomer, 1,4-dimethyl-4-vinyl*cyclo*hexene. The evolution of these dimers to aromatics at 450°–700° C is faster than that of 4-vinyl*cyclo*hexene and an almost fully aromatic liquid is produced already at 600° C. The monocyclic fraction (93 per cent by weight of the total liquid) contains benzene 5 per cent, toluene 30 per cent, xylenes 38 per cent, and also, characteristically, considerable amounts of C_9 alkylbenzenes (21 per cent), derived from the dimers by elimination of one carbon atom.

Furthermore, aromatization of *d*-limonene at 450° C was shown by Pines and Ryer[9] to proceed through the intermediate formation of *gem*-dialkyl*cyclo*hexadienes, which give by demethanation important amounts of C_9 alkylbenzenes.

Published data on the composition of tobacco smoke[1] support the view that aromatization based on thermal pyrolysis of isoprene occurs in burning tobacco. Indeed, isoprene and dipentene, as well as C_9 aromatics[1], that is, 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene, have been identified. 1,4(8)-Menthadiene[1], which could arise by double bond isomerization of dipentene, has also been reported.

Isoprene constitutes 50–80 per cent of the total unsaturated gaseous hydrocarbons present in tobacco smoke (0·87–4·2 mg/cigarette, as compared with 1 mg paraffins, 0·035–0·13 mg acetylene and 0·04–0·12 mg butadiene)[4]. Also, the temperature at which aromatization of isoprene occurs most readily (600°–800° C) corresponds to that found in burning tobacco.

Kennaway[10] has shown that isoprene tar is strongly carcinogenic. We have now established the presence of 3,4-BP in the tar.

100 g of isoprene was pyrolysed at 600° C in an empty stainless-steel tube. At 3-sec contact time 94 per cent of the diene reacted. The liquid product (67 per cent by weight) was distilled at 0·5 mm, up to 150° C, leaving 4·2 g of tar. Part of this material was subjected to elution chromatography on alumina[11] and the combined fractions containing 3,4-BP further chromatographed on acetylated paper. Finally, the amount of 3,4-BP was determined by ultra-violet spectrophotometry (analytical band, λ 385 mμ[11]) and found to be 0·1 per cent of the tar. In another experiment, carried out at 610° C and 4-sec contact time the concentration of 3,4-BP in the tar was 0·15 per cent by weight. The analytical procedure was checked with synthetic blends composed of anthracene, chrysene, fluorene, pyrene and 3,4-BP (0·8–7·0 per cent). It should be noted that the amount of 3,4-BP to be accounted for in the burning of one cigarette is only 0·005–0·025γ[4,12]. 3,4-BP is formed from isoprene already at a relatively mild temperature in accord with the established high tendency of dienes to undergo thermal aromatization[5,6]. In contrast, tobacco paraffins[1] do not yield any trace of this carcinogen at 600° C.

It should be pointed out that 3,4-BP content is only one of the factors determining the carcinogenicity of a tar[13]. Roe[12] has reported recently that the phenolic fraction of tobacco smoke condensate promotes the carcinogenicity of 3,4-BP (for mouse skin). This observation justifies in some respects the attention paid to the presence in tobacco tar of this polycyclic aromatic in spite of its low concentration. On the other hand, additional potent carcinogenic hydrocarbons are no doubt also present in the smoke, and 3,4 : 9,10-dibenzopyrene has actually been identified[1]. It is significant that 0·05 per cent of the latter compound is formed from 4-vinyl*cyclo*hexene at 700° C (ref. 6*b*).

Yields of isoprene ranging from 10–60 per cent have been found in the thermal decomposition of terpenic hydrocarbons[14] and alcohols[15] at 500°–750°. Obviously, terpenes present in tobacco should undergo depolymerization in a similar way. Solanesol[1] occurs in tobacco leaves to the extent of 0·4 per cent. Borneol and linalool have also been identified[1] in the leaves, whereas squalene (0·8γ/cigarette) has been found[1] in smoke condensate. Furthermore, essential oils are sometimes added to tobacco in the course of its processing.

Thus, taking terpenes as the precursors of isoprene, we propose the scheme given in Fig. 1 as an important pathway for the formation of polycyclic aromatics in burning tobacco.

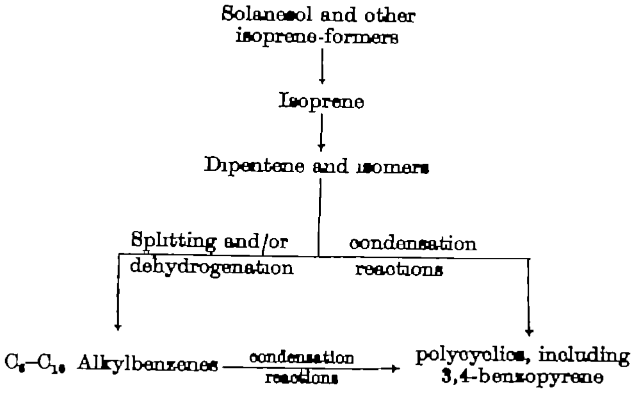
Solanesol and other isoprene-formers
Isoprene
Dipentene and isomers
Splitting and/or dehydrogenation
condensation reactions
C_8–C_{10} Alkylbenzenes
condensation reactions
polycyclics, including 3,4-benzopyrene

Fig. 1

We thank Miss C. Funt for carrying out the analyses of 3,4-benzopyrene.

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OXIDATIONS AND TRANSHYDROGENATIONS INVOLVING PYRIDINE-DINUCLEOTIDES CATALYSED BY BOVINE MILK XANTHINE OXIDASE

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THE oxidation of NADH (DPNH) by bovine milk xanthine oxidase was first reported twenty-three years ago[1] while more recently it has been stated that NAD+ (DPN) can act as electron acceptor for the enzyme when xanthine is the substrate[2,3]. These reactions have provoked relatively little interest despite the fact that the changes in the intracellular levels of both the co-enzymes and also xanthine oxidase have been cited as possible contributory factors of abnormal and malignant growth[4–6]. Attention has mainly been confined to ascertaining whether or not a contaminant of the bovine milk enzyme was responsible for the catalytic oxidation of NADH[7–13]. The following is a preliminary report on the results of recent studies on this enzymatic reaction: from these there remains little doubt that xanthine oxidase itself is the catalytic entity involved and that the flavin groups of this enzyme participate in the reactions.

Oxidation of NADH. When following the aerobic oxidation of NADH by the decrease in 340 mμ absorption, I have always observed an induction period[14], the change in optical density during this period being equivalent to the oxidation of about 5 moles of NADH per mole of enzyme (Fig. 1). It is believed that this delay in the attainment of maximum rate is due to a slow approach

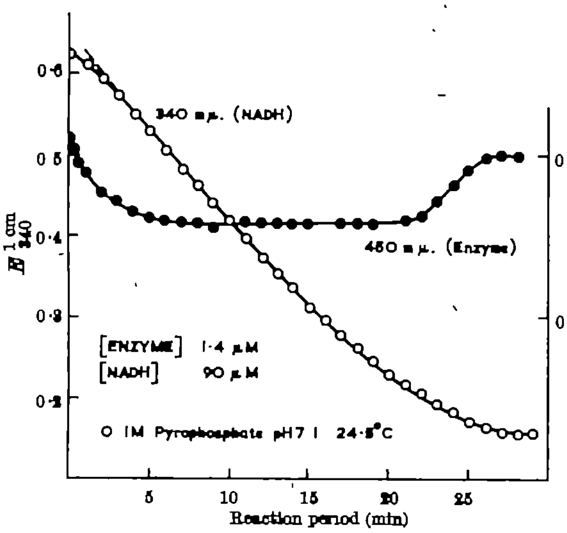


Fig. 1. Variation in extinction of the enzyme and NADH during oxidation of the latter. 5 μl. NADH was added to 2·0 ml. buffer, the reaction started by the addition of 100 μl. enzyme and the 340 mμ absorption followed in a 1 cm cell. In order to observe the changes in extinction of the enzyme at 450 mμ, the experiment was repeated using a 4 cm cell and volumes five times those given here

to the steady state. In agreement with this view is the fact that during this period there is a net partial reduction of the xanthine oxidase as judged from a decrease in the visible absorption of the enzyme (Fig. 1; cf. curve of Gutfreund and Sturtevant for the xanthine reaction[14]). Furthermore, the enzyme is either eventually re-oxidized or further reduced according to whether or not the NADH is in excess of the oxygen acting as electron acceptor.

The decrease in the 450 mμ absorption of the enzyme during the initial period of the reaction (that is, to the steady-state level) was found to obey first-order kinetics over at least 75 per cent of its course under all conditions so far adopted. From the magnitude of the changes that can be produced, it is clear that the flavin groups must be involved. The results also confirm the view that enzyme no longer active toward xanthine is still able to oxidize NADH (refs. 1 and 13). Using one particular enzyme sample, the rate coefficient for this change (k_s) and the steady state concentration of reduced enzyme ($[EH.P]_s$) were found to vary with the NADH concentration at all pH values near neutrality: under otherwise constant conditions, both were maximal at pH 6·7. The rate coefficient k_s was markedly influenced by the nature of the buffer ion, being much smaller in *tris* than in pyrophosphate at the same pH. The changes in the absorption of the enzyme in the region of 600 mμ did not parallel those occurring at 450 mμ; similar observations have been made by Gibson and Gilbert (unpublished) and confirmed by Bray[15] in the case of the xanthine reaction. It is possible that the changes at the higher wave-lengths reflect binding of the oxygen molecule to the enzyme since, in the case of NADH at least, it appears that the changes at 600 mμ occur only when the enzyme is reduced beyond the steady-state level.

In addition to the pre-steady-state investigations, I have also determined the effect of pH on the overall aerobic reaction. From the results obtained with one enzyme sample it was found that the pK$_m$ versus pH graph[16] shows a break at pH 5·9, while V_m and V_s (V_m/K_m—the rate at infinitely low substrate concentration[17]) were maximal at pH 5·7 and pH 6·8 respectively.

Although no qualitative inconsistencies have been observed when using different batches of enzyme and NADH, a wide variation in the magnitude of the Michaelis constant for the latter has been noted[8,19]. A similar variation has previously been found in the value of the Michaelis constant for xanthine (see Bray[19] for a discussion on this point). It seems likely that the factors affecting the magnitude of K_m for one substrate also affects the value of the constant for the other, but not necessarily in the same direction. Bray[19] has presented evidence (with which we are in accord) that some xanthine oxidase samples contain an inhibitor of the xanthine reaction, but he did not determine whether or not the NADH reaction was likewise affected. Previously, however, Bray[18] observed that the specific activity (toward NADH) of one batch of xanthine oxidase decreased as the concentration of the enzyme was increased. This is consistent with the presence of an inhibitor of the NADH reaction in the xanthine oxidase sample.

The addition of NADH to concentrated xanthine oxidase results in a slow decrease in the activity of the latter towards xanthine, the magnitude of this effect correlating with the changes in the visible absorption of the enzyme described here. Thus, the inhibition is spontaneously reversible at low concentrations of NADH (that is, as the latter is eventually oxidized): with higher concentrations of pyridine dinucleotide, gradual spontaneous inactivation of the enzyme becomes significant and somewhat obscures these effects. Since the diaphorase activity of the enzyme is unaffected by a number of processes or reagents which block the xanthine reaction, it is tentatively concluded that the inhibition effect just described, may be attributed to reduction of the flavin groups of the enzyme and not merely to the binding of the NADH molecule. NAD+ can also inhibit the xanthine reaction in a similar fashion although the effect is not so marked as that observed with NADH, except apparently when the enzyme is previously reduced by the substrates xanthine or NADH. Dissociation of the NAD+ by dilution is slow.

Transhydrogenations. Xanthine oxidase has been found to catalyse a number of transhydrogenation reactions utilizing NADH as substrate and various pyridine dinucleotides as acceptors. Thus 3-acetyl-pyridine- and 3-formyl-pyridine-adenine dinucleotides (APAD+ and FPAD+ respectively) and the corresponding hypoxanthine compounds (APHD+ and FPHD+) were all found to be good acceptors for NADH oxidation even under aerobic conditions. At pH 7·1, certain values were obtained with one enzyme sample, the reduction of the analogues being followed at 390 mμ where the contribution of the NADH is relatively small (Table 1).

Table 1

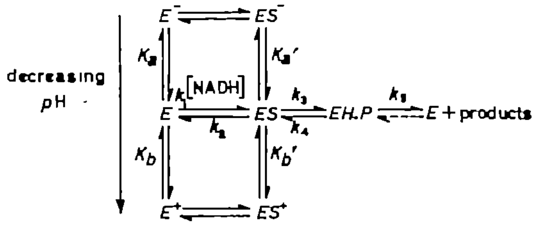
NAD+ analogue	K_m (μM)	V_m $\Delta E^{1\,cm}_{390}$/min	V_m (μmole/l./min) (relative)
APAD+	113	0·08	0·43
APHD+	382	0·08	0·43
FPAD+	222	0·13	1·00
FPHD+	665	0·13	1·00

[NADH] 147 μM. [Enzyme] 1·4 μM; 23·5° C. 0·1 M pyrophosphate pH 7·1 (aerobic system).

It can be seen that the rate of reduction of the NAD+ analogue is dependent on the nature of the pyridine substituent only while the Michaelis constant is also influenced by the purine moiety. The foregoing K_m values may be compared with a dissociation constant of 400 μM calculated for NAD+ when acting as an inhibitor of the xanthine reaction. The effect of pH on K_m, V_m and V_s has been determined in the case of FPAD+ reduction and the results found to be extremely similar to those obtained when following NADH oxidation with oxygen as acceptor. Thus the pK$_m$ versus pH graph shows a break at pH 6·2 while V_m and V_s are maximal at about pH 6·4 and pH 6·7 respectively. The results suggest that the reduced forms of the foregoing analogues are eventually re-oxidized by the enzyme although the reactions involving the isolated reduced compounds have not been studied.

Since NAD+ and NHD+ (nicotinamide hypoxanthine dinucleotide) both decrease the maximum rate of change in 340 mμ absorption when NADH is oxidized aerobically and also increase the induction period, it is concluded that these compounds also act as acceptors even in the presence of oxygen. The apparent inhibition arises because the visible spectra of the reduced forms of these compounds are identical to that of the substrate. Some simple 3-substituted pyridinium compounds did not react. Contamination of the NADH by NAD+ could thus presumably influence the observed kinetics and this factor may contribute toward the quantitative discrepancies referred to here[11,12].

Reaction scheme. The results available have been analysed, bearing in mind the quantitative differences mentioned earlier. All the results seem to be consistent with NADH oxidation proceeding according to the following partial reaction scheme, where $k_3 > k_1$ [NADH] $\gg k_2 \approx (k_4 + k_5)$:



In this scheme, the reactions in the vertical columns represent the ionizations of the enzyme due to the pH (assumed rapid) while those in the horizontal lines represent the rate-determining steps in the overall oxidation process. Thus *ES* is the enzyme–substrate complex, the enzyme still being in the oxidized form: reduction of the enzyme and oxidation of the substrate occurs to give the reduced enzyme–product complex, *EH.P*. The coefficient k_3 must be complex (and may be pH dependent) but was pseudo first-order under the conditions used. From anaerobic studies (when k_5 must be zero) it is concluded that k_4 cannot be zero. The following values have been calculated from the pre-steady-state data already referred to: k_1/k_2 = 500 l./mole; k_3 = 0·2 min^{-1}; $(k_4 + k_5)$ = 0·14 min^{-1} (0·1 M pyrophosphate pH 8·0, 23·5° C). The Michaelis constant for this sample of enzyme was not determined directly but the value calculated from the coefficients just given (800 μM), although rather high, is of the same order of magnitude as some values determined experimentally. It would thus seem that the foregoing reaction scheme may be accepted as a guide for future studies.

In the case of bovine milk xanthine oxidase and possibly other mammalian forms of this enzyme, the rates of oxidation of NADH, or reduction of NAD+, seem to be too low for the reactions to be biologically significant. If, however, these coenzymes can reach the xanthine oxidase *in vivo*, it is possible that their level could control the rate at which purines and/or pteridines are catabolized.

I thank Prof. F. Bergel for his advice.

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INTERACTION OF CHLORAMBUCIL WITH CELL SURFACES

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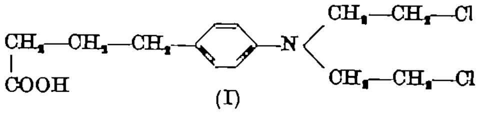
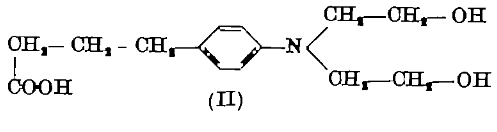
MANY of the cytotoxic and mutagenic actions of the alkylating agents suggest that the nucleic acids of the cell nuclei are the probable sites for the primary chemical reaction which is responsible for these biological effects[1]. We have found that interaction takes place between chlorambucil and suspensions of human erythrocytes, or of Ehrlich ascites tumour cells. No loss of chlorambucil from solution was evident. The reactions that occurred have been deduced from changes in the solubility of chlorambucil and from the conditions that caused lysis of the red cells, which indicated that only the cell surface was involved.

The compound chlorambucil in the non-ionized form (I) is soluble in benzene and in ethyl acetate. The hydroxyl derivative (II) is insoluble in benzene but is soluble in ethyl acetate. From an aqueous solution of (I) and (II) at pH 3·6 the form (I) present is extractable by benzene and the amount of form (II) is the difference between the amounts extracted by ethyl acetate and by benzene. Both forms (I) and (II) are soluble in water at pH above 7, and in ethanol[2,3].


(I)


(II)

To prepare the reaction mixture, freshly drawn human blood, or ascites fluid from mice bearing Ehrlich ascites tumours, was centrifuged and the cells were washed once in isotonic saline. Approximately 5 ml. of packed red cells or 3 ml. of packed ascites cells was obtained. 4 mg of chlorambucil (I) was dissolved in 4 ml. of 0·5 M sodium bicarbonate and this was diluted to 40 ml. with 0·11 N sodium chloride. The cells were added to 20 ml. of this chlorambucil solution. The remaining 20 ml. was used as a

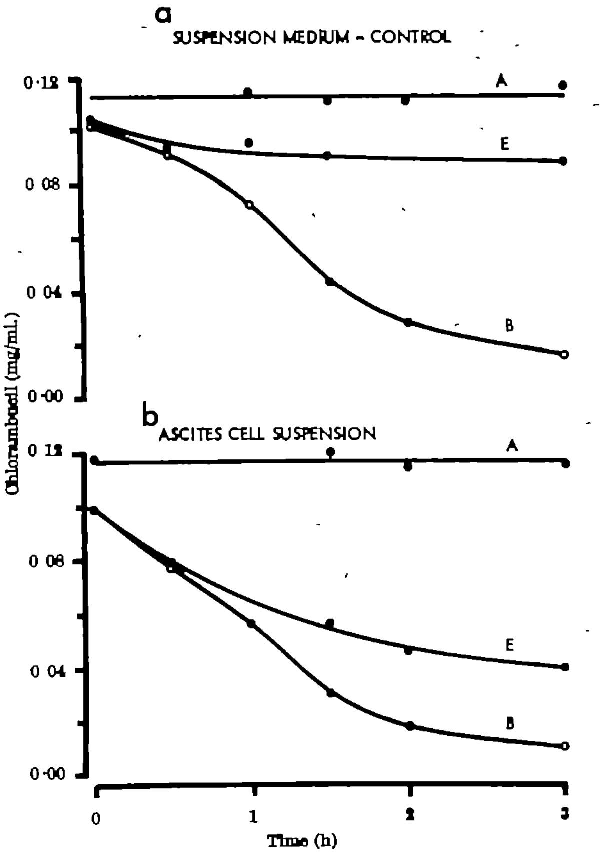


Fig. 1. *a*, Concentrations of derivatives of chlorambucil recovered from a solution of 0·05 M bicarbonate and 0·10 M saline at pH 8·0. *b*, Concentrations of derivatives of chlorambucil recovered from the medium in the presence of a suspension of ascites cells · (*A*) total concentrations by recovery in alcohol; (*E*) combined concentrations of chloro and hydroxyl forms, by recovery with ethyl acetate; (*B*) concentrations of chloro form by recovery with benzene

control. Both mixtures were adjusted to pH 8·0 with a few drops of 0·5 N hydrochloric acid, and were incubated in a 37° C bath with occasional inversion.

To follow the course of the reaction, three 1·0-ml. samples were withdrawn from each mixture at measured intervals of time. 9·0 ml. of ethanol was added to each sample to remove the protein material. The samples were centrifuged, the supernatant alcohol was evaporated from two of the samples from each mixture and the residues were partitioned between 0·1 M phosphate buffer of pH 3·6 and three 5·0-ml. volumes of either benzene or ethyl acetate[2]. The organic solvents were removed under reduced pressure and the residues were dissolved in 10 ml. of ethanol. The absorbance of the three alcohol solutions from each mixture was measured at 258 mμ. The amounts of chlorambucil recovered by the three solvents from a suspension of ascites cells at pH 8·0 and from a control of pH 8·0 in the absence of cells are shown in Fig. 1.

Similar results were obtained when samples in excess of 1·0 ml. were withdrawn from the reaction mixture, and were centrifuged to obtain 1·0 ml. of supernatant solution. Two samples of supernatant fluid from the suspension and two from the control were adjusted to pH 3·6 with about 0·5 ml. of 0·05 N hydrochloric acid, then made up to 5·0 ml. with water. These were extracted as described here. A third 1·0-ml. sample from each source was made up to 10 ml. with ethanol. With this second method, we studied the interaction of chlorambucil with red cells, red cell 'ghosts' and ascites tumour cells, and compared this with control solutions of the same bicarbonate and saline content. We also investigated the effect of a phosphate buffer in the absence of cells. The results are listed in Table 1.

Table 1*. PERCENTAGE OF CHLORAMBUCIL DERIVATIVES PRESENT AFTER 3 H IN CONTACT WITH CELLS IN SUSPENSION AND IONS IN SOLUTION AT pH 8 AT 37° C, RELATIVE TO BICARBONATE–SALINE CONTROL SOLUTION

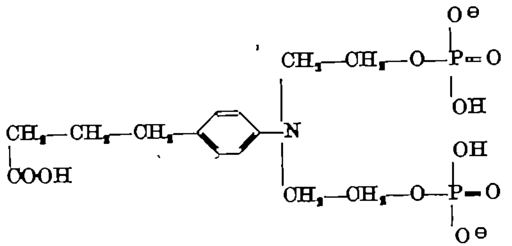
Reaction mixture		Derivatives recovered		
Reactant	Reagent	Chloro (I)	Hydroxyl (II)	Hydrophilic
Bicarbonate 0·05 M	(I)	27	73	0
Red cells 1·5 × 10^6/mm³	(I)	29	38	33
Red cell ghosts ,,	(I)	25	39	36
Red cells ,,	(II)	8	85	7
Ascites cells 3 × 10^4/mm³	(I)	16	6	68
Phosphate pH 7·5 0·05 M	(I)	9	8	83

* The results are expressed as a percentage of the amount initially extracted from the aqueous phase. This was about 0·1 mg/ml. as calculated from the samples in alcohol and about 10 per cent less in the ethyl acetate and benzene extractions[2]. Fig. 1 also refers to this.

When corrected for the (88 per cent) efficiency of extraction by the water-immiscible solvents, the control solutions which contained bicarbonate buffer converted 64 per cent of the chloro form of chlorambucil (I) to the hydroxyl derivative (II), in 3 h. 20 per cent was recovered as the benzene soluble chloro form. The remaining 16 per cent may be accounted for by assuming a reaction with the bicarbonate ion in solution to form an alcohol-soluble or 'hydrophilic' derivative, because 100 per cent of the initial chlorambucil concentration was recovered in alcohol (Fig. 1*a*).

In the presence of cell suspensions, 100 per cent of the initial chlorambucil concentration remained in the alcohol-soluble form but the amount recoverable by ethyl acetate diminished with time relative to the control solution (cf. Fig. 1*a* with *b*). Little effect was evident on the amount recovered by benzene. The decrease in the amount recovered by the ethyl acetate extractions, in the presence of cells, may also be attributed to the formation of a more hydrophilic form of chlorambucil which was soluble in alcohol and water but not in ethyl acetate. This derivative was not attached to the cell and had the same absorbance as chlorambucil because the recovery in alcohol throughout the reaction was equal to the initial concentration of chlorambucil. The decrease with time in the amount recoverable by ethyl acetate, relative to the bicarbonate–saline control, was a measure of the rate of formation of this hydrophilic derivative.

Table 1 indicates that the hydroxyl derivative of the chlorambucil did not interact with the red cell surface; some of the chloro form (I) most probably was present in the hydrolysed solution. An extensive reaction occurred with the phosphate buffer 'control' solution. The reaction with ascites cells was more extensive than with the red cells. Of the solutions and suspensions tested, bicarbonate ion was the least reactive. The nature of the hydrophilic derivatives is indicated by the extensive reaction which occurred in the phosphate buffer 'control' solution, which may have produced a compound of type (III).



(III)

The results suggest that a hydrophilic derivative of chlorambucil was formed at the cell surface by an alkylation reaction involving replacement of the chloro atoms by nucleophilic groups. The hydrophilic product was, moreover, present in solution. Therefore, it is probable that the chlorine atoms in the chlorambucil were replaced by small nucleophilic molecules or anions from the cell surface.

The cell envelope appeared to be weakened by the reaction with chlorambucil. A series of 5·0-ml. volumes

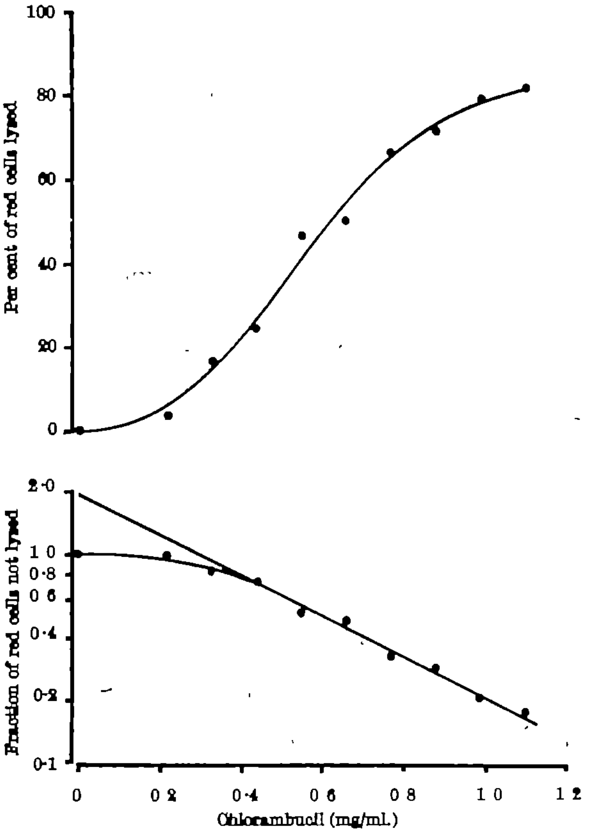


Fig. 2. (Above), Extent of lysis, determined by the absorbance of the supernatant solution after 0·5 h at 37° C, of a suspension of 0·5 ml. of packed red cells in 5·0 ml. of isotonic saline–glucose at pH 8·5 in the presence of increasing concentration of chlorambucil. (Below), The data of above curve plotted as logarithm of the fraction of cells surviving in the presence of increasing concentrations of chlorambucil

of solution were prepared which were 0·09 M sodium chloride, 0·02 M glucose, 0·05 M sodium bicarbonate and contained concentrations of chlorambucil varying from 0·2 to 1·0 mg/ml. 0·5 ml. of washed red cells were added to each volume and the suspensions were incubated at 37° C for 0·5 h with inversion every 5 min. The extent of hæmolysis was determined by measuring changes in absorbance at 550 mμ. Lysis was apparent at chlorambucil concentration greater than 0·3 mg/ml. (0·001 M) and the degree of lysis increased rapidly with increase of concentration, refer to Fig. 2 (above). No lysis was caused by the hydroxyl derivative (II). The derivative of (I) known as phenylalanine mustard reacted as did (I) up to 0·4 mg/ml. The nitrogen mustard cyclophosphamide (Endoxan), which is inert *in vitro*, behaved as (II).

The effect of chlorambucil which caused lysis of the red cells appeared to be additive. Two successive treatments of the same cells (7 × 10^8/ml.) with chlorambucil concentrations of 0·19 mg/ml. for 0·5 h at 37° C lysed only 2·0 per cent of the cells. A third treatment lysed another 14 per cent of these cells. One treatment with chlorambucil at 0·55 mg/ml. lysed 45 per cent of cells from the same stock.

These results, together with the shape of the curve of Fig. 2 (above) and the fact that weak solutions of chlorambucil caused no lysis over periods of 3 h at 37° C, indicated that more than one interaction of the drug with the cell must occur to cause lysis. In Fig. 2 (below) the data of this figure are plotted as the fraction of surviving cells against the chlorambucil dose. By analogy with the action of X-rays on cells[4,5], the existence of the shoulder in Fig. 2 (below) constitutes evidence for a multiple hit mechanism of lysis. Back extrapolation of the first slope of the curve indicated that two hits are required.

The results suggest that a primary action of chlorambucil may be the removal of a simple chemical component from the cell surface.

This work was supported by a grant from the National Cancer Institute of Canada.

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MEASUREMENT OF MOISTURE BOUNDARY LAYERS AND LEAF TRANSPIRATION WITH A MICROWAVE REFRACTOMETER

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THE refractive index (n) of air at microwave frequencies is determined chiefly by the air temperature (T), the total pressure (P) and the partial pressure of water vapour (e) present. The dependence of the index on the last quantity is sufficiently large that instruments (refractometers) which measure the index can be used to study variations in water vapour pressure. For practical purposes the index can be expressed as:

$$N \equiv (n - 1) \times 10^6 = a\frac{P}{T} + b\frac{e}{T^2}$$

The sensitivities of N to the factors are given by their partial derivatives, which for typical laboratory conditions have the following approximate values:

$$\frac{\partial N}{\partial T} = -1{\cdot}2\ N/°\mathrm{C}, \quad \frac{\partial N}{\partial P} = 0{\cdot}3\ N/\mathrm{mb}, \quad \frac{\partial N}{\partial e} = 4\ N/\mathrm{mb}$$

Since refractometers have been developed with resolutions of the order of 0·1 N and accuracy of the order of 1 N, measurements of water vapour pressure can be made to corresponding values of 0·025 and 0·25 mb (approximately 0·1 and 1 per cent relative humidity at a temperature of 20° C), if the temperature and pressure of the sample can be properly determined. In many cases total pressure is effectively determined by local gross atmospheric conditions. It is also relatively easy to maintain the temperature nearly constant. Consequently, the refractometer is readily adapted to experiments in which changes of water vapour pressure are to be studied. Several reports are available describing some of these applications.

In the case to be described here, the refractometer is used for examining the moisture profile found near a free water surface or near the surface of organic materials such as leaves, animal skins, etc.

Several types of microwave refractometers have been developed[1–4], and some of these can be purchased commercially at a cost of between 5,000 and 10,000 dollars. All these operate basically by responding to changes which occur in the resonant frequency of a cavity resonator as the index of refraction of its contents change. The

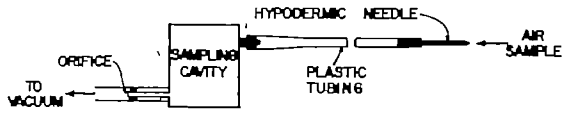

Fig. 1. Diagram of the sampling technique to draw moist air through the hypodermic needle and the plastic connecting tubing into the refractometer sampling cavity

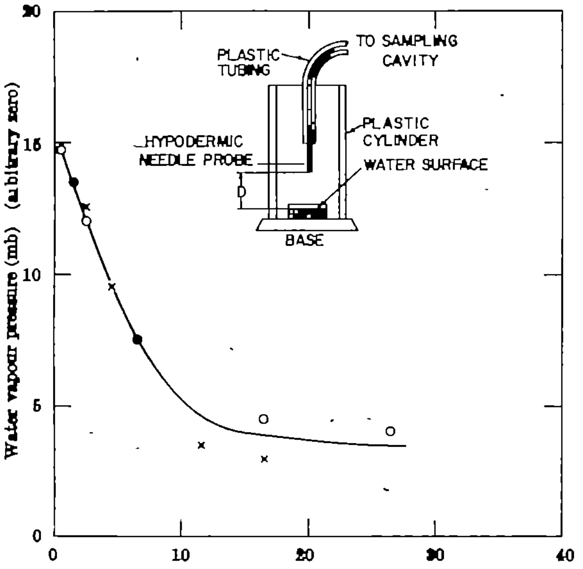

Fig. 2. Method for observing the moisture profile near a free water surface and the observed moisture profile of vapour pressure as a function of the distance above the water surface

experiments described in the following paragraphs were performed using the types described in refs. 1 and 4. These instruments operate at about 9,400 Mc/s. The sampling cavity is a right circular cylinder about 4·5 cm in diameter and 3·0 cm long. Each instrument measures the change of resonant frequency of the sampling cavity by comparing it with a similar reference cavity the contents of which are controlled (usually hermetically sealed and containing dry argon). In the system developed by Birnbaum this comparison is made by sweeping the frequency of a klystron oscillator and noting the relative time between resonance of the two cavities. In the Vetter refractometer the reference cavity is tuned by a servo-operated probe to track the frequency changes in the sampling cavity.

Because of the size and shape of the sensor (sampling cavity) the desired spatial resolution was obtained by connecting the cavity to a vacuum pump (or chamber) and drawing the air sample to be measured into the cavity through a flexible plastic tube fitted with a hypodermic needle on the outer end (Fig. 1). The needle was mounted on a rack and pinion so that its position with respect to the surface could be easily and closely adjusted. By using an appropriate orifice in the gas sampling path a suitable compromise can be made between sampling speed (response time) and the disturbance which the probe creates in the field to be measured. With the arrangement shown in Fig. 1 the time constant of the system was about 30 sec; however, a delay in response of about 5 sec occurs because of the volume of the intake tubing. The time constant is determined by the size of the cavity and the time required to replace the air in the cavity.

Moisture profile above a free water surface. The first measurements were of the moisture profile above a free water surface. The water was contained in a flat dish which was placed inside a closed box in order to avoid local convection effects (Fig. 2). The hypodermic needle probe (observed through a plastic window) was lowered towards the free water surface a few mm at a time and each setting was maintained for approximately 30 sec. The change in water vapour pressure which was observed is shown in Fig. 2. The scatter of the points obtained at the larger distances is believed to be due to the circulation of air within the container.

Transpiration of plant leaves. It is important to be able to measure the transpiration from plant leaves without disturbing the environment of the leaf or the boundary layer near the surface. By placing the hypodermic needle against the lower surface or just off the lower surface, the moisture near the surface can be monitored with relatively little perturbation. For some of the measurements the entire plant was placed within the box, while for others the leaf of the plant was placed inside the box with the stem entering through a small opening in the side. Measurements were first made on the free leaf with the needle against the lower surface. However, some difficulty was encountered with small movements of the leaf during extended periods of measurement which made it advisable to support the leaf with a flat piece of wood against which the leaf was taped along the edges.

An entire bean plant was placed within the chamber and the measurements were made with the needle set against the lower surface of a leaf. A tracing of a recording of the transpiration from the bean leaf is shown in Fig. 3*a*. The plant was initially in complete darkness. The plant was illuminated with approximately 600 ft.-candles of fluorescent light incident on the leaf being investigated.

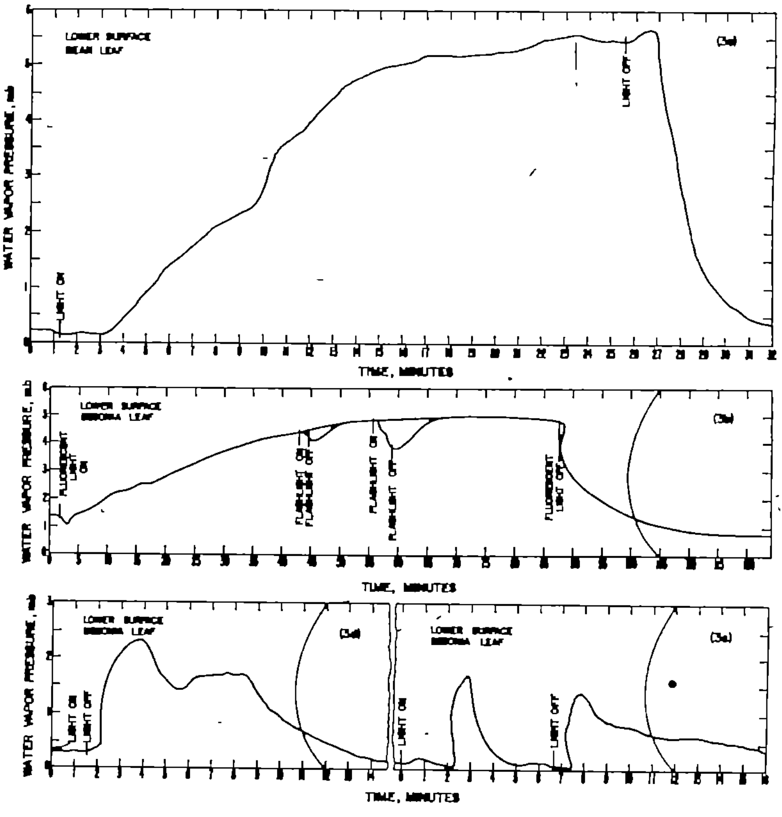

Fig. 3. The water vapour pressure next to the lower surface of a begonia leaf (3*a*) as a function of time after a fluorescent light is turned on and off. Vapour pressure near a bean leaf (3*b*) and some responses of the begonia leaf (3*c*) and (3*d*)

The immediate response was an apparent decrease in transpiration for 30 sec, followed by a slight increase for 1 min and a small decrease for another 30 sec. Then nearly 2 min after the initial stimulus by the fluorescent light an increase in transpiration began which reached a steady state about 20 min later which was about 21 *N* units greater than the initial level. This represented a change of about 21 per cent of relative humidity at 20° C, or an increase in vapour pressure of about 5·5 mb in the boundary layer near the leaf surface. When the light had been turned off the transpiration diminished abruptly for 4–5 min and then levelled off at a level just above the initial starting-level.

Fig. 3*b* shows tracings of direct recordings of the index of refraction of the air drawn into the refractometer cavity from the lower surface of a begonia leaf. The measurements were started with the leaf in total darkness. A fluorescent light was turned on the leaf through the clear acetate window of the box and the leaf illuminated with approximately 640 ft.-candles while the main plant remained in the ambient fluorescent light of the room. The immediate initial response was an apparent decrease in transpiration which lasted for approximately 1 min. Then 2–2·5 min following the initial stimulus by the fluorescent light, which had remained on, the transpiration steadily increased until it reached a steady-state level 52 min later at 13 *N* units above the initial level representing a change of vapour pressure of 3·25 mb or 13 per cent relative humidity at 20° C. Turning the fluorescent light off and placing the leaf in total darkness resulted in an initial slight drop in transpiration for nearly 30 sec, then a precipitous drop followed by diminishing transpiration for the next 30 min or more.

A particularly interesting response of the begonia leaf to light was observed when the leaf was transpiring at its maximum rate under an illumination of 640 ft.-candles of fluorescent light when an additional illumination of about 600 ft.-candles was put on to the leaf with a flashlight. The transpiration rate dropped abruptly rather than increasing as expected. When the flashlight was turned off the transpiration rate returned to its original value or trend. The change in vapour pressure was about 1 mb or 4 per cent relative humidity at 20° C. The precise stomatal action involved here must be explored further, but the response was clearly evident.

Some interesting responses were observed for a begonia plant when the entire plant was placed in the chamber and the air sampled from the lower surface of one of the leaves. The record of this behaviour is shown in Fig. 3*c*. With the fluorescent light turned on the entire plant, an initial small increase in transpiration appeared followed by a slight decrease. 2 min after the light was turned on an abrupt increase in transpiration occurred which amounted to a total change of about 6·5 *N* units (6·5 per cent relative humidity at 20° C). Although the lamp remained on, something shut off the transpiration just as abruptly as it had begun and the moisture concentration in the boundary layer dropped to its original level prior to the stimulus by the light. 1 min after the light was turned off, there was once again a sudden apparent increase in transpiration and the moisture concentration of the boundary layer increased by about 5·4 *N* units (5·4 per cent relative humidity at 20° C) followed by a gradual decrease.

Fig. 3*d* also illustrates an example of illuminating the entire begonia with light from the fluorescent lamp and then after 1·5 min turning off the light. The plant continued with its transpiration action, but after transpiring for about 6 min returned to its dark state in about another 5 min. The air was sampled from the lower surface of a single leaf although the entire plant was within the chamber.

Conclusions. It is shown that a microwave refractometer can be an extremely sensitive and responsive device for detecting the moisture concentration within a boundary layer near a moist surface. The air must be drawn very slowly into the refractometer. A hypodermic needle was used as a probe in the experiments described here. The boundary layer profile was measured over a free water surface. The transpiration from leaves was observed while they were subjected to various stimuli of light and darkness. Changes in moisture concentration amounting to a variation as little as 0·1 per cent relative humidity (at 20° C) can be detected. By reducing the size of the microwave cavity (for example, going to *K*-band rather than *X*-band) it should be possible to reduce the response time by about one order of magnitude and increase the sensitivity.

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ROLE OF THE PHOSPHORYLASE ENZYMES IN CARDIAC CONTRACTION: A PROPOSED THEORY FOR THE RHYTHMICAL PRODUCTION OF ENERGY IN THE HEART

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RECENTLY, considerable evidence has been obtained that alterations in heart function are associated with biochemical changes in the myocardium. Of the many biochemical events which may be of importance in cardiac contraction, particular attention has been focused on the phosphorylase reaction. In the heart[1], as well as in skeletal muscle[2], the enzyme phosphorylase exists in two forms: phosphorylase *a*, which is active in the presence or absence of adenylic acid (AMP), and phosphorylase *b*, which although inactive by itself, becomes active when AMP is present as a co-factor. From the work of Sutherland, Cori *et al.* it is known that adrenaline in minute amounts activates liver phosphorylase[3] and produces a transformation of phosphorylase *b* to phosphorylase *a* in skeletal[4] and heart muscle[1]. Sutherland and Rall[5] isolated a new coenzyme, 3′,5′ cyclic AMP, demonstrated that this compound was formed in tissues in response to adrenaline and found that it stimulated the formation of phosphorylase *a*.

The work of Krebs, Graves and Fisher[6] showed that one of the actions of cyclic AMP is to increase the activity of phosphorylase *b* kinase, which catalyses the synthesis of phosphorylase *a* from the *b* form of the enzyme. In the heart the positive inotropic action produced by adrenaline and other sympathomimetic amines has been shown to be correlated with an increase in the activity of phosphorylase *a* [7-11]. The increased force of contraction following stimulation of sympathetic nerves to the heart[10] or administration of ganglion stimulating drugs[12] has been found also to be associated with an increase in the activity of phosphorylase *a*. Most recently, Hess, Shanfeld and Haugaard[13] showed that in the open-chest rat preparation vagal stimulation or administration of acetylcholine were followed by a significant decrease in the activity of phosphorylase *a*. It is very likely that the changes in phosphorylase activity seen when the heart is stimulated or depressed reflect variations in the intracellular concentration of cyclic AMP.

We do not at the present time know the exact role that cyclic AMP or the phosphorylase reaction may have in the regulation of cardiac activity. However, the correlation between changes in phosphorylase activity and changes in function demonstrated in the many investigations already quoted, appear to justify the adoption of the working hypothesis that there is indeed a direct relationship between phosphorylase activity and some stage of the contraction cycle of the heart. Such a hypothesis is strengthened by the observations of Ellis and Vincent[14] that adrenaline and acetylcholine produce changes in glycogen content of the perfused heart.

Because of the special properties of the phosphorylase enzymes, there are two ways by which phosphorylase activity may be regulated: (1) by changes in the equilibrium phosphorylase *b* ⇄ phosphorylase *a* produced by cyclic AMP or other factors influencing this reaction, or (2) by changes in the concentration of AMP followed by an increase or decrease in the activity of phosphorylase *b*. As already mentioned, there is considerable experimental evidence that the first of these two mechanisms is involved in the action of the autonomic nervous system on heart function. It is proposed here that the second mechanism operates in the normal contractile cycle of the heart and that AMP may play a unique part in the regulation of heart rhythmicity.

It is proposed that AMP, produced during any stage of the contraction cycle, causes an increase in phosphorylase activity. In turn the stimulation of phosphorylase leads to an increase in glycolysis (or complete oxidation of glucose units), bringing about phosphorylation of ADP and through the intervention of the myokinase reaction, the disappearance of AMP. The essential role of AMP is that it produces an activation of phosphorylase and by this action causes its own destruction. By such a mechanism a discontinuous or rhythmical formation of high-energy phosphate bonds can be envisaged.

Cain and Davies[15] recently demonstrated in convincing experiments that in skeletal muscle, in which creatine phosphoryl transferase was inhibited by dinitrofluorobenzene, the energy for contraction came from ATP. It is therefore likely that in cardiac muscle also the primary source of energy for contraction is ATP. Assuming this to be the case, one may construct a picture of the possible chemical events in the contractile cycle of the heart that includes the proposed role of the phosphorylase reaction (Fig. 1). (1) During systole ATP is split and ADP is produced; (2) the ADP formed is transformed partially to AMP and ATP by the intervention of the myokinase reaction; (3) during diastole the AMP formed from breakdown of ATP stimulates phosphorylase *b*, causing an increased metabolism of glycogen (to lactate or carbon dioxide + water) and an increase in the rate of high-energy phosphate formation with consequent synthesis of ATP from ADP and AMP; (4) the concentration of AMP and the activity of phosphorylase *b* decreases and the concentration of ATP is restored. A new cycle of contraction and relaxation may now start again.

This description of the possible biochemical reactions during the cycle of cardiac activity represents no more than a simplified picture of a very complicated set of events. Ultimate sources of energy other than glycogen as well as the role of ions, for example, have been ignored. Whatever the actual chemical events in cardiac contraction and relaxation may prove to be, what is emphasized here is that AMP may serve as a link between the reactions which cause a splitting of ATP and the reactions involved in the synthesis of ATP. By the intervention of myokinase or possibly by direct dephosphorylation of ADP, AMP is formed when ATP is acted on by ATP-ase. The stimulation by AMP of phosphorylase *b* may be considered a feed-back mechanism whereby ATP is rapidly restored. According to such a concept, the enzyme phosphorylase *b* is involved in a rhythmical formation and transfer of pulses of high-energy phosphate, while phosphorylase *a* produces a steady rate of glycolysis and high-energy phosphate formation. This concept allows a role for both forms of muscle phosphorylase and gives more meaning to the experiments which have demonstrated that alterations in cardiac function produced by a variety of means are associated with changes in the equilibrium phosphorylase *b* ⇄ phosphorylase *a*.

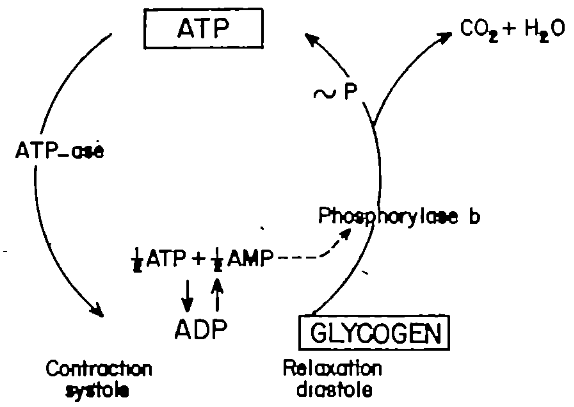

Fig. 1

Although at the present time there is little direct evidence to support the theory proposed here, there is much information about cardiac metabolism which is consistent with it. It has been shown that, except during strong sympathetic stimulation, the major proportion of phosphorylase is present in the *b* rather than the *a* form. In the open-chest rat, for example, control values of cardiac phosphorylase *a* range from 30 to 40 per cent of total phosphorylase[13] and in the isolated perfused heart the activity of phosphorylase *a* is even lower[12]. For AMP to play a part, such as that proposed here, its average concentration in the heart must be near the concentration for half maximal stimulation of phosphorylase *b* (5×10^{-5} M). Accurate values for tissue concentrations of AMP are, unfortunately, extremely difficult to obtain, since the slightest breakdown of ATP or ADP during the analytical procedures will produce erroneously high results. Nevertheless, it has been found by Fleckenstein *et al.*[16] that the concentration of AMP in the heart is extremely low, compared with the values for ADP and ATP. In our own laboratory, using rapid freezing and enzymatic methods, values for AMP in rat heart ranging from 0·1 to 0·2 μM/g (10^{-4} M) were obtained (Haugaard, N., and Corson, S. L., unpublished work). If some AMP exists in a bound form within the cell the average concentration of free AMP may be close to 0·05 μM/g, the amount that produces half maximal stimulation of phosphorylase *b*.

Another indication that the concentration of AMP is low in heart is the observation that AMP is an extremely efficient acceptor of phosphate in this tissue[17]. In heart homogenates, oxidizing glucose or glycogen, rates of phosphorylation of AMP up to 7,000 μM/g/h (wet weight) have been recorded[17]. An additional factor which will tend to maintain a low concentration of AMP in the heart is the presence of a very active 5′-nucleotidase associated with microsomes (Inesi, G., and Haugaard, N., unpublished work). Although the foregoing considerations provide indirect support for the proposed role for AMP and phosphorylase *b* in cardiac contraction, it is obvious that more direct evidence is needed.

Efforts are being made in this laboratory to determine the true concentrations of AMP in the heart under a variety of experimental conditions and to attempt to correlate changes in AMP concentration with alterations in function. In the meantime, it is hoped that the concepts outlined here, even if eventually rejected, will help stimulate work leading to a better understanding of the role of the phosphorylase enzymes in cardiac contraction.

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SYNTHETIC CYTOLIPIN *H* AND OTHER LACTOSIDES

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The hapten cytolipin *H* has been isolated from a cultured human epidermoid carcinoma by Rapport, Graf, Skipski and Alonzo[1]. Chemical degradation of the hapten characterized it as a simple, non-reducing glycolipin composed of sphingosine, a fatty acid, glucose and galactose in equimolar ratios. The carbohydrate moiety was identified as lactose[2], pointing to a ceramide lactoside as a probable structure for cytolipin *H*. This structure could be proved by synthesis. Synthetic analogues of similar structure might exhibit competitive inhibition at some site of the cytolipin *H* metabolism, and thereby be useful in the determination of any metabolic role of cytolipin *H* in cancer cells.

Ceramide was obtained by enzymatic hydrolysis of sphingomyelin[3], which had been extracted from beef heart lecithinsphingomyelin sludge. Purified ceramide and α-acetobromolactose were reacted under the conditions used by Sowden and Spriggs[4] for the synthesis of β-gentiobiose. A lactoside was obtained, which melted with decomposition at 86° C, which had a carbohydrate content of 32 per cent (measured on the acid-hydrolysis products of 100 μg of the compound[5]), and an optical rotation of + 2·1° (0·49 per cent in pyridine). The infra-red spectrum of the compound is practically identical to that of an authentic sample of cytolipin *H* isolated and given to us by Dr. M. M. Rapport (Fig. 1).

The same conditions of synthesis were used for preparing the lactosides of methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-hexadecyl, and isopropyl alcohols, and for the preparation of the lactosides of some fatty acid amides of 2-hydroxyethylamine. The lactosides of phenol, *p*-phenylphenol, *p*-nitrophenol, and *p*-aminophenol were prepared by the method of Babers and Goebel[6].

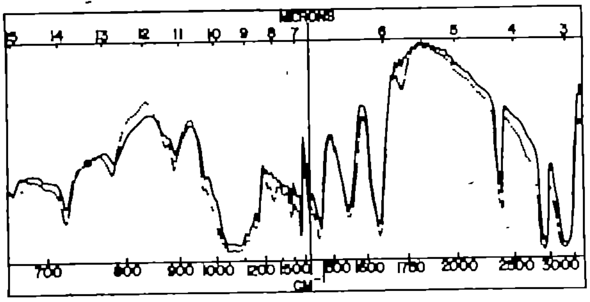

Fig. 1. Infra-red spectra of cytolipin *H* (solid line) and ceramide lactoside (broken line), pressed in potassium bromide

For immunological investigations, the *p*-aminophenollactoside was diazotized and coupled to bovine serum albumin and to egg albumin according to the method of Goebel and Avery[7]. Rabbits were immunized with a single intramuscular injection of 10 mg diazophenollactoside bovine serum albumin, homogenized in Freund's adjuvant and hyaluronidase[8], and boosted 6 weeks later with an intravenous injection of 65 μg of the same preparation of diazophenollactoside bovine serum albumin.

Rapport *et al.*[1] showed in inhibition investigations that the carbohydrate residue of cytolipin *H* had the configuration of lactose[2]. Cytolipin *H* is non-reducing, and therefore the lactose moiety must be linked through an acetal bond to the ceramide moiety. Of the two hydroxyl groups on the sphingosine molecule, the terminal (primary) hydroxyl group is most likely the one involved in the bond. This is the kind of structure assigned to cerebrosides, and the infra-red spectrum of cytolipin *H* (Fig. 1) is very similar to that of cerebrosides[9].

The synthesis of galactosphingolipids reported by Shapiro and Flowers[10] would yield a dihydrosphingosine analogue of cytolipin *H*. A preliminary investigation of the infra-red spectrum of cytolipin *H* revealed an absorption maximum at 969 cm⁻¹, which is characteristic of the *trans* configuration of a HC=CH double bond. Therefore, it appeared that natural cytolipin *H* must contain sphingosine, and another method was chosen. The fatty acid residue of cytolipin *H* has not been entirely characterized; but it is unlikely that a specific fatty acid is required for haptenic activity[11]. As the fatty acid is apparently linked to the sphingosine moiety through an amide bond, ceramide was the obvious starting material, even though ceramide prepared from natural extracts will contain a mixture of fatty acid residues.

Since the synthetic ceramide lactoside contains about 32 per cent carbohydrate, this compound has only one lactose entity per molecule. A lactoside linked to the secondary hydroxyl group of ceramide is rather unlikely, since the hydrogen of a secondary hydroxyl group is less readily replaced than that of a primary hydroxyl group, especially in this case where the secondary hydroxyl group is a sterically hindered allylic hydroxyl group. This argument is substantiated by the fact that the lactoside of *N*-(2-hydroxyethyl)-docosanoamide (which is the lactoside of a primary hydroxyl group) has an infra-red spectrum very similar to that of cytolipin *H* and of ceramide lactoside.

The main differences between the spectra of cytolipin *H* and of ceramide lactoside are found in three places

(Fig. 1): around 1,725 cm^{-1}; between 1,200 and 1,400 cm^{-1}; and between 850 and 950 cm^{-1}. The absorption at 1,725 cm^{-1} is due to a trace of ester, attributed to some acetate residue on the carbohydrate moiety. As ester carbonyl absorption is very strong, the intensity of the peak shows that only a trace of acetate is present. The differences in the 1,200–1,400 cm^{-1} region are probably due to differences in the fatty acid residues of the two compounds. It has been our experience that lactosides of different fatty acid amides of 2-hydroxyethylamine differ in that part of the spectrum. As an example, Fig. 2 shows the relative differences in the spectrum of the lactosides of *N*-(2-hydroxyethyl)-docosanoamide (line *D*) and of *N*-(2-hydroxyethyl)-oleamide (line *C*) in comparison with the corresponding part of the spectrum of ceramide lactoside (line *B*) and of cytolipin *H* (line *A*). The single peak present at 1,252 cm^{-1} in lines *B* and *D* strongly suggests a saturated straight-chain fatty-acid residue for ceramide lactoside. Indeed, ceramide obtained by the same enzymatic hydrolysis and from the same source has been shown to contain mostly lignoceric acid[3]. The peak at 969 cm^{-1} for both cytolipin *H* and ceramide lactoside shows that both compounds do have some degree of unsaturation (sphingosine), barring interference from the carbohydrate absorption, which is so heavy in that part of the spectrum.

The absorption maxima at 874 and 892 cm^{-1} in the spectrum of ceramide lactoside are due respectively to the D-galactosidic and β-glucosidic links[12]. The spectrum of lactose itself (Fig. 3, line *E*) shows those two peaks at 876 and 897 cm^{-1}. They are present in all the lactosides which have been synthesized, though the intensity of the β-glucosidic bond absorption is increased in the lactosides, presumably by stabilization of the acetal bond after replacement of the hydrogen by another radical (methyl, etc.).

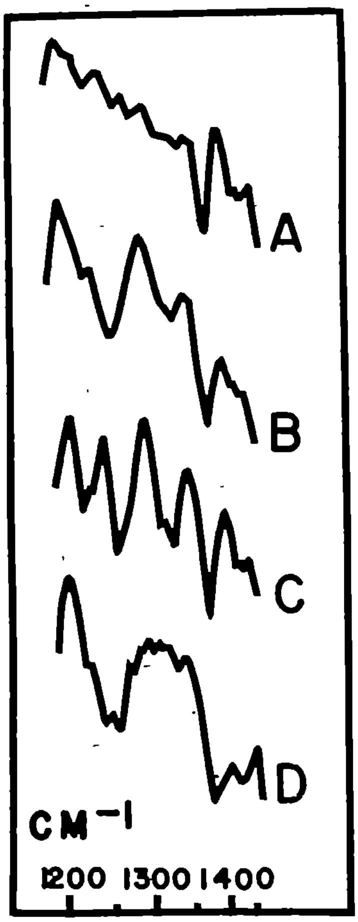

Fig. 2. Infra-red spectra in the region of 1,200–1,400 cm^{-1}, of cytolipin *H* (line *A*); of ceramide lactoside (line *B*); of the lactoside of *N*-(2-hydroxyethyl)-oleamide (line *C*); and of the lactoside of *N* (2-hydroxyethyl)-behenamide (line *D*); pressed in potassium bromide

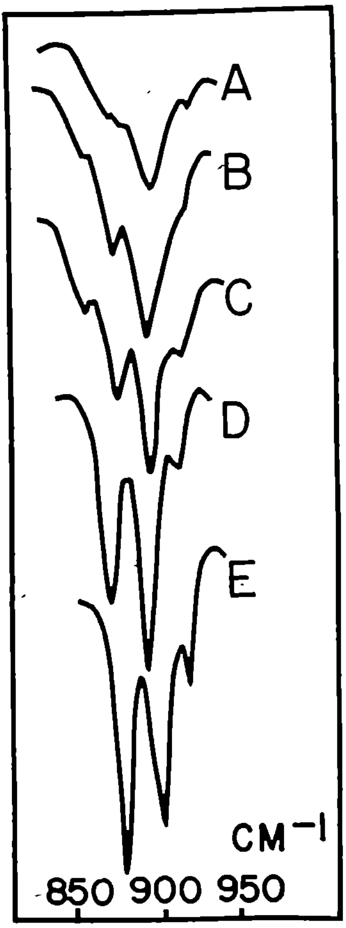

Fig. 3. Infra-red spectra in the region of 850–950 cm^{-1}, of cytolipin *H* (line *A*); of ceramide lactoside (line *B*); of the lactoside of *N*-(2-hydroxyethyl)-palmitamide (line *C*); of *n*-hexadecyl lactoside (line *D*); and of β-lactose (line *E*); pressed in potassium bromide

The methyl lactoside had an optical rotation of +6·4°. β-Methyl lactoside has been reported to have an optical rotation of +6·3° (ref. 13), showing that the β-lactoside had been obtained exclusively. The other lactosides all exhibited relatively negative optical rotations, varying from −12·7° (*n*-propyl lactoside) to +6·4° (methyl lactoside). Since all showed the characteristic maximum absorption in their infra-red spectrum between 889 and 892 cm^{-1}, the optical rotations support the labelling of the compounds as β-lactosides.

The complement-fixation tests which were so useful in the isolation and characterization of cytolipin *H* cannot prove the identity of cytolipin *H* and ceramide lactoside. Since it was found that lactose is the determinant site of this hapten any lactolipid would elicit a positive response[3].

Skin tests of ceramide lactoside and other lactosides were positive only in rabbits immunized against the diazophenollactoside bovine serum albumin, as one would expect from animals immunized against a lactoside hapten. This showed the presence of a lactoside group in the compounds which elicited a positive skin reaction Ring precipitin tests proved that the lactose structure had not been affected during the handling in the synthetic procedures: lactose and all lactosides tested exhibited inhibition of the precipitin reaction between antiserum and diazophenollactoside 'egg albumin, while other sugars were inactive.

Absolute proof of identity of cytolipin *H* and ceramide lactoside has not been established. But the accumulation of data from synthesis, infra-red spectrum analysis, and immunology shows that the synthetic compound is a ceramide-β-lactoside, and is very similar, if not identical, to cytolipin *H*.

We thank Mr. A. P. Boer and Mr. V. Jurevics for their assistance. We also thank the Lederle Laboratories (Pearl River, New York) for gifts of lecithinase *D*, from *Clostridium perfringens* type *A* toxin; Mr. S. Rosenberg (Sylvana Chemical Co., Orange, New Jersey) for gifts of lecithinsphingomyelin sludge; and the Wyeth Pharmaceutical Co. for gifts of hyaluronidase ('Wydase').

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STRUCTURAL REQUIREMENTS FOR PUROMYCIN INHIBITION OF PROTEIN SYNTHESIS

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THE antibiotic puromycin[1] has been found to inhibit protein synthesis in a wide variety of organisms and tissues[2–5]. Its structure (Fig. 1) has a striking resemblance to that of aminoacyl-*s*RNA, as first recognized by Yarmolinsky and de la Haba[6], who showed that the antibiotic inhibits amino-acid transfer from *s*RNA into proteins. Along with inhibition of protein synthesis puromycin induces the formation of small peptides from the amino-acids originally attached to *s*RNA[7,8]. Although growth of the peptide chain on the ribosome appears to be interrupted by puromycin[9–11], its action is not yet entirely understood in biochemical terms. In the course of studies on protein synthesis from aminoacyl-*s*RNA in the *E. coli* ribosomal system[12], we have examined the effect of analogues and isomers of puromycin in this system in the hope that a definition of the structural requirements for inhibition would help in understanding its mode of action.

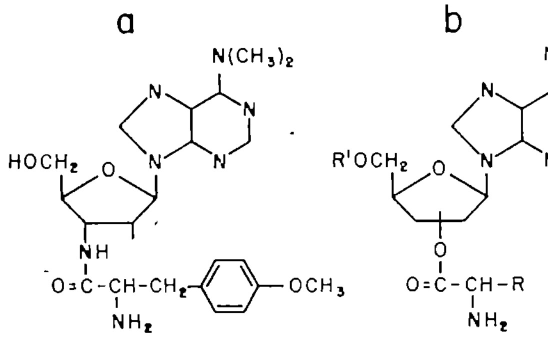

Fig. 1. Structure of puromycin (*a*) and the adenosine end of aminoacyl-*s*RNA (*b*)

Most of the compounds tested were the gift of Dr. Leon Goldman of the Lederle Laboratories, American Cyanamid Corporation, Pearl River, New York. The remainder (synthetic puromycin and the L-phenylalanyl, L-prolyl and L-phenylalanyl-*p*-methoxy-L-phenylalanyl analogues) were synthesized from the aminonucleoside of puromycin (or in the case of the peptidyl analogue, from puromycin) and the corresponding carbobenzoxy amino-acid by the method of Baker *et al.*[13]. The 2′-isomer of puromycin was obtained by coupling the corresponding 2′-aminonucleoside (9-(2′-deoxy-2′-amino-β-D-ribofuranosyl)-6-dimethylaminopurine, obtained from Dr. Goldman) with carbobenzoxy-*p*-methoxy-L-phenylalanine. The product, which was not crystallized owing to the small amount of starting material available, had the same R_F as puromycin in three chromatographic systems and the same electrophoretic mobility at *p*H 4·7. These properties differed markedly from the starting 2′-aminonucleoside, the mobility of which was identical to that of the 3′-aminonucleoside. Also, the absorption spectrum of the 2′-isomer of puromycin, purified by paper chromatography in butanol–acetic acid–water, was the same as that of puromycin.

Results of the inhibition studies are given in Table 1. Each compound was tested both in the regular system with *s*RNA esterified with all amino-acids, including ^{14}C-leucine[12], and in the polyuridylate system of Nirenberg and Matthaei[14] with ^{14}C-phenylalanyl-*s*RNA. Though the latter system seems somewhat less sensitive to puromycin, the results in the two cases are practically identical. In addition, many of the compounds were tested with rat liver preparations[15] and similar results obtained. As indicated in Table 1, the effect of each compound on the enzymatic formation of peptides and free amino-acids ('alcohol-soluble products') from aminoacyl-*s*RNA (ref. 8) has also been determined.

With the reservation that in some instances only a single compound of a given type has been tested, the major conclusions with regard to the structural specificity of puromycin may be summarized as follows: (1) Both the aminonucleoside and amino-acid are required. (Yarmolinsky and de la Haba previously observed that removal of the purine base resulted in loss of activity[6].) (2) The aminonucleoside must be substituted at the 3′-position. (3) The amino-acid must be of L-configuration. (4) Aromatic amino-acid derivatives are by far the most potent, whereas derivatives with certain amino-acids are inactive. (5) Compounds with a dipeptide side-chain are inactive.

The great differences in activity of compounds with different amino-acids were unexpected. One might have anticipated that any analogue with a natural amino-acid would be equally effective since each of these compounds would resemble an aminoacyl-*s*RNA. As noted, however, substitution with some amino-acids (glycine, proline) leads to analogues with no activity; substitution with others (L-leucine) gives slight activity; and the presence of phenylalanine (with or without a *p*-alkoxy group) or tyrosine results in marked activity. The basis for this specificity is unknown, but could reflect a tighter binding of the aromatic aminoacyl derivative to the inhibited site.

The fact that the phenylalanyl analogue is a more potent inhibitor of both phenylalanine and leucine incorporation than is the leucyl analogue suggests that the amino-acid substituent does not confer specificity for the inhibition of incorporation of that particular amino-acid.

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Table 1

Compound	Conc. (M)	Phenylalanine incorporation % inhibition	Phenylalanine incorporation % in alcohol-soluble products	Leucine incorporation % inhibition	Leucine incorporation % in alcohol-soluble products
Puromycin	10^{-4}	72	20	83	28
	10^{-3}	96	21	93	33
Puromycin (synthetic)	10^{-4}	66	20	84	39
	10^{-3}	95	27	91	37
Altered aminonucleoside					
2′-puromycin isomer	10^{-4}	0	0	3	1
	10^{-3}	0	0	2	0
5′-puromycin isomer	10^{-4}	0	0	0	0
	10^{-3}	0	0	0	0
N-diethyl analogue	10^{-4}	23	7	63	30
	10^{-3}	60	14	81	36
3′-aminonucleoside of puromycin	10^{-3}	—	—	0	0
Altered side-chain					
L-phenylalanyl analogue	10^{-5}	—	—	49	—
	10^{-4}	55	13	79	—
	10^{-3}	94	20	90	40
O-butoxy-L-phenylalanyl-analogue	10^{-4}	22	7	60	18
	10^{-3}	63	16	78	27
D-phenylalanyl analogue	10^{-4}	0	0	17	3
	10^{-3}	19	0	35	18
L-tyrosyl analogue	10^{-4}	14	3	65	19
	10^{-3}	67	12	81	—
L-tryptophanyl analogue	10^{-4}	0	0	0	0
	10^{-3}	6	0	12	7
L-leucyl analogue	10^{-4}	0	0	10	6
	10^{-3}	0	0	35	17
L-prolyl analogue	10^{-4}	—	—	10	0
	10^{-3}	—	—	9	2
Glycyl analogue	10^{-4}	1	0	7	2
	10^{-3}	0	0	4	4
Peptidyl side-chain					
Glycyl-*p*-methoxy-L-phenylalanyl analogue	10^{-4}	11	0	—	—
L-phenylalanyl-*p*-methoxy-L-phenylalanyl analogue	10^{-4}	6	2	—	—

Incubation conditions were similar to those given previously[12]. In a volume of 0·10 ml. the following components were present: 0·4 mg of ribosomes (phenylalanine) or 1·0 mg (leucine); 10 μg of transfer factor; 0·0005 M GTP; 0·01 M phosphoenolpyruvate; 3 μg pyruvate kinase; 0·01 M glutathione; 0·012 M $MgCl_2$; 0·08 M KCl, 0·05 M *tris* HCl pH 7·4. In the phenylalanine experiments 0·2 μg of polyuridylate and 25 μg of sRNA charged only with ^{14}C-phenylalanine (2,890 c.p.m.; 1·2 × 10^6 c.p.m./μmole) were also present; and in the leucine experiments, 12 μg of aminoacyl-sRNA containing 5,360 c.p.m. ^{14}C-leucine (1·6 × 10^6 c.p.m./μmole). After 10 min at 30°, 2 volumes of cold ethanol containing 1/20 volume of 20 per cent potassium acetate was added and an aliquot of the supernatant counted. The precipitate was then extracted with 5% TCA at 90° for 15 min, washed, and counted as described previously[12]. In the absence of inhibitor, 1,250 c.p.m. were incorporated into the TCA precipitate and 580 c.p.m. were found in the alcohol supernatant with phenylalanyl-sRNA; and 2,070 c.p.m. in the precipitate and 1740 c.p.m. in the alcohol supernatant, with leucyl-sRNA. Per cent inhibition refers to the incorporation of isotopic amino-acid into acid-precipitable form; per cent in alcohol-soluble products refers to the per cent of added ^{14}C-amino-acid found in this fraction minus that due to chemical deacylation.

Table 2

Compound	Conc. (M)	Per cent inhibition leucine	Per cent inhibition proline
Puromycin	10^{-4}	80	85
Leucyl analogue	10^{-3}	57	67
Prolyl analogue	10^{-3}	13	0

Incubation conditions were similar to those given in the legend of Table 1.

Also consistent with this is the observation (Table 1) that the tyrosine analogue inhibits phenylalanine incorporation in the polyuridylate system. This lack of specificity is again seen in the experiment reported in Table 2; the leucyl analogue is equally effective in reducing leucine and proline incorporation, whereas the proline analogue has little or no effect in either instance.

The striking difference in activity between the 2′ and 3′ substituted nucleosides deserves comment, since it might be related to the specific position of the amino-acid on the adenosine of sRNA[16]. Such an interpretation, however, must await the determination of the exact position of the amino-acid in esterified sRNA.

As shown in Table 1, the amount of alcohol-soluble compounds formed in the presence of various analogues correlates well with the degree of inhibition of protein synthesis by a given compound, thus substantiating the conclusion that the enzymatic formation of small peptides from aminoacyl-sRNA is intimately related to the mechanism by which puromycin inhibits protein synthesis.

Finally, the specific structural requirements for inhibition suggest that puromycin acts by binding to the active centre of an enzyme or to a ribonucleoprotein involved in the last steps of protein synthesis. In considering the reactions which occur[7], the possibility that puromycin interacts with the peptide bond-forming enzyme is an attractive hypothesis.

We thank Dr. Fritz Lipmann and Dr. Heinrich Waelsch, in whose laboratories this work was carried out, for their interest and advice.

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EFFECT OF RNA FROM NORMAL HUMAN BONE MARROW ON LEUKÆMIC MARROW *IN VIVO*

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It is now generally accepted that RNA, conveying DNA's information, directs specific proteinopoiesis. This notion, derived mainly from cytochemical and biochemical evidence, is being utilized to demonstrate specific proteinopoietic activities of native ribonucleic acids extracted from different cellular systems. It has been shown that RNA from yeast cells adapted to copper specifically protects unadapted strains against lethal doses of copper[1], and that a new type of RNA appears in yeast cells when catalase synthesis is induced by exposure to oxygen[2].

True redifferentiation of mouse ascites tumour cells by RNA from normal calf liver may have been obtained by Niu[3–5]. On the other hand, normal mouse kidney cells treated by RNA from the ascites tumour cells were able to induce local tumours in mice[5]. We have shown that RNA from human leukæmic and tumour cells can be cytopathogenic for cultures of primary lines of human cells[6,7] and oncogenic and leukæmogenic for animals[8]. The interplay of normal and malignant RNA with malignant and normal cells obtained with the Novikoff tumour model points towards the possibility of redifferentiating human malignant cells with human normal RNA[9-12]. RNA information transfer between sickle hæmoglobin-producing and normal human erythroblasts was apparently obtained by Weisberger[13]. Significant reduction in tumour takes were obtained by Harel *et al.*[14] by the effect of normal mouse liver RNA on *FLS* ascites carcinoma cells of the Swiss mouse. Proof of penetration of labelled RNA into normal and tumour cells was obtained by Schwarz *et al.*[21].

The foregoing evidence led to the hypothesis that RNA from malignant cells is biologically different from RNA from non-malignant cells and that the degree of differentiation or undifferentiation of the malignant cells might reflect the relative amounts of normal and abnormal RNA. Abnormal RNA would be unable to direct specific protein synthesis; enzyme deletions and antigenic simplification, both found in malignant cells, could be the indirect result of the unfitness of the abnormal RNA[15]. Of the two classes of RNA now described by Sibatani[16] the high metabolically active, low in guanidine content and the less metabolically active, high in guanidine content, the last was reported a few years ago to be predominant in malignant cells[17].

A pattern for alterations of normal RNA under carcinogenic influences (viral, physical, chemical) may have been set by the experiments of Tsugita and Fraenkel-Conrat[18] and Siegel[19], and the findings of Alderson[20]. The transformation of cytosine into uracil in the RNA of the tobacco mosaic virus by nitrous acid resulted in a mutant producing a protein differing from the natural TMV protein in the replacement of proline by leucine in a certain location. It is recalled that a similar difference in one single aminoacid is all that distinguishes hæmoglobin *A* from sickle hæmoglobin and other hæmoglobins. Similar mutagenic alterations on the TMV RNA were brought about by 4-nitroquinoline-N-oxide[22], a substance which is leukæmogenic for the mouse by mechanisms suggesting endovirogenesis[23].

Departing from the hypothesis that malignant cells remain undifferentiated because of a deficiency of normal proteinopoietic RNA, an attempt was made to supply these cells *in situ*, with RNA extracted from normal cells. Leukæmic marrow and RNA from normal bone marrow seemed an ideal system for such experiment.

It must be emphasized that the aim of the work recorded here is to examine some biological properties of the RNA from normal marrow, specifically to determine if the hæmatopoietic information it contains can be transferred by a sort of sub-cellular homografting of a template to other cells. This work by no means contains a therapeutic or pharmacological implication, and it is presented for the possible biological consequences rather than for its content of clinical repercussions.

RNA from human normal bone marrow. Non-leukæmic bone marrow was obtained from a 48-year-old white male presenting a history of epigastric distress which on X-ray was shown to be due to herniation of gastric mucosa through the pylorus. The following were his hæmatological parameters: Hæmatocrit value 52; hæmoglobin 15·30 g per cent; red blood cells 5,200,000 per mm³; white blood cells 8,000 per mm³; differential count in the peripheral blood: neutrophils 57 per cent, lymphocytes 42 per cent, and monocytes 1 per cent.

25 ml. of marrow were drawn from a sternal puncture in 2·5 ml. of 3·8 per cent sodium citrate. This marrow contained a total of 1·75 × 10⁶ cells per ml. and the myelogram was within normal limits.

RNA from this marrow was extracted by phenol. The alcohol-precipitate was redisolved in isotonic phosphate-buffered saline at *p*H 7·1. The final concentration of RNA was adjusted to 90 μg/ml. This preparation was filtered through 03 Selas porcelain filters and kept at −20° C for 24 h when it was thawed to 2° C for use.

Leukæmic patients' histories. H. M., 57-year-old white male. Admitted for the first time on October 28, 1959, because of petechiæ and small ecchymoses over chest and limbs. Three weeks before admission started noting easy fatigue and tiredness, pallor of skin and mucosæ, petechiæ and small ecchymoses and papullar infiltrations over the skin of chest and legs. His blood data were: hæmoglobin 8·65 g per cent; red blood cells 2,560,000/mm³; white blood cells 11,400/mm³; 52 per cent stem cells, 30 per cent lymphocytes, 11 per cent neutrophils, 1 per cent plasmocytes; 40,000/mm³ platelets, *O* reticulocytes. The sternal bone marrow was hypoplastic and paucicellular with partial megaloblastic transformation of the erythropoiesis. The leukæmic cells, representing about 15 per cent of all elements, were of myeloblastoid resemblance.

The treatment from October 30, 1959, to March 14, 1960, had consisted of transfusions, methyl-prednisolone and 6-mercaptopurine.

During the period of treatment the hæmoglobin oscillated between 8 and 12 g per cent, the leucocyte count from 14 to 40,000/mm³ and the platelets from 40 to 80,000/mm³ with 25–90 per cent of leukæmic cells. No remissions were ever achieved, although the general condition was satisfactory, the patient was asymptomatic, ambulatory and even resumed part-time work. Two weeks before this experiment, all treatment was discontinued. Tables 1 and 2 contain marrow data a few minutes before the experiment started.

Table 1. MYELOGRAMS OF PATIENT H. M. (% OF 500 CELLS COUNTED)

Place of instillation	Before normal RNA		2 days after 1st installation of normal RNA	10 days after normal RNA
Sternum	Leukæmic cells	84	63	91·5
	Erythroblasts	9	21	6
	Myelocytes	7	14 5	2·0
	Megakaryocytes	0	1·5	0·5
Place of instillation	Before buffered saline		2 days after buffered saline	10 days after buffered saline
Iliac bone	Leukæmic cells	92·5	92	92
	Erythroblasts	5	6·5	5·5
	Myelocytes	2·5	1·5	2·5
	Megakaryocytes	0	0	0

Table 2. MYELOGRAMS OF PATIENT M. H. (% OF 500 CELLS COUNTED)

Place of instillation	Before normal RNA		2 days after normal RNA	10 days after normal RNA
Sternum	Leukæmic cells	99	55	99
	Erythroblasts	1	30	0 5
	Myelocytes	0	13·5	0·5
	Megakaryocytes	0	1·5	0
Place of installation	Before buffered saline		After buffered saline	10 days after buffered saline
Iliac bone	Leukæmic cells	99·5	99	100
	Erythroblasts	0·5	0·5	0
	Myelocytes	0	0·5	0
	Megakaryocytes	0	0	0

M. H., 71-year-old white male. Admitted on April 1, 1960, because of weakness, dizziness and faintness. The laboratory findings on admission were: hæmoglobin 7·5 g per cent; red blood cells 2,160,000/mm³; white blood cells 4,100/mm³; of the last, 50 per cent were myeloblastoid anaplastic stem cells and 5 per cent basophils. The bone marrow was cellular and monomorphic, 95 per cent of its cells being leukæmic cells. Treatment consisted, until 2 weeks before this experiment, of transfusions and antibiotics.

Intra-osseous instillation of normal marrow RNA. A puncture was made in the middle of the second sternal piece. Marrow was aspirated for morphological study. Through the same needle 1 ml. of the normal RNA preparation was injected. At the same time a puncture was made in the right iliac crest at a point 1 cm behind the anterior superior spine at an angle pointing to this spine. Marrow was obtained for morphological study followed by injection of 1 ml. of phosphate-buffered saline as control. Later aspirations of marrow were made in the same marked places.

Differential counts represent percentages of cells in Wright stained smears. Each value is the average of three different counts. Absolute total counts were made from a suspension of 2 ml. of marrow diluted to 10 ml. with citrate-buffered saline. Counts were made in Levy–

Hausser counting chambers. Each value represents an average of three counts in 64 large squares. Absolute figures for leukæmic cells, myelocytes and megakaryocytes were calculated from the percentages in smears and total counts. Absolute counts of erythroblasts were obtained directly in cell suspensions with the violet light microscope method described by Wilkins and us[11,12] making use of the specific hæm absorption in the Soret band. This method permitted to check the accuracy of the indirect absolute estimations which we found to represent an approximation of ± 7 per cent.

Tables 1 and 2 summarize the cellular composition of the marrows obtained before and after instillation of normal marrow RNA and control-buffered saline.

Because of the slight, though clear, change in the hæmopoietic direction and the quick reverse to the previous full leukæmic stage after this first RNA instillation, an amount of normal marrow RNA 10 times larger was reinstilled in patient H. M. 900 μg of the same RNA in 10 ml. of buffered saline was instilled in the sternal manubrium 30 days after the first RNA instillation in the second sternal piece. Samples of marrow were taken before and 2, 6, 10 and 14 days after this reinstillation. Table 3 gives the results.

Table 3. RELATIVE AND ABSOLUTE DIFFERENTIAL CELL COUNTS OF MARROW CELLS OF PATIENT H. M. AFTER SECOND REINSTILLATION OF 900 μG OF NORMAL HUMAN MARROW RNA IN THE STERNAL MANUBRIUM

	Time after RNA instillation									
	0 days		2 days		6 days		10 days		14 days	
Cells	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.
Leukæmic cells	91	3 7	2	0·1	20	0·6	40	1·2	90	3 6
Erythroblasts	6·5	0·25	65	2	56	1·6	36	1	6	0·2
Myelocytes	1·5	0 1	31	0·9	22	0·6	13	0·4	4	0 2
Megakaryocytes	0	0	2	0·6	2	0·1	0·3	0 001	0	0
Total absolute cell numbers	4 05		3·6		2·9		2·6		4·0	

Relative counts: Percentages in 1,000 cells in smears
Absolute counts. Millions in 2 ml. of marrow

Fourteen days after the second instillation of RNA, when the local marrow had reverted back again to a full leukæmic stage, intravenous injections of 1,800 μg of RNA were made three times at two-day intervals. No change was detected in the peripheral blood counts or in an iliac marrow sample taken 8 days after the last intravenous injection.

Morphological studies and differential cell counts of the marrows of the two leukæmic patients clearly indicated a local hæmopoietic response following instillation of the RNA from normal marrow. For reasons not well understood, this hæmopoietic response either fades away or it is completely obscured by the leukæmic proliferation. There seems to be a relationship between the amount of RNA instilled and the degree of hæmopoietic response as well as the period of time for reversion to the previous leukæmic stage. When 90 μg of RNA were instilled, only a slight hæmopoietic response was noted with reversion to the *statu quo ante* within 10 days. When 10 times that amount was re-instilled, a full hæmopoietic response was obtained in 2 days with progressive decline within 14 days.

Failure of intravenous administration of RNA to induce marrow changes may probably be accounted for by quick inactivation in the circulation by ribonuclease or inhibition by strong combination of the RNA with plasma proteins. Data on this problem are apparently conflicting. Gierer and Schramm[23] have indicated that highly purified RNA from tobacco mosaic virus is largely inactivated by proteins in serum. However, injections of tumoral and leukæmic RNA have been successful in inducing disease in mice[8,24-26], thus indicating persistence of activity after injection. It may be that these preparations are not so highly purified as those of Schramm and that residual protein affords some degree of protection. Ellem[27] and we found[7] that in testing the cytopathogenicity of RNA preparations, it is very important to wash off the medium of the tissue culture and to operate in conditions of osmolality, *p*H, temperature and time for absorption which proved the most favourable to the nucleic acid, while extracellular. The foregoing conditions were to a certain extent reproduced in the method of intra-osseous instillation and this fact probably accounts for the success of local application and the failure of the intravenous administration of the RNA.

While it is possible to obtain these conditions *in vitro*, it will probably be necessary to learn how to reproduce them *in vivo* before we will be able to bioassay specific proteinopoietic activities of the ribonucleic acids from different sources.

A third patient provided the opportunity for further probing into the possibilities of protecting RNA intravenously injected.

W. B., a 40-year-old male, was diagnosed of acute stem-cell leukæmia about three months after the first symptoms of weakness and fatigue. The patient had not received any form of treatment, not even transfusions. By the time of the experiment the hæmatological findings were: sternal bone marrow: 95 per cent leukæmic cells; hæmoglobin 7·00 g per cent; red blood cell 2·8 × 10^6/mm³; white blood cell 4 × 10^4/mm³ of which 80 per cent were leukæmic cells, reticulocytes 0 per cent, platelets 2 × 10^4/mm³.

An intravenous infusion of 1 g of calcium disodium versenate in 150 ml. of 5 per cent glucose in water was dripped for 1 h. Urine collected before, during this time, and in the following 2 h was assayed for calcium content, and showed an elimination of 1/5 of the total previous blood calcium. Blood calcium-levels, however, did not show appreciable differences due to bone calcium mobilization in accordance with reported findings[18]. Magnesium in the blood dropped from 3 to 0·7 mg per cent.

The last was particularly the one intended to be decreased since it is the activating oligoelement of ribonuclease.

Immediately following the last drops of the chelating agent 1,480 μg of RNA in buffered versenate saline obtained from pooled normal human bone marrow were injected intravenously.

The same procedure was repeated for 3 days. 1–2 h after the EDTA–RNA administration, chills and temporary elevation of temperature were noted. Table 4 summarizes the hæmatological findings in the first 2 weeks of observation. The hæmatopoietic response by the systemic RNA, as shown by the moderate reticulocyte elevation and hæmoglobin build up, was maintained for about the same time as that observed in the local stimulation by regional instillation of RNA.

Table 4. EFFECT OF INTRAVENOUS EDTA FOLLOWED BY NORMAL MARROW RNA ON LEUKÆMIC BLOOD

Day	Hb (g %)	RBC (10^6 mm³)	WBC (10^3 mm³)	Leukæmic cells (% of WBC)	Platelets (10^4/mm³)	Reticulocytes (%)
0	7·0	2·8	50	80	2	0
0·5	7·0	2 8	57	85	2	0
1	7·0	3·0	28	40	4	0·1
2 2·5	7·8	3·2	18	30	5	1·0
3 3·5	8·10	3·4	13	10	6	1·4
4	8·80	3·5	12	10	6	2·7
5	9·1	3·7	12	10	6	3 1
6	10·1	3·8	10	6	6	4
7	10	3·9	10	6	6	4 2
15	8	3·2	26	15	4	3·1

The question of whether the phenomena described represent specific hæmopoietic redifferentiation of the leukæmic cells by a hæmopoietic RNA template or if they represent non-specific hæmopoietic stimulation of reticulum cells by the preparation cannot be solved until RNA from organs other than bone marrow is used in the same way. Local changes of megaloblastic into normoblastic marrow were induced by Horrigan[28] following local instillation of vitamin B_{12}. In 1927 Larsell *et al.*[29] injected nucleic acid preparations from erythrocytes of the fowl intravenously into rabbits and into 11 anæmic patients. Marked hæmopoietic stimulus was noted as judged by peripheral blood cell counts. With the method of preparing the nucleic acids used by these authors, degradation of the nucleic acids and heavy protein contamination was to be expected and thus transfer of intact templates may probably be excluded. Non-specific stimulation of the hæmopoiesis by thymidine residues and analogues was probably the mechanism of action in this case. This and similar works established the basis for the therapeutic use, now discontinued, of nucleotides in aplastic anæmia to stimulate hæmopoiesis. However, one problem is to stimulate hæmopoiesis and another is to redifferentiate anaplastic leukæmic stem cells. Further evidence for true redifferentiation of the leukæmic cells may have to be gained by the use of normal RNA on cultures of leukæmic cells in the absence of potentially hæmopoietic normal reticulum cells.

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CHANGE OF HUMAN CHROMOSOME COUNT DISTRIBUTIONS WITH AGE: EVIDENCE FOR A SEX DIFFERENCE

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In previous communications we reported that the proportion of aneuploid cells in cultures of human leucocytes increased with the age of the subject[1], and that, at any given age among adults, the proportions did not differ appreciably between persons with and without cancer[2]. We now have much more data, and also have evidence that there is a difference between the sexes both in the manner in which the proportions of aneuploid cells change with age, and also in regard to the chromosomes affected.

Altogether data are available for 8,380 cells from 247 subjects with normal karyotypes inclusive of those reported previously. These cells have been obtained from blood cultures prepared by the technique of Moorhead *et al.*[3]. For each aneuploid cell an attempt has been made to describe the missing or additional chromosomes. The distribution of chromosome counts is recorded in Table 1 for each age-group, and divided according to chromosomal sex. The proportion of aneuploid cells (expressed as a percentage of the diploid cells) increases with age in both sexes; but the rate of increase in the proportion of hypodiploid cells differs between the sexes. Comparison of the regression coefficients in the linear regression formulæ (Table 1) shows the coefficients for men and women to differ significantly ($P < 0·01$). Furthermore the male data are satisfactorily explained by a linear regression, whereas inspection suggests that the female data would be better fitted by a cubic curve in which the rate of increase is low under the age of 45 years, high between the ages of 45 and 64 years, and low again at ages over 65 years. Under 45 years of age, the trend in the female data is similar to that for males, and can be fitted by the linear equation $y = 0·056x + 2·03$.

Table 1. DISTRIBUTION OF CHROMOSOME COUNTS BY AGE AND CHROMOSOMAL SEX

	Age group (yr.)	No. of subjects	Mean age*	No. of cells	No. of cells with counts of <46	46	>46	Expressed as % of diploid cells <46	>46
XY Chromosomal sex	0–4	10	1·10	300	11	287	2	3·83	0·70
	5–14	13	9·98	370	13	354	3	3·67	0·85
	15–24	22	20·84	989	36	948	5	3·80	0·53
	25–34	25	29·61	990	40	941	9	4·25	0·96
	35–44	8	37·72	256	13	239	4	5·44	1·67
	45–54	14	49·77	597	34	556	7	6·12	1·26
	55–64	10	58·50	300	19	275	6	6·91	2·18
	65–74	10	70·47	320	23	292	5	7·88	1·71
	75+	11	80·67	349	30	307	12	9·77	3·91
	All ages	123	36·15	4,471	219	4,199	53	5·22	1·26
XX Chromosomal sex	0–14	9	7·22	270	6	262	2	2·29	0·76
	15–24	33	20·08	968	33	930	5	3·55	0·54
	25–34	28	29·74	900	29	860	11	3·37	1·28
	35–44	13	39·08	452	19	427	6	4·45	1·41
	45–64	16	57·07	571	33	533	5	6·19	0·94
	65–74	11	68·09	330	37	291	2	12·71	0·69
	75+	14	81·72	418	46	356	16	12·92	4·49
	All ages	124	39·66	3,909	203	3,659	47	5·55	1·28

Regression on age of the percentage of: (i) hypodiploid cells (*XY*) $y = 0·082x + 2·26$; (ii) hypodiploid cells (*XX*): $y = 0·161x - 0·84$; (iii) hyperdiploid cells (*XY*): $y = 0·360x - 0·04$; (iv) hyperdiploid cells (*XX*): $y = 0·031x + 0·05$. Each of the regression coefficients differs significantly from zero. For (i)–(iii), $P < 0·01$; for (iv) $P = 0·04$.

* Weighted for the number of cells counted.

In examining the types of chromosomes involved in the formation of the aneuploid cells, an attempt has been made to assign the missing or additional chromosomes to one or other of the following six groups, based on the Denver classification. Where this has been possible a cell is said to have been fully analysed. In a number of cells a full analysis was not possible, but a partial analysis could be made. Such cells in males were included with those fully analysed even though the chromosome could only be ascribed to the M/L group, and in females where the chromosome could only be ascribed to the S group (S_1–S_4).

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Chromosomes	Group
21, 22 and Y	S_1
16, 19 and 20	S_2
17 and 18	S_3
13, 14 and 15	S_4
6–12 and X	M
1–5	L

In group L, autosomes Nos. 1, 2 and 3 can of course be recognized as individual entities, but so few cells contained an abnormal number of L autosomes that this refinement was omitted. In the subsequent analyses of the aneuploid cells, those cells have been omitted which were of too poor a quality for full analysis, together with all cells carrying structural chromosomal abnormalities. Furthermore, cells with a chromosome count of 43 or less have also been discarded as many or all are likely to be artefacts due to rupture of the cell. The distributions of the counts in the remaining cells are given in Table 2; the results show that the proportion of hypodiploid cells still increased with age, and that the regression coefficients still differed significantly ($P < 0{\cdot}01$). In this and the subsequent analyses the numbers of hyperdiploid cells were found to be too small for any valid conclusions to be drawn.

Table 2. DISTRIBUTION OF CHROMOSOME COUNTS BY SEX AND AGE AFTER EXCLUSION OF CERTAIN GROUPS OF ANEUPLOID CELLS*

Chromosomal sex	Age group (yr.)	No. of cells with chromosome counts <46	>46	Percentage of diploid cells: Cells with hypodiploid counts	Cells with hyperdiploid counts
	0–14	17	3	2·65	0·25
	15–24	28	5	2·95	0·52
	25–44	45	3	3·81	0·57
XY	45–54	24	2	4·22	0·47
	55–74	21	1	3·70	0·34
	75+	20	10	6·52	2·81
	All ages	155	24	3·60	0·64
	0–24	26	4	2·18	0·62
	25–34	20	2	2·09	0·21
XX	35–54	18	10	3·30	0·85
	55–64	21	5	4·90	0·90
	65–74	20	5	6·87	0·88
	75+	32	7	8·99	2·28
	All ages	137	33	3·65	0·79

Regression on age of the percentage of:
selected hypodiploid cells, sex XY : $y = 0{\cdot}036x + 2{\cdot}39$
" " " " XX : $y = 0{\cdot}104x - 0{\cdot}40$
Both regression coefficients are significantly different from zero ($P < 0{\cdot}01$)

* See text for definition. The number of subjects, their mean ages and the numbers of diploid cells counted in each group are given in Table 1.

Inspection of the data suggested that in males an undue proportion of the hypodiploid cells was missing an S_1 chromosome, whereas in females an undue proportion was lacking an M chromosome. In fact the proportion of these cells not only increases with age (Table 3), but also these cells account for nearly all the regression on age previously observed. If these cells are excluded then the regression of the remaining hypodiploid cells on age becomes extremely small, and the coefficients do not differ significantly from zero in either sex (males, $y = 0{\cdot}006x + 2{\cdot}15$; females, $y = 0{\cdot}005x + 1{\cdot}41$).

The results reported here are open to criticism on the grounds that the observations were not made on a representative sample of the general population. The subjects were comprised of healthy volunteers, hospital in-patients, parents of children with chromosome abnormalities, etc. To offset this difficulty a study is in progress of a group of subjects, selected at random from the lists of general practitioners. This investigation will eventually include men and women of all ages from 15 years and upwards, but so far data only referable to those of 65 years and more are available. These, however, confirm the findings already noted for this group, and in this study every cell has been fully analysed. Examination of 1,050 cells from 35 males (mean age 71·69 years) shows 9·26 per cent to be aneuploid and that the proportions increase progressively for the age-groups 65–69, 70–74 and 75 years and over, being respectively 7·14, 10·09 and 12·15 per cent. 48 cells with 45 chromosomes were available for analysis, 27 of which were missing an S_1 chromosome (expected number on the basis of random loss, 5·22). In 12 of these cells the missing chromosome could confidently be said to be a Y chromosome, whereas only 1·04 such cells would be expected if all chromosomes had an equal chance of being lost. The findings from the analysis of 1,290 cells from 43 females (mean age 73·49 years) again confirmed the earlier findings. The proportion of aneuploid cells was 11·50 per cent and this proportion did not change progressively for the age-groups 65–69, 70–74, 75–79 and 80 years and over, the relevant proportions being 11·37, 12·68, 11·94 and 10·20 per cent. 95 cells with a count of 45 chromosomes were available for analysis, of which 81 were missing an M chromosome (expected number on the basis of random loss, 33·04). The substantial agreement between the earlier and later findings for ages of 65 years and more does indicate that it is unlikely that any serious bias has been introduced into the earlier findings due to the composition of the study population.

Table 3. DISTRIBUTION OF HYPODIPLOID CELLS LACKING S_1 AND M CHROMOSOMES BY AGE AND CHROMOSOMAL SEX

Chr. sex	Age group (yr.)	Cells lacking only S_1 chromosomes: Number	% of Diploid cells
	0–14	4	0·62
	15–24	4	0·42
	25–44	6	0·51
XY	45–54	8	1·44
	55–74	8	1·41
	75+	10	3·26
	All ages	40	0·95
		Cells lacking only M chromosomes	
	0–24	9	0·76
	25–34	5	0·52
XX	35–54	10	1·88
	55–64	10	2·33
	65–74	15	5·16
	75+	27	7·58
	All ages	76	2·02

Regressions on age of the percentage of
(i) XY cells : $y = 0{\cdot}030x - 0\ 120$
(ii) XX cells : $y = 0{\cdot}096x - 1{\cdot}823$
Both regression coefficients differ significantly from zero ($P < 0{\cdot}01$)

It seems reasonable to postulate that the observed increases in the proportion of aneuploid cells with age, which in males is mainly due to the loss of an S_1 chromosome and in females to the loss of an M chromosome, is in fact largely due to divisional errors involving the Y chromosome in men and the X chromosome in women. Direct evidence is available for the Y chromosome as in good preparations it is identifiable, and therefore its absence is recognizable. Direct evidence for the involvement of the X chromosome is not yet available, but it may become so from techniques using tritiated thymidine labelling. It may well also be relevant that the major change involving an M chromosome in women occurs at the time of a sharp decline in sexual activity. Both in males and females the findings could be explained: (1) if there was a special liability for cells to lose a sex chromosome, or (2) if all chromosomes were liable to be involved, but that the cells with an abnormal number of autosomes were at a disadvantage and failed to survive, or (3) if both possibilities occurred together.

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CHEMICAL COMPOSITION AND TERMINOLOGY OF SPECIALIZED ORGANELLES (MELANOSOMES AND MELANIN GRANULES) IN MAMMALIAN MELANOCYTES

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Development of Melanin Granules

In the past few years, investigations of the fine structure of the melanocyte have altered the concept of the nature of this cell. Birbeck, Mercer and Barnicot[1] were the first to point out that melanocytes contain a system of membranes similar to those seen in secretory cells. They have also observed that the melanin granules present in the cytoplasm of the melanocyte are not of uniform structure, but appear at any given moment to be in varying stages of development. "The granules appear to start as hollow vacuoles in which a tenuous material appears in the form of a folded lamella. . . . This material is rapidly thickened and defined by the deposition of more dense material. . . ."[2]. Later investigations of the fine structure of melanin granules have confirmed and extended these findings[3-9].

At the fifth International Pigment Cell Conference in New York, an investigation was described[10] in which particles isolated from pigmented tissues by ultracentrifugal, density-gradient methods had been studied by both biochemical techniques and electronmicroscopy[3,4]. The results of this work have made it possible to outline the successive steps in the development of melanin granules. Because melanin granules were found to be specialized organelles which differ from mitochondria not only in structure and development but also in chemical and enzymatic composition, a set of new terms was proposed for the various stages of their development. Introduction of these new terms is justified by inadequacy of the single, inclusive term, 'melanin granule', to describe with precision the structural and chemical metamorphoses which take place in the granule during melanization. In Fig. 1 are outlined the essential features of pre-melanosomes, melanosomes and melanin granules—the three specialized organelles found in melanocytes.

It is believed that three stages can be recognized in the formation of the *melanin granule*—the final product of the melanocyte (Fig. 1).

(I) A first stage in which polypeptides are synthesized and condensed into the secondary and tertiary structures of the enzymatic protein molecules.

(II) An intermediate stage in which the enzyme molecules are arranged in structural form—the *pre-melanosome*. The enzyme molecules are aligned in an ordered pattern.

(III) A third stage in which melanin biosynthesis begins and melanin accumulates inside the pre-melanosome. During this stage, the granule contains tyrosinase activity and is a *melanosome*.

The final product, in which melanization is complete, and in which no tyrosinase may be detected, is a *melanin granule*.

During stage II, the arrangement, shape and size of the pre-melanosomes are thought to be both species- and site-dependent. Melanin is not yet synthesized at this stage. During the early parts of stage III, the lamellar pattern of the pre-melanosome can still be seen in electronmicrographs, but as the process of melanization continues, the lamellar pattern gradually becomes obliterated by deposited melanin and an amorphous *melanin granule* eventually results. As melanin gradually forms within the melanosome, the tyrosinase activity of the melanosome correspondingly decreases : in other words, melanin formation and tyrosinase activity are inversely related[11].

Melanin appears to be synthesized *in vivo* predominantly during stage III : nevertheless, active tyrosinase may be recovered *in vitro* from the small-granule fraction. It is possible that extraction of the enzymatic protein results in its activation. There is reported to exist in insects an inactive enzyme called pro-tyrosinase which may be activated both *in vivo* and *in vitro*[12-15]. Melanin cannot be seen in electronmicrographs of rough or smooth membranes, yet small amounts may be present, but not detectable, because melanin and the other cytoplasmic structures have a similar density.

This series of developmental steps must hereafter be considered in any study of the regulatory mechanisms of melanin formation, whether they are hormones, ultraviolet light or disease states.

Chemical Composition of Melanosomes, Melanin Granules and Mitochondria

Stein[16] has examined the chemical composition of melanin granules isolated from the choroid of the eye of the ox and has compared his data with those obtained from the mitochondria of rat and mouse liver. It has seemed worth while to compare the chemical composition of the 'melanosomal' and 'mitochondrial' fractions isolated by differential and density-gradient techniques as a means of clarifying the biochemical characteristics of these two distinctive components of the melanin-forming cell. In evaluating results, however, one must remember that the so-called 'melanosomal' fraction may contain both melanin granules (as defined here) and melanosomes.

The *B*-16 mouse melanoma and the Harding–Passey mouse melanoma used in the experiments reported here were serially transplanted in *C*-57 and Swiss strain mice respectively. The entire, actively growing tumours were excised when their diameter reached 1·0–1·5 cm and promptly homogenized in 0·25 M sucrose at about 0° C. All subsequent processing occurred in a cold environment (about 3° C). The homogenate was centrifuged at 700*g* for 10 min. The resulting 'low-speed' supernatant, when centrifuged at 11,000*g* for 10 min, yielded a sediment which was resuspended in 0·25 M sucrose and recentrifuged at 15,000*g* for 10 min. This sediment was again suspended in 0·25 M sucrose to make the 'large-granule' preparation used as starting material for density-gradient centrifugation. Density-gradient sucrose columns were prepared in the tubes of the Spinco swinging-bucket rotor (*SW*-39-*L*) by layering 0·5 ml. of eight different concentrations of sucrose solution in serial order with the most concentrated

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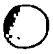
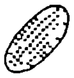
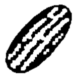
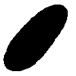
Fig. 1. STAGES IN THE DEVELOPMENT OF MELANIN GRANULES

Name and size	Morphology	Biochemical composition		
Ribosome (100–150 Å)	•	RNA + protein	Site of polypeptide biosynthesis	Stage I Biosynthesis of protein
Golgi vesicle (0·05μ)	•	Phospholipid + protein	Condensation (?)	
Intermediate vesicle (0·5μ)		Phospholipid + protein	Tyrosinase arranged in structural form	Stage II Development of organelle
Premelanosome (0·7 × 0·3μ*)		Phospholipid + protein tyrosinase	End of tyrosinase arrangement Final product of albino melanocyte	
Melanosome (0·7 × 0·3μ*)		Tyrosinase + melanin	Specific site of melanin formation	Stage III Biosynthesis of melanin
Melanin granule (0·7 × 0·3μ*)		Melanin + no measurable tyrosinase activity	Final product of melanocyte	

* Typical size of human brown melanin granules.
Adapted from *Subcellular Localization of Melanin Biosynthesis*, by Seiji, M., Shimao, K., Birbeck, M. S. C., and Fitzpatrick, T. B. Chapter in *The Pigment Cell: Molecular, Biological and Clinical Aspects*, edit. by Riley, V., and Fortner, J. G., 100, 497 (New York Acad. Sci., 1963).

layer at the bottom of the tube. They were then allowed to stand overnight (about 18 h) so that the gradient might become smooth. The sucrose concentrations used for the separation of melanosomes were 2·6 M, 2·4 M, 2·2 M, 2·0 M, 1·8 M, 1·6 M, 1·55 M, and 1·5 M; for the separation of mitochondria, the concentrations were 2·0 M, 1·8 M, 1·6 M, 1·55 M, 1·5 M, 1·4 M, 1·2 M, and 1·0 M. Immediately before centrifugation, 1 ml. of freshly prepared large-granule suspension from mouse melanoma was layered carefully over the top of each tube. After centrifugation at 103,000*g* for 60 min, the melanosomal fraction and the mitochondrial fraction were isolated from their respective centrifuge tubes by a centrifuge-tube cutter. The fractions thus obtained were then subjected to chemical analysis and their tyrosinase, succinoxidase and glutamate oxidase activity were measured.

The analytical technique of Schmidt and Thannhauser[17], modified by substituting perchloric acid for trichloroacetic acid, was used for the extraction of lipid and ribonucleic acid. Phospholipid content was estimated from the phosphorus content of the lipid fraction. Phosphorus content was determined by Fiske–Subbarow's method[18]. Ribonucleic acid content was calculated from the phosphorus content of the RNA fraction. To determine the protein–nitrogen content, the protein was precipitated by adding an equal amount of 10 per cent trichloroacetic acid to each sample. The precipitate was spun down, washed once with 5 per cent trichloroacetic acid, and dissolved in 1 *N* sodium hydroxide solution. The nitrogen content of this alkaline solution was determined by the method described by Johnson in 1941[19]. The zinc content was estimated by Vallee's method[20,21]. Tyrosinase activity was estimated manometrically by measuring the oxygen consumption, using a 10:1 mixture of L-tyrosine and L-*dopa* as substrate (1·77 μmoles) in M/10 phosphate buffer (*p*H 6·8)[22]. Succinoxidase activity was estimated respirometrically by measuring oxygen consumption in the presence of cytochrome *c*, as described by Umbreit[23]. Glutamate oxidase activity was estimated respirometrically by measuring oxygen consumption in the presence of ATP, DPN, nicotinamide, magnesium chloride and cytochrome *c* (ref. 24).

Table 1 contains analytical data obtained from melanosomal and mitochondrial preparations isolated from *B*-16 and Harding–Passey mouse melanoma by methods described here[25]. As can be seen, the composition of the melanosomal fraction contrasts sharply with that of the mitochondrial fraction as regards content of each component studied. The melanosomal fraction contains much more zinc than the mitochondrial fraction. Although the role of zinc has not yet been clarified, the fact that it does play a part in the formation of melanin is increasingly evident[26]. The high zinc content of the melanosomal fraction seems to be specific for that fraction. The RNA content of the mitochondrial fractions examined agrees well with the figures reported by Schneider and Hogeboom[27]. Especially significant is the great contrast between the low content of RNA and phospholipid in the melanosomal fraction and the high content of these substances in the mitochondrial fraction. RNA has been shown to be essential for biosynthesis of protein within the

Table 1. ANALYTICAL DATA SHOWING THE MARKED DIFFERENCES BETWEEN MITOCHONDRIAL AND MELANOSOMAL FRACTIONS OBTAINED BY CENTRIFUGAL TECHNIQUES

Component	Type of melanoma	Melanosome (μg/mg protein N)	Mitochondria (μg/mg protein N)
RNA–P	Harding–Passey	2·2 ± 0·32*	32·5 ± 0·87*
	B-16	4·7 ± 1·2	20·2 ± 1·38
Phospholipid-P	Harding–Passey	13·1 ± 0·9	158·2 ± 2·9
	B-16	19·1 ± 1·2	115·0 ± 1·2
Zinc	Harding–Passey	26·5 ± 0·5	<1
	B-16	39·0 ± 2·6	1·62
Enzymes		Specific activity (μl. O_2/h/mg protein N)	
Tyrosinase	Harding–Passey	1,028 ± 7·1	0
	B-16	243 ± 4·8	0
Succinoxidase	Harding–Passey	0	2,017 ± 11·0
	B-16	0	2,134 ± 13·6
Glutamate oxidase	Harding–Passey	0	713
	B-16	0	640

* Average value ± *S.E.*

cell[28]. The low RNA content of the melanosomal fraction is evidence that, so far as protein biosynthesis is concerned, melanosomes are probably very inert components of the melanin-forming cell.

A recent examination of the *in vivo* incorporation of leucine labelled with carbon-14 provides more direct evidence that the role of melanosomes in protein biosynthesis is very small as compared with that of mitochondria. In these experiments, animals were treated and fractionation carried out by methods already described[25]. The specific radioactivity of melanosomal and mitochondrial fractions obtained from melanoma excised 1 h after injection of leucine-^{14}C was 37·8 and 308 c.p.m./mg protein respectively. In other words, much less leucine-^{14}C was incorporated by melanosomes isolated from *B*-16 mouse melanoma than by mitochondria from the same tissue.

Tyrosinase, the oxidative enzyme responsible for melanin formation in mammals, is localized for the most part in melanosomes. Measureable amounts of tyrosinase activity were not found in the mitochondrial fraction isolated from mouse melanoma.

The amount of succinoxidase activity found in the mitochondrial fraction from the same source (Table 1) is comparable with that reported in hepatoma by Schneider[29]. In comparing the figures shown in Table 1 with the figures obtained from other normal and neoplastic tissues, it is necessary to be aware that Harding–Passey and *B*-16 mouse melanoma are composed of both melanoma cells and phagocytes, and that cell particles isolated from these tumours may be derived from both types of cells. The tissue may contain 40–60 per cent phagocytes. In a few of the experiments reported here, succinoxidase and glutamate oxidase activity were detected in melanosomal fractions isolated by centrifugation, but in almost negligible quantities. When melanosomal fractions containing this very small amount of succinoxidase and glutamate oxidase activity were purified again by density-gradient centrifugation (sucrose concentrations 1·5–2·6 M), a very faint white band could be observed at the position in the tube of fraction 2 (ref. 24). After centrifugation in the high-density gradient (1·5–2·6 M sucrose), fraction 2 was found to contain mitochondria. No succinoxidase or glutamate oxidase activity was observed in the purified melanosomal fraction. Therefore, the traces of succinoxidase and glutamate oxidase activity detected in the melanosomal fraction may be interpreted as evidence of contamination with mitochondria.

The results reported here support the concept that melanosomes are distinctive sub-cellular particles which differ from mitochondria; are unique in their localization within the cytoplasm of melanin-forming cells; contain a specialized metabolic pathway in which tyrosine is converted to melanin; and appear to be quite inert sub-cellular particles without biological function except in the formation of melanin.

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ENCEPHALOPATHY IN MICE FOLLOWING INOCULATION OF SCRAPIE SHEEP BRAIN

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A CHRONIC, progressive, degenerative disease of the central nervous system has been induced in mice inoculated with brain material obtained from a sheep with naturally acquired scrapie. There was a remarkable parallel between the bizarre clinical manifestations and course of the experimentally induced disease in mice and the clinical pattern of scrapie in sheep. Furthermore, the neurological signs and histopathological lesions seen in our affected mice were similar to those observed by Chandler in mice inoculated with passage material from goats with induced scrapie[1,2]. In addition, Chandler's observations of a scrapie-like degenerative disease of the central nervous system in mice inoculated with scrapie goat brain material was, in the course of this work, confirmed.

A Cheviot ram which belonged to a flock in Illinois first showed signs of scrapie at 4 years of age and died with advanced disease on October 1, 1961, after 5 months of illness. Post-mortem examination was performed promptly and materials obtained for virus isolation and histopathological study. Microscopic examination of the brain revealed demyelinization, neuronal degeneration, satellitosis, neuronophagia, gliosis and vascular thickening. Occasional neurones showed vacuole formation. These findings were considered consistent with the histologic lesions of scrapie and confirmed the clinical diagnosis in this instance. The brain of this Cheviot ram, which had been stored at − 70° C for 40 days, served as the inoculum for viral studies. A finely ground 10 per cent suspension of the brain in physiological saline was clarified by low-speed centrifugation and the supernatant used immediately after preparation. Mice of 5 different breeds weighing 8–10 g, and, in addition, suckling Syrian hamsters, were injected intracerebrally with 0·03 ml., or intraperitoneally with 0·1 ml., of the test material. After 9 months, when none of the hamsters had developed disease, they were inadvertently discarded. Table 1 summarizes the results obtained in mice inoculated with the sheep material.

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Table 1. INCIDENCE AND INCUBATION PERIODS OF SCRAPIE-LIKE DISEASE IN MICE OF FIVE BREEDS INOCULATED WITH SCRAPIE SHEEP BRAIN FROM NATURALLY OCCURRING DISEASE

Breed of mice	No. of mice inoculated	Incubation period Earliest	Incubation period Latest	Mortality after 1 year from scrapie-like disease	Survivors
Swiss, *GP*	9	3½	8	8/9*	1 Sick
Swiss, *NIH*	9	2½	8	8/9†	1 Sick
CFW	15	2½	8	15/15	0
*CDF*₁	14	7	8	10/14‡	4 Sick
*C*57/Black	10	6	10	6/10§	0

* 2 of these affected mice were killed for passage and histological examination.

† 1 of these 8 affected mice was killed for passage and histological examination.

‡ 3 of these 10 affected mice were killed for passage and histological examination.

§ 2 of the 6 affected mice were killed for passage and histological examination; 4 other clinically well mice killed at 10 months are not included among the 6 dead from scrapie-like disease.

Disease appeared as early as 2½ months after inoculation in some mice, and as late as 10 months after inoculation in others. In the CDF_1 and *C*57/Black mice the shortest incubation period was twice as long as in the Swiss and *CFW* mice. A few of the animals were killed for pathological and auto-immune hypersensitivity investigations before the disease developed, and others were killed for passage and pathological examination in advanced stages of illness. All other mice receiving this sheep brain inoculum developed a scrapie-like syndrome.

The first signs in affected mice were a hyper-excitability evidenced by rapid jerky movements and increased speed of the normal activities, particularly those of grooming. The animals tended to run and jump and to execute circular movements, changing irregularly from clockwise to counter-clockwise direction. This was more evident when the animals were disturbed by handling or by exposure to bright light or to noise. Vibration or touch stimulation resulted in excessive response in the affected mice as compared with unaffected animals. However, this circling also occurred spontaneously without provocation. In addition to these manifestations an increase in scratching, scraping and licking activities suggested a parallel to the reactions seen in sheep with scrapie and attributable to itching[3]. These activities of the mice led to excoriation of the ears, lumbosacral region and snout, with frequent loss of hair, bloody scabbing and ulceration. Hyperextension of the neck was common, and the gait became waddling, with lowered hindquarters. This latter was associated with spastic hind limbs which were held in strong adduction when the mouse was suspended by the tail, while the fore-limbs retained normal muscle tone. The tail was stiff and when bent into abnormal shapes maintained these for a minute or so. With the onset of neurological signs, a progressive deterioration of nutrition developed. The mice became emaciated and hyper-excitability decreased. Soiling of the abdominal coat, suggesting urinary and fæcal incontinence and persistent priapism were often present.

The brains of several of the mice have been examined histologically and, after being stained with Cajal's gold chloride, revealed a marked astrogliosis and moderately severe loss of neurones, with some vacuolated neurones and others pyknotic and shrunken in cerebrum, cerebellum and medulla oblongata. Inflammatory lesions, such as are usually associated with acute encephalitis, were not present. Dr. Philip Paterson of the New York University, in a personal communication, stated that he found no detectable anti-brain complement-fixing antibodies to normal mouse brain antigen in the sera of inoculated mice in varying stages of the disease. Moreover, he saw no evidence of histological change suggesting allergic encephalomyelitis in the animals (see Table 1 for list of mice killed).

Inocula prepared from brain tissue of 6 other American sheep, diagnosed as having scrapie, have thus far failed to produce scrapie-like disease in our mice. In addition, mice inoculated with non-scrapie sheep brain, as well as non-inoculated shelf mates, have remained free of neurological disease for more than a year, and have served as controls. None of these animals has exhibited neurologic signs of illness; nevertheless, they are still under observation, some for 13 months since inoculation.

Brain material from an experimentally infected scrapie goat, received from Mr. I. H. Pattison of the Agricultural Experiment Station, Compton, Berkshire, was inoculated into mice of the Swiss *NIH* and Swiss *GP* breeds. This represented ninth passage of the scrapie agent in goat brain from an animal affected with 'drowsy' type of disease (in contradistinction to the scratching type); this passage was one beyond that from which Chandler had induced disease in mice. This Compton material in our hands induced a degenerative disorder of the central nervous system associated with clinical signs like those described by Chandler[1-4]. The disease followed either intraperitoneal or intracerebral inoculation with incubation periods ranging from 6 to 17 months at dilutions up to 10^{-4} of the original goat brain. Carefully removed supernatant from 16,000 r.p.m. centrifugation for 30 min produced similar disease at 10^{-3} but not at greater dilution. Seitz filtrate of this high-speed supernatant produced disease at only 10^{-1} dilution.

Remission and exacerbation of the disease has been evident in two mice inoculated with goat brain material. These developed the syndrome of clumsy gait with spastic hind extremities and the slow movements and 'drowsy' behaviour of the scrapie-like disease, and then, after a period of 64 and 46 days, respectively, of this progressive disease appeared to recover completely. The first of these animals remained well for 74 days and then again developed the same syndrome. This recrudescence lasted for only 7 days, when the animal died of the disease. The other animal appears well 157 days after the initial recovery.

Second passage in mice of mouse brain material from both the sheep brain and goat brain-induced diseases has yielded no disease as yet; however, the longest observations have thus far been for only 4 months since inoculation. There is, as yet, no serological or immunological test for scrapie, hence it has not been possible to relate our experimental encephalopathy in mice to the natural disease in sheep. However, Chandler has recently demonstrated that material from mice suffering from his scrapie goat brain induced encephalopathy produces typical scrapie when inoculated back into goats[4].

Breeding experiments to determine whether the disease in mice can be genetically or congenitally transmitted are in progress. We are also performing experiments to determine whether neutralizing antibody develops in affected sheep or mice and to investigate whether immune tolerance may exist to the scrapie agent inoculated *in utero* or early in neonatal life, as is the case in lymphocytic choriomeningitis in mice with subsequent persistent infection[5].

The apparent genetic or congenital transmission of scrapie in nature in sheep, the marked differences in breed susceptibility, the remarkable stability of the scrapie agent, especially its resistance to boiling, and slowly progressive character of the essentially non-inflammatory disease in the central nervous system, and some clinical, epidemiological and neuropathological similarity between scrapie in sheep[3] and kuru disease in man in New Guinea[6,7] account for the intense interest of scrapie in medicine, virology, genetics and veterinary medicine. This chronic, progressive, degenerative disease of the central nervous system in mice inoculated with scrapie sheep brain (or scrapie goat brain), which resembles naturally occurring scrapie closely in both clinical aspects and neurohistopathology, offers a challenging experimental model for the study of the etiological agent and for the pathogenesis of slow infections of the central nervous system.

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ROLE OF PHOSPHATIDES IN NON-ENZYMATIC BROWNING

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In investigations into the browning reactions taking place in dried foodstuffs, it has become clear that more than one type of reaction is involved. At moisture-levels above 5 per cent sugar/amino reactions probably predominate in most cases, and the pathways involved have been dealt with elsewhere[1-5].

When the products are very dry it appears that oxidative effects due to splitting of unsaturated fatty chains may occur, probably by free radical reactions.

Vegetables contain various quantities of phosphatides, but little seems to have been reported on them or their effects on browning reactions[6].

Channon and Chibnall[7] found that cabbage contained about 0·14 per cent 'phosphatidic acid' (converted to a dry-weight basis), but they used an extraction process whereby phosphatides in physical association with carbohydrates or proteins were not extracted. Rewald[8] stated that potatoes contained up to 0·17 per cent phosphatides, calculated on the dry matter. Hanahan and Chaikoff[9], in their examination of dehydrated carrot, found that on dehydration the unsaturated fatty acid figure was reduced, but in their extraction of phosphatides from cabbage, they found a phosphatide content of about 1·5 per cent, corrected to a dry-matter basis, and, moreover, that the phosphatidic acid isolated earlier by Channon and Chibnall was an artefact produced from the phosphatides. Phosphatides can occur closely bound to both sugars and proteinaceous compounds, and should the fatty chains split on oxidative attack at a double bond, the resultant carbonyl compounds will react with any amino-groups present in the system, and join in a polymer-forming reaction of the type already described elsewhere[3,4,10,11]. Much of the browning occurring in phosphatide systems is probably, therefore, of the carbonyl/amino type (cf. Lea on unsaturated fatty compound browning[12]), which is perhaps the reason why sulphur dioxide and sulphites were found, by us, to be good retarding agents for the browning of phosphatide systems. The unsaturated fatty acids will undergo browning reactions when incubated with aqueous glycine; for example, linoleic and linolenic acids gave chromophores on incubation at 40° C which were more soluble in the fatty acid than in the aqueous phase, whereas the saturated caprylic acid underwent no colour change whatsoever. The presence of potassium metabisulphite retarded colour development in the unsaturated fatty acid systems.

In our experimental work on phosphatide browning the reactions were carried out in aqueous dispersion. Lecithin was used as the phosphatide after isolation in crystalline form, from egg-yolk, as described by Saunders[13]. After recrystallization from methyl ethyl ketone the lecithin was measured volumetrically in solution in this solvent, the solvent being blown off rapidly by a stream of air passed over a known volume of the solution in the reaction bottle.

The browning system consisted of lecithin, either alone or together with glycine, aliquots of each being treated separately in separate vessels with one of a range of additives respectively. These additives had previously been found to be, or were suspected by us as being accelerators or inhibitors of chromophore development in browning systems. They were added to the lecithin dispersions to give a concentration of 80 mg lecithin per 5 ml. of water and 800 mg glycine was added to the appropriate mixtures where the effect of glycine was to be examined. Potassium metabisulphite was added in 10-mg and 50-mg quantities, as were ascorbic acid, potassium pyrophosphate and ferrous sulphate. Calcium chloride (100 mg), aneurine (5 mg), ascorbic acid (100 mg) and sodium acetate (5, 20, and 50 mg) were also tested. In further experiments glycine was replaced by its ethyl ester, and by phenyl glycine, which was largely insoluble in the solutions. The reaction vessels were tightly stoppered and incubated at 40° C.

The rate of colour development was estimated visually, mainly because of the non-homogeneity of the system, and since both we and Hodge[14] support the view that the eye can be a more sensitive evaluator of the browning than the colorimeter in such systems, where the hues obtained are often different and the degree of dispersion may vary.

Using the same additives, there were marked differences in chromophore development to that occurring in the corresponding glucose/glycine system, some of which appear to be indicative of mechanisms operating in browning reactions. In the presence of added phosphate, the rate of colour development in sugar/amino systems increased, whereas a slight reverse effect was observed in the phosphatide/amino system. The accelerating effect noted in the sugar system[10,15] paralleled the higher polarographic readings obtained in the presence of phosphate by Cantor[16] and also the subsequent faster development of conjugated unsaturation[17], and thus seems to be a reflexion of the initial sugar stability in the solution.

In this respect it is noted that Pomeranz *et al.* have found[18], by an empirical method, the same order of browning for various sugars as we had already found and predicted[10] on theoretical grounds. The presence of calcium chloride had little or no effect on the phosphatide/amino system whereas it had a retarding effect on sugar/amino browning[10]. This again indicates that the main effect of calcium chloride is connected with its reaction with the sugar component. Simon[19] has shown that calcium chloride retards the browning of certain dehydrated vegetables, but no indication has so far been given as to how the calcium compound achieved this result. Calcium chloride may form complexes (believed to be in certain ring forms[20]; or presumably conformations) with the sugars, but it may also combine with diketones, amino compounds, pyrrole derivatives and phosphates.

These differences between the effect of phosphates and calcium chloride on the two systems in aqueous solution appear, therefore, to be important, since they suggest that the main effects of calcium and of phosphate are more closely connected with the changes—probably rate-determining—occurring in the sugar in the early stages prior to chromophore development[16], than with the potentialities in reactions occurring later in the browning sequence. If calcium is present in the early stages as a sugar complex, it will most certainly affect the later rate of dehydration of the sugar residue. Further evidence for believing that the effect of calcium chloride is mainly connected with sugar structure is derived from our investigations on its effect on straight carbonyl/amino systems, which parallels the results described herein on the phosphatide/amino reaction, but which is dissimilar to the effects exerted on aldose/amino systems.

The addition of sulphites or sulphur dioxide retarded the phosphatide/amino reaction, the degree of retardation being greater the larger the quantity of sulphite used. This was expected, since it probably acts in the manner discussed by Burton, McWeeny and Biltcliffe[2-4] in the carbonyl/amino system formed, the sulphite protecting the carbonyl group and reacting with centres of unsaturation.

Experiments in which lecithin was incubated at 40° C in glucose and in glycine separately showed quite clearly that the colour development was much more rapid in the presence of glycine. Lecithin and glucose, without additive, gave no colour after 3 months at 40° C, but in the presence of glycine the initial chromophores appeared within five days and even in presence of 10 mg of sodium metabisulphite the initial chromophore developed in 20 days, and had become a deep orange shade in 30 days. The importance of the nitrogen function for the rapid development of browning in carbonyl systems has been emphasized previously[2,4,10,11]. In the glucose/glycine 2·0 M w.r.t. glucose and 0·5 M w.r.t. glycine the degree of browning was greater with rising lecithin content over the concentrations studied, that is, 10–100 mg lecithin/7·5 ml. glucose/glycine.

The addition of ascorbic acid caused more browning to develop; in the absence of glycine, however, the effect of the ascorbic acid was much less than in its presence, demonstrating quite clearly that ascorbic acid browns quite rapidly with glycine, and fitting in with our previous findings that the browning of ascorbic systems is rapid in the presence of a nitrogen function. Ferrous sulphate caused an even more rapid browning reaction in the lecithin/glycine system, an insoluble brown pigment forming within a few days. Esterification of glycine led to a reduced rate of browning, while phenylglycine reacted in heterogeneous mixture with lecithin to give a dark solid which eventually became a black tar; metabisulphite retarded this reaction for a very long time, which may be explained as a normal reaction of sulphite at carbonyl and unsaturated centres, coupled with the lower availability of the amino compound for reaction in the solid state. Attempts to retard browning by pretreatment of lecithin with sulphur dioxide led to unknown products which slightly decreased the time before colour developed.

We are now aware that sulphite can very easily become attached at sites of unsaturation; Dodge[21] thought that the hydrolysability of the sulphonates depended on the location of the double bond, and we have been able to show that sulphite, added to a system containing unsaturated compounds, readily reacts at the unsaturated centre, and may become organically bound in a stable manner[11,22,23]. This appears to be the explanation for the otherwise unaccountable loss of sulphite after addition to foodstuffs when assayed by the Monier-Williams procedure, since it is not converted to sulphate[2,10,11].

It is of interest to mention that an unaccountable loss of sulphur dioxide (about 30 per cent) occurred during sulphitation and drying of cabbage at the Low Temperature Research Station, Cambridge. We have checked this experiment using sulphur-35 as a tracer, and have found that there was no loss of volatile sulphur-35 compounds, despite the low Monier-Williams recovery figures. In view of Hanahan and Chaikoff's results, it might well be that the added sulphite had combined with unsaturated lipids, known from their work to be present in cabbage, to a much greater extent than in other vegetables dehydrated at the time. There seems little doubt that the fatty acids and chains become substituted at unsaturated centres, and more sulphite is needed to prevent cabbage from browning than is needed, for example, for potatoes.

In experiments not reported here we found that sulphur-35 metabisulphite, added to other vegetables during scalding (blanching), appeared in part in the lipid fraction very soon after scalding, and in model systems not only become attached to the unsaturated compounds, but also retarded their browning with glycine.

It would seem that the browning reactions of phosphatides and presumably of unsaturated fatty acids are largely due to the production of chromophores following a carbonyl/amino reaction, and that its inhibition by sulphite is due to interference by the sulphite in this reaction to prevent chromophore development[2,4,11].

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EFFECT OF 2:4-DINITROPHENOL ON CARBON DIOXIDE FIXATION BY A *THIOBACILLUS*

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THIOBACILLUS rapidly oxidizes thiosulphate and other sulphur compounds to sulphate, the oxidation being coupled to carbon dioxide fixation[1]. Carbon dioxide fixation is mainly by reactions similar to those of the Calvin cycle in photosynthetic cells[2-4], and presumably requires a supply of reducing power, such as reduced pyridine nucleotide, and adenosine triphosphate[5]. It has been suggested that thiosulphate is first oxidized to tetrathionate and that other polythionates are formed as intermediates in the oxidation to sulphate[6–8]; adenosine triphosphate might then be formed by coupled oxidative phosphorylation. Peck[9], however, has recently proposed that the first step in thiosulphate oxidation is the reductive cleavage of thiosulphate to sulphide and sulphite. Sulphite combines with adenosine monophosphate and is finally oxidized to sulphate, the oxidation being accompanied by a substrate phosphorylation, which is insensitive to 2:4-dinitrophenol. If these reactions occur in intact cells, the sulphide formed from thiosulphate must also be oxidized to sulphate by an unknown pathway. Since the substrate phosphorylation studied by Peck is insensitive to dinitrophenol, while any oxidative phosphorylation might be expected to be sensitive to this inhibitor, it was of interest to investigate the effect of 2:4-dinitrophenol on carbon dioxide fixation by a *Thiobacillus*.

The organism used was isolated from canal water and most closely resembles *Thiobacillus thioparus*[1]. Cultures were grown in a liquid medium containing 1 per cent thiosulphate and aerated for 12 h after thiosulphate exhaustion. The cells were collected and suspended in 0·1 M phosphate buffer, pH 7·0 or 7·2, to give either a thin suspension (about 0·2 mg dry wt./ml.) or a thick suspension (about 2·5 mg dry wt./ml.). Experiments were carried out in Warburg flasks at 30° C in the presence of ^{14}C-bicarbonate and were started by tipping known quantities of thiosulphate or sulphide. Experiments were terminated when oxidation ceased and an aliquot of cell suspension from each flask was pipetted into an equal volume of ethanol containing 5 per cent (v/v) acetic acid. Carbon-14 fixation was measured by drying portions of this mixture to give infinitesimally thin samples for counting. At the end of some experiments aliquots of cell suspension were taken for sulphate determination[10].

No carbon dioxide is fixed unless thiosulphate is added and fixation ceases when the thiosulphate has been completely oxidized (Fig. 1). Carbon dioxide fixation is inhibited by low concentrations of 2:4-dinitrophenol which either do not affect or stimulate rate of oxygen uptake (Fig. 2).

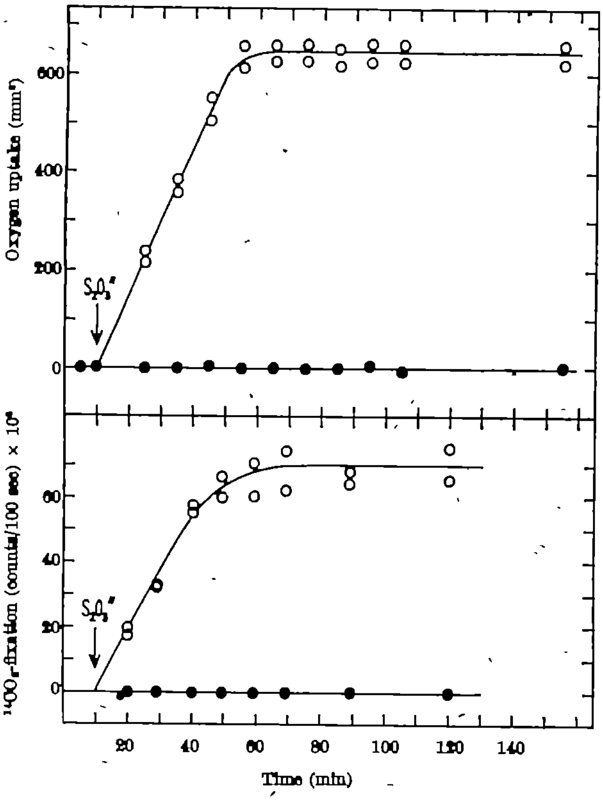

Fig. 1. Coupling of $^{14}CO_2$-fixation to thiosulphate oxidation by a thin cell suspension. Upper graph: oxygen uptake: lower graph: $^{14}CO_2$-fixation. Controls (black circles) received no thiosulphate. Theoretical oxygen uptake for complete oxidation = 672 mm³

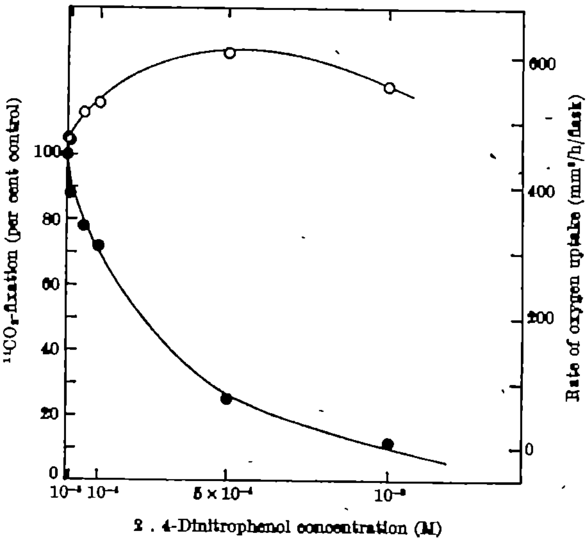

Fig. 2. The effect of 2:4-dinitrophenol on the rate of oxygen uptake and $^{14}CO_2$-fixation by thin cell suspensions oxidizing thiosulphate. Open circles, oxygen uptake; black circles, $^{14}CO_2$-fixation

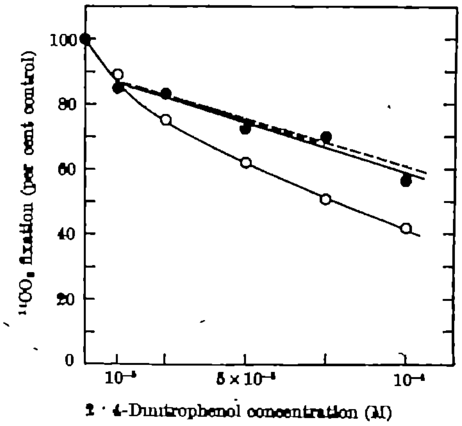

Fig. 3. The effect of 2:4-dinitrophenol on $^{14}CO_2$-fixation coupled to sulphide (open circles) or thiosulphate (black circles) oxidation by thick cell suspensions. 4·0 μmoles of each substrate were oxidized. The broken line shows the calculated $^{14}CO_2$-fixation coupled to thiosulphate oxidation

Sulphide is also rapidly oxidized provided that not too much is added and that thick cell suspensions are used. This oxidation can also be coupled to carbon dioxide fixation but the fixation differs in two respects from that coupled to thiosulphate oxidation.

First, less carbon dioxide is fixed per molecule of substrate oxidized when sulphide is the substrate instead of thiosulphate. In experiments in which care was taken to add equimolar quantities of sulphide and thiosulphate and complete oxidation was checked by sulphate analyses at the end of the experiment, carbon dioxide fixation with sulphide as energy source was 62·5 ± 4·9 per cent of that with thiosulphate as substrate. Yet both a molecule of sulphide and one of thiosulphate require two molecules of oxygen for complete oxidation to sulphate. Secondly, carbon dioxide fixation linked to sulphide oxidation is more sensitive to inhibition by 2:4-dinitrophenol (Fig. 3 and Table 1). For example, 10⁻⁴ M dinitrophenol gives 60 per cent inhibition of carbon dioxide fixation with sulphide as substrate, but only 40 per cent inhibition with

Table 1. EFFECT OF 2:4-DINITROPHENOL ON $^{14}CO_2$-FIXATION COUPLED TO THE COMPLETE OXIDATION OF SULPHIDE OR THIOSULPHATE BY THICK CELL SUSPENSIONS OF THIOBACILLUS

The percentage inhibition, compared with controls without dinitrophenol, is given. 4·0 μmoles of each substrate were oxidized.

	Exp. 1		Exp. 2		Exp. 3	
Substrate / DNP Concentration	S″	S_2O_3''	S″	S_2O_3''	S″	S_2O_3''
0	0	0	0	0	0	0
5 × 10^{-5} M	38	—	—	—	—	—
2·5 × 10^{-4} M	95	71	72	61	82	69
5 × 10^{-4} M	—	—	90	80	94	88

Sulphate analysis at the end of experiment 2 gave 113 per cent recovery of sulphur from both sulphide and thiosulphate. At the end of experiment 3, recovery was 107 per cent.

thiosulphate. The difference is consistent and extends over a range of dinitrophenol concentrations.

The inhibition of carbon dioxide fixation by low concentrations of dinitrophenol, which do not inhibit the oxidation of sulphur compounds, suggests that oxidative phosphorylation is involved in the coupling between carbon dioxide fixation and substrate oxidation. If, as appears not unreasonable, carbon dioxide fixation can be used as an index of high-energy phosphate production, oxidative phosphorylations, inhibited by dinitrophenol, may be relatively more important in the oxidation of sulphide than of thiosulphate.

If Peck's scheme for thiosulphate oxidation is correct[9], our finding that carbon dioxide fixation during sulphide oxidation is 63 per cent of that with an equimolar amount of thiosulphate as substrate, suggests that two-thirds of the phosphorylation coupled to thiosulphate oxidation occurs during the oxidation of sulphide to sulphate and one-third during the oxidation of sulphite. Moreover, if we assume that all the phosphorylations coupled to sulphide oxidation are sensitive to dinitrophenol, and that therefore only two-thirds of those coupled to thiosulphate oxidation are similarly sensitive, we can calculate the carbon dioxide fixation expected by cells oxidizing thiosulphate in the presence of dinitrophenol; the calculated values are not dissimilar from those observed (Fig. 3, broken line).

Thus our results suggest that both oxidative and substrate-phosphorylations are coupled to the oxidation of thiosulphate by *Thiobacillus*.

We thank the Department of Scientific and Industrial Research for a grant to one of us (D. P. K.).

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A THERMOSTABLE HÆMOLYTIC FACTOR IN SOYBEANS

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IT is a well-known fact that raw soybean oil meal is not utilized efficiently by the living organism. The nutritional value of the meal can be raised by adequate heat processing, as soybeans contain growth-depressing materials which are destroyed by heat. Among these alleged anti-nutritional factors are the saponins, which are generally characterized by their bitter taste and foam-forming and hæmolytic activities[1]. The aim of this work was to examine the effect of heat processing, under conditions found as most favourable for elevating the nutritional value of the meal for chicks and rats, on the hæmolytic activity of soybean saponin extract. Since soybean saponin extract partly inhibits the proteolytic activity *in vitro* of *Tribolium castaneum* larval midgut enzyme solution and also trypsin and α-chymotrypsin[2], it was of interest to examine the effect of autoclaving on these inhibitory activities as well.

Soybean saponin extract was prepared from ether-extracted soybean flour, Lincoln variety, by a combination of two methods[3,4] as follows: 200 g soybean flour were suspended in 2 l. ethanol 80 per cent, stirred for 12 h at 50–60° C and the residue was re-extracted after filtration with another 2 l. ethanol 80 per cent. The ethanol was removed from the combined filtrates by distillation in vacuum to 400 ml. Accompanying materials and pigments were removed by extracting the solution with 200 ml. portions of ethyl ether until the latter was colourless. To the remaining aqueous solution 20 g sodium chloride and hydrochloric acid to pH 4·0–5·0 were added and the solution was then shaken with 300 ml. and 150 ml. butanol, successively. The combined butanol extracts were washed twice with 50 ml. sodium chloride 5 per cent. The washed butanol phase was concentrated to dryness yielding 4 g crude material. 1 g of the dried preparation was suspended in 100 ml. water and treated with 1 g calcium monoxide. The precipitate formed was separated by filtration, suspended in 50 ml. hot methanol 80 per cent and neutralized with hydrochloric acid. The resulting turbid solution was filtered and the filtrate concentrated to 0·75 of its volume. 50 ml. water were then added and the soybean saponin that precipitated was collected by filtration, washed with acetone and dried. The same procedure was applied for preparation of soybean saponin extract from autoclaved soybean meal. Heat processing of soybean meal and of soybean saponin extract was performed by autoclaving at 15 lb. pressure for 20 min. The following designations were used for the various soybean saponin extracts: $SBSE_1$, extract from raw soybean meal; $SBSE_2$, extract prepared from autoclaved soybean meal; $SBSE_3$, autoclaved $SBSE_1$. The nitrogen content of each preparation was about 0·18 per cent. All three preparations could form foam easily when shaken in an aqueous solution.

The hæmolytic activity of soybean saponin extract was determined by Kofler's method[5] as modified by O'Dell *et al.*[6]. 1 ml. solution of soybean saponin extract in 0·3 M phosphate buffer pH 7·4 was mixed in a test tube with 1 ml. suspension of previously washed ram red blood cells. The mixed suspension was allowed to stand at room temperature for 20 h. Full hæmolysis was defined, according to Büchi *et al.*[7], as absence of precipitate at the bottom of the test tube, and the hæmolytic index, as weight of reaction mixture divided by weight of soybean saponin extract assayed.

The inhibitory effect of soybean saponin extract on the proteolytic activity of *Tribolium castaneum* larval midgut enzyme solution and on α-chymotrypsin (crystallized 3 times, salt-free, Worthington Biochemical Corp.) has been examined by the casein digestion method at 280 mμ[8]. Larval midgut enzyme solution was prepared according to Birk and Applebaum[9].

Paper-chromatographic separation of soybean saponin extract was performed on Whatman No. 3 *MM* filter paper. The paper was washed, prior to chromatography, with solvent—butanol/ethanol/water (6 : 2 : 3) and dried in the hood. Soybean saponin extract solutions in 80 per cent ethanol were then applied and chromatographed (ascending direction) in the same solvent. The chromatograms were dried in the hood at room temperature and then at 100° C for 30 min. The spots were detected by dipping the paper in the Carr–Price reagent (saturated solution of antimony trichloride in chloroform) and heating for 5 min at 100° C. Corresponding spots were cut out from the duplicate chromatograms, which had not been dipped in the reagent, and eluted with warm 75 per cent ethanol. The ethanol was removed by distillation and the residues were examined on hæmolytic activity and on inhibition of larval midgut enzyme solution and α-chymotrypsin.

The comparative detrimental activities of $SBSE_1$, $SBSE_2$ and $SBSE_3$ are shown in Fig. 1. It appears from this figure that $SBSE_2$ (prepared from autoclaved soybean meal) exhibits even higher hæmolytic activity than $SBSE_1$. However, autoclaving of $SBSE_1$ resulted in a considerable decrease in hæmolytic activity as can be seen from the value of $SBSE_3$. It seems that the hæmolytic activity of soybean meal is protected, when *in situ*, against the possible diminution caused by heat processing. No considerable difference has been found between the three soybean saponin extracts concerning their inhibitory effect on the proteolytic activity of *Tribolium castaneum* larval midgut enzyme solution and α-chymotrypsin.

The paper chromatographic behaviour of $SBSE_1$, as compared with that of $SBSE_2$ and $SBSE_3$, is shown in Fig. 2.

Spots *B*, *C*, *D* of each soybean saponin extract preparation possess foam-forming activity. It may be concluded from Fig. 2 that heat processing does not affect the chromatographic behaviour of soybean saponin extract. All three preparations can be separated, each, into four well-defined spots with similar R_F values. Spots *A* from the various origins have no hæmolytic activity. All the other three spots of each preparation are hæmolytic. The hæmolytic index of the spots designated *D* is about 2,000 and of those designated *C* about 400. Component *B*, however, seems to be affected by heat processing, as the hæmolytic index of *B* from $SBSE_1$ is more than 10,000, the hæmolytic index of *B* from $SBSE_2$ is even higher, and that of *B* from $SBSE_3$ is about 5,000. These results indicate that the sensitivity of component *B*, when $SBSE_1$ is autoclaved, accounts for the lower hæmolytic activity of $SBSE_3$ (Fig. 1). Spots *B*, *C* and *D* of all three soybean saponin extract preparations exhibit considerable inhibition of the proteolytic activity of *Tribolium* larval enzymes and chymotrypsin.

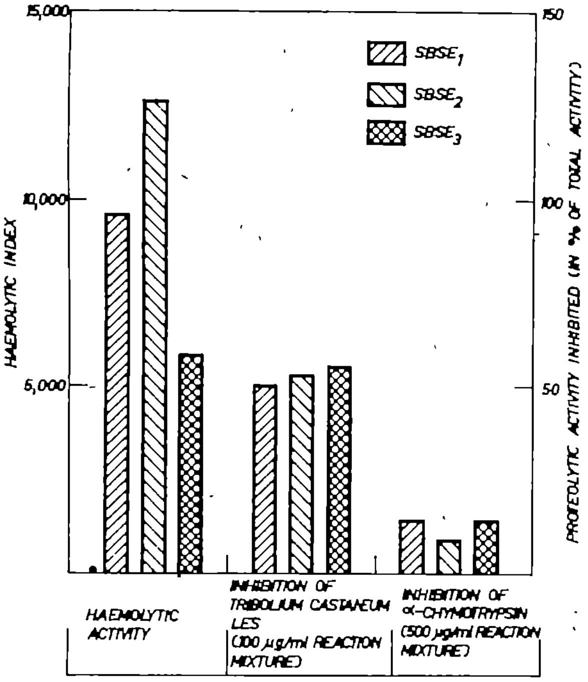

Fig. 1. Comparative hæmolytic and proteolysis-inhibiting activities of raw and autoclaved soybean saponin extracts

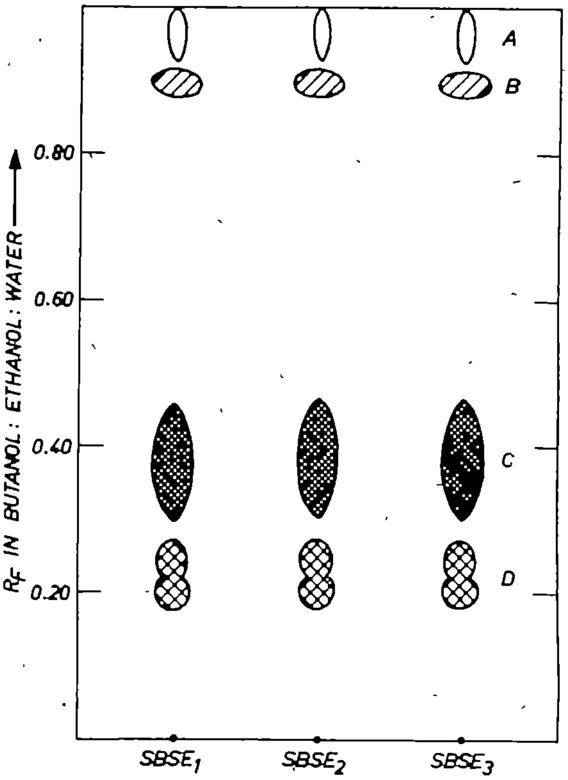

Fig. 2. Comparative chromatographic behaviour of $SBSE_1$, $SBSE_2$ and $SBSE_3$. The colours of the spots are as follows. *A*, brown; *B*, brown; *C*, purple; *D*, purple. The distribution of chromatographed soybean saponin extract per spot is: White, ~5 per cent, single hatching, ~10 per cent; double hatching, ~30 per cent; deep hatching, ~55 per cent

Summarizing its hæmolytic activity, it appears safe to claim that soybean saponin extract is highly hæmolytic (hæmolytic index ~ 10,000) as compared with saponins from other sources[10]. This activity is not affected at all when soybean meal is autoclaved under optimal conditions and may still be classified as very high in $SBSE_2$.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Deficiency of Metals in Population II Stars

I PROPOSE the following as an explanation for the deficiency of heavy elements in Population II stars.

Consider the ionization potentials of the elements. A brief list is given in Table 1 for representative elements. If we assume that there is a certain amount of ionization of interstellar matter, it is clear that the heavy elements, with rare exceptions, will have a considerably higher probability of being ionized than will the light elements. It is commonly believed that the galaxy possesses a magnetic field of 10^{-5}–10^{-6} gauss[1]. Therefore, elements which are ionized are effectively contained in the galactic disk, where the field exists, while neutral particles have relatively little difficulty in escaping from the surface of the galactic disk towards the halo regions. This escape is particularly easy for hydrogen and helium atoms in the high velocity tail of the Maxwell velocity distribution; gravitational attraction somewhat aids the magnetic field in preferentially retaining heavier elements. Moreover, the elements which are retained need not be ionized for all time—should they become ionized at any instant of time, the magnetic field will halt any outward motion they might have. Consequently, it is reasonable that interstellar matter should be increasingly depleted in the metals as the distance from the disk increases, and stars formed in the halo region must be deficient in the metals. This, in fact, is what is observed since the metal-poor Population II stars[2] show a deficiency in the metals which increases with galactic latitude[3]. There is a similar dependence on the space velocity of stars[3].

This interpretation removes a major difficulty which attends explanations depending on nucleogenetic developments, for those explanations require a steady growth with time in the proportion of helium and heavy elements in the galaxy[3,4–7]. In fact, old Population I stars, such as those of *M* 67 (refs. 8–10), and very young stars, have essentially identical metal contents[11–13]. In addition, there is some evidence that Population II stars have a normal abundance of helium[13] which is incompatible with the nucleogenesis theory of stellar chemistry. I argue that the paucity of metals in Population II stars is not evidence that the galactic abundances have changed with time.

The present model suggests that neutral gas escaped from the galactic disk, but this admittedly presents a serious problem of how that (low-density) gas could condense into stars. A more reasonable picture is that of Eggen *et al.*[3], who discuss formation of the Population II stars in terms of matter left behind as the nascent galaxy collapsed. I suggest that the collapsing gas contained a magnetic field which helped drag ionized matter, that is, the metals, into what is now the disk. In this case, the metals were left behind whenever they became neutral, so that some metals were present in the matter which formed the Population II stars. Ionization became increasingly important as stars formed because of the hot radiation from them. Thus all elements with small ionization potentials have remained trapped in the galactic disk since the earliest days of star formation, and the distribution of the metals with height above the galactic plane is readily understood.

I thank Prof. G. P. Kuiper and Miss B. M. Middlehurst for stimulating discussions of this topic.

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PHYSICS

Mean Life and Mass of Elementary Particles

THE weak interaction constant G may determine the mean life of some elementary particles. If the muon mean life of $2{\cdot}212 \times 10^{-6}$ sec is equated to $(1/2\ \pi a)\ (\lambda_e^2\hbar/G)$ then G equals $1{\cdot}48 \times 10^{-49}$ erg cm^3 (a is the fine structure constant and λ_e is the Compton wave-length of the electron). The charged pion mean life equals $1/4\ (\lambda_e^2\hbar/G)$ or k while the Σ^- hyperon mean life equals $a\,k$. Three types of particles may exist corresponding to the presence of a with exponents of -1, 0, or $+1$ in a function giving the mean life of the particle.

The weak interaction constants may be similar to the Rydberg constants ($R_\infty = a^2/2\ \lambda_e$) in that they depend on a centre of mass factor. However, for present purposes this can be neglected.

Table 1 lists the observed mean life of some elementary particles together with the mean life calculated from the multiple of k listed.

Table 1. PARTICLE MEAN LIFE (SEC)

Particle	Observed*	Calculated	Interaction factor
π^+	$2{\cdot}55 \times 10^{-8}$	$2{\cdot}54 \times 10^{-8}$	k
K_2^0	$6{\cdot}1 \times 10^{-8}$	$5{\cdot}1 \times 10^{-8}$	$2\,k$
K_1^0	1×10^{-10}	$0{\cdot}9 \times 10^{-10}$	$a\,k/2$
Σ^-	$1{\cdot}6 \times 10^{-10}$	$1{\cdot}85 \times 10^{-10}$	$a\,k$

* *Handbook of Chemistry and Physics* (1962).

Particle mean life and particle structure should be related. If dimensional units are defined so that the mass of the electron equals 2π then the components of particles can be represented by rest masses of $\pi/2a$, $1/2\ a$ and 2π (refs. 1 and 2). The neutral kaon with two different lifetimes may consist of two different arrangements of these units. For example:

$$K_1^0 = 4(6\pi/2a + 1/2a) + 4\pi/2a + 1/2a = 4 \text{ muons} + 4\pi/2a + 1/2a$$

$$K_2^0 = 4(8\pi/2a) - [4(\pi/2a - 2\pi) - 1/2a] = 974 \text{ electron masses}$$

Table 1. FIRST IONIZATION POTENTIALS (I.P.) (eV) FOR VARIOUS ELEMENTS

Element	H	He	N	O	F	Ne	Si	Cr	Mn	Ga	Sr	I	Ba	Eu	Hg
I.P.	13·6	24·6	14·5	13·6	17·4	21·6	8·1	6·8	7·4	6·0	5·7	10·5	5·2	5·7	10·4

The pions and the charged kaon may be similar to K_2° in structure (ref. 2).

The hyperons may have structures similar to the K_1° particle because of the similarity in mean life. For example:

$$\text{neutron} + \text{muon} + 4\pi/2a = \Lambda = 2{,}182{\cdot}5 \text{ electron masses}$$

$$\text{neutron} + \text{muon} + 2(4\pi/2a) + 1/2a = \Sigma^{\circ} = 2{,}330{\cdot}4 \text{ electron masses}$$

$$\text{neutron} + \text{muon} + 2(4\pi/2a + 1/2a) = \Sigma^{-} = 2{,}341{\cdot}3 \text{ electron masses}$$

$$\text{proton} + \text{muon} + 2(4\pi/2a) + 1/2a = \Sigma^{+} = 2{,}327{\cdot}9 \text{ electron masses}$$

The resonances can also be represented as combinations. For example:

$$\text{proton} + 3(4\pi/2a + 1/2a) + 4\pi/2a = 1{,}235 \text{ MeV} = N^{*}$$

$$\text{proton} + 4(4\pi/2a + 1/2a) + 2(4\pi/2a) = 1{,}383 \text{ MeV} = Y^{*}$$

$$2\pi^{\circ} + 4(4\pi/2a) = 550 \text{ MeV} = \eta^{\circ}$$

$$2\pi^{\circ} + \pi^{+} + \pi^{-} + \text{mu}^{+} + \text{mu}^{-} = 760 \text{ MeV} = \rho^{\circ}$$

The pion resonance does not involve charge formation. Eliminating a charge gives:

$$3(4\pi/2a - 1/2a) = 193 \text{ MeV}$$

The proton may consist of two resonant states represented by an average mass of:

$$4[2((4\pi/2a) - 1/2a) + \text{muon}] = 938{\cdot}2 \text{ MeV} = \text{proton}$$

Apparently a charge with rest mass of 2π can acquire a rest mass of $4\pi/2a$ or $1/2a$. Combinations of these units form the resonances and the elementary particles.

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Seasonal Variations of Cæsium-137 in Air at Ground-level during 1960 and 1961

MEASUREMENTS on radioactive fall-out in air and rainwater in different parts of the world from 1954 onwards showed that during late winter and spring an increase in specific activity was observed. This increase was attributed by Stewart[1] and Machta[2] to seasonal effects while it was attributed by Martell[3] mainly to the testing schedule of polar explosions. The presence of 'spring peak' was observed in India and other countries from the measurements made in the spring of 1960 as there were no tests between November 4, 1958, and February 13, 1960.

The spring increase in the concentration of cæsium-137 in the air at ground-level recorded at Bombay during 1957–60 has already been published[4]. The peaks in cæsium-137 activity were observed at Bombay during the period February–May in the years 1957–60. In ref. 4 the peaks in cæsium-137 activity observed at Srinagar, Delhi, Nagpur, Calcutta, Bangalore and Ootacamund during 1960 were also reported. As two French tests were conducted in February and April 1960, respectively, the spring increase observed in 1960 had to be confirmed by similar measurements in 1961.

Since the level of activity in the air during 1961 was very small, samples from 4 northern stations (Srinagar, Delhi, Gangtok and Calcutta) and 4 southern stations (Nagpur, Bombay, Bangalore and Ootacamund) were pooled to determine whether the seasonal effects could be observed during 1961. Fig. 1 gives the levels of cæsium-137 in the ground-level air for the northern stations, the southern stations and the average levels for all the stations. It can be seen from Fig. 1 that in the month of September 1960 the levels of cæsium-137 in the air had reduced to less than 10^{-15} c./m³ air. After September 1960 an increase is observed. In the northern stations the peak is observed in the month of April 1961 and in the southern stations two peaks are observed in January and May 1961 respectively. It is significant that the peak-levels of activity during 1960 and 1961 are nearly the same in the southern stations and the 1961 peak-levels in the northern stations are even higher than the 1960-levels.

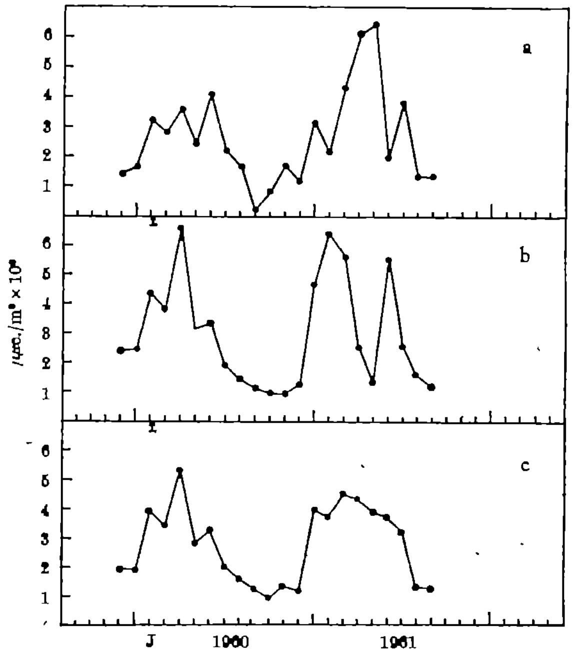

Fig. 1. Cæsium-137 concentrations in the air at ground-level. *a*, Northern stations average, *b*, southern stations average; *c*, average for all stations

The mechanism of spring increase is not fully understood and different explanations have been offered. Lindblom[5] has observed spring peaks at Stockholm and Edsvalla in Sweden during 1961 and the peak levels at these two stations during 1961 were as high as the levels during 1960. Kuroda[6] has also observed an increase in strontium-90 concentration in spring rains during 1961, the magnitude of which is comparable with that in 1960. He has attributed this to the descent of radioactive fall-out from upper stratosphere to the lower stratosphere.

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Production of Liquid Drops

DURING an investigation into methods of producing small, uniform-sized drops the following observations were made on the mechanism of drop formation when influenced by the application of high voltage.

Drops of water were produced from a vertical stainless-steel, hypodermic needle, 0·27 mm bore and 0·48 mm outside diameter. The tip of the needle was ground flat and thoroughly cleaned before use. Water pressure was provided by a constant liquid head.

In the case where gravitational forces alone applied drops grew to a diameter of about 4 mm before becoming detached from the tip, this occurring once every 3–4 sec. On the application of smoothed, positive, high voltage (about 4 kV) there was a marked change in the mechanism of drop production. In ordinary light water appeared to leave the tip of the needle in a continuous, narrow jet.

This effect was reported by Vonnegut and Neubauer[1] in a qualitative paper which did not attempt to describe the formation of the individual drops.

When such a jet is viewed in stroboscopic light it is apparent that it is composed of a series of small drops (about 300μ diameter) produced at a definite frequency. The frequency is sometimes caused to vary by factors such as change in the liquid properties and the condition of the atmosphere into which the drops are sprayed. However, the frequency can be maintained constant by making fine adjustments to the applied voltage.

Observation of the tip of the needle under a low-power microscope, with stroboscopic back lighting, indicates that the process is highly repetitive. This is evident from the sharpness and steadiness of the image when the stroboscope is synchronized with the object motion. Photographs 1–13 (Fig. 1) record the peculiar manner in which the drops are produced. These photographs are not all of one particular cycle but are a collection of stills taken at random over a period of 5 min and put into order after printing. They represent a sequence of events which recur at a rate of 300/sec.

The water behaves in a manner which would be expected of a liquid of very low surface tension, there being only a very short retention time at the tip before the drop is ejected. The shape of the liquid at the tip also shows a great departure from sphericity.

The process can be explained qualitatively by considering the opposing effect to surface tension of electrostatic charge at the surface.

In the early stages (1–2) the shape of the advancing liquid front is almost spherical but continual flow of the water and gravitational force on the accumulated liquid soon produce an elongation, creating a small radius of curvature at this front (3–5). Here a higher charge density at the high curvature reduces surface tension more than elsewhere on the surface. Consequently water flows through this weak area at an increasing rate (6–7), creating a sharp-nosed filament (8–9). As this lengthens the more usual process of jet break-up commences and 'necking' occurs (9–10). When the filament breaks away, the curvature of the liquid remaining on the needle is suddenly decreased (11) and the liquid withdraws for a brief period (12) until flow of liquid through the needle causes the process to start again (13–1).

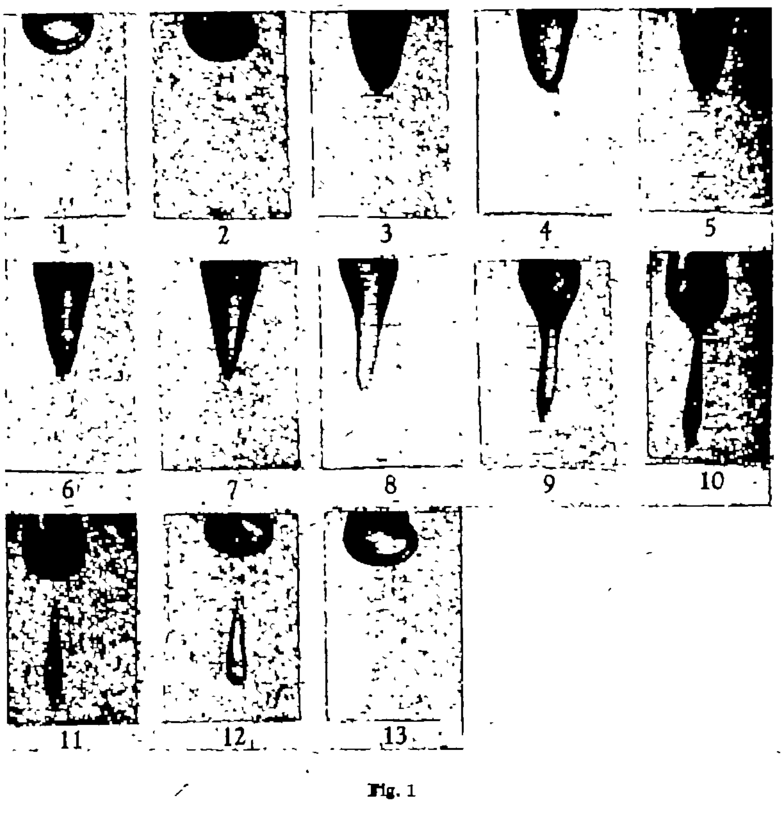

Fig. 1

Secondary drops which may be produced from the thin tail of the parent drop are repelled, by electrostatic forces, at an angle to the primary drop stream depending on the relative drop sizes. This angle is large enough to allow the secondary drops to be removed without affecting the primary stream. Thus, the system produces a continuous stream of uniform-sized drops of controllable frequency. The drop size may be varied by altering the flow rate.

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Occurrence of Maximum and Minimum in the Principal Magnetic Susceptibility versus Temperature Curves

IN an examination of the temperature variation of principal magnetic susceptibilities of paramagnetic salts of the iron group of elements from room temperature down to about 80° K, it has been found in certain cases that the square of the effective magnetic moment (p^2) value comes down so rapidly with the decrease of temperature that the corresponding susceptibility actually shows a maximum in the susceptibility versus temperature curve[1]. The condition for the occurrence of such a maximum can be easily investigated.

By definition, $p^2 = \frac{3k}{N\beta^2}\chi T$, where χ is the gram molecular susceptibility. Now $\frac{dp^2}{dT} = \frac{3k}{N\beta^2}(\chi + T\frac{d\chi}{dT})$. At the temperature corresponding to the maximum susceptibility $d\chi/dT$ should be zero. Thus when the slope of the p^2–T curve exceeds the value p^2/T, the thermal coefficient of susceptibility, namely, $d\chi/dT$, should be positive, whereas ordinarily it is negative. This criterion can be expressed geometrically in the following manner. Consider any point Q on the p^2–T curve. Then the temperature variation of susceptibility at Q will be positive if the slope of the curve exceeds the line joining Q and the origin ($T = 0$, $p^2 = 0$). This condition is satisfied for the lowest of the three principal magnetic moments in the hexahydrated cobalt selenate crystal, and the corresponding susceptibility shows a maximum at about 130° K, below which the susceptibility decreases with the lowering of temperature. Another crystal, namely, copper acetate monohydrate, also shows similar maximum in all three principal susceptibilities owing to the fact that the effective moments for the three directions come down rapidly with the fall of temperature. The p^2–T curves for all the three principal directions of this crystal have slopes higher than that given by the foregoing equation.

Besides having a maximum susceptibility in the susceptibility versus temperature curve described here, the curve can also exhibit a minimum under suitable conditions. Such a minimum has been observed, for example, in samarium salts at about 400° K. The ground-state of Sm^{+++} is $^6H_{5/2}$. The multiplet interval between the lowest components for samarium is not large, so that it is not only the level for which J is $5/2$ but the immediately higher level ($J = 7/2$) will also be occupied. Now the higher level corresponds to a considerably larger magnetic moment than the lower level. Starting from low temperatures, as the temperature is raised continually there will come a stage when the population in the upper level will increase rapidly. The average moment per ion will thus increase sufficiently quickly with the rise of temperature to overbalance the direct influence of temperature. If p_1^2 be the value characteristic of the lower level and p_2^2 of the higher level, then as the temperature is raised the values of p^2 will rise from p_1^2 to $\frac{1}{2}(p_1^2+p_2^2)$. Suppose that in the p^2–T curve at the point Q of inflexion the slope of the curve is greater than the critical value deduced here, then evidently there will be two points A and B where the slope will have the critical value. If T_A and T_B be the corresponding temperatures, then $d\chi/dT$ will be normal, that is, negative for temperature $T < T_A$ or $> T_B$, whereas for temperatures in the interval $T_A < T < T_B$ $d\chi/dT$ will be positive. The temperatures T_A and T_B will thus be the positions of the minimum and maximum of the curve. If p_1^2 is zero, that is, if the lower level is non-magnetic, the susceptibility will fall continually as we lower the temperature from T_A without exhibiting a minimum.

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GEOPHYSICS

Examination of Irregular Sub-surface Structures by Seismic Refraction Methods

THE great volume of modern refraction data has revealed the inadequacy of what was, until recently, the usual method of interpretation, that is, the process of fitting a series of straight lines to the travel-time observations, and of interpreting the resulting diagram in terms of a plane-stratified crustal model. Sometimes the artificiality of such models is revealed within the area of a single survey. On other occasions, discrepancies have appeared when structures postulated to explain data from neighbouring areas have failed to agree at the survey boundary.

One approach towards greater generality is that which has been discussed by Willmore, Scheidegger and Bancroft[1,2] from which the postulate of plane stratification is eliminated. Instead of this, it is assumed that there is a surface in the ground below which the travel-time is a function of distance only, and that one can identify the packet of energy which passes downwards from the source to this 'marker-layer', is refracted into it, and is then refracted again to the receiver. If the marker-layer is not assumed to be plane, one has to solve sets of equations of the form:

$$t_{ij} = a_i + a_j + f(\Delta_{ij}) \tag{1}$$

where the subscripts i and j refer to the source and received respectively, t_{ij} is the travel time, and Δ_{ij} is the distance between the feet of the perpendiculars drawn from the source and receiver to the marker-layer. It is shown that the constants a_i, a_j are simply related to the lengths of the corresponding perpendiculars, and that they are almost independent of azimuth. In a preliminary solution, we use the great circle distance between the ith source and the jth receiver as a first approximation to Δ_{ij}, and the solution yields a first approximation to the velocity in the marker-layer and to the contours of the interface. In successive approximations, corrections can be introduced to allow for the effects of quite steep dips in the marker-layer, and for curvature of the refracted rays. The equations can be solved for extended networks of sources and detectors, without requiring that the elements of the net should conform to any particular pattern.

In another approach, which has been discussed by Hagedoorn[3], Hawkins[4] and others, the sources and receivers are laid out in the form of a linear profile shot from both ends. The quantities a_i, a_j are derived, in this special case, by a very simple arithmetical procedure, and the configuration has the advantage that all the ray paths lie close to a single vertical plane. By subtracting a_i and a_j from the travel-time to each detector in turn one can follow the progress of the refracted wave front along a particular line in the surface of the refractor. It is assumed here that there is no significant average increase of velocity with depth, which would cause the wave to plunge deeply below the boundary. When this is done, any irregularity in the apparent progress of the wave front can be accounted for by postulating local variations in the velocity below the marker-layer. However, the completeness of this solution is obtained at the cost of losing control over the experimental uncertainties, because the number of constants fitted to the system has become equal to the number of observations available.

It now appears possible to combine the advantages of both systems by having a two-dimensional net, with a sufficient number of sources and receivers to give good statistical control over the constant terms, and deriving an average marker velocity in a first approximate solution. For any pair of detectors which are approximately in line with a given shot, one can derive an estimate of the local crustal velocity by forming the quantity:

$$\frac{\delta(t_{ij} - a_j)}{\delta(\Delta_{ij})} \tag{2}$$

Similarly, one can derive the local velocity below pairs of shot points as being equal to:

$$\frac{\delta(t_{ij} - a_i)}{\delta(\Delta_{ij})} \tag{3}$$

If these local estimates fit into a regional pattern, successive approximations to the structure can be built up.

All these methods depend on the possibility of recognizing onsets from a particular crustal stratum and this will not necessarily be easy in the general case. It is therefore very desirable to include small groups of fixed elements in the system, and so to plan the operation that observations from these groups of elements are obtained for waves which have travelled a number of significantly different distances. Special studies of the data from these small groups will determine the apparent velocity of any given phase, and whether this apparent velocity depends on distance. If this scheme is to be successful, adequate attention must be paid to the resolving power of the fixed-element array in terms of apparent velocity. This requires that conservative estimates of error in determining arrival times should be combined with information theory to yield the necessary array-length, sampling point interval and sampling density to avoid ambiguity. In this way, we may strengthen the method of distinguishing non-linear terms in $f(\Delta_{ij})$ from the effects of regional variations in velocity.

It is unnecessary to elaborate further at this stage, our object being mainly to emphasize the fact that the numerous special techniques which are now being developed all embody the same general principles. In particular, the theory indicates that all methods which yield a complete solution must satisfy the following conditions:

(*a*) Each recording site should be occupied for the observation of waves from at least two sources in different azimuths, the distances being such as to permit the observation of corresponding phases for all sources. Conversely, each source should be observed by a number of detectors.

(*b*) To eliminate an arbitrary constant which appears in the equations, one or more source points should be used as recording sites for waves from other sources.

The literature contains numerous descriptions of investigations in which structural conclusions are advanced, but in which the foregoing conditions have not been satisfied. In all such cases, assumptions such as plane layering, or zero average dip of the marker, have been introduced, even if they have not been recognized explicitly. We should further emphasize that, if the conditions cannot be satisfied in a single field operation, the possibility of combining data from past or projected operations should be borne in mind.

It would appear that something analogous to this approach can be applied to any set of data in which an observed quantity can be represented as the sum of a continuous function of another observed quantity and a number of constant terms. In particular, we might observe the surface waves spreading from a number of sources. At any one frequency, the phase should vary linearly with distance, and discrete phase-shifts may exist at each source and receiver. Each 'phase term' should be capable of further analysis into a part characteristic of the source mechanism or the detecting equipment, and a part characteristic of the crustal structure. The phase velocity extracted from the analysis should have the same significance as that derived in conventional phase-velocity studies, but should gain in accuracy from the localization of effects at the sources and receivers. The amplitude of a given phase should also be susceptible to study in the same way.

The three types of study which have been suggested are all applicable to the calibration of the permanent seismograph arrays which are now being used for the detailed investigation of seismic wave trains. On a larger scale, appropriate investigations should yield time, phase and amplitude corrections applicable to the conventional seismograph stations of the world network.

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GEOLOGY

A Potassium/Argon Age Determination from a Lewisian Inlier

THE potassium/argon age of pyroxene from an eclogite occurring within the Glenelg Lewisian inlier has been determined.

The eclogite from which the pyroxenes were extracted occurs on the north-east shore of Loch Duich, Ross-shire (Grid ref. —*NG*903236). Here the rock forms a rounded knob projecting from the beach above high-water mark. The relationships between the eclogite and the surrounding rocks are obscured by vegetation and beach material at this particular locality, but at other occurrences in the neighbourhood the eclogites form the cores of large-scale augen structures in garnet–biotite–gneiss.

The specimen of eclogite collected is a very striking bimineralic rock consisting of emerald green clinopyroxene (omphacite) and bright red garnets. The texture is equigranular with individual crystals about half a centimetre in diameter. This particular specimen was selected for dating because neither of the minerals shows any signs of alteration, unlike most specimens of eclogitic rocks collected in the same area which show retrograde metamorphic effects[1]. The rock was crushed and the pyroxenes extracted by the use of a Franz electro-magnetic separator. No heavy liquids were used in the separation.

Argon extraction and estimation were made by the standard technique of isotope dilution. Potassium oxide content was determined using a flame photometer. Six separate analyses were made, the figure 0·060 per cent K_2O given in Table 1 being the average of all six.

Table 1. RESULTS OF AGE DETERMINATION

Material	Ref.	K_2O (per cent)	Per cent atmospheric contamination	Vol. of radiogenic argon (mm³ N.T.P.)/g	Age and experimental error
Pure clinopyroxene	2032/30 *KA*/416	0·060	7·1	0·004616	1,515 ± 104 M. yr.

$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$. $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

Apart from errors arising from uncertainties in potassium oxide and argon content, it has been recently shown[2] that pyroxenes can sometimes contain excess radiogenic argon-40. It is thought that this argon is incorporated in lattice defects at the time of crystallization, though not all pyroxenes exhibit this effect.

If this sample of clinopyroxene does contain a significant amount of excess radiogenic argon its true age would be less than the 1,515 ± 104 million years yielded by the measurement. It could be supposed that the mineral was last recrystallized in the Caledonian (420 ± 15 million years) folding, during which it incorporated excess argon-40 from the surrounding rock. Work on micas from Morar, Knoydart and southern Ross has shown that certain parts of the area were affected little by the last (420 ± 15 million years) folding (Brown and Miller, in preparation). It is considered, therefore, that this particular clinopyroxene has not been contaminated with excess radiogenic argon and has yielded an age which corresponds to a specific geological event.

This new age determination is of interest for the light it throws on the history of the Lewisian rocks of the Glenelg inlier and its relationship to the history of the Lewisian rocks of the Caledonian foreland.

In the Glenelg inlier the Lewisian and Moinian rocks outcrop as alternating belts running in a north–south direction (Geol. Surv. Sheet 71, Scotland). The locality from which the eclogite used in the age determination was obtained lies within the eastern outcrop of Lewisian rocks where eclogitic rocks are associated with paragneisses.

With the evidence now available[3–6] it is possible to construct an outline of the sequence of metamorphic and structural events which affected the Lewisian rocks of the eastern part of the Glenelg inlier:

(1) Early metamorphism—granulite facies (eclogites)—1,515 ± 104 million years (this communication).

(2) Retrograde metamorphism—amphibolite facies (eclogites amphibolitized[1]).

(3) First folding (F_1)—responsible for the interbanding of the Lewisian and Moinian rocks *circa* 740 million years[4]. Certainly older than 560 million years (Brown and Miller, in preparation).

(4) Later folding (F_2 and F_3)—about 420 million years[5,6].

The episodes of folding (3) and (4) here affect both the Moinian and the Lewisian rocks so that they can be considered to form part of the Caledonian orogeny. The retrograde metamorphism (2) has also been considered a result of the Caledonian orogeny by Clough and Alderman but direct evidence is lacking. The early metamorphism (1) has been previously considered to be of Scourian age[3], on the supposition that the high-grade metamorphic rocks, including the eclogites, found at Glenelg could be correlated with the pyroxene granulites of Scourie in the Lewisian of the foreland.

The new date from a Glenelg eclogite falls well within the range of dates given by Giletti *et al.*[6] for the Laxfordian of the Loch Laxford area. This evidence therefore introduces the possibility that the early high-grade metamorphism of the Glenelg area should be correlated with the Laxfordian rather than the Scourian.

We are aware that a single date from an area with a complex structural and metamorphic history does not constitute proof of age and the date given in this communication can only be considered a minimum age. But it is emphasized that the minerals of the specimen of eclogite used in this age determination show no signs of retrograde alterations, unlike most of the eclogitic rocks in the same neighbourhood.

The possibility of a Laxfordian age for the Lewisian rocks in the eastern part of the Glenelg inlier invites comparison with the Lewisian rocks of South Harris recently described by Dearnley[7]. The marked similarities in lithology and metamorphism between the Lewisian rocks of South Harris and those of Glenelg have long been appreciated. The recent work of Dearnley and the new data from Glenelg make possible a closer comparison (Table 2).

Table 2

	South Harris[7]	East Glenelg
Scourian	Sedimentation First granulite facies metamorphism Intrusion of igneous complex	Sedimentation* Not definitely identified Intrusion of basic and ultra-basic rocks
Early Laxfordian	Second granulite facies metamorphism	Granulite facies metamorphism
Late Laxfordian	Retrograde metamorphism	Retrograde metamorphism
Caledonian		Folding (F_1, F_2, and F_3) with associated metamorphic effects

* The evidence from Glenelg does not preclude a post-Scourian age for the deposition of the Glenelg sediments.

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Geographical Variations of Oxygen-18/Oxygen-16 Ratio in Surface Snow and Ice from Queen Maud Land, Antarctica

THE work recorded here is a continuation of that reported in *Nature*[1,2] on the isotopic composition of Antarctic snows.

The ratio of oxygen-18 to oxygen-16 has been measured in some hundred samples of surface snow and ice in the region of the King Baudouin Station (70° 26′ S., 24° 19′ E.), and taken from an area extending from the coast to the southern slopes of the Sor-Rondane Mountains, situated 300 km to the south. Information concerning the geodesy and morphology of this region has been published[3,4].

These samples were collected during the Belgian Antarctic Expeditions of 1959 (Commandant F. Bastin) and 1960 (Commandant G. Derom).

Detailed results will be published elsewhere. We report here only those results relative to samples of which the thickness represents one or more years of accumulation, in order to minimize the seasonal variations.

The ratio of oxygen-18 to oxygen-16 is expressed, as in previous publications, by δ, in parts per thousand of relative variation with respect to the standard mean ocean water (SMOW).

Fig. 1 shows the locations at which samples were taken and the values of δ. Each value corresponds, in general, to the average of several neighbouring samples of similar isotopic composition.

Fig. 2 shows the variations of δ as a function of the altitude at which the samples were taken. We have added two points taken from other publications: the point from the South Pole according to Epstein and Sharp[5], and a group of points corresponding to Victoria Land, and measured by Roth, Nief and Botter. The latter points have been estimated from the average content of deuterium reported by Lorius[6], and the altitudes reported by Stuart and Heine[7]; the deuterium concentrations have been translated into δ according to the relation found at the King Baudouin Station in 1958[8].

Despite the poor statistical value of samples of which most represent only one year, the values of δ follow well the general scheme of variations of isotope composition of precipitations[8,9]. It is known that the value of δ depends principally on the temperature of precipitation. In the Antarctic, the average annual temperature is essentially a function of the altitude.

Table 1 indicates that the four classical geographical zones of the Antarctic are characterized by clearly different values of the δ of precipitations.

TABLE 1 AVERAGE OXYGEN-18/OXYGEN-16 RATIO OF SURFACE SNOW FROM THE FOUR GEOGRAPHIC ZONES—PRINCESS RAGNHILD COAST

Zone	δ (SMOW) averages
Sea ice* and ice-shelf (68°–70° S.)	−22
Continental slope (70°–71° 30′)	−22 to −26
Mountains (71°–72°)	−26 to −37
Polar plateau (72°–90°)	−37 to −50

* Snow precipitated on the ice-pack. The sea ice itself shows δ of + 1.

The most-pronounced difference is between the ice formed on the plateau and that formed on the continental slope. The variation of δ in the 300 km separating the coast from the mountains is as great as the variation over the whole of the polar plateau. This corresponds to the increasing spacing of the curves of equal elevation, equal temperature and equal accumulation when proceeding from the coast toward the centre of the continent.

It will be important to make a network of measurements of δ of surface snows extending throughout the continent, as well as measurements of δ as a function of the depth at diverse points of the Antarctic ice-cap. These data could provide important information on the large-scale displacements of the ice-cap (see, for example, ref. 10 in reference to Greenland).

We take from the present work two examples of information which can be derived from the application of isotope methods on a more local scale:

(1) The sample of ice *LD* comes from a region of the continental slope where the bare ice is exposed over large areas, at more than 60 km from any rocky outcrops. The origin of these fields of bare ice is still controversial. In this particular case, the isotopic composition ($\delta = -22$) indicates that this ice does not come from the polar plateau, but that it must have been formed at the expense of snow precipitated in the same zone (assuming that it has never experienced strong evaporation).

(2) The entire zone of the Sor-Rondane Mountains shows traces of important glacial recessions. Although most of the glaciers of the northern slopes no longer connect with the plateau, it is probable that in the past the ice-cap ran over the mountain range.

It can be seen (Figs. 1 and 2) that all the samples taken from the northern slope of the range have about the same isotopic composition ($\delta = -30$), whether it is bare ice from the ablation zones, or surface snow representing recent precipitation. These ices, then, were formed from local precipitations, and do not represent possible remainders of the ice-cap, dating from before the recession.

Only the sample *GU* has a clearly more negative δ (-39). This value is in good agreement with the geographical facts. In fact, this sample is the only one which comes from an outlet glacier (the Gunnestadbreen), still in connexion with the plateau and presenting a measurable surface flow (maximum displacement of the order of

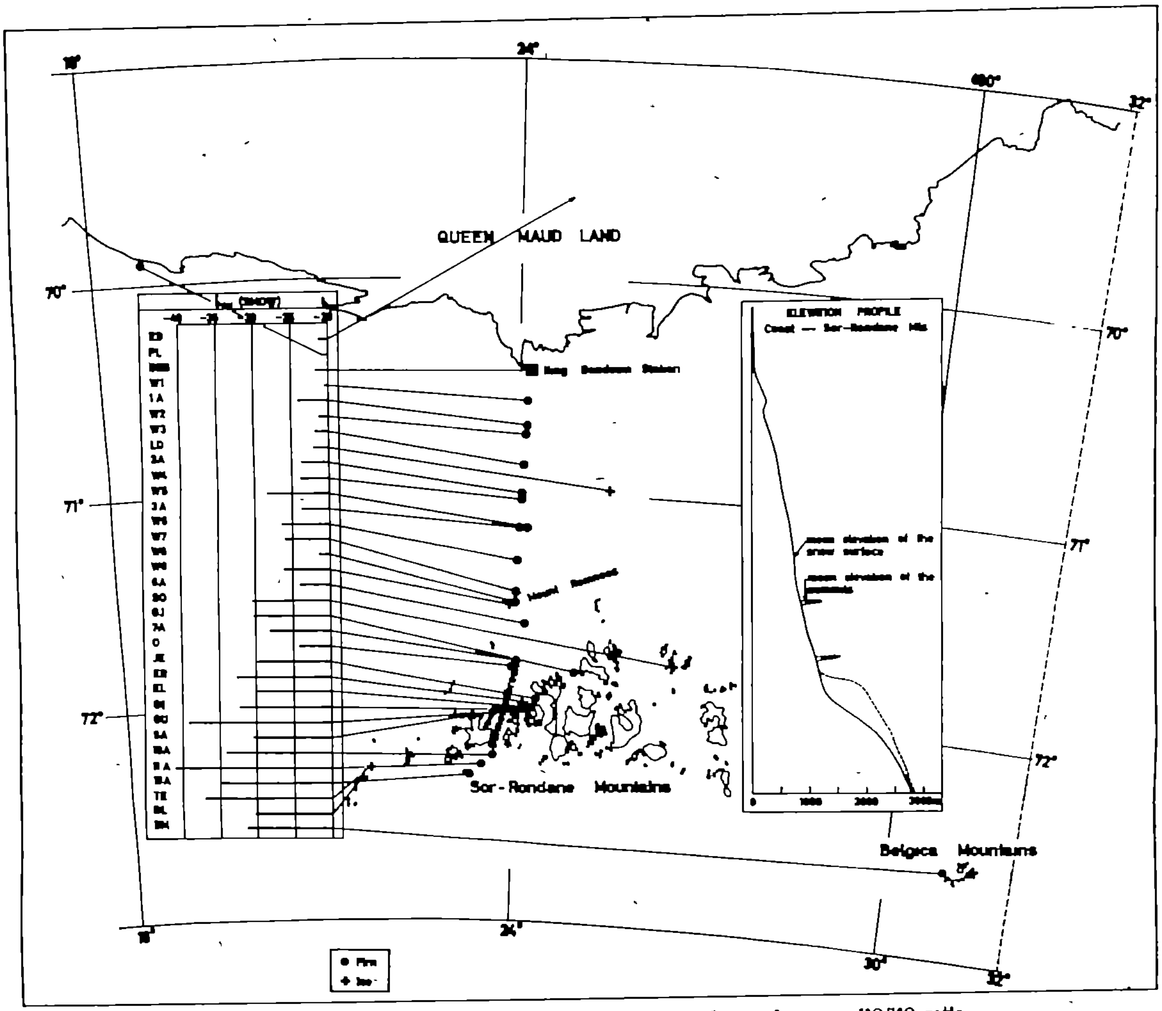

Fig. 1. Geographical distribution of surface samples and annual average $^{18}O/^{16}O$ ratio

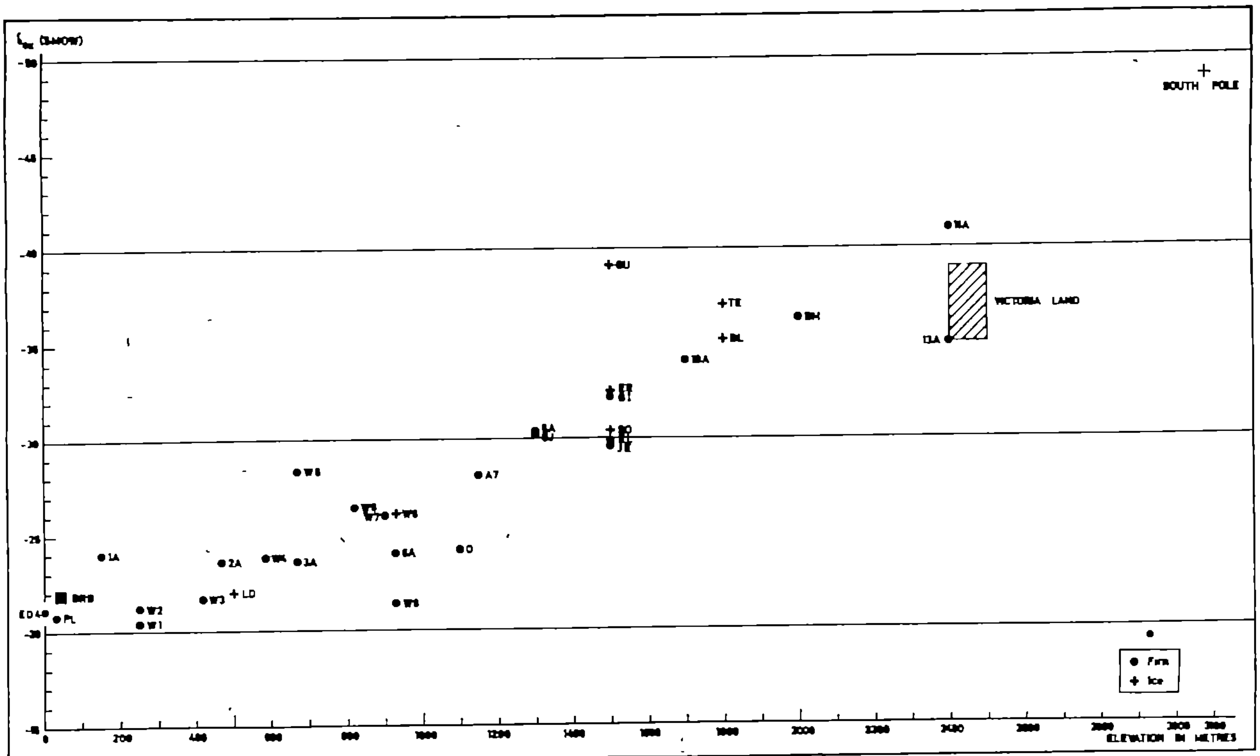

Fig. 2. Annual average $^{18}O/^{16}O$ ratio of surface samples as a function of altitude (Victoria Land and South Pole from Lorius (ref. 6) and Epstein and Sharp (ref. 5))

10 m/year, according to K. Blaiklock and T. Van Autenboer).

We thank the members of the Belgian Antarctic Expeditions of 1959 and 1960 and of the summer journeys which participated in the collection of samples, notably F. Bastin, T. Van Autenboer, J. Giot and J. Loodts.

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Yoredale Cyclothems of Northumberland

IN discussion of Yoredale cyclothems insufficient attention has been paid to the degree of change between successive environments of deposition in thinly bedded sequences. There is general agreement that the study of cyclothemic and cyclical sedimentation is primarily an investigation of the sequence of the environmental changes that take place. If it is accepted that this is so regardless of how long a new environment may persist or how widespread it may be, then the full implications of such a statement must be appreciated. For example, in the case of a sandstone–limestone junction the change from a sandstone to a limestone two feet thick is exactly equivalent to the change from an identical sandstone to an identical limestone thirty feet in thickness. That is to say, the change in environment is of the same degree in both cases and that this change occurred in a distinct period of time. The duration for which the new conditions were maintained is of no consequence in this context since this indicates the history of the rock after the all-important event had taken place.

The Yoredale Series is interpreted as a succession of cyclothemic units each of which approaches, or can be related to, an 'ideal' or 'complete' cyclothem in which the beds succeed one another in a series from limestone, through shale, sandstone, and seat-earth to coal. To illustrate the difficulties encountered in attempting to interpret the Yoredale sediments of Northumberland in terms of such a concept an example is taken from the section of Middle Limestone Group strata at Beadnell (*National Grid*, ref. 46 236293) on the Northumbrian coast. In Fig. 1, a plot is made above the mid-horizontal line of the various rock types arranged in succession from one environmental extreme to the other in accordance with the standard cyclothem, and the changes of environment as inferred from the succession of rock types are consecutively numbered 1–28 along the top of the diagram. Below the mid-horizontal line the thicknesses of the beds are indicated by vertical lines. It would appear that this sequence can be interpreted in several different ways as detailed here:

(1) The traditional interpretation would be to consider the beds from 1 to 27 as a single cyclothem; this same sequence might more recently be referred to as a 'major' cyclothem.

(2) Two cyclothems may be distinguished, from beds 1–11 and 12–27 respectively, that is, beginning a new cyclothem with each limestone that follows a change of high degree in environment.

(3) Three cyclothems may be recognized, from beds 1–11, 12–17, and 18–27, if the fossiliferous calcareous sandstone is interpreted as a marine horizon.

(4) Four cyclothemic units from beds 1–11, 12–17, 18–23, and 24–27, may be recognized, if in addition to the last case beds 24–27 are considered to represent a cyclothem with the calcareous member missing.

In the first of the interpretations made here, all the sediments from one thick limestone to the next are grouped as a single cyclothem in the traditional manner. Such a disordered grouping cannot be accepted as a cyclothemic unit. In recent years[1], distinction has been made between 'major' and 'minor' cyclothems, whereby relatively thin or minor cyclical repetitions of strata are conceded near the top of a larger and embracing major cyclothem. This interpretation is equally difficult to accept since the thin limestones of the 'minor' cyclothems should be regarded as equally significant environmentally as the thick limestone which defines the 'major' cyclothem. Any grouping of beds 1–27 in terms of a cyclothem, 'major' or otherwise, is identified only by the presence of thick limestones; thick limestones are named, are important commercially, are conspicuous in the field, are important stratigraphical marker horizons, but they do not represent especially important units in a cyclical series.

The three remaining subdivisions of the sequence shown in Fig. 1 form a series recognizing in turn a thin limestone, a fossiliferous calcareous shale, and finally the position where a marine horizon might have occurred, as logical points in the succession to define the limits of cyclothemic units. Such interpretations realize the overall importance of the changes which occur in the environment and are not primarily subject to considerations of thickness. However, following this line of argument, further cyclothems might be recognized wherever

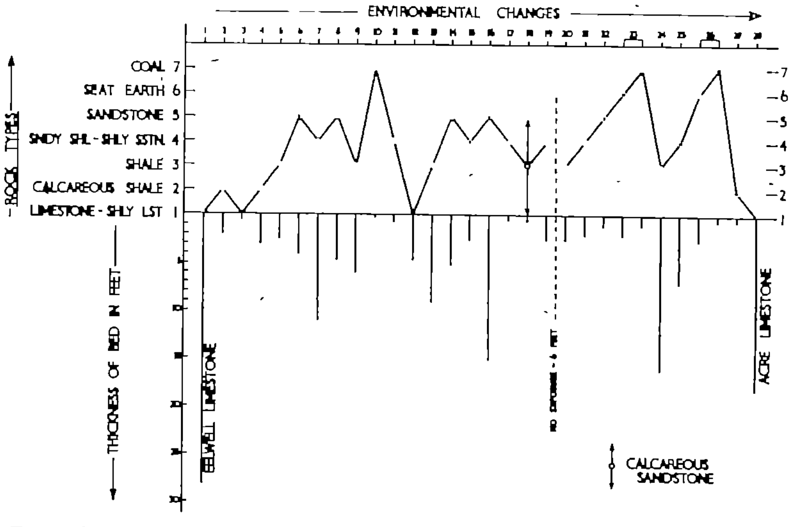

Fig. 1. Sequence from the Belwell to the Acre limestones at Beadnell, Northumberland

there is an interruption of the 'ideal' gradual change. It is clear that once cyclothems are referred to with certain members 'missing' or 'absent' then a process is begun which if taken to its logical conclusion fragments the sequence to the point of meaninglessness. Nevertheless, in the literature to-day, cyclothems are described as 'modified' or 'reduced'; beds are referred to as 'missing', 'restricted', 'absent', etc. Reference is made to 'typical' or 'usual' and 'unusual' cyclothems in which the standard taken is the 'ideal' sequence and in no sense is this a comparison with an actual rock sequence or an 'average' rock sequence. Repeatedly authors point out the inadequacy of their successions to realize theoretical conceptions and so one is led to the conclusion that attempts to apply the cyclothem hypothesis to such successions might mistakenly distinguish sequences of rock types as equatable or comparable entities by admitting variation in 'detail' from an ideal, instead of realizing the full implication of the actual existence of such 'modified' sequences. That is to say, perhaps in certain cases it is not the successions which are 'inadequate' but that the application of such 'ideals' is inappropriate.

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METEOROLOGY

A Secondary Source of Water Vapour in the Upper Atmosphere

The early pioneering work of Dobson, Brewer and Cwilong with frost point hygrometers indicated that over South England the water-vapour content of the stratosphere between 12 and 15 km is extremely small in all seasons, being about 2 mg/kg (frost point temperature being about − 80°). Subsequent measurements of humidity between the same levels over a geographical region extending from Norway to equatorial Africa revealed the interesting fact that in all seasons and in most of the locations the moisture content of the lower stratosphere is very small. On the basis of such observations, which led to the concept of a uniformly dry stratosphere, Dobson *et al.*[1] postulated the existence of a meridional circulation in the stratosphere involving the poleward movement of very cold saturated air near the equatorial tropopause and its descent over high latitudes. This theory has been amplified further by Ramanathan[2] and Murgatroyd and Singleton[3]. On the other hand, a large number of observations made over the North American continent and Greenland during 1959 and 1960 using automatically registering frost point hygrometers indicated a very wide range of frost point temperatures from − 67° to − 80° C (refs. 4, 5). Similar observations made over Japan indicate that on many occasions the water-vapour mixing ratio increases with height in the stratosphere[6]. Rohrbough[7], using a balloon-borne sampling system, has recently reported high humidity mixing ratio of 320 mg/kg at a height of 30 km over the United States, this height being a record for the determination of humidity. These recent observational results, which indicate a rather complex distribution of water-vapour, suggest that the earlier concept of a uniformly dry stratosphere needs re-examination.

Quite apart from the data provided by individual soundings involving elaborate equipment, no routine climatological data on humidity in the stratosphere were available until very recently. The monthly mean humidity data (based on at least ten individual observations each month) of a large number of stations from the U.S.S.R. and a few from Sweden for levels up to 100 mb have been given in the *Monthly Climatic Data for the World* published by the U.S. Weather Bureau. An analysis of these data covering the period January 1961–May 1962 reveals the following interesting features:

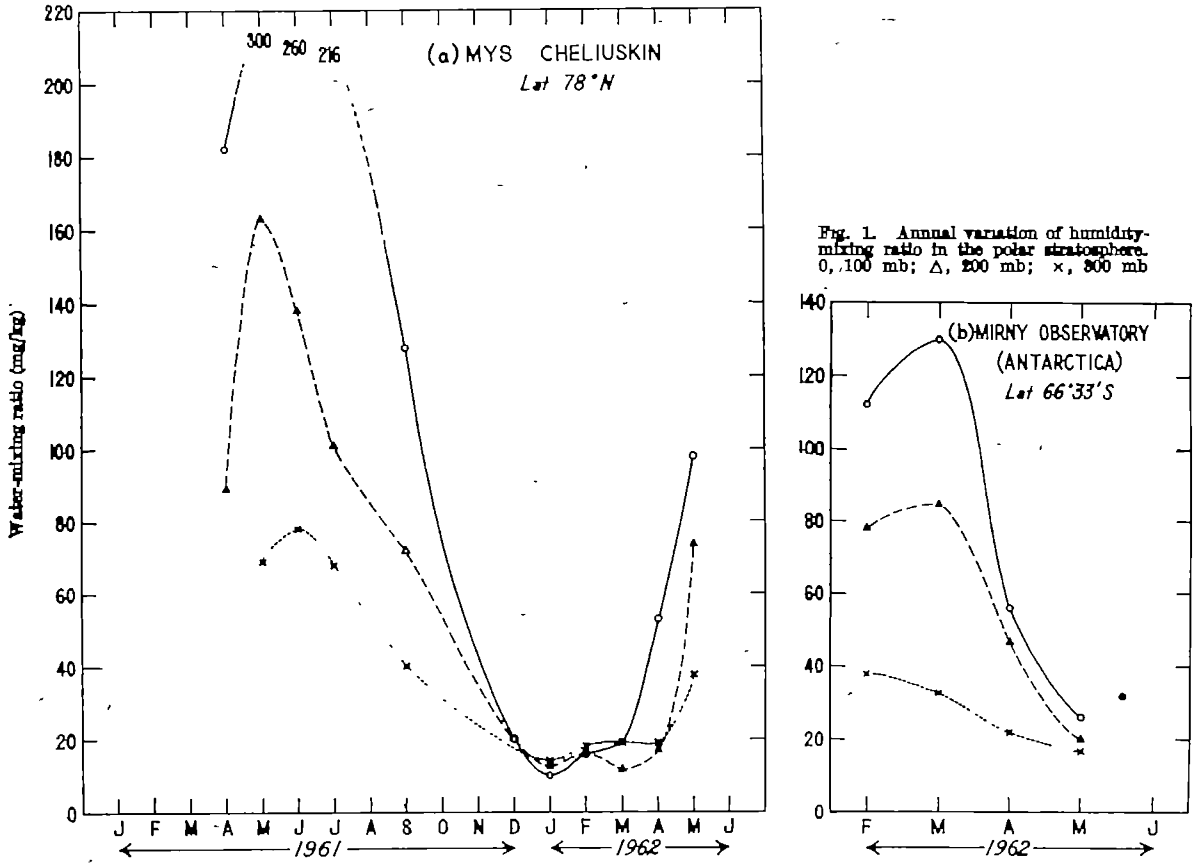

Fig. 1. Annual variation of humidity-mixing ratio in the polar stratosphere. O, 100 mb; △, 200 mb; ×, 300 mb

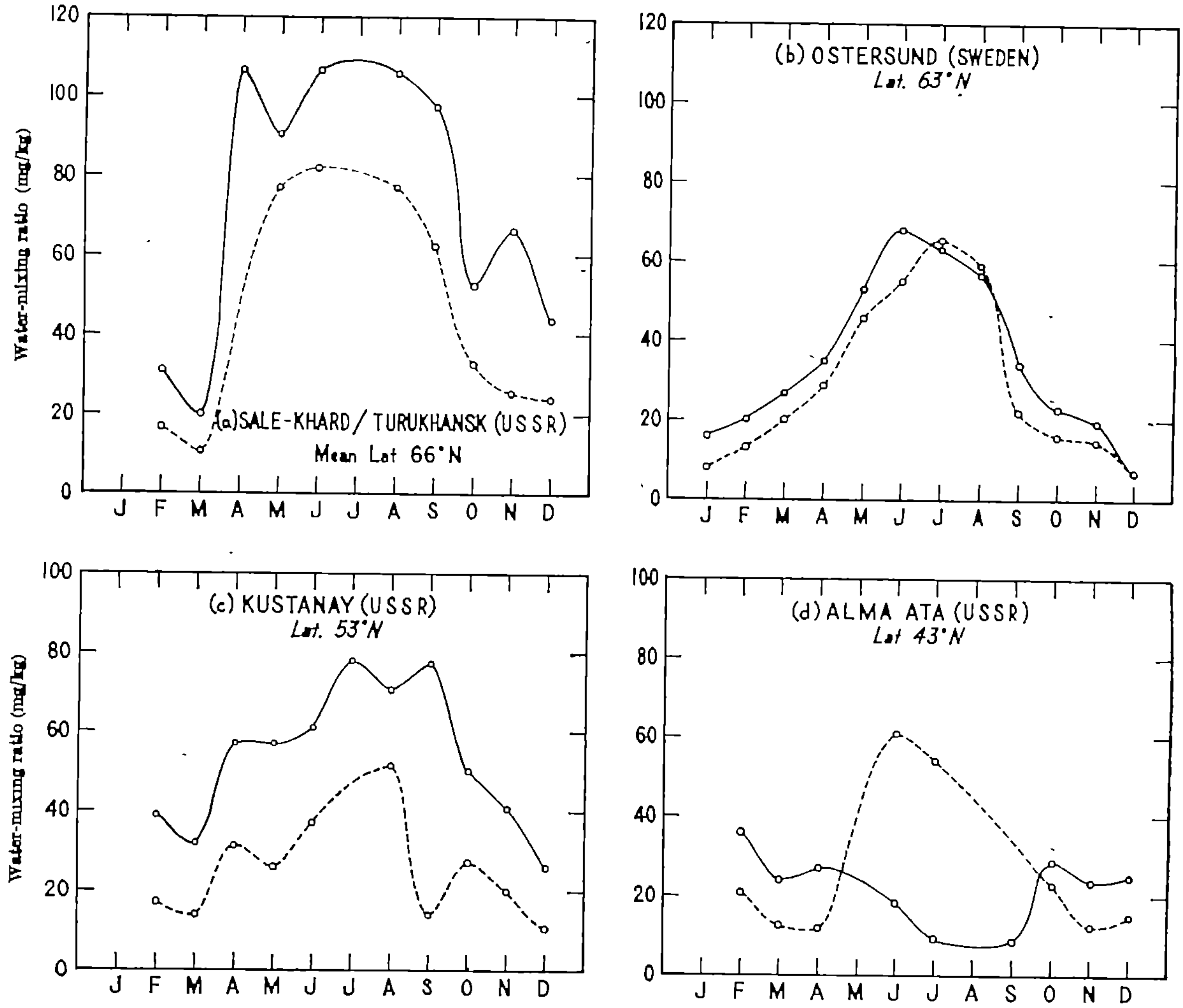

Fig. 2. Annual variation of humidity-mixing ratio at four stations at different latitudes (1961). ——, 100 mb; – – –, 200 mb

(1) The highest concentration of water-vapour in the lower stratosphere is found near the summer pole and attains values of about 200–300 mg/kg at the 100-mb level (16 km).

(2) There is a systematic annual cycle in the water-vapour concentration at all stations to the north of lat. 45° N. with a maximum in summer and a minimum in winter.

(3) The amplitude of the annual variation is the highest near the summer pole and decreases with decreasing latitude.

(4) Water-vapour mixing ratio increases with height for the greater part of the year at the high-latitude stations.

(5) The annual cycle of mixing ratio in the Antarctic stratosphere is similar to those in the Arctic in the appropriate season.

(6) At stations to the south of about lat. 45° there is a reversal in the phase of the annual cycle.

Fig. 1*a* shows the annual variation of the monthly mean mixing ratio at Mys Cheliuskin (77° 43′ N., 104° 17′ E.) during the period April 1961–May 1962 for three different levels, 300, 200 and 100 mb. The summer values of mixing ratio amounting to more than 300 mg/kg at 16 km in May is the highest ever reported anywhere in the lower stratosphere and is about 100 times greater than the value in the early British observations. Independent confirmation of the summer increase of humidity is afforded by Fig. 1*b*, which illustrates the data of Mirny Observatory, Antarctica (60° 83′ S., 93° 00′ E.), where summer conditions prevail in January and February. The decrease of annual amplitude with latitude is illustrated by Fig. 2, which shows the results in respect of 4 different stations covering the latitude range 66° N. to 43° N. It is interesting to find that at Ostersund (Sweden), where instruments of different makes are used, the annual pattern is the same as at the Russian stations to the north of lat. 45° N. At latitudes less than 45° N. the annual variation seems to follow a different pattern, as would be seen from Fig. 2*d*, which pertains to Alma Ata (43° 14′ N., 76° 56′ E.). The mixing ratio at 100 mb is a minimum during July–September whereas that at 200 mb is a maximum in the same period.

The features in respect of Alma Ata can be explained broadly in terms of the known features of upper air climatology of the Asian region together with some kind of meridional circulation. But the other observed features cannot be accounted for by the prevailing concept that the whole of the water-vapour in the lower stratosphere has its ultimate source in the Earth's surface. A clue to a possible secondary source of water-vapour in the upper atmosphere is afforded by the recent theory put forward by de Turville[illegible] that the entire hydrogen content of the Earth's oceans could be of solar origin. According to this, the protons contained in the solar wind get trapped by the Earth's magnetic field and after a process of accretion throughout the estimated age of the Earth finally get oxidized as water. Frith[illegible], following up this theory further. has suggested that the water-vapour formed at high levels could be transported downwards by turbulence. In support of this, it has been cited by him that the noctilucent clouds found in the mesosphere at 80 km as well as the mother-of-pearl clouds found occasionally in the middle stratosphere (25 km) could both be formed by the condensation of water-vapour carried downwards from the higher levels. Such a concept involving a secondary source of water-vapour in the upper atmosphere could qualitatively account for some of the new features revealed by the recent data on stratospheric humidity. Since relatively large values of humidity occur only to the north of lat. 60° and only during the sunlit period of the year, it is tempting to suggest a photochemical origin for the water-vapour in accordance with the equation:

$$\mathrm{OH} + \mathrm{H} \rightleftharpoons \mathrm{H_2O}$$

From available data on the spectrum of the night airglow, there is evidence for the presence of OH radical at about 70 kilometres where there is an appreciable amount of atomic hydrogen also. The dominant circulation in the upper stratosphere and the lower mesosphere during summer is a vast circumpolar anticyclone of great persistence[10]. The subsidence associated with this anticyclone might be effective in transporting the water vapour downwards from the mesosphere to the lower stratosphere.

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Barometer and Wind Fluctuations in North-West Europe, 1796–1950

Barometric series from manuscript and printed sources were recently utilized for the preparation of maps of annual anomalies. From these maps it was possible to determine the pressure at the Wash (53° N., 0½° E.) since 1706 and pressure gradients (since 1796) between positions 400 km south-south-west and north-north-east and 400 km east-south-east and west-north-west (corresponding to west and south calculated components of surface wind). In Fig. 1 the mean values for ten-year periods of pressure and pressure gradients are shown. The 'SW wind' is a measure of the closeness of the isobars directed west-south-west to east-north-east, and the 'NW wind' relates to a component of pressure gradient at right angles to this. The latter component is small. Indeed, the variation in direction from one thirty-year period to another of the prevailing wind, as shown in Fig. 1 (for example, 224°), is much smaller than has hitherto been supposed.

Wind observations of north-west Europe since 1635 were likewise combined into components, as explained in a recent issue of *Geografiska Annaler* (44, 303; 1962). The apparent mean wind in any decade, determined without taking velocity differences into account, was found to be directed along the isobars, on the average from west-south-west, with frequency components of the order of 18 per cent from west and 8 per cent from south. The 'true' surface wind at a typical land station comes from the south-west, not west-south-west. The vector anomalies of the wind, if one year is compared with another, were nevertheless found to follow Buys Ballot's law in the usual way, and are directed from the west-south-west when the isobaric anomaly lines lie west–east. Using empirical relationships with the wind components in percentages, it was possible to 'calibrate' the pressure gradient curve as indicated at the right-hand part of Fig. 1, and to determine the pressure-gradient fluctuations quantitatively in the seventeenth and eighteenth centuries.

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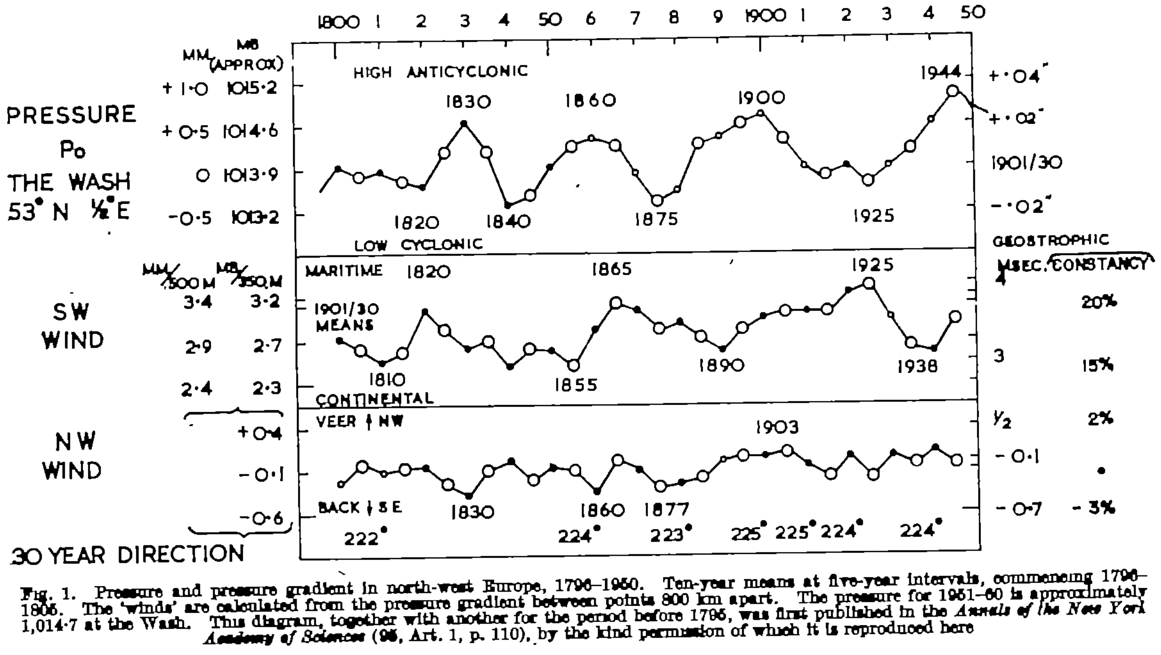


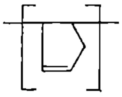
Fig. 1. Pressure and pressure gradient in north-west Europe, 1796–1950. Ten-year means at five-year intervals, commencing 1796–1805. The 'winds' are calculated from the pressure gradient between points 800 km apart. The pressure for 1951–60 is approximately 1,014·7 at the Wash. This diagram, together with another for the period before 1795, was first published in the *Annals of the New York Academy of Sciences* (95, Art. 1, p. 110), by the kind permission of which it is reproduced here

RADIATION CHEMISTRY

Radiation-induced Ionic Reactions: *Cyclo*pentadiene Polymerization

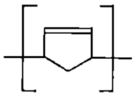
Recent investigations[1–3] on the radiation-induced polymerization of certain monomers have provided evidence for the production of ionic as opposed to radical initiation. The polymerization of such monomers can be initiated by Friedel–Crafts catalysts, and *cyclo*pentadiene[4] falls into such a class. Pure, rigorously dried (silica-gel treated) *cyclo*pentadiene was irradiated with cobalt-60 γ radiation (dose-rate $2\cdot27 \times 10^{17}$ eV g^{-1} min^{-1}). At $-78\cdot5°$ C a gel was formed after conversion of 1·6 per cent of the monomer to polymer. The results of carbon, hydrogen analysis fitted an empirical formula of C_5H_6. The gelling dose was $8\cdot5 \times 10^{17}$ eV g^{-1}, and the subsequent increased rate of polymer formation corresponded to the removal of $17{,}760 \pm 1{,}521$ (*S.D.*) monomer molecules per 100 eV. This was linear with dose up to at least 40 per cent conversion. At 0° C the yields were lower and suffered a high degree of irreproducibility (G(-monomer) range of 1,518–12,750). At 22° C a dimer impurity could not be excluded owing to its spontaneous thermal formation[4], but polymerization did ensue in one experiment with an overall G(-monomer) yield of 10,600 at 15 per cent conversion. In the absence of silica-gel drying the yields were substantially reduced. The efficiency of the reaction at low temperature, and the necessity for rigorous drying[9] to attain high yield are criteria for the non-participation of radical processes.

The infra-red spectrum of the radiation-induced polymer in a potassium bromide disk was similar to that of polymer prepared by stannic chloride catalysis in chloroform at −5° C (ref. 5). The repeating unit of the latter polymer has been formulated as:



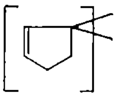
(I)

or



(II)

Nuclear magnetic resonance analysis of a solution of a sample of catalysed material in carbon tetrachloride favours structure I. A similar analysis of a radiation-produced sample which formed a swollen gel in the solvent suggests a structure:

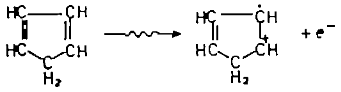


(III)

Owing to line-broadening, hyperfine structure was not observed so that the assignments must be regarded with some reserve.

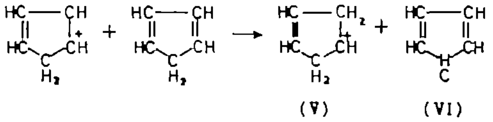
In formulating a mechanism for a radiation-induced polymerization it is primarily necessary to explain the modes of charge separation and stabilization. The production of a stable carbonium ion from an initial radical-ion has been accounted for by proton transfer[6], ion-molecule reactions[3] or ion re arrangements[2]. Capture of the conjugate electron on solid sub-microscopic particles[6,7] is one favoured postulate for production of a stable negatively-charged entity. Carbanion formation[2,8] has also been proposed as a possibility, and in the case under discussion such a negative ion can be envisaged in the following possible scheme.

The primary process is:



(IV)

followed by an ion-molecule reaction with monomer:



(V) (VI)

Afterwards VI captures the electron on its return to the neighbourhood of the positive ion to form the very stable *cyclo*pentadienyl ion which acts as the counter-ion. V or a re-arranged form then initiates propagation more readily than it undergoes neutralization. The energy requirements for charge separation may be derived from the energetic primary event and from the contribution of polarization forces. The rapid gelation and insolubility of the whole polymer in solvents suggests a cross-linked structure arising from branching during propagation[8]. This might result from hydride-ion transfer between propagating chains or between such chains and formed polymer[9]; an alternative would be the occurrence of proton transfer to the remaining double bond in a repeating unit of a neighbouring chain.

I thank Mr. T. L. Jones and Dr. R. K. Webster for carrying out the nuclear magnetic resonance analysis, and acknowledge an initial suggestion by Dr. T. F. Williams and the encouragement and advice of Dr. W. G. Burns and Dr. W. Wild.

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CHEMISTRY

Improved Efficiency in Gas Chromatography by Molecular Sieve Flour

IT has been demonstrated that columns packed with 5*A* molecular sieve flour dispersed on conventional 'Chromosorb' support perform better than columns packed with crushed molecular sieve pellets[1]. This is illustrated in Fig. 1 by the chromatograms showing the separation of air. Curve *A* was produced with a 2 m × ¼ in. column packed with 60/80 mesh 5*A* molecular sieve. Curve *B* was produced with an equal-dimension column packed with 28 per cent molecular sieve flour coated on 60/80 mesh red 'Chromosorb'. The molecular sieve column provides only 97 plates compared with 562 plates for the mixed column. Expressed as height equivalent to a theoretical plate, this represents a decrease from 20·6 mm to 3·5 mm and a six-fold increase in efficiency. Separations were at room temperature with flow-rates near 50 ml./min of helium. The sample was 2 ml. of air.

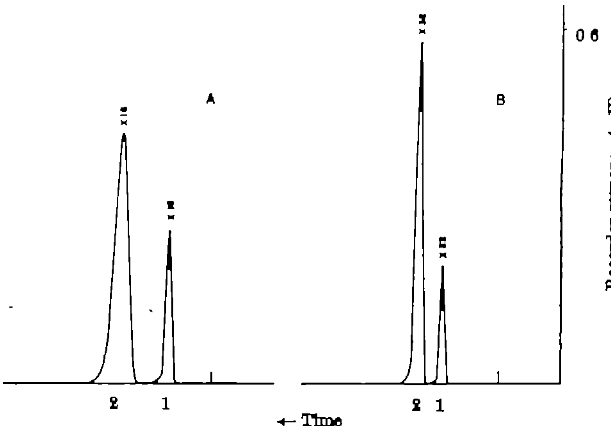

Fig. 1. Chromatogram showing the separation of oxygen (1) and nitrogen (2). *A*, Molecular sieve 60/80 mesh; *B*, molecular sieve flour coated on 'Chromosorb'

The mixed 'flour' column worked effectively with light hydrocarbons as well as with permanent gases.

The −200 mesh 5*A* molecular sieve flour was obtained from Chrom-Line Laboratories, Kansas City. It was dispersed on 'Chromosorb' by mixing two parts 'Chromosorb' with one part molecular sieve flour. The mixture was rolled in a cylindrical container for 18 h and then screened. The material which passed a 60 but was retained on an 80- mesh screen was used as the column packing. Of the original 33 wt. per cent added to the mixture, 28 wt. per cent of the flour was retained on the 'Chromosorb'.

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Electron Paramagnetic Resonance Spectrum of the Acetyl Radical

THE electron paramagnetic resonance (EPR) spectrum of the acetyl radical, CH_3CO, has been detected during and after photolysis of biacetyl at 77° K. Small (100 mg) samples of biacetyl were distilled into thin-walled quartz tubes and degassed. The samples were placed in the *V*-4546 quartz Dewar accessory of a Varian *V*-4500 spectrometer, and irradiated under liquid nitrogen with a low-pressure mercury arc.

A typical spectrum, recorded after 90 min irradiation, is reproduced in Fig. 1. This spectrum was analysed in terms of two radical species each exhibiting hyperfine interaction with three equivalent protons. The wider spectrum, in which the proton hyperfine interaction is 66 ± 5 Mc/s, was attributed to the methyl radical, since the observed hyperfine splitting and *g*-value (2·0027 ± 0·0008) are in excellent agreement with previous work[1,2] on the methyl radical. The features of the spectrum due to the methyl radical decreased slowly in intensity when the lamp was turned off, reaching half-intensity in approximately 15 min. The remaining spectrum, which is believed to be that of the acetyl radical, did not decay significantly after switching off the lamp, but on warming to approximately 140° K in a *V*-4547 variable temperature accessory, it decreased rapidly in intensity without change in shape. The hyperfine interaction is that of three protons with equal couplings of 47 ± 5 Mc/s, appreciably less than that to be expected from equivalent protons β to a π-electron radical centre[3]. Acetyl, which has not previously been detected spectroscopically, is a σ-electron radical analogous to the formyl radical reported recently[4]. The *g*-value of the acetyl spectrum was found to be 2·0055 ± 0·0008 and the comparatively high maximum-slope line-width of 15 Mc/s probably reflects a small anisotropy in the coupling constants, rather than in the *g*-value, since the spectrum of acetyl was quite accurately centro-symmetric.

The presence of both acetyl and methyl radicals in irradiated, condensed biacetyl is consistent with the mechanism of Noyes *et al.*[5,6], who proposed the primary decomposition step at 2700 Å to be:

$$CH_3COCOCH_3 + h\nu \rightarrow 2\alpha CH_3CO^* + (2-2\alpha)CH_3CO \qquad (1)$$

where CH_3CO^* decomposes almost immediately into $CH_3 + CO$. Their evidence suggested that α was 0·5, and therefore (1) was kinetically indistinguishable from:

$$CH_3COCOCH_3 \rightarrow CH_3COCO + CH_3 \qquad (2)$$

followed immediately by:

$$CH_3COCO \rightarrow CH_3CO + CO \qquad (3)$$

In our experiments the observed concentration of acetyl radicals was at least five times that of the methyl radicals, and even allowing for the disappearance of methyl radicals during irradiation, an upper limit of 0·25 can be placed on α of reaction (1). Therefore under our conditions reaction (2) cannot be the only primary decomposition step.

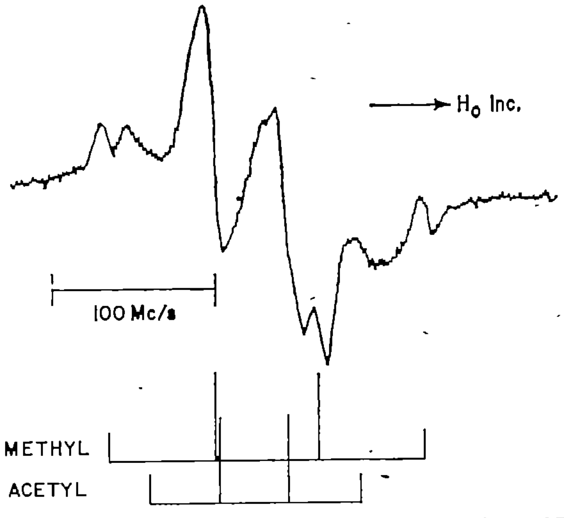

Fig. 1. EPR spectrum of ultra-violet-photolysed biacetyl at 77° K

If the acetyl radical concentration were sufficiently high, a methyl radical could be formed on absorption of a second quantum of energy by an acetyl radical:

$$CH_3CO + h\nu \rightarrow CH_3 + CO \qquad (4)$$

However, the build-up of methyl radicals on irradiation of biacetyl is simultaneous with that of the acetyl radicals, so that absorption by acetyl radicals cannot be the (sole) precursor to methyl radical formation.

If, as is generally believed, radical recombination reactions have very low or zero activation energies, the persistence of acetyl radicals in biacetyl at 77° K indicates that these radicals are rigidly held in the matrix. In contrast, the methyl radicals are presumably free to migrate. Further work to determine the fate of the methyl radicals is in progress, and the investigation is being extended to include higher ketyl radicals.

We thank Dr. R. J. Cvetanović for his interest.

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Estimation of Small Amounts of Sulphate by Precipitation with Radioactive Barium

IN the course of work on the metabolism of sulphur amino-acids in malnourished infants it was necessary to measure total sulphate concentration in large numbers of urine samples. The urine from these patients is very dilute, so that to obtain accurate results by the classical gravimetric or titrimetric methods it would be necessary to use large volumes. This is inconvenient, since for the oxidation of organic sulphur the urines have to be digested with concentrated acid. By precipitating with barium chloride containing radioactive barium, and measuring the radioactivity in the precipitate, the estimation could be made on 0·1–0·5 ml. urine.

To a sample of 0·5 ml. containing 0·5–20 μM sulphate are added 1–2 ml. of barium chloride reagent. 1 ml. of the reagent is enough for quantities of sulphate up to 5 μM; for quantities between 5 and 20 μM 2 ml. should be added. The reagent consists of 0·03 M barium chloride in 1 N hydrochloric acid and 60 per cent ethyl alcohol. To this is added $^{133}Ba\,Cl_2$, about 0·05 μc./ml., depending on the sensitivity of the counter. The alcohol is added to reduce the solubility of the barium sulphate[1]. If the solution is not acid, precipitation is incomplete. After this was done it was found that an excess of acid significantly increases the solubility of barium sulphate[2]. In practice, however, the effect seemed to be negligible, and is allowed for if sulphate standards are included in each batch of measurements.

The sample and barium chloride reagent are mixed by shaking, allowed to stand for 1 h, and centrifuged at 4,500 r.p.m. for 10 min. The supernatant is decanted and the tube allowed to drain. The sides of the tube and the surface of the precipitate are then rinsed with 1 ml. of non-radioactive barium chloride reagent. It was found that washing the precipitate decreased the recovery and made the results more irregular. In one experiment the amount of supernatant trapped in the precipitate was measured by precipitating sulphate with a solution of non-radioactive barium chloride containing radioactive silver nitrate. The activity trapped in the precipitate was negligible.

After rinsing, the activity of the precipitate is counted in a scintillation counter with a well crystal. The whole operation is carried out in one tube, so that the possibilities of loss are minimal. The amount of barium-133 added to the reagent is adjusted to give a count-rate of about 2,000 c.p.m./μM sulphur.

In 21 consecutive measurements of 2–10 μM sulphate the mean difference between duplicates was 1·4 per cent (max. 3·8 per cent). In 8 consecutive measurements of 0·5–2 μM sulphate the mean difference was 3·0 per cent, or 1·5 per cent if one aberrant pair of readings, differing by 12 per cent, is excluded.

In 3 experiments to test linearity, the results shown in Table 1 were obtained.

Table 1

μM SO_4 present	No. of samples	Counts per min	Percentage of counts at 0·5 μM	Percentage error
0·5	4	1,028	100	—
1·0	6	2,068	200	0·1
2·0	6	4,162	404	1·0
4·0	2	7,945	772	4·0

In acid solution the other constituents of urine did not interfere with the precipitation of sulphate. 8 samples of infants' urine were taken at random, and aliquots of 0·5 ml. added to 0·5 ml. of standard solutions containing 10 and 20 μM sulphate per ml. The mean recovery was 101 per cent.

It appears, therefore, that by this method amounts of sulphate down to 0·5 μM can be estimated with an accuracy of ± 2 per cent.

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Tetrahedral Co-ordination in *Bis-N*-isopropyl Salicylaldiminato Nickel II

SACCONI *et al.*[1] have recently reported that preliminary investigations indicate a tetrahedral arrangement of ligands about the nickel atom in the paramagnetic *bis-N*-isopropyl salicylaldiminato nickel II. This was based on the isomorphism of this compound with the cobalt and zinc analogues, and the large (~ 7 Å) distance between nickel atoms, which precludes a polymeric octahedral structure such as found for acetylacetonato nickel II (ref. 2), or *bis*-dimethylglyoximato nickel II (ref. 3).

We have since collected three-dimensional data by the equi-inclination Weissenberg technique and have proceeded with structure determination. We are still refining the structure and at this stage $R = 0·064$. The arrangement of the ligands about the nickel atom is a distorted tetrahedron.

Current bond distances about the nickel atom are:

Average Ni—O distance is 1·90 Å
Average Ni—N distance is 1·97 Å
N_1—Ni—O_1 angle is 94·0°
N_2—Ni—O_2 angle is 94·9°
N_1—Ni—O_2 angle is 112·6°
N_2—Ni—O_1 angle is 111·7°
N_1—Ni—N_2 angle is 121·0°
O_1—Ni—O_2 angle is 125·1°

The two salicylaldimine residues are essentially planar, the mean deviation of atoms from the least square planes of the two being about 0·025 Å. The angle between the planes is 81°.

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Apparent Molecular Weight of Insulin in Dilute Acid Solution

THE molecular weight of the smallest unit of the insulin molecule occurring in dilute acid solution has been in doubt for some time. Most data from experimental methods, such as ultracentrifugation[1], light scattering[2] and osmometry[3], indicate a minimum value of about 12,000. Some evidence, however, has accumulated for the existence of an insulin molecule of molecular weight of about 6,000 under acidic conditions[4]. This corresponds to the insulin unit the molecular weight of which obtained from the amino-acid sequence is 5,750 (ref. 5). A number of investigators have indeed observed such a value in non-aqueous solvent/water mixtures[6], in media favourable to dissociation[7] and in aqueous solution at alkaline *p*H values[8].

We have measured the apparent molecular weight of insulin in acid solution over a range of concentrations from approximately 0·8 to 0·02 g/100 ml. using the technique of sedimentation equilibrium. A Spinco model *E* ultracentrifuge, equipped with a Rayleigh interference optical system and both 12-mm and 30-mm cells, was used.

The results of our study are shown in Fig. 1, which refers to four different insulin solutions. The insulin was a crystalline preparation containing an unknown amount of zinc, prepared by the Commonwealth Serum Laboratories (batch number *A3*). The solvent in all cases was an aqueous glycine–hydrochloric acid–sodium chloride buffered solution of ionic strength 0·1 and *p*H 2·00. Solutions 1, 2, 3 and 4 had initial concentrations of approximately 0·5, 0·2, 0·1, and 0·05 g/100 ml. respectively and were centrifuged at speeds of 17,980, 17,980, 29,500 and 29,500 r.p.m., respectively, until equilibrium was reached. Since column heights of only 2–3 mm were used equilibrium was reached in less than 18 h. The temperature of the rotor was held constant at 25·0 ± 0·2° C. The four solutions were prepared from a concentrated (1 g/100 ml.) stock solution of insulin that had previously been dialysed for 72 h against a hundred times its volume of buffer with a change of buffer at 48 h. After dialysis the *p*H of both insulin solution and diffusate was 2·04. The refractive index increment of the stock solution compared with the diffusate was determined by means of the refractometer described by Cecil and Ogston[9].

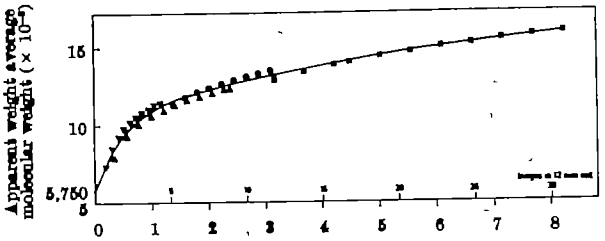

Fig. 1. Apparent weight average molecular weight of insulin as a function of insulin concentration at pH 2·04 and 25° C. ■, Solution 1; ●, solution 2; ▲, solution 3; ▼, solution 4

The four solutions for centrifugation were prepared by weight dilution of portions of the stock solution with diffusate. Diffusate was placed in one compartment of the double sector ultracentrifuge cell. Dow-Corning '555' silicone oil was added to the solution compartment to provide a curved bottom. The equilibrium Rayleigh patterns were photographed and the plates measured on a toolmakers' microscope. Schlieren photographs at 90° bar angle were also taken to facilitate determination of the positions of meniscus and cell bottom. From these results and knowledge of the refractive increment, the equilibrium concentration distribution of solute in the cell was calculated in terms of fringe number (J). By means of a least squares procedure, a quadratic expression for the dependence of $\ln J$ on r^2 was obtained. (r = distance in the cell from the centre of rotation, in cm.) Substitution into the first derivative of the latter expression allowed calculation of the apparent molecular weight for a series of r values and thus for a series of concentrations by means of the expression[10]:

$$M_{\text{app}} = \frac{2RT\text{d}\ln J}{(1 - \bar{v}\rho)\omega^2\text{d}r^2}$$

taking a value of 0·72 (ref. 11) for $\bar{v}$, the partial specific volume.

For a thermodynamically ideal polydisperse system of uncharged macro-molecules for which the refractive index increment depends linearly on the concentration of solute, this equation would yield a weight average molecular weight. These conditions do not strictly hold for insulin and we can consider that M_{app} is only an approximate measure of the weight average molecular weight.

It is apparent from Fig. 1 that over the concentration range studied the apparent weight average molecular weight of insulin is strongly dependent on concentration, ranging from about 7,500 at 0·02 g/100 ml. to about 16,000 at 0·85 g/100 ml. This behaviour is consistent with the belief that under the conditions of our experiments insulin exists in a state of dynamic association. It also appears that in the limit of zero concentration a molecular weight of about 6,000 would be obtained.

Marcker[12] has suggested that the insulin dimer (of molecular weight 12,000), which is the predominant form of the molecule in acid solution at moderate concentrations, is formed by means of a double disulphide bridge between two monomer (of molecular weight 6,000) units, with a disulphide exchange reaction allowing rapid equilibrium between monomer and dimer to occur. He also suggested that the reaction is mediated by mercaptide ions. The results presented here would seem to disagree with the disulphide exchange reaction explanation since the concentration of mercaptide ions would be very low at pH 2·04, yet a rapid, reversible equilibrium appears to exist under these conditions. Furthermore, in experiments with mixtures of cystine and oxidized glutathione at pH 2 Spackman[13] *et al.* have found no evidence for disulphide exchange between these model compounds.

We thank Dr. E. T. Adams, jun., of the University of Wisconsin, for discussing his results with us prior to publication, and Prof. D. O. Jordan, of the University of Adelaide, for his advice.

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BIOPHYSICS

Examination of Optically Excited Amino-acids by Electron Spin Resonance at Very Low Temperature

INVESTIGATIONS carried out in our laboratory[1] on the optical behaviour of serum-albumin protein and of its main constituents have not given any serious evidence of the photo-conductor behaviour of the protein. Things are exactly as if each amino-acid, taking off a fraction of the quantum it absorbs selectively, reacted independently from its partners. The time of radiative deactivation by slow fluorescence is the only factor varying with the association process and the chemical purity of the aggregate.

Combining electron spin resonance with optical excitation, Allen and Ingram recently demonstrated the existence of a metastable state that they considered characteristic of the condensed state of their compound (semiconductivity of hydrated proteins irradiated by ultraviolet at low temperature)[2].

Now combining electron spin resonance and optical excitation we experimented again: we wanted to see whether, under certain conditions, it was possible to obtain a signal for the amino-acid alone. The uniformity of the quantic behaviour observed during the purely optical experimentation then occurred again.

Samples of phenylalanine and tryptophan, in microcrystalline form, were placed in quartz tubes which were vacuum-tapered. Irradiation at temperatures close to that of liquid nitrogen was made directly in the cavity of the 100 kc/s Varian spectrometer for times varying from a few minutes to 1 h, the sources being mercury steam arcs at low pressure (emission maximum at 2537 Å) and at high pressure (emission maximum at 3650 Å). Interferential or chemical filters were added to determine more accurately the spectral zone chosen for examination.

The main results, mostly qualitative, were as follows: (1) Radiations centred on 2537 Å produced a progressive narrow electron spin resonance signal, not yet explained. of a factor g close to 2 (Fig. 1). This signal, obtained at liquid nitrogen temperature, remained after the end of the optical excitation and of the resulting radiative deactivation. It was thermostable and seemed to proceed from a non-reversible radiation damage, analogous to that producing ionizing radiations[3]. Under the conditions of our experiment, the signal was only produced by optical excitation at very low temperatures. Consequently, it seemed to result from the photochemical evolution of excited electronic states, artificially accumulated.

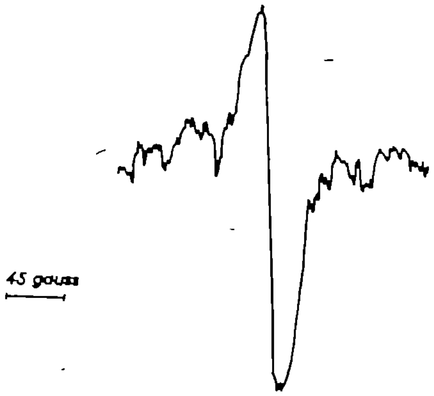

Fig. 1. Irradiation of phenylalanine (λ = 2537 Å)

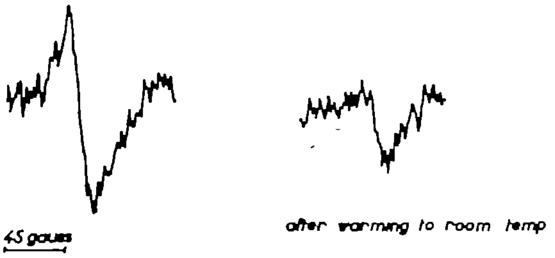

Fig. 2. Irradiation of tryptophan (λ = 3650 Å)

(2) Radiations centred on 3650 Å produced an electron spin resonance signal, similar to the first, conditions being equal. It could be imputed to the intervention of shorter wave-length radiations, insufficiently filtered. But its intensity now varied with temperature: if we heated the sample up gradually, a sharp decline occurred from −150° C. After heating up to normal temperature, a new measurement at −196° C revealed the existence of a residual signal expressing quite certainly an irreversible radiation damage (Fig. 2).

Indeed the chemical purity of our samples could be challenged: the primary importance of this factor in electron spin resonance is well known and especially when the spectrometer was used, as in this case, at a high degree of sensitiveness. But this restriction could be reduced by the reproducibility of the results with amino-acids of different origin and particular care given to the manufacturing of the quartz tubes.

To avoid radiation damage of the experiment with radiations centred on 3650 Å, we tried to improve their selection in limits possible within the sensitivity of the spectrometer.

If, as we hope, the electron spin resonance signals already obtained were specific for amino-acid molecules or for their crystalline state, the following conclusion can be drawn: radiations centred on 3650 Å produce a metastable dynamic configuration, different from the excited configurations producing quick radiative deactivation ineluctibly at −196° C, and also different from thermostable configurations resulting from the photochemical evolution of the preceding ones.

This conclusion accords with the theories inspired by the optical behaviour of amino-acids referred to at the beginning of this communication.

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BIOCHEMISTRY

Actomyosin-like Protein in Mitochondria of the Mouse Liver

Incubation of mitochondria isolated from normal cells or cancer cells liberate two substances into the medium—a lipoprotein and adenine nucleotide. When separated from the mitochondria and added to the soluble cell fraction, these have been found to exert a highly stimulating effect on glyoolysis[1,2]. In the case of mitochondria from normal cells, liberation of both these factors has been shown to depend on reversible changes in configuration of mitochondrial membranes. Pre-incubation of mitochondria with adenosine triphosphate, magnesium ions or ethylenediamine tetraacetic acid—that is, agents provoking shrinkage of mitochondria—proved to diminish or to prevent liberation of the stimulating factors and their retention within the mitochondria. On the contrary, pre-incubation with inorganic phosphate, calcium ions or potassium chloride, known to cause membrane relaxation and swelling of mitochondria, increased the release of the stimulating factors into the medium[1,2].

This evidence led to the assumption that release of glycolysis-stimulating factors within the cell was related to functioning of the enzymatic contractile system of mitochondrial membranes[1,2]. On the other hand, it had been suggested by Lehninger that intermediate enzymes of the coupled mechanism of respiratory phosphorylation, occurring in mitochondrial membranes, represented 'mechanoenzymes', similar to actomyosin[4]. This prompted our attempt to isolate actomyosin from mitochondria, the origin of which was not muscle tissue.

After mechanical disruption, mouse liver mitochondria were extracted with Weber's solution at 0° for 24 h. The extracted protein displayed considerable ATPase activity, increasing on the addition of dinitrophenol, magnesium ions or flavin adenine dinucleotide. The Weber extract was used in experiments with polymerization of actomyosin. Owing to the protein content and viscosity being low in the extract, we proceeded to determine the presence of actomyosin on the addition of extraneous myosin *A* as 'primer'. Rabbit muscle myosin *A*, added to the Weber extract of liver mitochondria, caused a drastic increase of viscosity (more than 5-fold). Fig. 1*A* shows viscosity of the resulting complex to be much higher than the total viscosity of its components. Addition of ATP to the solution caused the complex to dissociate and protein viscosity to diminish. Dissociation of the complex proved to be reversible, so that demonstration of the polymerization–dissociation reaction could be repeated with the same solution (Fig. 1*B*). When the actomyosin formed had been dropped into water, the addition of ATP produced a different mechanical effect—intense retraction of the gel and extrusion of water, apparent to the naked eye (Fig. 2). Addition of muscle actin to the extract from mitochondria failed to raise its viscosity.

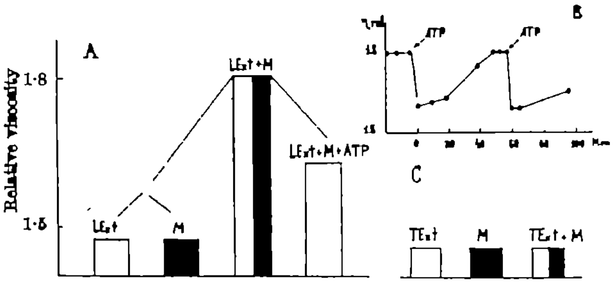

Fig. 1. Variations in relative viscosity with actomyosin polymerization dissociation. *A*, Extract from liver mitochondria (*LExt*), myosin *A* (*M*) and their mixture. Protein content: *LExt* 1 78 per cent; *M*, 0 062 per cent; KCl, 0·6 M; pH, 7 0; temperature, 22° C. *B*, Same; arrows indicate addition of ATP, 1 mM. *C*, Extract from cancer cell mitochondria

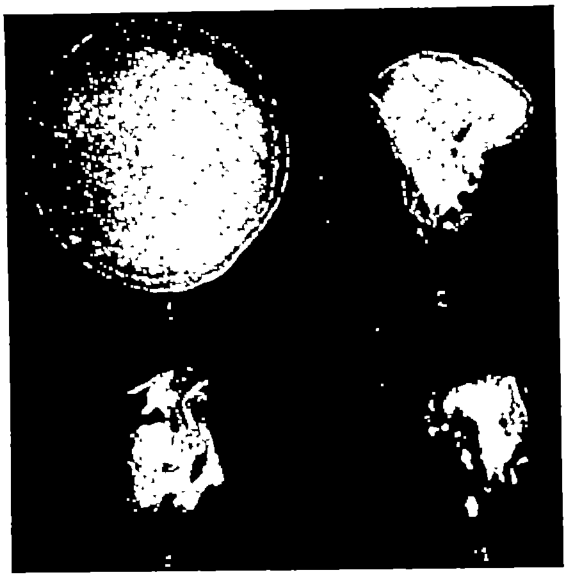

Fig. 2. Synaeresis of actomyosin–water gel exposed to ATP (× c. 4·5)

Although the actomyosin complex has only been revealed on addition of exogenous myosin *A*, it seems reasonable to consider the complex to be performed within the mitochondria, as: (*a*) polymerization occurs on the addition of myosin *A* in a very small quantity insufficient for forming a complex even with muscle actin; (*b*) the structural protein, extractable from mitochondria, displays ATPase properties, being activated by the same substances as the ATPase from actomyosin.

The presence of an actomyosin like protein in liver mitochondria must play a part of outstanding importance in their functioning. Owing to the reversibility of changes in configuration of actomyosin molecules, interaction between actomyosin and ATP may be accompanied by two correlated processes in mitochondrial membranes. In the first place respiratory energy coupling intermediate enzymes assume a kinetically favourable spatial distribution, promoting high rate of synthesis of ATP. On the other hand, this may affect regulation of the release from mitochondria of the glycolysis-stimulating factors and in all probability of other agents as well. When the ATP-level within mitochondria is high, stimulating factors are maintained by the actomyosin bond preventing their release from mitochondria, so that glycolysis fails to occur. As the ATP-level decreases, the configuration of the actomyosin molecule changes, the stimulating factors are released from the bond and liberated into the hyaloplasm, glycolysis sets in, resulting in restoration of a normal intracellular ATP-level.

Attempts to detect the actomyosin-like protein in mitochondria from cancer cells have failed (Fig. 1*C*), although tumour cell mitochondria display marked contractility. It may be assumed that, in tumour cells, mitochondrial membranes, as well as external membranes[5], have a genetically determined abnormal structure, their permeability being independent of contractility.

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Interaction of Œstrogens labelled with Carbon-14 with Pyridine Nucleotide in the Presence of Peroxidase

THE discovery by Stotz *et al.*[1,2] that the injection of physiological doses of œstrogens into ovariectomized rats produces large increases in the activity of uterine peroxidase and that several tissues with high rates of cell renewal also have high peroxidase activity has aroused interest in the possible role of this enzyme in cellular proliferation and growth. In work[3,4] on horseradish peroxidase, it has already been shown that this enzyme could catalyse the interaction between œstrogens and certain amino-acids and proteins under aerobic conditions, and this communication deals with a similar reaction involving the pyridine nucleotides.

(16–^{14}C) œstrone (310,000 c.p.m. in 10 μg) dissolved in 5 ml. phosphate buffer *p*H 7·4 was incubated under oxygen with DPNH (5×10^{-4} M), manganese chloride (10^{-4} M) and horseradish peroxidase (10 μg), for 24 h at 37° C, and the amount of radioactivity remaining in the aqueous medium after extraction with ether was determined. This was repeated under various experimental conditions and with different compounds. In addition, each ethereal fraction was examined for œstrone or its metabolites by chromatography followed by autoradiography as described previously[5]. The anaerobic incubation was carried out in an evacuated Thunberg tube.

The results in Table 1 show that horseradish peroxidase in the presence of DPNH or TPNH is able to convert œstrone (and other phenolic œstrogens) into water-soluble, ether-insoluble compounds in relatively high percentage yields. The ether extracts contained several unidentified radioactive metabolites and virtually none of the added œstrogen remained intact. No products of either kind were formed if the reduced pyridine nucleotides were omitted, showing that DPNH and TPNH are directly involved in the reaction and are not merely acting as water-soluble carriers.

Table 1. PERCENTAGE CONVERSION OF ^{14}C-ŒSTRONE TO WATER-SOLUBLE ETHER-INSOLUBLE PRODUCTS BY PEROXIDASE IN THE PRESENCE OF PYRIDINE NUCLEOTIDES UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental conditions	Percentage of added radioactivity remaining in aqueous medium after extraction with ether
Normal incubation*	39·0 43·5
DPNH omitted	1·1
MnCl$_2$ omitted	25·6 30·6
Incubated for 1 h	16·2
Incubated for 5 h	25·7
TPNH substituted for DPNH	38·4
DPN or TPN substituted for DPNH	1·2 1·6
Peroxidase solution boiled	0·7
Anaerobic incubation	1·4
Incubated with catalase (100 μg)	0·5
Incubated with H$_2$O$_2$ (10^{-4} M)	41·7
MnCl$_2$ omitted and incubated with H$_2$O$_2$ (10^{-4} M)	40·8
DPNH replaced by 'related' compounds† (2 mg) and incubated with H$_2$O$_2$ (10^{-4} M)	2·5
^{14}C-œstradiol substituted for œstrone (5 h incubation)	29·4
^{14}C-diethylstilbœstrol substituted for œstrone	31·0
Incubated with uterine 'oxidase-peroxidase' preparation (5 mg)	0·8

* Incubation mixture: peroxidase, 10 μg, œstrone, 10 μg, MnCl$_2$, 10^{-4} M; DPNH, 5×10^{-4} M, in 5 ml. Conditions as described in text.

† DPN, TPN, ATP, RNA, DNA, nicotinamide, adenine, guanine, thymine, cytosine, uracil.

No changes occurred under anaerobic conditions or if the peroxidase solution was boiled, and catalase was found to be strongly inhibitory, suggesting that hydrogen peroxide is involved in the reaction. In addition, the lower yield of water-soluble products obtained when the incubations were carried out in the absence of manganese could be restored by the addition of hydrogen peroxide (10^{-4} M). It appears, therefore, that horseradish peroxidase acting as an aerobic oxidase can utilize DPNH or TPNH to generate hydrogen peroxide and that afterwards, behaving like a true peroxidase, it can convert œstrone to metabolites which are either water-soluble themselves or capable of combining with the pyridine nucleotides. However, DPN or TPN could not replace DPNH or TPNH in the reaction in the presence of added hydrogen peroxide, and this finding deserves further investigation. A number of other compounds having features in common with the nucleotides (see footnote to Table 1) were tested but none had any effect.

Only when water-soluble products were formed could compounds other than the added œstrogen be detected in the ether extracts and these were all found to be more polar than œstrone and, with the exception of one compound, more polar than œstradiol-17β. Very little, if any, of this last steroid was formed.

Similar incubations with a lyophilized preparation (5 mg) of œstrogen-stimulated rat uteri, which in the presence of manganese and 2,4-dichlorophenol was shown to have high DPNH oxidase activity, did not result in the formation of water-soluble products from œstrone. In addition, this material did not give a positive reaction[6] with guaiacol, which suggests that the oxidase and peroxidase activities of œstrogen-stimulated rat uteri may be separate in contrast to horseradish peroxidase where both functions appear to be intimately connected. Hollander and Stephens[7] found that these two enzymatic activities were lost from uterine homogenates at different rates on storage, but their results indicate that the oxidase was more labile than the peroxidase.

Finally, it should be mentioned that the results with horseradish peroxidase are not in conflict with those obtained by Williams-Ashman *et al.*[8], who managed to recover virtually all their added œstradiol unchanged after incubation under similar conditions, since their experiments were of relatively short duration.

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Interference by Ammonium Sulphate with the Estimation of Proteins by the Biuret Reaction

THE procedure of Gornall, Bardawill and David[1] is widely used for the estimation of proteins, the only frequent modification being adjustment of the volumes to permit mixing of reagents and reading of the extinction directly in the cells of a spectrophotometer. Gornall, Bardawill and David[1] state that 'ammonium ion is a disturbing factor in the biuret reaction', but provide no quantitative data. Some information on the interference has been provided by Henry, Sobel and Berkman[2]; but these workers used two biuret reagents of different composition and instead of a pure protein used serum as a standard. The extent of the interference of ammonium ion with the biuret reaction does not appear to be widely known. Thus, Dixon and Webb[3] claim that "few of the non-protein materials likely to be present affect the estimation, and it is particularly useful because it is insensitive to ammonium salts".

Crystalline egg albumin (Sigma Chemical Co., St. Louis 18, Mo.) was dissolved in 0·15 M sodium chloride to a concentration of 10 mg/ml. Biuret reagent was prepared according to Gornall, Bardawill and David[1]. Protein solution (0·2 ml.), freshly filtered, saturated (20°–22°) ammonium sulphate and sufficient 0·15 M sodium chloride to bring the volume to 1·5 ml. were mixed and 1·5 ml. biuret reagent was added. The mixtures stood for 30 min at 20–22°. The extinction at 540 mμ was determined in a Shimadzu *QR* 50 spectrophotometer, using a blank consisting of 1·5 ml. 0·15 M sodium chloride and 1·5 ml. biuret reagent. The extinction values for duplicate tests with constant protein content and varying final concentrations of ammonium sulphate are shown in Table 1.

Table 1

Concentration of ammonium sulphate in final reaction mixture* as Per cent saturation	 Molarity	Extinction at 540 mμ of duplicate tests
0	0	0·191, 0·190
1	0·04	0·192, 0·191
2	0·08	0·185, 0·185
3	0·12	0·175, 0·175
4	0·16	0·151, 0·150
5	0·20	0·030, 0·028
6	0·24	0·033, 0·030

* 2 mg of protein per test.

It is clear from the results in Table 1 that a concentration of 0·08 M ammonium sulphate in the final reaction mixture already interferes appreciably with the recovery of protein, even though the level of protein selected for the tests was near the upper limit of the range in which extinction at 540 mμ is linearly related to the concentration of protein.

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Composition of Normal and Pathological Cutaneous Elastin

ELASTIN, a fibrous protein invariably associated with collagen[1,2], has been quantitatively measured in a variety of mammalian tissues[3,4]. The amino-acid composition of elastin prepared from various animal sources including aorta[5], lung[6] and ligamentum nuchæ[7] has been reported and the elastin content of human dermis has been determined[8,9]. The purpose of this work was to determine the amino-acid composition of human dermal elastin and compare it with that of the elastin-like proteins found in increased amounts in chronically sun-damaged skin (actinic elastosis) and pseudoxanthoma elasticum[8].

Human skin was obtained at necropsy from two Caucasian adults. Actinic elastotic skin was removed under local anæsthesia from the forearm of two volunteers with a Brown dermatome (obtained from Dr. C. C. Tindall, Kissimmee, Florida). Pseudoxanthoma elasticum skin was obtained from a 46-year-old White female who died of a cerebral vascular accident with progressive deterioration (obtained from Dr. S. Polt, Duke University Medical Center). All hair was carefully removed by shaving and subcutaneous fat trimmed away. Skin specimens were frozen in liquid nitrogen, ground in a Wiley mill, and extracted three times with 0·1 N sodium hydroxide in boiling water bath for 15 min[8]. Between the first and second extractions the skin specimens were ground in a glass homogenizer. The suspensions were centrifuged at 20,000*g* and the residues air-dried on filter paper after washing with acetone and ether. Aliquots were desiccated in a vacuum oven for 3 h and then *in vacuo* over phosphorus pentoxide at 110° C for dry-weight determination. Duplicate aliquots of pathological elastin were hydrolysed under nitrogen for 24 h at 110° C with redistilled 6 N hydrochloric acid. Duplicate aliquots of control samples were hydrolysed *in vacuo* at 110° with redistilled 6 N hydrochloric acid for 24, 48 and 72 h. All amino-acid analyses were carried out on a Spinco model 120 automatic amino-acid analyser. Total nitrogen was determined by micro-Kjeldahl as well as by combustion; total ash was determined by combustion (performed in the Galbraith Laboratory, Knoxville, Tennessee). Amide nitrogen was determined after hydrolysis at 100° and atmospheric pressure for 2 h, with N hydrochloric acid followed by steam distillation and titration.

Table 1. AMINO-ACID COMPOSITION OF HUMAN DERMAL ELASTIN

Amino-acid	g Amino-acid/ 100 g protein	g Amino-acid residue/100 g protein	Nitrogen as as percentage of total
Alanine	25·10	20·02	23·02
Arginine	1·01	0·91	1·90
Aspartic acid	0·61	0·53	0·37
Glutamic acid	3·39	2·97	1·87
Glycine	28·85	21·93	31·40
Hydroxyproline *	1·66	1·43	1·02
Isoleucine	4·04	3·48	2·50
Leucine	8·57	7·39	5·29
Lysine	1·20	1·06	1·30
Phenylalanine	4·06	3·62	1·98
Proline	15·02	12·67	10·61
Serine	1·53	1·27	1·16
Threonine	1·65	1·40	1·11
Tyrosine	4·55	4·10	1·98
Valine	17·43	14·75	12·10
Amide †	0·27		1·32
Total	118·94	97·53	98·94

Values are extrapolated to zero time or maximal for 24, 48 and 72 h hydrolysis.

* Determined separately by the method of Neuman and Logan (ref. 13).

† See text for details of assay.

Table 2. RESIDUES PER 1,000 TOTAL RESIDUES

	Normal human dermal elastin	Actinic elastosis	Pseudo-xanthoma elasticum	Bovine ligamentum nuchae *
Alanine	232	230·5	212	237
Glycine	319	330	309	360
Valine	123	108	106	135
Leucine	54·2	60·8	61·6	69
Isoleucine	25·5	24·7	26	29·5
Proline	108·5	99·4	98	111·7
Phenylalanine	20·4	21·6	25·7	37
Tyrosine	20·1	9·2	22·5	8
Serine	12·1	18	19	13·5
Threonine	11·5	16·5	15·5	13
Arginine	4·8	7	24	8
Lysine	6·7	13	3·5	3·5
Aspartic acid	3·8	15	12	8
Glutamic acid	23·5	54	55	31
Hydroxyproline †	10·5	10·5	10·5	12
Amide	13·4	—	—	—

Amino-acid composition of normal and pathological human dermal elastins. Elastotic and pseudoxanthoma elasticum values are average of two samples. Controls represent best or extrapolated values from 24-, 48- and 72-h duplicate hydrolysis.

* Analysis reported by Partridge and Davis (ref. 7).

† Performed separately by the method of Neuman and Logan (ref. 13).

Kjeldahl nitrogen on a dry-weight basis gave 16·20 and 16·70 per cent for two control samples. Total nitrogen by combustion gave 16·04, 16·20 and 16·08; 16·04 on duplicate samples of two control specimens. Ash was 3·32 and 3·28 per cent respectively. Amino-acid analyses in all instances gave 95 per cent or better nitrogen recovery by weight of material put on the analyser; typical results are presented in Table 1. Table 2 summarizes the results and compares these results with published analyses on bovine ligamentum nuchae elastin[7].

The identity of the abnormally increased dermal fibres stained by classic elastic tissue stains in actinic elastosis and pseudoxanthoma elasticum has been the subject of considerable controversy[1,2,10]. The non-extractable collagen (insoluble collagen) in actinic elastosis has reduced amounts of proline and hydroxyproline relative to nitrogen suggesting that it is degraded[11]. In pseudoxanthoma elasticum, however, there is a slight reduction in the total collagen, but no evidence for degradation[12]. The fibrous proteins isolated in this work by hot alkali extraction of normal human skin and human skin involved with actinic elastosis and pseudoxanthoma elasticum have quite similar amino-acid compositions. These amino-acid compositions are also quite similar to elastins from other sources prepared differently. Based on solubility characteristics, amino-acid composition and elastase susceptibility[8,12] it seems unlikely that the elastin-like proteins found in actinic elastosis and pseudoxanthoma elasticum are derived from collagen.

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Glucuronide Formation and Transport of Various Compounds by Gunn Rat Intestine *in vitro*

THE ability of the mucosa of the gastrointestinal tract to form glucuronides has been previously reported[1-3]. The function of such conjugate formation in the intestinal mucosa has not yet been clarified. It has been variously suggested that glucuronide formation by this tissue may play an important part in detoxification[3], in mucopolysaccharide formation[4], or in the absorption of various substances from the intestinal lumen[5,6]. A genetically determined deficiency of the enzyme responsible for the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to various acceptors has been detected in the livers of homozygous Gunn rats[7,8]. The work reported here was undertaken to determine whether there is a similar deficiency of this enzyme (UDP transglucuronylase) in the intestine of the Gunn rat. The results provide further evidence for the similarity between

Table 1. GLUCURONIDE FORMATION BY PREPARATIONS OF SMALL INTESTINAL MUCOSA FROM NORMAL AND GUNN RATS *in vitro*

Source of intestinal mucosa			*O*-Aminophenol glucuronide formed (μM/g)	4-Methyl umbelliferone glucuronide formed (mμM/mg nitrogen)
Normal Wistar rat				
Mucosal	stripping	(10)*	0·063 ± 0·021	68 ± 19
	homogenate	(8)	0·007 ± 0·002	28 ± 12
Fractions	nuclei	(6)	0	0
	mitochondria	(6)	0·001	10
	microsomes	(6)	0·004 ± 0·002	18 ± 8
	supernatant	(6)	0	0
Gunn rat				
Mucosal	stripping	(10)	0·004 ± 0·002	10 ± 6
	homogenate	(8)	0	0
Fractions	nuclei	(6)	0	0
	mitochondria	(6)	0	0
	microsomes	(6)	0	0
	supernatant	(6)	0	0

* No. of animals.

Incubation media. For strippings, substrate plus 0·05 M *tris* buffer, *p*H 7·8 and 0·02 M glucose; for homogenates and cell fractions, substrate plus 0·025 M $MgCl_2$, 0·025 M *tris* buffer, *p*H 7·8, UDPGA $2 \cdot 1 \times 10^{-3}$ M and EDTA 0·001 M.

Table 2. TRANSPORT OF VARIOUS COMPOUNDS BY EVERTED SACS OF NORMAL AND GUNN RAT INTESTINE

	Triiodothyroacetic acid		Testosterone		L-Monoiodotyrosine	
	Gunn	Control	Gunn	Control	Gunn	Control
Radioactivity in / Radioactivity out	1·55	2·97	1·5	2·6	11·4	9·4
Moles transported/0·2 ml./100 mg	$1·1 \times 10^{-8}$ M	$2·35 \times 10^{-8}$ M	$1·8 \times 10^{-8}$ M	$2·9 \times 10^{-8}$ M	$7·6 \times 10^{-8}$ M	$6·0 \times 10^{-8}$ M
Moles glucuronide/100 mg	$0·9 \times 10^{-8}$ M	$2·1 \times 10^{-8}$ M	$0·8 \times 10^{-8}$ M	$2·5 \times 10^{-8}$ M	$0·96 \times 10^{-8}$ M	$1·1 \times 10^{-8}$ M

Incubation medium: 0·134 M NaCl, 0·011 M KCl, 0·008 M sodium phosphate buffer pH 7·4, 0·0001 M $CaCl_2$, and 0·02 M glucose, ^{131}I-labelled triiodothyroacetic acid, ^{131}I-labelled L-monoiodotyrosine, and ^{14}C-labelled testosterone, each was added at a concentration of 2×10^{-8} M.

glucuronide synthesis in the intestinal mucosa and liver[3] and for the importance of glucuronide formation in the transport of various substances by rat intestine *in vitro*.

Various preparations of mucosa from normal and Gunn rats were examined for their ability to form glucuronides of *O*-aminophenol and of 4-methylumbelliferone. Male Wistar and homozygous Gunn rats weighing 100–120 g were fed until they were killed by decapitation. The small intestinal mucosa was stripped by scraping and 75–150 mg of tissue was incubated with either 0·2 μM of 4-methyl umbelliferone or 0·55 μM of *O*-aminophenol (in 0·01 M ascorbic acid) for 60 min at 37·5° C in 5 per cent carbon dioxide : 95 per cent oxygen. Whole homogenates and cell fractions of mucosa were prepared in 0·25 M sucrose and aliquots were incubated with the same two substrates for 40 min at 37·5° C in an atmosphere of nitrogen. 4-methyl umbelliferone glucuronide was estimated by a fluorometric technique[9] and *O*-aminophenol glucuronide by the method of Levvy and Storey[10]. The results are presented in Table 1. All the preparations from mucosa of Gunn rats had a markedly reduced ability to form glucuronides. Following homogenization of strippings of normal mucosa, glucuronide synthesis was reduced by about 85 per cent. An increased UDPGA requirement following homogenization probably results from hydrolysis of UDPGA by UDPGA pyrophosphatase[11]. Ethylenediamine tetraacetic acid was found to retard the hydrolysis of UDPGA as previously noted by Pogell and Leloir[12].

The active transfer of a number of substances from the mucosal to the serosal surface of everted sacs of rat intestine has been shown to be dependent on glucuronide conjugation[5,6,13]. With a number of thyroxine analogues, such as triiodothyroacetic acid (TRIAC), an ethereal glucuronide is formed, and all the radioactivity accumulating in the serosal medium is in the form of this conjugate[5]. With testosterone, the accumulation of radioactivity in the serosal medium results from the formation of a glucuronide of 3-α,17β-androstanediol[13]. In the case of the amino-acid L-monoiodotyrosine (MIT), 5–30 per cent of the radioactivity in the serosal medium can be recovered as an ethereal glucuronide of MIT. It has been suggested that the formation of a labile *N*-glucuronide may also be involved in the transport of this amino-acid although no direct evidence of such conjugation has been obtained[5,6]. The ability of sacs of Gunn rat intestine to transport these various substances has been examined to test further the hypothesis that their active transfer is dependent on glucuronide conjugation.

Everted sacs of normal and of Gunn rat intestine were incubated with TRIAC, MIT, or testosterone as previously described[5,6,13]. Glucuronide production was estimated following separation by paper chromatography (using secondary butanol/ammonia (150 : 50) for TRIAC and MIT and Bush B_1 solvent system for testosterone). The results are presented in Table 2. Using both TRIAC and testosterone, the final ratio of radioactivity in the serosal medium to that in the mucosal medium of sacs from Gunn rats was less than 50 per cent of normal. With MIT, on the other hand, the concentration gradients were consistently greater with sacs from Gunn rats although the quantity of phenolic glucuronide of MIT which could be detected in these sacs was less than normal. These results do not resolve the question of whether the formation of labile *N*-glucuronides is involved in the transport of amino-acids, since the ability of Gunn rats to form *N*-glucuronides has recently been shown to be normal[14].

These investigations provide support for the view that the biosynthesis of glucuronides in intestinal mucosa is similar to that in liver. They also provide further evidence that glucuronide formation plays a major part in the active transfer of thyroxine analogues and certain steroids by the mucosal cell.

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Solvent-Induced Changes in the Absorbancy of Isolated Chloroplast Fragments

IN a previous paper[1], we described changes in absorbancy which occur when leaves of green plants are subjected to certain alcoholic or acetonic treatments. In order to obtain further evidence as to the nature and possible significance of these changes in absorbancy, this work has been extended to a treatment of isolated chloroplast fragments.

Chloroplasts were prepared from leaves of *Nicotiana tabacum* (Habana 38) according to the method described by Arnon *et al.*[2]. The final pellet containing the 'purified' chloroplasts was then suspended in 0·05 M phosphate buffer at *p*H 6·7, precipitated by centrifugation, and resuspended in the same buffer. The resulting suspension was checked by microscopic examination to ascertain a low starch and debris contamination. Such preparations gave essentially the same response to alcoholic treatment after as long as 12 days' storage at 2 to 4° C. Absorbancy measurements were made in a recording Beckman *DK*-2 spectrophotometer at room temperature. The absorb-

Table 1. CHANGES IN THE HEIGHTS OF ABSORBANCY MAXIMA OF CHLOROPLAST FRAGMENTS AT 662–678 mμ AND 740 mμ AS A FUNCTION OF TIME OF SEVERAL TREATMENTS

Time in min	Ethanol 52% (v/v)		Methanol 65% (v/v)		Methanol 65% (v/v) + $CuCl_2$*		Boiled, then methanol 65% (v/v)		Methanol 75% (v/v)	
	662–78	740	662–78	740	662–78	740	662–78	740	662–78	740
0	0·300	0·000	0·290	0·000	0·280	0·000	0·300	0·000	0·290	0·000
15	0·090	0·090	0·090	0·110	0·270	0·000	0·300	0·000	0·090	0·000
30	0·070	0·120	0·060	0·140	0·250	0·000	0·300	0·000	0·060	0·000
45	0·050	0·150	0·050	0·150	0·250	0·000	0·300	0·000	0·050	0·000
60	0·040	0·180	0·040	0·160	0·250	0·000	0·300	0·000	0·050	0·000
75	0·040	0·180	0·040	0·160	0·250	0·000	0·300	0·000	0·060	0·000
Equal vol. of ethanol added†	0·290	0·000	0·295	0·000	0·280	0·000	0·295	0·000	0·300	0·000

* 5×10^{-4} M cupric chloride.

† Absorbancies multiplied by the dilution factor so that values are directly comparable with preceding measurements. Ethanol added following the 75-min treatment.

ancies were corrected for light scattering as previously described[1].

When redistilled methanol is added to a chloroplast preparation at room temperature to a concentration of 65 per cent (v/v), results such as shown in Fig. 1 are obtained. After a short lag, the main absorbancy band of chlorophyll in the red region decreases within 1 h to a value often representing as little as 15–20 per cent of the original peak absorbancy. Results of a similar experiment are shown in Table 1. After about 30 per cent of the absorbancy of the chlorophyll in the red region has been lost, a new absorbancy maximum at a wave-length near 740 mμ begins to appear. At the completion of this reaction, as much as 30 per cent to 50 per cent of the original chlorophyll absorbancy has thus shifted. None of these changes is observed with methanol concentrations above 80 or below 60 per cent. Similar results are obtained with ethanol at a concentration of 52 per cent. The rate of appearance and amount of 740-mμ absorbing material formed is affected by *p*H. At *p*H 5 or below little 740 mμ absorbing material is formed, while a *p*H of about 8·5 appears optimum.

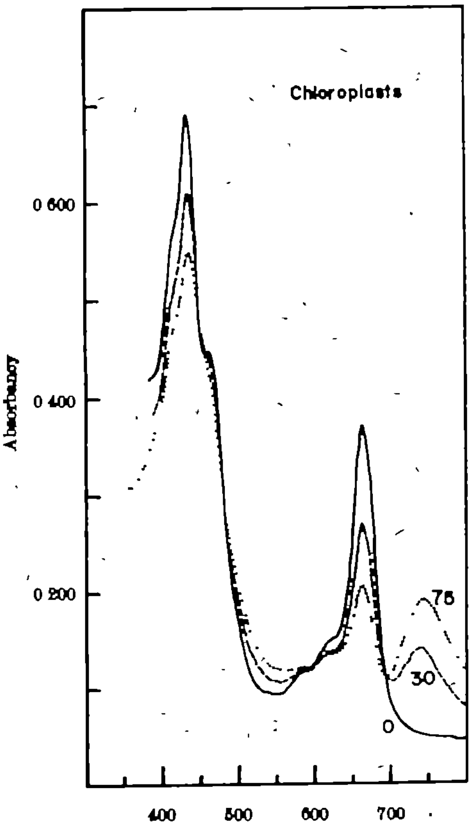

Fig. 1. Changes in absorbancy as a function of time when isolated chloroplast fragments are suspended in 65 per cent methanol: solid line, absorbancy immediately after addition of methanol; dashed line, 30 min after addition; dotted line, 75 min after addition

At methanol concentrations between 70 and 75 per cent a similar loss in chlorophyll absorbancy near 662 mμ is observed but without the formation of a 740 mμ absorbancy maximum (Table 1). At these concentrations of methanol, the absorbancy of the chlorophyll is shifted from about 678 mμ in buffer alone to 662–663 mμ in the presence of the alcohol. At 65 per cent methanol, where a 740-mμ maximum is formed, the chlorophyll absorbancy maximum is shifted to only about 670 mμ. Since the absorbancy of chlorophyll at these higher wave-lengths is due to its binding in the plastid structure[3], these results indicate that the changes resulting in the formation of the 740 mμ absorbancy maximum proceed without completely extracting the chlorophyll from its natural environment.

The validity of this conclusion was demonstrated in the following way. Chloroplast fragments were suspended in 75 per cent methanol and allowed to bleach (Table 1). These mixtures were then centrifuged at 2,500*g* for 15 min and the precipitate suspended in 65 per cent methanol. The addition of ethanol to the supernatant and to a portion of the resuspended precipitate indicated that most of the 'bleached' chlorophyll precipitated with the chloroplast fragments and was, therefore, still in a bound form. The material in the precipitate, however, on resuspension in 65 per cent methanol gives rise to a 740 mμ absorbancy maximum. Thus, chlorophyll remains bound to portions of the chloroplast structure at higher alcohol concentrations than those which are required to produce the 740 mμ absorbancy maximum.

The loss in chlorophyll absorbancy when these chloroplast fragments are suspended in 75 per cent methanol may be as great as 80 per cent of the initial value and is completely reversible. The reversal may be accomplished by increasing the concentration of the organic solvent which brought about the bleaching or by extracting the chlorophyll with any suitable solvent. Since no 740-mμ absorbing material is formed under these conditions, these results demonstrate that a bleached form of chlorophyll is being formed in these mixtures and is reversed by the addition of more solvent. If, following such a treatment in which the chlorophyll absorbancy first disappears and then reappears, the mixture is rediluted with water to an appropriate solvent concentration, the formation of the bleached form of chlorophyll proceeds anew.

An additional change in chloroplast fragments treated with alcohol under these conditions is observed on microscopic examination of the material. By doing particle counts on equal dilutions of these preparations and estimating the size of the particle examined, it is possible to show that the chloroplast fragments from non-treated preparations are three to four times larger than those receiving alcoholic treatment. Such a result is consistent with the requirements for obtaining a crystallization of the materials in these fragments as discussed in a previous paper[1].

Since the formation of a bleached form of chlorophyll appears to be either an oxidation or a reduction of the parent molecule[4], the ability to obtain such a change with a natural constituent of the chloroplast in the presence of methanol might be of considerable importance in the study of the initial stages of photosynthesis. This is true whether this change be mediated through enzymatic action or through direct chemical processes. Some evidence has been obtained which suggests that the formation of this bleached form of chlorophyll in these experiments may be an enzymatic process (Table 1). Chloroplast fragment preparations placed in a boiling water bath for 5–7 min do not show this loss in chlorophyll absorbancy or the formation of 740 mμ absorbing material. The initial chlorophyll absorbancy peak nevertheless shows a shift from 678 mμ to 670 mμ in response to the addition of 65 per cent methanol, just as it does in non-boiled samples. Cupric chloride, at a concentration of 5 × 10^{-3} M or higher, also inhibits the loss of chlorophyll absorbancy and prevents the appearance of material absorbing at 740 mμ. A similar concentration of this salt has been found necessary to inhibit the 'Hill reaction'[4]. No absorbancy changes occur when chloroplast–methanol mixtures are placed at – 26° C. Although the high concentration of alcohol necessary to obtain these changes appears to be very unphysiological, it should be recalled that at least two other enzymes attributed to the chloroplast require similar conditions for activity. They are phosphatidase c[5,6] and chlorophyllase[7].

These results provide conclusive evidence that the solvent-induced changes described in a previous paper are due to chlorophyll and fragments of the plastid structure. The decrease in size of these chloroplast fragments which accompanies the formation of the 740 mμ absorbancy maximum is consistent with the assumption that this change involves a crystallization of the chlorophyll molecules. A reversible bleaching of chlorophyll as a result of these treatments is clearly demonstrated in these investigations. The relation of this bleached form of chlorophyll to the formation of a 740 mμ absorbing band is such that the bleached form may be an intermediate. The effects of boiling, cupric chloride and low temperature suggests that an enzymatic reaction is responsible for the production of this bleaching.

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Argininosuccinic Acid in Germinating Seeds of *Vicia faba* L.

THE ornithine cycle amino-acids have been determined in ungerminated seeds and 6- and 14-day-old seedlings of *Vicia faba* L. grown without an external source of nitrogen.

The amino-acids were identified by: (1) their elution characteristics from 'Zeo-Karb 225' (200–300 mesh, × 9 per cent); (2) their R_F values on two-dimensional paper chromatograms using 80 per cent (w/v) aqueous phenol as the first solvent and *n*-butanol/acetic acid/water (58 : 180 : 20 v/v/v) as the second; (3) colour tests for arginine[3]; argininosuccinic acid[4]; citrulline[5].

Argininosuccinic acid was separated from the free amino-acids of extracts of 500 g ungerminated beans using a 50 × 2·8 cm column of 'Zeo-Karb 225' (200–300 mesh, × 9 per cent), desalted by the method of Mueller[6] and identified using the following criteria.

(1) It was eluted from cation exchange resins as the anhydride forms and emerged as a discrete peak between leucine and tyrosine[7].

(2) Chromatography with 80 per cent (w/v) aqueous phenol gave two spots with ninhydrin, the R_F values of which, 0·27 and 0·47, corresponded to those of 'active' argininosuccinic acid and its anhydride respectively[4].

(3) The anhydride gave a colour in the Jaffe reaction[4].

(4) It behaved as a dicarboxylic amino-acid on paper electrophoretograms using *p*H 6·5 pyridine, acetic acid, water, buffer (10 : 0·4 : 90 v/v/v) and a potential of 12 V/cm (20 m.amp).

(5) It was acid stable, but on refluxing with strong barium hydroxide gave equal amounts of aspartic acid and ornithine[4].

Table 1. CHANGES IN MG NITROGEN IN THE ORNITHINE CYCLE AMINO-ACIDS OF 100 G FREEZE-DRIED UNGERMINATED BEANS, 6- AND 14-DAY-OLD SEEDLINGS OF *Vicia faba*

The amino-acids were determined colorimetrically (ref. 1) after separation on cation exchange resins under conditions similar to those used by Moore and Stein (ref. 2)

Amino-acid	0 days	6 days	14 days
Arginine	1,123	662·32	197·83
Argininosuccinic acid	2·46	6·53	78·84
Citrulline	0	0	62·71
Ornithine	0	0	31·69

In view of these findings, it is of interest that the enzyme argininosuccinase, which reversibly catalyses the formation of argininosuccinic acid from arginine and fumaric acid, has been shown to be present in peas[8], jack bean meal[9], and green pea, ryegrass and wheat[10]. The occurrence of argininosuccinic acid together with the other acids of the ornithine cycle strongly supports the previous suggestion that part, at least, of the ornithine cycle is involved in the metabolism of plants as well as animals.

When arginine labelled with carbon-14 was vacuum-infiltrated into disks of 10-day old cotyledon, subsequent extraction and chromatography of the free amino acids showed that argininosuccinic acid and proline were the first amino acids to become labelled. With increasing length of time of incubation of the tissue with ^{14}C-arginine, ornithine, citrulline, glutamic acid, aspartic acid and finally alanine and γ-aminobutyric acid, became radioactive. The importance of these amino acids in the metabolism of germinating seeds of *Vicia faba* L. will be discussed fully in a later publication.

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Alteration of Steroid Dehydrogenase Synthesis in a Mutant of *Pseudomonas testosteroni*

Marcus and Talalay have described two nicotinamide-adenine dinucleotide (NAD) dependent steroid dehydrogenases obtained from induced culture of *Pseudomonas testosteroni* (American Type Culture Collection 11996)[1]. The α-enzyme catalyses the oxidation of 3α-hydroxy steroids while the β-enzyme reacts with the 3β- and 17β-hydroxy steroids. Extracts of the cells grown in the presence of testosterone exhibit both types of activities. The enzymes may be separated from one another by careful ammonium sulphate fractionation and can then be used as reagents for quantitative determination of their respective substrates.

A culture of the organism was obtained from the American Type Culture Collection in July 1958, and several lots of cells were prepared and assayed by the methods in the reference. Androsterone was used as the type substrate for α-activity, testosterone for β. (The reaction mixture contained 100 μmoles sodium pyrophosphate, *p*H 9·0, 0·5 μmole NAD, 15 μg steroid in 0·1 ml. methanol, 0·1 ml. cell extract in a total volume of 3·0 ml. The control cuvette contained all components except that 0·1 ml. methanol was substituted for the steroid. The reaction was initiated by addition of steroid. Absorbances were read at 30-sec intervals at 340 mμ in a Beckman *DU*.)

From October 1959 until April 1961 stock slants of the organism on basal medium plus testosterone were stored without transfer. When a transfer was made in April 1961 the growth was examined and found to be contaminated with other bacteria. The culture was plated out by dilution and the *Pseudomonas* isolated and recultivated in pure culture. When a batch of cells from this isolate was collected, dried, extracted and assayed for dehydrogenase activity, it was found that the cells were no longer active with testosterone while androsterone activity remained intact.

Repeated transfers of the organism produced cells which, when extracted, showed only the α-activity. After storage of these cultures on slants from April 1961 until October 1962, fresh transfers still produced the same enzyme pattern. Another culture obtained from the American Type Culture Collection in April 1961 and stored on agar slants has continued to yield both enzymes.

In testing, extracts of cells were prepared by grinding 100 mg dried cells with an equal weight of alumina and 5·0 ml. 0·03 M phosphate, *p*H 7·2, 0·001 M ethylenediamine tetraacetic acid. 0·1 ml. of such an extract when diluted 1–10 produced an absorbance change at 340 mμ of approximately 0·010–0·050/min with either steroid. The extracts from the altered cells, while always active on androsterone, never produced any change in absorbance with testosterone, even when undiluted. Extracts of fresh cells from slants showed the same behaviour as the dried cells.

It was concluded from the foregoing observations that a mutant strain had been formed in the contaminated culture which maintained its genetic marker for the lack of the β-activity. Its usefulness seemed apparent in the application of these enzymes as steroid reagents as expressed here.

However, further testing of extracts of the mutant cells, prepared as described here, revealed, to our surprise, distinct activity in dilutions of 1–25 with dehydroepiandrosterone (3β-hydroxy-Δ^5-androstene-17-one), producing an absorbance change of 0·020–0·030/min at 340 mμ. Repeated tests, under identical conditions, with testosterone as the substrate, failed to reveal any activity.

The chance contamination of the dehydroepiandrosterone was ruled out by purity determinations, including chromatography.

These results suggest that the mutant cells contain a β-steroid dehydrogenase, different from, and more highly specific than, that reported previously or that the earlier described β-steroid dehydrogenase contains two components, each specific for the 3β- and 17β-hydroxyl groups respectively. On the other hand, the explanation may be more complicated, for example, the mutant may produce a completely different enzyme such as an isomerase specific for the 3-position and the product may then be subject to the α-steroid dehydrogenase. Finally, there is the remote possibility of elaboration by the mutant of a factor inhibitory for the testosterone enzyme. These matters are presently under study.

We thank Mr. Nick Denny for culturing the cells and performing the isolation of the mutant.

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Zinc Toxicity in Brewer's Yeast

Zinc toxicity is known to reduce the levels of the hæm enzymes cytochrome oxidase and catalase in the rat[1] and in the larvæ of the rice moth *Corcyra cephalonica*[2]. This communication reports observations made on cytochrome oxidase levels in and fermentation by *Saccharomyces carlsbergensis* after growth in media containing toxic concentrations of ionic zinc.

S. carlsbergensis was grown aerobically at 30° C in either Walters and Thistleton's medium[3] (control culture), or in this medium containing 450 μg/ml. zinc as the acetate ('zinc toxic' culture). Preliminary experiments in which growth results were determined turbidimetrically indicated that this concentration of zinc was sufficient to reduce the rate of growth by half. After 24-h incubation the yeast was centrifuged down, washed in and suspended in 5 vol. of de-ionized water.

Studies on oxygen uptake, and aerobic and anaerobic fermentation demonstrated that all three systems were affected by ionic zinc.

Table 1. OXYGEN UPTAKE AND FERMENTATION IN YEAST

	Normal culture	'Zinc toxic' culture
Oxygen uptake (μl. O_2/mg dry weight)*	27·8	16·5
Aerobic fermentation (μl. CO_2/h/mg dry weight)	63·4	28·4
Anaerobic fermentation (μl. CO_2/h/mg dry weight)	32·6	16·0

Manometer vessels contained 1 ml. yeast suspension plus 1 ml. 6 per cent (w/v) glucose in the main compartment, and 0·2 ml. 2 N potassium hydroxide in the centre well for oxygen uptake studies; for aerobic fermentation, potassium hydroxide was removed and replaced with 2 N sulphuric acid; for anaerobic fermentation, 0·1 ml. potassium cyanide was added to the yeast suspension.

* All figures are arithmetic means of levels in three separate cultures.

Cytochrome oxidase assays were performed on a cell-free extract of yeast obtained using a Hughes press. The results demonstrated a decreased level of the oxidase in zinc toxicity.

Table 2. CYTOCHROME OXIDASE LEVELS IN YEAST

	Cytochrome oxidase* (μl. O_2/h/mg protein (ref. 4))
Normal culture	32·1
'Zinc toxic' culture	15·1

Manometer flasks contained the following reaction mixture: 0·2 ml. 0·01 M diaminoethane tetraacetic acid, neutralized to *p*H 7·0 with sodium hydroxide, 0·2 ml. 7 × 10^{-4} M cytochrome *c*, and 0·2 ml. of yeast extract in the main compartment, and 0·2 ml. 5 per cent (w/v) sodium ascorbate in side-arm. The final volume was made to 2·0 ml. with 0·1 M *tris*, *p*H 7·3. Mixture was equilibrated for 10 min, contents mixed, and oxygen consumption measured over 25 min.

* All figures are arithmetic means of levels in three separate cultures.

This communication provides evidence that excessive zinc may interfere with the levels of hæm enzymes in plant as well as animal species.

I thank the Swan Brewery Co., Ltd., of Perth, Western Australia, for supplying cultures, and Prof. E. J. Underwood for his advice.

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PHYSIOLOGY

Influence of the Œstrous Cycle and Ovariectomy on the Phospholipid Content of the Pineal Gland in the Rat

AMONG the various roles attributed to the pineal gland in mammals the most important seems to be its influence on the function of the hypophysis and gonads. However, the results obtained by different investigators are contradictory[1]. In order to obtain some knowledge of the possible relationship between the pineal gland and the gonads, the histological and histochemical picture of the gland was examined during the œstrous cycle and following ovariectomy.

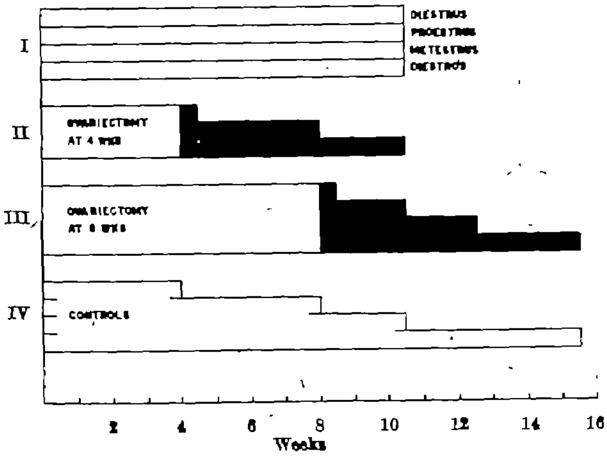

Fig. 1. Outline of the experiments; each bar represents six animals

In the experiments recorded here 4 series of virgin female rats were used (Fig. 1). In series I, 24 animals were killed at the age of 10·5 weeks in different phases of the œstrous cycle: 2 groups of 6 animals at di-œstrous, 6 at pro-œstrous and 6 at met-œstrous. In series II, 18 animals were ovariectomized at the age of 4 weeks and killed in groups of 6 rats each: the first group 3 days, the second 28 days, and the third 45 days after ovariectomy. In series III, 24 animals were ovariectomized at the age of 8 weeks and killed in 4 groups of 6 rats each: the first one 3 days, the second 17 days, the third 31 days and the fourth 52 days after ovariectomy. In series IV, 24 animals, serving as controls to series II and III, were killed at di-œstrous in groups of 6 rats each: the first group at the age of 4 weeks, the second 8 weeks old, the third 10·5 weeks old and the fourth 15·5 weeks old.

The pineal glands were stained with sudan black *B*, after previous controlled chromation[2]. This technique is supposed to be specific for phospholipids. By means of a Zeiss integration eyepiece following the method described by Hennig[3], the phospholipid content expressed as a surface percentage was determined in sections of the pineal gland. The integration eyepiece contains a grid showing a number of testing points. The number of times phospholipid droplets were hit by these testing points was counted, and from this number the surface percentage could be calculated. The way the method has been used in this problem must be looked on as being semi-quantitative. The results of the measurements in series I are plotted in Fig. 2; that of the measurements in series II, III and IV in Fig. 3. For each group of 6 animals the mean value of the phospholipid content pro-pineal gland was calculated and indicated in the graph by a dot. Besides this the highest and the lowest value in a group were also plotted.

As is shown in Fig. 2, just before ovulation the phospholipid content of the pineal gland is lowest. It increases after ovulation, reaching its maximum at di-œstrous. In Fig. 3 the animals of series IV demonstrate that the phospholipid content of the pineal gland decreases wit increasing age, at least during the period of life investigated. Ovariectomy at 4 and 8 weeks gives a steep ris of the phospholipid content though this rise is mor pronounced after ovariectomy at 8 weeks (series III than it is after 4 weeks (series II). Apart from an increase i pineal phospholipids due to ovariectomy, series II an III show that the phospholipid content decreases durin the ageing of the animals. The curves of the ovariectomized animals run fairly parallel to that of th controls.

Although interpretation of the results is rathe premature, some remarks seem worth while. As can b seen from Fig. 1, the obvious fluctuation in the phospholipid content of the pineal gland is linked to the œstrou cycle, that is, to the state of activity of the ovaries. Thi is supported by the fact that ovariectomy invariabl causes an increase in pineal phospholipid content. It remains to be investigated whether this effect is due t either a primary gonadal or a primary hypophysea influence.

Each first group of series II and III was killed 3 day after ovariectomy. It is remarkable that the increas of pineal phospholipids is much less in series II (ovariectomy at 4 weeks) than it is in series III (ovariectom at 8 weeks). The mean values for controls and experimental animals at 4 weeks are 32·72 per cent and 36·2 per cent respectively; at 8 weeks 26·60 and 32·60 per cent the differences being 3·5 and 6 per cent and the ratio being 1·4/1·3 and 1·6/1·3 respectively. Apparently th influence of ovariectomy is less at 4 weeks. Probably this difference is due to the fact that the endocrin apparatus, responsible for reproduction is less activ at 4 weeks than it is at 8 weeks.

As stated before, the curves of the ovariectomize animals and the controls run fairly parallel in Fig. 3 Apart from the animals belonging to series III whic were killed at 10·5 weeks, the mean values of th phospholipid content of the pineal glands of both experim

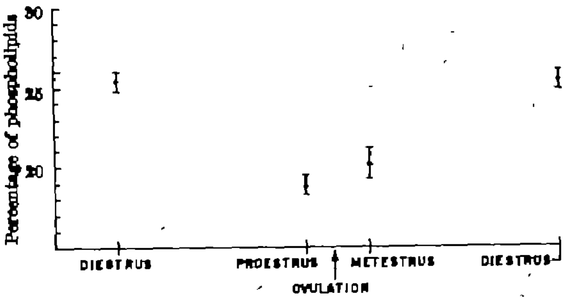

Fig. 2. The pineal lipid content in different phases of the œstrous cycle

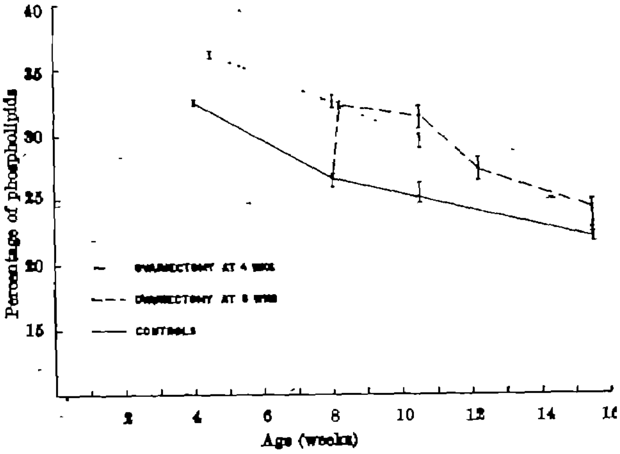

Fig. 3. The pineal phospholipid content after ovariectomy and in control animals

ental series can be connected by an almost straight line. Apparently ovariectomy causes a change in activity of the pineal, as concerns the accumulation of phospholipids, which is not merely due to operation trauma. The results obtained strongly suggest that it is due either to deprivation of ovarian hormones or to overproduction of gonadotrophic hormones, as is shown by the fact that the effect is not a temporary one. The exact interrelation between epiphysis, hypophysis and gonads, as well as the part the phospholipids played in this interrelation, remains still to be investigated.

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Regulation by Calcium of Parathyroid Growth and Secretion *in vitro*

Evidence that the concentration of calcium in the blood directly controls parathyroid activity has been obtained in whole animals[1,2]. Gaillard[3] found that parathyroid glands could be maintained in organ cultures and that the medium in which these glands had been cultivated could stimulate bone resorption. These findings prompted me to examine the effect of changes in calcium concentration in the medium on parathyroid growth and secretion *in vitro*.

Parathyroid glands, dissected from 13-day chick embryos, were cultivated on a layer of agar held at the surface of a fluid medium by a metal screen cut to fit a small embryological watch glass. The culture fluid consisted of 30 per cent rat serum in a chemically defined nutrient medium[4]. Parathyroid glands were also dissected from adult male rats (140–200 g). These were placed on millipore filter and cultured as above except that 50 per cent rat serum in a modified Eagle's medium[5] was used. Concentrations of calcium, inorganic phosphorus, magnesium, and strontium were varied, sodium bicarbonate was 25 m.moles/l., and the explants were cultivated under 5 per cent carbon dioxide, 95 per cent oxygen. After 48 h the glands were fixed in Zenker's fluid and serial sections were cut. These were examined for mitotic rate, cell size, and the ratio of cytoplasmic to nuclear volume (*C/N* ratio) as determined by Chalkley's method[6].

Table 1. Effect of Calcium Concentration in the Medium on Relative Abundance of Cytoplasm (*C/N* Ratio) in Parathyroid Glands

	Medium calcium concentration (m.moles/l.)	No. of glands	*C/N* ratio (mean ± *S.E.*)	*P* value
Exp. 1 Embryonic chick	1·0	4	2·18 ± 0·14	< 0·01
	3·2	4	1·27 ± 0·07	
Exp. 2 Embryonic chick	0·8	4	2·50 ± 0·15	< 0·01
	3·3	5	1·28 ± 0·08	< 0·01
	0·7*	3	2·55 ± 0·09	
Exp. 3 Adult rat (dialysed serum)	0·2	4	1·43 ± 0·12	< 0·05
	0·7	3	1·83 ± 0·04	< 0·01
	2·1	4	1·59 ± 0·04	< 0·02
	2·7	3	1·19 ± 0·04	
Exp. 4 Adult rat	0·9†	4	2·22 ± 0·10	> 0·05
	0·9	7	2·65 ± 0·14	< 0·05
	1·6	4	2·11 ± 0·11	
Exp. 5 Adult rat	1·3	4	3·11 ± 0·06	< 0·01
	3·1	4	2·01 ± 0·15	

* Plus 2·5 mM/l. magnesium chloride. † Plus 3·1 mM/l. strontium chloride.

Although the *C/N* ratio varied in different experiments, in any one experiment it provided an index of the relative stimulation or suppression of cytoplasmic growth by the media used (Table 1). After cultivation in a medium low in calcium concentration, explants of parathyroids from chick embryos and adult rats showed abundant cytoplasm (Fig. 1). A high medium calcium concentration caused a marked decrease in the amount of cytoplasm with but

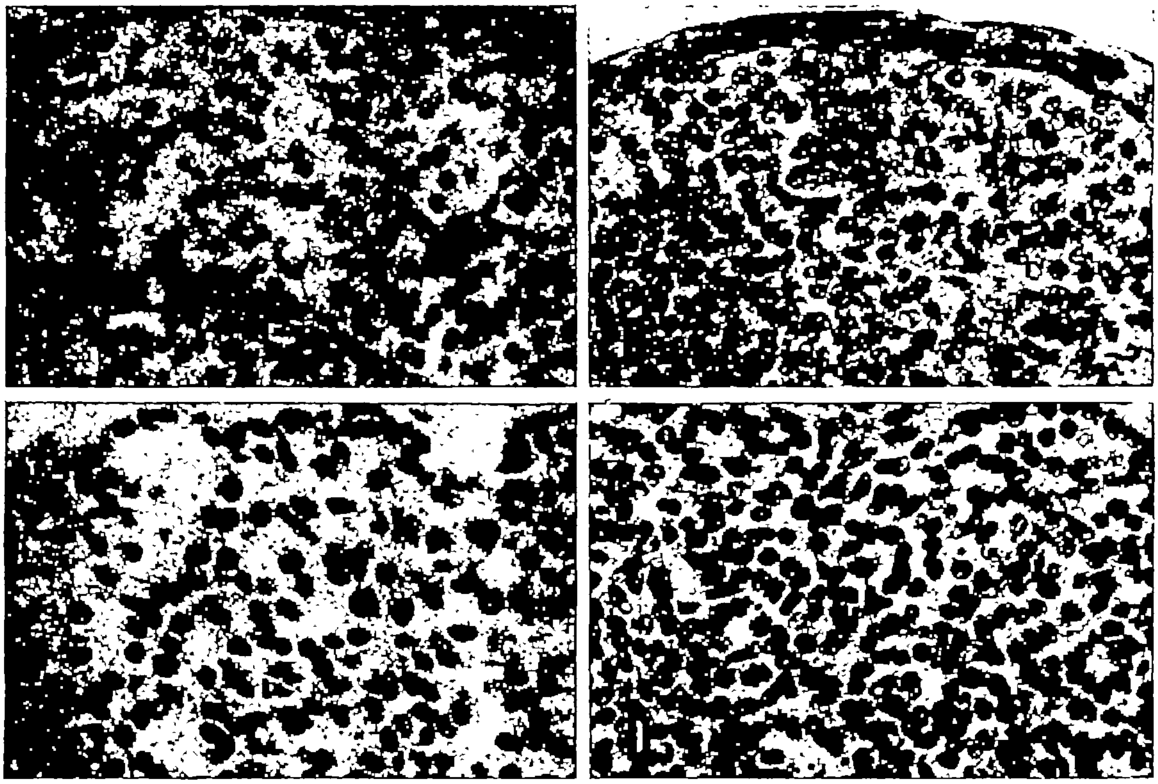

Fig. 1. Effect of low calcium (*A*) and high calcium (*B*) concentration in the medium on paired parathyroid gland cultures from a chick embryo (experiment 1). Note mitoses (arrows) in *A*. Effect of low calcium (*C*) and high calcium (*D*) concentration is also seen in parathyroids from an adult rat (experiment 5). (Hæmatoxylin and eosin stain, × 200)

little change in nuclear size. In embryonic chick glands a significant difference in mitotic rate was observed. In Exp. 1 there were 1·3 ± 0·1 mitoses/1,000 cells in glands cultivated in low calcium medium, compared with 0·6 ± 0·1 mitoses/1,000 cells in high calcium medium. In adult rat glands mitoses were rare. When calcium concentration was decreased to 0·2 m.mole/l. using dialysed serum (Table 1, Exp. 3) cell disruption and a decrease in cytoplasmic volume occurred. At higher calcium concentrations there was an inverse relationship between calcium and *C/N* ratio. The cytoplasm was less abundant in this experiment than in others, perhaps because some growth factor was lost from the serum during dialysis. Changes in calcium concentration did not affect *C/N* ratio in cultures of either embryonic or adult thyroid, kidney, and lung. Increasing inorganic phosphate concentration from 1 to 3 m.moles/l. when calcium concentration was low did not result in further stimulation of the glands. Replacement of the calcium by magnesium to maintain a constant divalent cation concentration did not prevent the low calcium effect (Table 1, Exp. 2). Addition of 3·1 m.moles/l. of strontium had less suppressive effect than a much smaller (0·9–1·6 m.mole/l.) increase in calcium concentration (Table 1, Exp. 4).

The secretion of cultivated parathyroid glands was measured by an *in vitro* assay technique recently developed in our laboratory[7]. The assay is based on the ability of parathyroid extracts to stimulate resorption of embryonic bone in organ cultures. The embryos were obtained from pregnant rats which had been injected with calcium-45 three days prior to death. Paired shafts of 18–19-day radius or ulna were cultivated. Resorption was measured by the release into the medium of the calcium-45 which had previously been incorporated in the embryo bones *in vivo*, and also by the change in stable calcium concentration in the medium at the end of 72-h cultivation. In these experiments adult rat parathyroids were grown in media of 0·9–3·1 m.moles/l. calcium concentrations. At the end of cultivation of the parathyroids, the media were mixed 1 : 1 with fresh control medium of varying calcium concentration to bring the concentration of the mixture to 2·1 m.moles/l. These reconstituted media were then tested against fresh control media which had never been in contact with parathyroid glands (Table 2). Media originally low in calcium (0·9–1·6 m.mole/l.) which had stimulated or incompletely suppressed parathyroid growth were found, when reconstituted, to contain bone-resorbing activity. Medium originally high in calcium which had suppressed parathyroid growth contained no more bone-resorbing activity than did control medium. The active media caused the release of 80–150 per cent more calcium-45 than did control medium. This effect lies between that obtained with 0·1 and 1·0 units/ml. of parathyroid extract[7].

Table 2. EFFECT OF RECONSTITUTED MEDIA FROM PARATHYROID GLANDS ON CALCIUM RELEASE FROM EMBRYONIC BONES

	No. of bones	Medium calcium concentration Initial	Reconstituted (m.moles/l.)	Final	^{45}Ca release (c.p.m./bone)
Medium from Exp. 4	6	0·9	2·1	2·51 ± 0·11*	1,398 ± 99†
Medium from Exp. 4	4	1·6	2·1	2·23 ± 0·05	1,260 ± 80†
Fresh control medium	6	—	2·1	2·21 ± 0·07	553 ± 42
Medium from Exp. 5	6	1·3	2·1	2·25 ± 0·03*	2,052 ± 105†
Medium from Exp. 5	6	3·1	2·1	2·11 ± 0·02	1,158 ± 178
Fresh control medium	6	—	2·1	2·08 ± 0·01	1,150 ± 89

* Significantly different from control, $P < 0·05$.
† Significantly different from control, $P < 0·01$.

The experiments recorded here provide direct evidence that a low concentration of calcium stimulates and a high concentration suppresses the cytoplasmic growth and secretion of the parathyroid glands. This response appears to be relatively specific for parathyroid tissue and for the calcium ion. The system used is a convenient model for analysis of the mechanism by which calcium regulates the parathyroid glands.

This work was begun at the Strangeways Research Laboratory, Cambridge, England, while I was a Special Research Fellow of the U.S. Public Health Service. I thank Dr. Honor B. Fell, director of the Laboratory, for her encouragement and advice.

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Release of Catecholamines from the Atrium of the Dog by Tyramine

IT was suggested in 1958 by Burn and Rand[1] that tyramine acts by releasing catecholamines from tissue 'stores'. Direct evidence to support this has been sought, but with inconclusive results.

In the work reported here, an effort was made to demonstrate the release of endogenous catecholamines from the isolated right atrium of the dog by tyramine. The atrial β receptors for catecholamines were previously blocked by dichloroisoproterenol (DCI). The isolated right atrium of the dog, bathed in balanced salt solution at 31° C, was incubated with tyramine for 15 min. At the end of the incubation period the presence of catecholamines in the bathing fluid was determined by transferring the bath fluid to another tissue-bath containing a spiral aortic strip from a reserpinized rabbit. Suitable controls were devised to exclude the possibility that the response of the aortic strip was due to spontaneous release of a vasoactive substance from the atrium, or to the direct action of tyramine on the arterial strip itself.

The release of small quantities of a vasoactive material by tyramine was repeatedly demonstrated in six such experiments on the atria of the dog (Fig. 1). The constancy of the magnitude of response relative to the

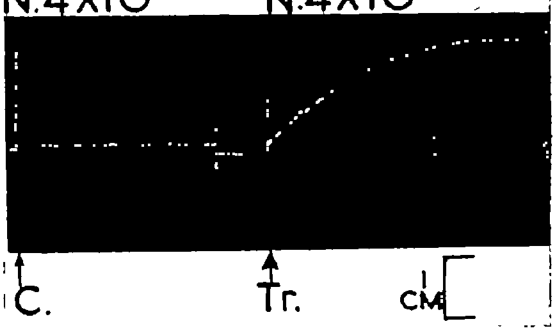

Fig. 1. Responses of reserpinized rabbit aortic strip to (*N*) noradrenaline, (*C*) control and (*Tr*) atrial bath fluid after incubation with tyramine

catecholamine sensitivity of the strip suggests that the vasoactive substance was itself a catecholamine[3].

Cyproheptadine[3] (an anti-histamine and an anti-5-HT agent) decreased greatly the response of the aortic strip to added 5-HT, and abolished the response to added histamine. There was no detectable change in the response to added noradrenaline or to the vasoactive material.

Observations on a rat fundal strip and on an isolated guinea pig ileum indicated that tyramine does not release 5-HT or histamine in amounts adequate to stimulate the spiral aortic strip. The observations on the guinea pig ileum also suggested that a vasoactive polypeptide, for example bradykinin, was not responsible.

It was concluded that the vasoactive material was almost certainly a catecholamine released from the tissue 'stores' of the atrium by tyramine.

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Antiserum Inhibition of the Œstrous Cycle in Normal Rats

Evidence for the neutralization of pituitary gonadotrophic hormone activity in mammals by immunological procedures has been reviewed in several articles. The specificity of the hormone neutralizing (antihormone) effect of the antiserum has been suggested by many of the investigations reviewed, but there has been some question as to the specificity of this effect, because of the possible role of impurities or trace antigenic contaminants in the hormone preparations used[1–4]. A recent report[5] describes the successful inactivation of a purified pituitary follicle-stimulating hormone (FSH); the marked follicular stimulation obtained by the injection of sheep FSH into immature hypophysectomized female rats could be completely inhibited with rabbit antiserum to the homologous FSH. This inhibitory effect was found to be dependent on the dosage of antiserum used. Other recent studies have reported the neutralization of endogenously secreted pituitary interstitial-cell stimulating hormone (ICSH) in the normal immature[6–8] and mature[8] male rat and in the immature female rat[9], with the use of antiserum prepared against purified sheep ICSH.

This is a preliminary report showing that rabbit antiserum to purified sheep ICSH can inhibit the œstrous cycle in adult female rats. Supporting evidence is presented to indicate the specificity of the observed antihormone effect. A detailed report of the inhibition (with the same antiserum) of testicular development and maturation in the normal male rat is now awaiting publication[10].

Antiserum was prepared by a method previously described[8]. Young albino male rabbits were immunized by injecting them subcutaneously with purified sheep ICSH (N.I.H.-*LH*-*S*1, received through the courtesy of the Endocrine Study Section of the National Institutes of Health: this preparation has been fully characterized; the assay data have been presented elsewhere[10]) in Freund's adjuvant, over a period of 3–5 months (total dose of 4–6 mg per animal). The last injection (0·25 mg) was given in saline 8–10 days prior to bleeding. The antiserum was absorbed to remove non-specific antibodies against homologous (sheep) serum proteins.

Before antiserum treatment was started, the vaginal cycling patterns of adult female Long–Evans rats were followed for a period of 16 days. Animals were selected for the experiment on the basis of a normal 4–5 day cycling pattern, as determined by examination of their vaginal smears (stained with Giemsa). The antiserum injections were given in gradually increasing dosages (0·5 ml. to 3·0 ml. per day) over an 11-day period, with an average daily dose of approximately 1·5 ml. All antiserum-injected rats showed sustained dioestrus vaginal smears after the second or third day of treatment. Regardless of the particular phase of the vaginal cycle in which the animal happened to be at the time that the injections were commenced, the antiserum administration resulted in a complete cessation of cycling, stopping it in the same phase in all animals. The vaginal cycling of animals treated with normal rabbit serum (NRS) showed no significant differences from that of the non-treated control animals. It was of interest to note that the antiserum-injected animals commenced to cycle again approximately 10 days after treatment had been discontinued. Histological examination of the ovaries of antiserum-treated rats revealed that the interstitial cells were as deficient in appearance as those in hypophysectomized animals. The recovery of normal vaginal cycling was accompanied by a restoration of the appearance of these cells to normal.

Rats in one of the antiserum-treated groups were injected 24 h before death with 1 µc. of iodine-131 to determine the effect of antiserum treatment on thyroid function. It was found that the uptake of iodine-131 by the thyroid glands from the antiserum-injected rats was not significantly different from that for animals injected with NRS. This finding suggested that the ICSH antiserum did not contain antibodies against thyroid-stimulating hormone, as demonstrable by this method.

There was no evidence to indicate toxicity of the antiserum, as judged by the lack of effect on gain in body-weight and adrenal or thymic weights. If anything, the antiserum administration resulted in a slight but definite increase in body-weight gain. The foregoing findings suggested that the antiserum did not contain any detectable antibodies against rat pituitary growth hormone or adrenocorticotrophic hormone. Still another test which indicated the specificity of the antihormone effect was the observation of precipitin reactions in agar diffusion plates[11]. Using a technique previously described[12], it was found that the minimum detectable amount of the homologous hormone (sheep ICSH, N.I.H.-*LH*-*S*1) was of the order of 2 µg, yet the same antiserum did not give a positive precipitin reaction with varying doses (2–200 µg) of any of the other known anterior pituitary hormones in highly purified form. All hormones had been obtained from the homologous (sheep) species except for thyroid-stimulating hormone which was bovine in origin. The results of the various tests mentioned here strongly suggested that the antihormone effect of the antiserum may be dependent on a specific antigen–antibody interaction. A detailed report of these studies in female rats will be presented elsewhere.

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HÆMATOLOGY

Coagulation of Blood in Temperature Acclimatization

PROLONGED clotting and prothrombin times as a function of lower blood temperature have been observed in various species *in vivo*[1–4] and *in vitro*[5]. The *in vivo* observations were made in æstivating and hibernating mammals[2–4] and with human blood from blood vessels where a differential in temperature had been demonstrated[1]. By contrast, Tadzer[6], using the rat in a study of hypothermia of short duration and uncomplicated by shock, did not find prolonged thrombin times; Lasch *et al.*[7], working with dogs, even obtained shorter clotting times on cooling, and a prolongation, due to an increase in heparin, on rewarming.

Comparatively little has been reported on the effect of prolonged adaptation to a hot or cold environment on blood coagulation, particularly when body temperatures remain near normal. Mills *et al.*[8] reported an increase in hæmorrhagic deaths in vitamin K-deficient rats exposed to the equivalent of tropical heat, as compared with animals kept in temperate coolness. Sutherland and Campbell[9] reported shorter prothrombin, but markedly increased clotting times in cold-adapted rabbits. The investigation reported here discusses the prothrombin and clotting time of adult Leghorn chickens maintained at three different ambient temperatures.

A total of 55 female and 24 male birds, approximately 1 year old, were available. They were kept in individual cages in temperature-controlled rooms at, respectively, 32° C and 60 per cent relative humidity ('hot'), 21° C ('control'), and 0° C ('cold'). In the second and third rooms the humidity was not controlled. At the time of testing all birds had been in their respective environments in excess of three months. Blood samples were taken by venipuncture from a brachial vein with siliconized syringes and whole blood clotting and prothrombin times measured, the latter by a one-stage method, using acetone dehydrated chick brain powder as a source of thromboplastin. Details of the methods have been described elsewhere[10], except that citrate rather than oxalate was used as an *in vitro* anticoagulant.

Table 1. PLASMA PROTHROMBIN AND WHOLE BLOOD CLOTTING TIMES OF ADULT BIRDS KEPT AT THREE ENVIRONMENTAL TEMPERATURES*

Environmental temperature (° C)	Prothrombin time			Blood coagulation time		
	$\text{sec}^{-1} \times 10^4$	sec		$\text{min}^{-1/2} \times 10^4$	min	
Females						
0	612 ± 10†	16·3	(19)‡	29·6 ± 2·6	11·4	(20)
21	600 ± 9	16·7	(17)	29·8 ± 3·1	11·2	(19)
32	568 ± 11	17·6	(19)	28·6 ± 2·0	12·2	(20)
Males						
0	632 ± 9	15·8	(5)	36·6 ± 3·3	7·5	(5)
21	611 ± 10	16·4	(10)	27·6 ± 1·7	13·1	(10)
32	586 ± 19	17·1	(9)	25·0 ± 1·7	16·0	(9)

* Since prothrombin and blood clotting times do not follow a normal distribution, appropriate transformations of individual values were used for all calculations, and mean values (columns 2 and 4) reconverted to conventional values (columns 3 and 5).

† Arithmetic mean ± *S.E.*

‡ No. of birds sampled.

Table 1 shows a general trend towards hypocoagulability with increasing environmental temperature. Plasma prothrombin times of females kept at 32° C were significantly longer ($P < 0·01$) than at the control and cold environment. Equivalent changes in the males fell short of statistical significance, probably due to the smaller number of males available. The whole blood clotting time of the males kept in the hot environment, however, was significantly longer ($P < 0·01$) than that of the control birds. Although changes in clotting time in the hens maintained at 32° C showed the same trend, they were not statistically significant.

Prolonged exposure to high and low ambient temperatures was accompanied by certain physical and physiological changes. Thus, compared with the controls, both the 'hot' and 'cold' birds lost body-weight: 13 and 11 per cent, respectively, for the females, and 7 per cent in both environments for the males; the females also decreased in productive activity (21 and 28 per cent reduction in egg production), but diverged in feed consumption, which was 67 and 63 per cent of normal for females and males in the hot environment, and 106 and 152 per cent of normal, respectively, for the birds in the cold. The smaller increase in the females is probably associated with decreased egg production due to cold-stress. Hæmatocrits (microtubes), measured in the females only, increased with decreasing ambient temperature (23·2, 25·4 and 29·0 in the (hot), (controlled) and (cold) environment, respectively), but deep body temperature, determined with a flexible thermistor inserted 10 cm through the cloaca, was not significantly different among the three groups. Thus, differences in blood temperature, as observed in the dormant state or in hypothermia, could not explain our observations.

Mills[1] considered the possibility of a disturbance in the production of vitamin K by intestinal bacteria under conditions of tropical heat; as evidence he cites data indicating a much higher prevalence of hæmorrhagic disease of the new-born in southern than in northern U.S. cities. At the present state of knowledge it must be considered doubtful, however, that intestinal synthesis plays a significant part in the provision of vitamin K for the chicken. In our work, there is a possibility that, in connexion with the lower hæmatocrit of the 'hot' birds, hæmodilution might promote decreased levels of prothrombin and possibly other clotting factors. Lastly, consideration should be given to a possible relationship between the coagulation picture and the changes in the rate of overall metabolism, as indicated by the differences in feed consumption and in body-weight data among the various groups.

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Hæmoglobin in a Flatworm

ALTHOUGH the presence of hæmoglobin in free-living flatworms (Turbellaria) has been recorded by several authors[1–4], the pigment has not yet been investigated in detail. A large sample of the rhabdocœl, *Phaenocora unipunctata* (Orsted 1843), was obtained from mud from Coe Fen, Cambridge. These worms were found to contain hæmoglobin, and a preliminary examination was made of the properties of this pigment and its distribution within the tissues of the animal.

The worms, the largest of which were about 5 mm in length, were kept in the laboratory in shallow dishes containing about 0·5 in. of mud under 1 in. of water. They crawled and burrowed actively, usually feeding on the oligochæte worm *Tubifex*, present in the mud. The reddish colour of the worms was due not only to their own hæmoglobin but also to the presence of food hæmoprotein in their gut and their red-brown tanned eggs. Worms were collected from the dishes as required with a Pasteur pipette and transferred into clean water. The worms were left in clean water for several days before proceeding to other studies to avoid interference from food hæmoprotein.

Glass beakers, each containing ten worms, were set up either without food, or with food free from hæmoprotein ('Farex' baby food). Those beakers for experiment were filled with de-gassed tap-water and immediately covered with liquid paraffin. The control beakers were filled with tap-water equilibrated with air. No obvious change in colour or activity of the worms was noted after a week, except that worms without food became much shrunken at the posterior end, having apparently metabolized a considerable portion of their reserve substances. The disappearance of some worms and the appearance of winter eggs in all beakers indicated that all the worms had been under some stress. Visual examination, however, failed to reveal any obvious difference in hæmoglobin content among the various batches of worms, although those in de-gassed water had concentrated in or near the liquid paraffin layer. The results suggest that this worm does not increase its hæmoglobin content under conditions of oxygen deficiency, as concluded by Fox[4] with an unidentified species of *Phaenocora*.

The presumed function of the hæmoglobin as an oxygen carrier was tested by confining a number of worms in a small volume of water at the bottom of a Thunberg tube. A close-fitting rubber plug was used to keep them at a suitable density for spectroscopic examination, that is, 25–30 worms in 1 ml., and a preliminary examination showed the characteristic bands of oxyhæmoglobin at about 580 and 540 mμ. After 2 min, the normal activity of the worms was reduced and the oxyhæmoglobin spectrum had been replaced by an absorption band of cytochrome at about 560 mμ. This suggests that a *B* type of cytochrome is the major component of this system present in the worm. The β-band of this cytochrome component could not be seen, showing that the cytochrome is in much lower concentration than the hæmoglobin. When sufficient air was allowed to enter the Thunberg tube, the cytochrome band was replaced by those of oxyhæmoglobin. This cycle was repeated several times. The worms recovered their normal activity and the oxyhæmoglobin spectrum returned permanently when air was finally readmitted.

The distribution of the pigment was examined by a sodium nitroprusside–benzidene technique which gives a reaction with hæmatin compounds[5]. This was compared with the distribution of ferric iron found with Perl's technique[6]. For the latter technique, worms were fixed for two days in 5 per cent formalin in 0·9 per cent sodium chloride, embedded in paraffin wax and sectioned at 7μ. Complete worms were used for the hæmatin investigation after fixing for 24 h in 70 per cent ethanol. Hæmatin was found to be most concentrated on each side of the pharynx. It was also present between the eyes and extended dorsally over the whole length of the gut, but it was absent above the pharynx. The concentration appeared to be greatest at the anterior end, in agreement with visual observation, but it was also present in the posterior portion of the body. Ferric iron was found to be distributed evenly in the tissues of the anterior part of the worm and it was equally concentrated just beneath the dorsal epidermis throughout the whole length of the worm, except in the region over the pharynx. Comparison with the hæmatin distribution suggested that some ferric iron was separate from the hæmatin compounds. Prenant[7] examined several species of turbellarian and found that they did not contain an appreciable amount of hæmatin, although the parenchymal tissues were rich in iron.

The small amounts of hæmoglobin present and the lack of a suitable means of culturing large numbers of the animals made it difficult to determine the physico-chemical properties of this pigment. We calculated that in spite of their distinct redness each worm provided only 1 or 2 μg of extractable hæmoglobin. We therefore offer the following observations merely as a guide for further work.

The pigment was extracted as a yellow solution by homogenizing up to two hundred worms at a time in a 'Teflon' homogenizer. The solution, clarified by centrifugation, showed the characteristic bands of oxyhæmoglobin. These became undetectable when the air pressure was reduced to 2 mm of mercury. A sample of extract was analysed for us by Mr. B. Boon, of the Biochemistry Department, in a Spinco model *E* ultracentrifuge. Although the experiment was not completely satisfactory because of the low concentration, it nevertheless suggested that the coloured material moved in association with the main peak. This seemed to comprise all the sedimenting material and had an uncorrected S_{20} of 2·3 ± 0·4. A similar sample was electrophoresed on polyacrylamide gel at *p*H 8·6 and was found to move two and a half times as fast as a control sample of horse hæmoglobin.

We thank Mr. S. Prudhoe of the British Museum (Natural History) for identifying the flatworm. He also informed us that the whole body of another aquatic flatworm, *Rhynchomesostoma rostratum*, has a pinkish coloration[8]. Moseley[1] mentioned that *Dendrocoelum lacteum* has a red colour. The only previous reference to hæmoglobin in *Phaenocora* was given by Fox[4], but Gilbert[9] describes red pigmentation in three species of this genus, *P. highlandense*, *P. virginiana* and *P. kepneri*. In describing *P. highlandense*, Gilbert states that the anterior end is coloured a rich red as far back as the posterior edge of the pharynx where the colour is graded off into the colour of the body proper. He also records that he has occasionally found the same region in the other two species coloured a delicate rose, but never a deep red as in *P. highlandense*.

Our observations on the properties of the pigment suggest that it has a relatively high oxygen affinity, a low isoelectric point and a low molecular weight. The latter might be anticipated, since all known tissue hæmoglobins are of low molecular weight, usually about 18,000. It confirms, however, that we were not dealing simply with contaminating *Tubifex* hæmoglobin, the molecular weight of which is 3×10^6.

It appears that *Phaenocora* is not one of those invertebrate types in which the hæmoglobin concentration varies according to the oxygen tension of the environment[4]. It seems more likely that it is connected with the normal physiology of the animal. In this worm the pigment is concentrated around the muscular pharynx, and respiratory pigments often occur in or near muscular tissues in which sustained or powerful contractions take place.

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HISTOCHEMISTRY

Electron Microscopy of Post-mortem Changes in Porcine Muscle

MARKED changes were noted in porcine muscle during the initial 24-h post-mortem period. These changes were of particular interest in relation to muscle structure and water-binding, as described by Briskey and Wismer-Pedersen[1].

Muscle samples from the longissimus dorsi were removed and fixed in osmic acid immediately as the animal was stunned, at the onset of rigor mortis[3] and 24 h *post mortem*. Afterwards, the samples were passed through a graded series of alcohol, embedded in methacrylate (1-part methyl to 4 parts butylmethacrylate) sectioned with the Porter-Blum ultramicrotome and examined in a Siemens electron microscope. Pictures were taken at an original magnification of 8,000. The longissimus dorsi muscle samples were removed from an animal that was subjected to 120° F temperature for 40 min *ante mortem*. The immediate post-mortem temperature of the longissimus dorsi was 110° F. The onset of rigor mortis began 15 min *post mortem* when the *p*H of the muscle was 5·6. Following the 24-h chilling period, the muscle exuded large quantities of moisture, exhibited a pale colour and appeared to be quite open in structure.

Fig. 1 shows the soft-watery muscle at the time of death. The muscle appeared to be in a contracted state with only a small portion of the I band visible. Only 15 min later a drastic change had occurred in the muscle as seen in Fig. 2. Even though some sectioning marks are present on the myofibrils, the I bands are clearly visible and the *M* line can be seen in some cases. Most dramatic are the ruptured mitochondria, the remains of which are scattered between the myofibrils. The appearance of the muscle 24 h *post mortem* is seen in Fig. 3. A complete banding pattern is shown; however, this does not mean the muscle structure is complete but may indicate an extraction of material from the muscle, thereby rendering the bands clearly visible. The appearance of the *A* band is quite important; the definite granular appearance may indicate that a disruption of the myosin filaments has occurred in this muscle, which had a rapid onset of rigor at a low *p*H, turned a pale colour, and exuded large quantities of liquid. There seems to be no evidence of precipitated proteins on the myofibrils, as suggested by Bendall and Wismer-Pedersen[3], for this type of muscle. However, it is possible that sarcoplasmic proteins could have collected on the myofibrils and washed off during fixation and dehydration.

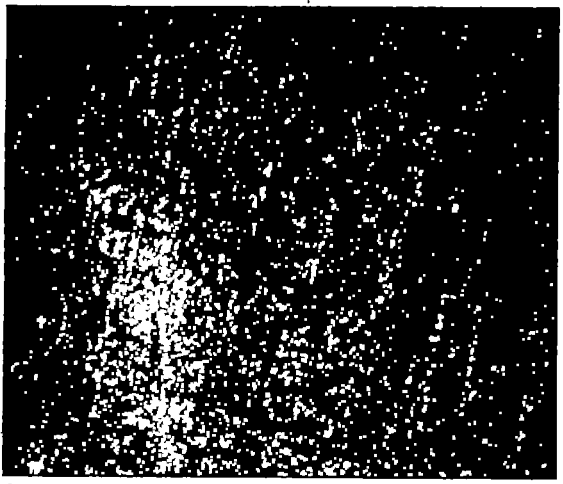

Fig. 1

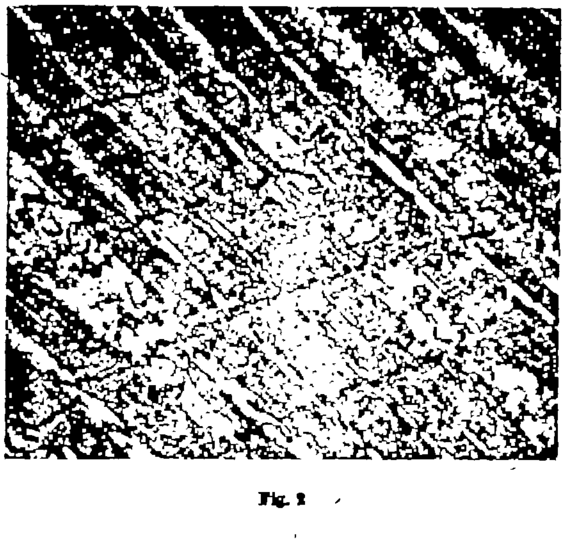

Fig. 2

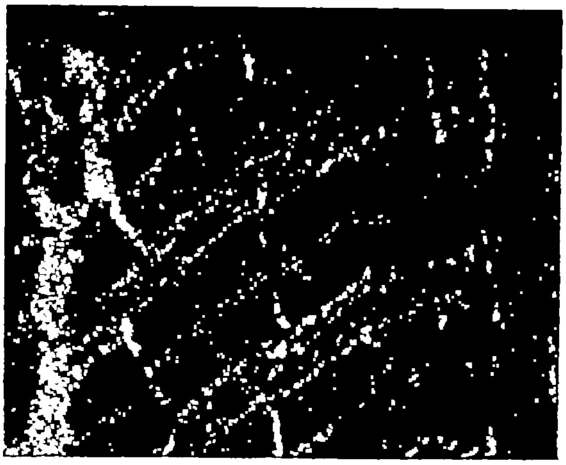

Fig. 3

The problem remains to relate the post-mortem changes in the ultrastructure of the muscle to its gross appearance and biochemical properties.

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A Colorimetric Method for the Quantitative Measurement of Total Lipids in Tissues

It is well known that the sudan and related dyes used for demonstrating lipids in histochemical technique dissolve in the lipid and can be re-extracted with fat solvents. Such oil-colorants are more soluble in oil than in the solvent used, and if an oil is shaken with a great excess of colorant solution, the oil will become saturated with colorant. Gomori[1] has introduced triethyl phosphate as a solvent for oil colorants in histochemistry for it has the advantage of being miscible with water. If the colorant is dissolved in triethyl phosphate, therefore, excess colorant may be removed by addition of water without re-extraction of colorant from the oil. The amount of oil will be proportional to the amount of colorant dissolved, and this may be measured photometrically.

It has been found possible to perform the whole procedure within a separatory funnel. The lipid extract is transferred in diethyl ether to a perfectly clean, dry funnel. A funnel of globular shape with little or no neck and short stem has been found best, for the funnel may be laid on its side so that the lipid solution is not brought into contact with the tap. By placing such a funnel on its side in a beaker of hot water the ether may be boiled off and the vapour removed with a current of air, or removed by reduction of pressure. A large excess of oil colorant dissolved in 60 per cent triethyl phosphate is then added and the flask gently shaken. The colorant should form a very concentrated but not necessarily saturated solution, and contain five drops of 'Teepol' or similar wetting agent per 100 ml. The funnel is allowed to stand and the excess hypophase run off. If the quantity of lipid is such that the colour of the phosphate solution is visibly reduced, a fresh portion is then added and the funnel shaken as before to ensure saturation of the oil with colorant. Water is then added, the funnel gently rotated, allowed to stand and the hypophase removed. This procedure is repeated. The coloured lipid which remains may be dissolved in acetone, and the solution made up to a known volume when the optical density may be measured photometrically or spectrophotometrically at the absorption maximum of the colorant used.

Oil red O has an absorption maximum around 512 mμ in most solvents and has been found to be best, for it is more soluble in aqueous triethyl phosphate than most oil-colorants, and is less easily precipitated on addition of water. The precipitate which forms is prevented from adhering to the lipid droplets and to the sides of the funnel by the wetting agent: too much of this must not be added nor the funnel shaken too vigorously or the lipid may be emulsified. Too little will cause adherence of the precipitate and introduce considerable error. The triethyl phosphate solution must also be free from precipitate, and should be filtered if necessary before use, for any precipitate it contains will remain in the oil after washing. The use of acetone in the final step has the advantage of making drying unnecessary since the acetone will take up any water left in the funnel. The absorption maxima of carotenoids which may be present in the oil are also well removed from that of oil red O. If carotenoids are present in quantity their effect may be reduced by exposure to light, oil red O being relatively stable. The anthraquinone oil-colorants such as sudan blue while preferable in having absorption maxima near or above 600 mμ are much less soluble in aqueous triethyl phosphate and tend to form troublesome precipitates. The procedure may be hastened by using a funnel that can be placed in a centrifuge.

The estimate of the quantity of oil present is dependent on the laws governing solution, so that different kinds of oil cannot strictly be compared. In practice for a given tissue and within a narrow range of temperature the method gives consistent results within an accuracy of 2 per cent down to at least 2 mg oil.

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IMMUNOLOGY

Ribonucleic Acid Metabolism in the Plasma Cell Sequence

Examination of the immune response of rats to *Salmonella* antigens has confirmed a large body of evidence that plasma cells (mature and immature) are the chief producers of antibody[1]. Both in the primary and secondary responses, these specialized effector cells arise by multiplication and differentiation of primitive lymphoid blast cells, and do not seem to divide[2,3]. As each cell usually forms only one antibody[4], its ribosomes presumably are geared primarily to the production of a single protein. The ultimate source of the information for this synthesis is unknown, but presumably during production it must be encoded in ribonucleic acid (RNA). The present experiments were designed to approach this problem by examination of RNA metabolism in the different members of the plasma cell family *in vitro*. Tritiated uridine, a precursor of both deoxyribonucleic acid (DNA) and RNA, was used, the differential uptake into each being evaluated by digestion of the cells with the appropriate nucleases.

Popliteal lymph node cells from rats immunized with *Salmonella adelaide* flagella were suspended in a modified Eisen's[5] medium supplemented with amino-acids, vitamins and 5 per cent fœtal calf serum. After a short period of equilibration at 37° C., tritiated uridine was added to a concentration of 5 or 20 μc./ml. Two approaches were used:

(*A*) *Continuous presence of isotope*. Cells were kept in medium containing tritiated uridine for the whole of the experiment.

(*B*) *Short pulse of isotope*. Cells were incubated for 5 min in the presence of tritiated uridine, rapidly chilled in medium containing an excess of carrier uridine, washed and resuspended for further incubation in uridine-free medium. In either case, cell samples were taken at intervals, chilled, spun at 1,000*g*, resuspended in fœtal calf serum, smeared on gelatin-coated slides and fixed in 89 per cent methanol and 1 per cent acetic acid. Some smears were digested with ribonuclease, others with deoxyribonuclease and others with both enzymes *seriatim*. Then all smears, including undigested ones, were processed for autoradiography by Pelc's technique[6] using 'Kodak *AR* 10' stripping film. After 14-days' exposure, the mean counts of grains overlying the nucleus and cytoplasm of cells in the different categories were determined.

The most striking finding was that while plasmablasts incorporated tritiated uridine rapidly, mature plasma cells (the chief antibody-formers in micro-drop studies) exhibited little RNA turnover. Fig. 1 shows the result of a typical experiment using cells 4 days after secondary stimulation, with continuous presence of isotope. At all stages blast cells were labelled far more heavily than any other cell category, both in the nucleus, and, at later time points, in the cytoplasm. Mature plasma cells sometimes showed less incorporation into RNA than small lymphocytes. Pulse labelling experiments showed that most of, if not all, the RNA synthesis in blast cells occurred in the nucleus and that some RNA later moved to the cytoplasm. Counts continued to rise for about 1 h after

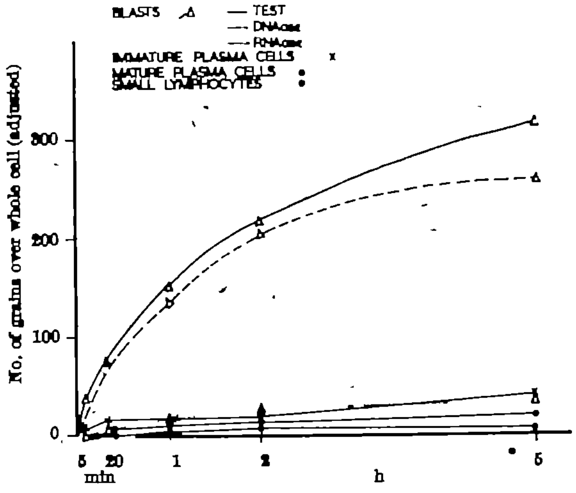

Fig. 1. Incorporation of tritiated uridine into lymph node cells of a 4-day secondary response. In this experiment 5 μc./ml. of tritiated uridine was continuously available to the cell culture. —— represents counts over cells from smears not digested by enzymes; – – – represents DNase digested blasts, and · · · · represents RNase digested blasts

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removal of the isotope, indicating progressive incorporation into macromolecular RNA[7]. Then total counts fell rather rapidly for the next hour and more slowly thereafter, suggesting a fraction with a rapid turnover rate, as well as a more stable fraction.

To determine whether the low RNA-metabolism in plasma cells was due to unphysiological conditions, *in vivo* experiments using foot-pad inoculation of tritiated uridine were performed. These completely confirmed the *in vitro* results. As had previously been noted by Schooley[8], mature plasma cells showed virtually no label. Schooley claimed that mature plasma cells merely released, and did not actually synthesize, antibody. To study this point, tritiated leucine incorporation was measured in our *in vitro* system. These experiments are subject to the reservation that no enzymatic digestions are available to determine whether the leucine actually entered protein, but it seems likely that free amino-acids would be removed from the cells by fixation and the extensive subsequent washing procedures. The results of a typical experiment are shown in Fig. 2. When tritiated leucine is continuously available to cells from 4-day secondary nodes, the initial rate of incorporation into mature plasma cells is two-thirds that into blast cells. Moreover, the kinetics suggest that the blasts are synthesizing predominantly stable protein, as the grain counts continue to rise; whereas the mature plasma cells appear to be turning over protein rapidly, since counts later than one hour show no further rise. This strongly suggests active antibody synthesis and export by mature plasma cells. The results are in close agreement with the overall picture presented by Helmreich *et al.*[9].

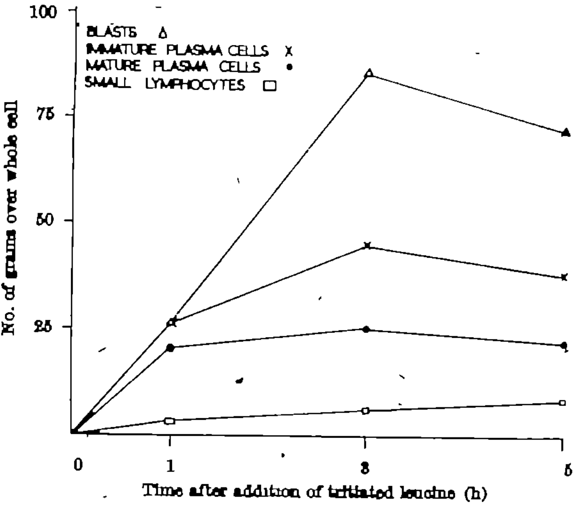

Fig. 2. Incorporation of tritiated leucine into lymph node cells of a 4-day secondary response. The medium contained 10 μc /ml of tritiated leucine

Accepting the results of the leucine experiments at face value, for every molecule of leucine taken into protein blast cells turn over thirty times as much RNA as do mature plasma cells.

In considering the source of nucleic acid templates for synthesis of antibody protein by plasma cells, we postulate that the ancestral blast cells manufacture the information during their active proliferative phase, and that this is passed down the maturation scale to the mature plasma cells. We feel that these preliminary results agree with the conclusion of Arnstein *et al.*[10] that where a specific protein is being produced, and all the efforts of the cell are directed towards the one function, any RNA analogous to the bacterial 'messenger'-RNA[11] is a stable entity attached to the ribosomes early in the cell's functional maturation.

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BIOLOGY

A Specimen of *Morganucodon* from Yunnan

IN early July of 1948, while I was Rector of the Catholic University of Peking, I asked the Rev. Edgar T. Oehler, SVD, associate professor of chemistry in the University, to undertake an expedition to Yunnan in quest of *Bienotherium* fossil material, since I wanted to section and examine the otic region of the animal. The expedition was financed by Father Ralph Thyken, SVD.

On August 10, Father Oehler arrived at Lu Feng, and, with local help, set about his prospecting. In late September he found a skull about 1 in. in length, which is fairly complete as to lower jaw, upper jaw and cranium. It was found at Yang Tsao Ti (Goat Grass Place), a poor grazing area 25–30 min walk north-east of Lu Feng, in beds which were Bien's lower beds of the lower Lu Feng series. They are of a somewhat compacted red clay with purplish and light blue-green streaks and contain many calcareous nodules. The beds also contain *Bienotherium*.

A week after finding this fossil, Father Oehler sent it to me in Peking. It was eventually brought out of China, after my arrest by the Chinese Communists, by Dr. Wilhelm Brull, of the Catholic University, who gave it to me in February 1956.

I did not find time to describe this fossil as I had wished because at first I was recovering from more than four years of imprisonment in Peking; and then in July 1957 I went to Liverpool in England, where I was beset with many administrative duties. Around the beginning of 1960, I deposited this skull with Dr. K. A. Kermack, of University College, London, whose assistant, Mrs. Frances Mussett, undertook the delicate work of cleaning it.

Dr. Kermack has sent me stereoscopic photographs of diagnostic portions of this fossil. It is quite evident that this fossil comes close enough to the type of *Morganucodon* to place it in the same genus. I plan eventually to describe this fossil fully and in detail. Due to circumstances there may be a long delay before I can do this, and in view of the importance of the specimen a preliminary description seems justified. It is the first complete skull of any Mesozoic mammal of pre-Upper Cretaceous age. It has also been possible to observe in it the reptilian jaw joint between the quadrate and articular postulated in *Morganucodon* by Kuhne[1] and Kermack and Mussett[2] but not previously seen.

Morganucodon oehleri sp. nov. *Derivation of name.* In honour of Rev. Edgar Theodore Oehler, the discoverer of the specimen.

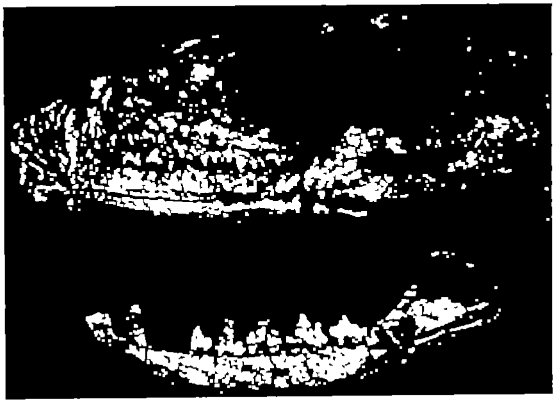

Fig. 1. *Morganucodon oehleri* sp. nov. Lateral view of skull and lower jaw and medial view of detached right ramus of lower jaw. (× *c*. 3)

Material. Holotype at present in the Zoology Department, University College, London. The partial brain-case mentioned by Patterson and Olsen[3] as *C.U.P.5* is almost certainly referable to the same species.

Horizon and locality. Lower beds of Lower Lu Feng Series at Yang Tsao Ti, Lu Feng, Yunnan, China.

Diagnosis. Lower molariform teeth larger than in *Morganucodon watsoni* (three fully erupted teeth together measure 5·5 mm in length). Dental formula differs from any of the specimens from Wales referred to *Morganucodon* by Kermack and Mussett[4] by having four premolariform and three molariform teeth in the upper jaw. In the lower jaw the formula is the same as in some of the Welsh forms, three premolariform and four molariform.

The presence of *Morganucodon* in the Lower Lu Feng Beds would suggest that they are of the same age as the Welsh fissures containing this genus—Rhaetic or Basal Liassic (Harris[5]).

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Introductions of Exotic Fish

AT the tenth session of the Indo-Pacific Fisheries Council held in Seoul, Korea, recently, it was recommended that a register of introductions of exotic fish should be compiled by the Food and Agriculture Organization and that institutions and scientific workers should be asked to assist in this. I have undertaken to compile a list of all the introductions of *Tilapia* spp. into countries where they are not indigenous. *Tilapia* has already been introduced extensively in a number of countries, and if records are not compiled soon it will not be possible to ascertain the identity of any particular fish. This has already proved the case in some parts of Africa, where fish identified as a particular species in one area have proved different from fish given the same specific name in another area.

The *Tilapia* spp. are proving valuable fish in pond culture, and they can be considerably improved by judicious genetic selection and hybridization. For such a programme to be successful it is essential that stocks should be standardized and of known origin, and a start has been made in this Institute to standardize our own stocks. Since in many countries distribution of fish fry for pond culture is carried out by Fisheries Departments, there is no reason why these fish should not be improved standardized strains. In addition *Tilapia* spp. have become increasingly important as laboratory animals for behavioural and physiological research and it is essential that identity of the strains used should be known, as is the practice with other laboratory animals.

I would therefore request all those who have introduced *Tilapia* spp. into countries where they are not indigenous to co-operate in this compilation by sending me details of the origin, date of introduction and place of introduction of any species of *Tilapia*. In addition I would urge all those working with *Tilapia* spp. to bring about the standardization of their stocks by careful selection. Copies of the compilation on *Tilapia* will be deposited with the Food and Agriculture Organization for further compilation into a larger register.

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Spiralization in Tails of Mammalian Spermatozoa in Hypotonic Media

IT was noted by Kölliker[1] that treatment of mammalian spermatozoa with water brings about coiling and spiralization of their tails. The present investigation, chiefly made on ejaculated bull spermatozoa, but supplemented by observations on sperms from man and rabbit, confirms these observations. Hypotonic solutions (calcium-free Ringer solution plus distilled water) induce in spermatozoa a very intense swimming without rotation around the longitudinal axes, and—with increasing hypotonicity—a progressive curving and bending of their tails which later coil into spirals (Fig. 1).

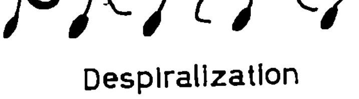

Fig. 1

Despiralization (Fig. 1) sets in after spiralization, and proceeds gradually at a higher rate at higher degree of hypotonicity. Maximum frequency of spiralized spermatozoa (up to 80 per cent), when counted after 20 min, is obtained in the hypotonic medium, 1 + 4 (one part isotonic Ringer + four parts distilled water). After 30–45 min in (1 + 4) rather good swimming still results, when the cells are brought back to isotony.

The process leading to spiralization seems not to depend on pure swelling. This appears from experiments in which 3·5–10 per cent of polyvinylpyrrolidone or 6–18 per cent of the polysaccharide dextran were added to the (1 + 4) medium, making it isotonic or hypertonic without affecting the phenomenon studied. On the other hand, repeated washing, cold shock, ageing, and storing are inhibitory to the spiralization, as is pretreatment of the cells with 10^{-3} M mersalyl or *p*-chloromercuribenzoate, substances which also cause despiralization of spermatozoa in hypotonic media. Cysteine abolishes the action of these —SH reagents. In a series of ejaculates taken immediately after each other, the frequency of spirals in the (1 + 4) medium first rises, then decreases. Thus the state of physiological maturation[2] seems to be of importance. Semen samples of high viability, that is, long survival in isotonic Ringer's solution at room temperature, give higher frequencies of spiralized cells than such of low viability. Low temperature very markedly reduces the durability of the spirals. Furthermore, curving and bending of the sperm tails as described here are immediately evoked also by hypertonic solutions of glycerol (15–20 per cent in isotonic Ringer), yet without spiralization. No tested hypertonic concentrations of sodium chloride, saccharose, or urea give this result. Remarkable glycerol effects on other fibrillar systems have been reported[3,4].

The work was based on the hypothesis that the spiralization caused by hypotony is a result of a flux of ions or other low-molecular substances into the medium, causing disturbances in the contraction–relaxation mechanisms. Present work in this field[5–7] indicates that these mechanisms are in close agreement with those in muscle.

I thank Prof. P. E. Lindahl for valuable criticism.

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Validity of the Species *Cirratulus norvegicus* Southern

DURING the course of an investigation into the ecology of the cirratulid worm, *Cirriformia tentaculata* (Montagu) it was noticed that the young of this animal were remarkably similar to the worms described by Southern[1] as being of the species *Cirratulus norvegicus*. Previous workers have not been eager to accept this species. Fauvel[2], for example, regarded the worm as a variety of *C. tentaculata*. Wilson[3], while not denying the existence of a separate species (since Southern reported a mature female), directed attention to the fact that the features used by Southern to distinguish *C. norvegicus* from *C. tentaculata* were in general those that might occur in the juveniles of the latter species.

The following characters are those by which Southern distinguished *C. norvegicus*: (*a*) it was of smaller size and had fewer segments; (*b*) there were fewer tentacular filaments and these were on the fourth chaetigerous segment, those of *C. tentaculata* being on the sixth or seventh; (*c*) the crotchets appeared on a more anterior segment.

Several hundreds of young stages of *C. tentaculata* were examined between December 1959 and December 1961. Of these, many possessed all the features ascribed to *C. norvegicus* by Southern. A young polychaete is of smaller size and has fewer segments than the adult, thus it is not difficult to explain Southern's first distinction. With regard to the other distinguishing characters, the data collected during the two-year period showed that very young *C. tentaculata* possess tentacular filaments and crotchets on the anterior chaetigerous segments. As the worms grow, the filaments and crotchets are progressively lost from the early setigers and replaced by others farther back. Clearly, during their development the animals must pass through a stage in which the tentacular filaments an crotchets are positioned as in Southern's *C. norvegicus*. I addition, specimens of *C. tentaculata* which have reache this 'identical stage' in their physical development ar sexually mature. Hence, it is not difficult to account fo the existence of the gravid female reported by Southern

It would appear, therefore, that the doubts expressed b Wilson as to the validity of the species *C. norvegicus* ar well founded, and the animals recorded by Southern wer only physically immature specimens of *C. tentaculata*.

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Decay and Recovery of Luminescent Ability in *Gonyaulax monilata*

SOME species of dinoflagellates respond to mechanica stimuli by emitting visible light. When stimuli of constant intensity are applied at regular time-intervals progressively lower light-intensities result. This is caused by a temporary exhaustion of the bioluminescent system o the organisms. Recovery can be complete provided the organisms are permitted to rest for a suitable period o time. A mathematical model is proposed to describe the response of *Gonyaulax monilata* to mechanical stimuli of 1/2-sec duration occurring at regularly spaced time intervals. The results of measurements are compared with the mathematical model.

It is assumed that the intensity of luminescence produced on stimulation is proportional to the number of dinoflagellates, Σ, able to respond at the beginning of the time interval, and that during and after stimulation the ability to respond will be recovered at a constant rate. The net rate of recovery for the interval of stimulation is then given by $\frac{d\Sigma}{dt} = \gamma - \delta\Sigma$, where γ is the recovery constant and δ is the decay constant.

The solution of this equation yields, subject to the condition that:

$$\Sigma = \Sigma_0 \text{ at } t = 0, \quad \Sigma = \frac{\gamma}{\delta}(1 - e^{-\delta t}) + \Sigma_0 e^{-\delta t}$$

In investigating the validity of this expression, agreement between experiment and a mathematical model was improved by assuming a period of inactivity (14·5 sec) immediately following stimulation. Values of 0·04 and 0·14 were chosen for γ and δ respectively. The time-intensity relationship of luminescence was determined as follows: at $t = 0$, let $\Sigma_0 = 50$, an arbitrary value. The value of Σ is calculated for $t = 0·5$ sec. The next 14·5 sec are considered dead time, leaving 15 sec for recovery at the constant rate γ, for a 30-sec period. The recovery is added to the calculated value of Σ, giving the Σ_0 for the next period. This process is repeated in conformity

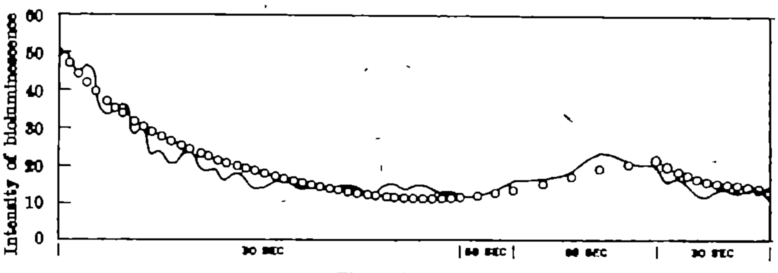

Fig. 1. —, Observed; ○, calculated

with the experimental procedure which includes 58- and 38-sec periods.

The observed and calculated responses are shown in Fig. 1.

No attempt was made to reproduce the fluctuations in the experimental curve. These are believed purely random and are consistent with variations in the observed responses of different cultures of *Gonyaulax monilata*.

We thank Dr. Giorgio Soli of the Naval Ordnance Test Station for the experimental data.

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Cœnocytic Vessel Elements in *Dioscorea alata* L.

DURING the course of a morpho-histogenic study on the cultivated species of *Dioscorea* of Gujarat State, India, certain observations were made on the development of the vessel elements in the stem of *D. alata* (Kamodio variety). The vessel elements of the plant were found to be unusual in being very broad with long scalariform plates which often exhibit a branching of the bars in the plate[1,2].

The metaxylem vessel elements are 1·3 cm long, and have perforation plates varying from oblique, elongated and scalariform to transverse, rounded and reticulate. The tracheids are branched and show scalariform pitting. Intermediate types, such as vessel elements with only one perforation plate at one of the ends and 'vessel-tracheids', are also present. These latter are tracheid-like elements with a small perforation plate composed of a few branched and unbranched bars. It is doubtful if any membrane is present between them. The vessel-tracheids have small, simple, elongated pits on the other parts of the wall, and are obviously of a different category from those defined by Fahn[3].

The protoplast of the vessel element of *D. alata* is cœnocytic. As many as 7–9 or more nuclei were observed in vessel elements, 0·5 cm long. The multinucleate phase in vessel differentiation is due to divisions of the nucleus of the mother cell (Figs. 1 and 2). This contradicts Hill and Freeman's observations according to which the multinucleate condition in the central vessels of the root of *D. prehensilis* is due to an obliteration of the transverse and lateral walls of the elements[4]. The nuclei are scattered throughout the length of the vessel elements during the phase of elongation.

Later the protoplast becomes fragmented into disconnected strands. Pirotta and Buscalioni (1898)[5] and Němec (1910) reported that in the Dioscoreaceae the perforation of the end walls occurs after the disintegration of the nuclei; but in my material the cœnocytic protoplast has been found to persist even after the formation of the perforation plate (Figs. 3 and 4), which is most unusual. Eames and MacDaniels[6] and Esau[7] now suggest that in the development of the vessel the protoplast must persist at least until the completion of the perforation plate.

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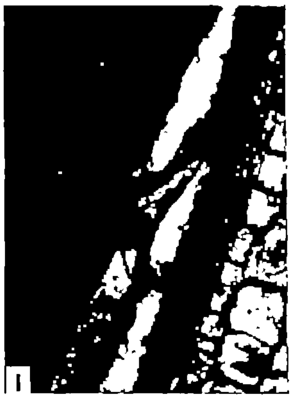

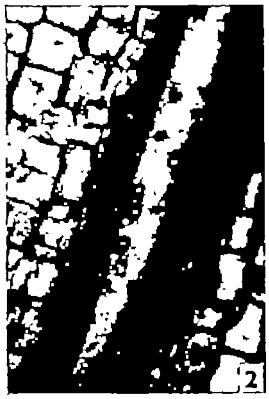

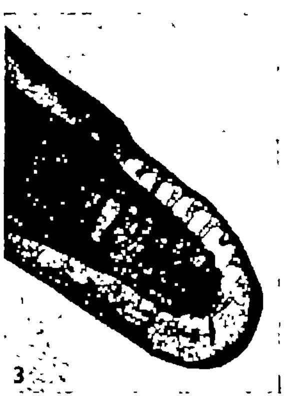

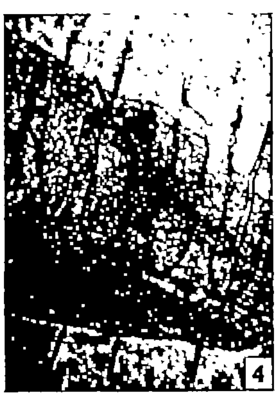

Fig. 1. Two differentiating vessel elements, note nuclei lying against end wall in upper cell (× 250)

Fig. 2. An elongated vessel element near apical region of the stem showing a row of four nuclei (× 250)

Fig. 3. Vessel element with perforation plate, showing persistent protoplast. The lower four bars were damaged because of the pressure applied to the macerated element (× 63)

Fig. 4. Persisting nuclei at end of the vessel element. Note the broken bars of the perforation plate (× 250)

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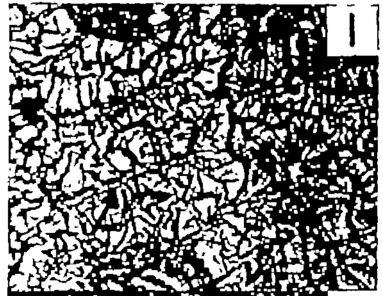
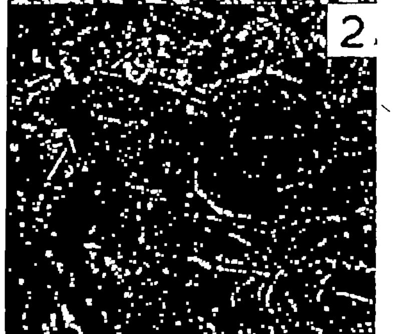
Reticulate Cuticle on Leaf Epidermis in *Hevea brasiliensis* Muell.

VARIATIONS of the surface patterns of cuticles have attracted the attention of both plant morphologists and physiologists. The formation and the functions of cuticle have been previously discussed[1-3]. Electron microscopic studies have revealed much greater details[4,5]. Observations under the light microscope show three main types of cuticles, namely, lamellate, striate and reticulate[1,6,7], and the effects of cell growth are mentioned as chief causes for the formation of different types[1,4]. Of these three, occurrence of the lamellate cuticle appears to be more common than the other two. Different genera that possess striated cuticles have been listed[3,7].

Martens[6,8] examined in detail the cuticle pattern on the floral parts of *Tradescantia*. In most instances he observed lamellate and striate cuticles and only in few cases the reticulate type. Scarcely any other report has been made about the occurrence of reticulate cuticle in plants. The present observations illustrate the occurrence and nature of reticulate cuticle on the lower epidermis in *Hevea brasiliensis*.

Leaves of *H. brasiliensis* were collected from plants growing in the University compound. Peels of upper and lower epidermis of leaves were made and observed under the microscope. Some of these were stained with 1 per cent safranin. Transverse as well as paradermal sections of leaves, 5–8µ thick, were prepared and stained with safranin and green. The cells of the upper epidermis, in the surface view, are angular in outline and covered with thick lamellate cuticle. Lower epidermal cells are wavy





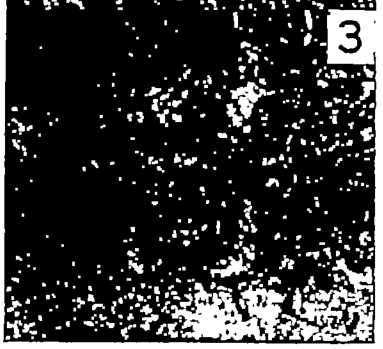


Fig. 1. Surface view of lower epidermal peel showing reticulate cuticle

Fig. 2. Paradermal section of epidermis showing central ridge and arms

Fig. 3. Transverse section of leaf showing a part of leaf with lower epidermis and cuticle. Arrows indicate the central ridges and their arms (× 200)

in outline. On each cell there is a thick ridge of cuticle, median in position and oriented parallel to the long axis of the cell (Figs. 1 and 2). From this central ridge several arms originate with gradual tapering ends. Some of these extend to the neighbouring cells and establish connexion with the main ridge and arms thereof, forming a loose reticulum (Fig. 1). In no instance were they observed to extend beyond the neighbouring cells. The refractive reticulum was best focused differently from the epidermal cells (Fig. 2).

The stomata are present only on the lower epidermal cells and are of paracytic type[9] (Fig. 2). The median ridge of cuticle was usually absent on the subsidiary cells and this was better evidenced in the paradermal sections (Fig. 2). In case of *Cestrum* it was noticed that the stomata were focii of origin for the development of striations[7]. In the present case, however, the ridge and the arms developed on all the epidermal cells except guard or subsidiary cells. The transverse section of the leaf shows the extracellular position of reticulate branches and superficially they resemble the fibrous thickenings of anther endothecium (Fig. 3).

Young leaves were also studied for developmental stages. Even a young leaf that measured 2·0 × 1·0 cm showed the formation of cuticular reticulum on its lower surface and it was more prominent near the midrib region than on the lamina. Leaves collected from different parts of Malaya were also examined. All of them possessed reticulate cuticle, which led to the suggestion that the formation of cuticle was not affected by environmental factors.

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ENTOMOLOGY

Wild Pigs as Hosts of *Glossina vanhoofi* Henrard and *Trypanosoma suis* Ochmann in the Central African Forest

IN 1951 we found in *Glossina brevipalpis* from the Institute of Scientific Research in Central Africa's Mosso field station, Burundi, north-east of Lake Tanganyika, a trypanosome infection of the salivary glands similar to that noted by Burtt in 1942 near Amani, Tanganyika. Our rate of infection was very low; two infected flies out of 700 dissections of salivary glands (0·28 per cent) as compared with the low rates found also by Burtt (0·84 per cent), by Whitnall in 1934 (0·62 per cent) and by Brown in 1938 (1·14 per cent). Elsewhere we have stated[1] regarding the salivary gland infections that "we do not believe that this trypanosome is necessarily a polymorphic one as assumed by Burtt. Its morphology might be completely different from the trypanosomes of the salivary glands in *G. palpalis* and *G. morsitans*". As many as 6,397 *G. brevipalpis* were examined during the period April 1952–April 1953 by E. Peel and M. Chardome in our laboratory. They discovered 1·09 per cent infection of the salivary glands and isolated strains which when transmitted cyclically to domesticated pigs, showed a monomorphic trypanosome identical with *T. suis* Ochmann. This trypanosome was originally recorded in East Africa, first by Ochmann (1905) from the domestic pig, and later in 1912 by Geisler and Mayer from the warthog: all these authors gave an inadequate description of this trypanosome. Preparations showing *T. suis* from the Congo were demonstrated by L. van den Berghe[2] at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene in January 1954, and it was finally adequately described by Peel and Chardome in 1954[3] (preliminary announcement in 1953[4]) and its revival announced by Hoare the same year.

In 1955[1] we were fortunate in discovering in the Irangi dense forest station of the Institute of Scientific Research in Central Africa, west of Lake Kivu, Congo, a large fly belonging to the fusca group, *Glossina vanhoofi* Henrard, which was known as a museum species and had never been observed in its habitat. The study of the biology of *G. vanhoofi* proved very difficult in comparison with our previous field work with *G. brevipalpis* in the Mosso field station. The population of a forest species of *Glossina* seems far more dispersed than the one of a savannah fly. The limited range of visibility and the very poor light are also factors which rendered the discovery of *G. vanhoofi* particularly arduous in the dense Irangi forest. This fly very rarely feeds on man, another factor which makes it difficult to capture.

In the course of 1960 we were able to ascertain one important fact in the biology of *G. vanhoofi*. While for years we had never been able to discover more than one or two replete flies, we finally secured a few (six in all) gorged males from distant sites around the station. The stomach contents, sent to Weitz in London, demonstrated in all cases blood from wild pigs. *Potamochoerus* are common in the Irangi forest but *Hylochoerus* are also found in the region. It seems that the wild pigs of the forest are the preferred, if not the only, hosts of *G. vanhoofi*. This is in complete agreement with the findings made approximately at the same time (1952–55) by several authors studying the stomach contents of the *G. morsitans* group in the eastern savannah belts (Lovemore[5] in Southern Rhodesia, Weitz and Jackson[6] in Tanganyika, and van den Berghe and Lambrecht[7] in Rwanda). A *Potamochoerus* host preference was also established in West African forest (Nigeria) for *G. fusca*, *G. nigrofusca* and *G. medicorum*.

Infections of the salivary glands of *G. vanhoofi* had already been discovered by us in 1955, providing an identical picture to that found in the Mosso savannah region with *G. brevipalpis*. *T. suis* Ochmann was identified in the laboratory at Lwiro from stained metacyclic forms[8] and its geographical distribution, therefore, considerably extended from the East African savannahs to the dense central African forest.

The investigations were made at the Laboratory of Medical Protozoology and Zoology, Institute of Scientific Research in Central Africa, Lwiro (Bukavu), Congo, and at its forest station (Irangi, Maniema).

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MICROBIOLOGY

Nitrification by *Arthrobacter globiformis*

A VARIETY of heterotrophic micro-organisms have now been shown to effect the biological oxidation of nitrogen. Bacteria and actinomycetes have been reported to produce nitrite in the presence of ammonia[1,4], pyruvic oxime[2,3,5], and ethylurethane[6,7]. The fungus, *Aspergillus flavus*, produces hydroxylamine, nitrite and nitrate from ammonia or amino compounds[8,9]. The ecological significance of heterotrophic nitrification in complex natural environments has, however, been viewed with some reservation[10]. The observation reported here that a strain of *Arthrobacter globiformis*, a ubiquitous soil inhabitant, oxidizes ammonia to hydroxylamine, nitrite, nitrate, and possibly a gaseous intermediate may, therefore, be of some ecological interest.

A. globiformis strain 616 was used in these investigations. It was grown in a medium containing, per litre distilled, de-ionized water: NH_4Cl, 2·0 g; KH_2PO_4, 1·0 g; K_2HPO_4, 1·5 g; $MgSO_4.7H_2O$, 0·6 g; iron chelate[11], 4·0 ml; $ZnSO_4.7H_2O$, 0·03 g. Succinic acid was the sole source of carbon; it was provided in the form of a succinic acid–sodium hydroxide buffer, *p*H 6·2, separately sterilized by Seitz filtration and added so as to give a final concentration of 0·05 M succinate. The final *p*H of the medium was 6·9–7·0. Culture vessels were vigorously aerated with sterile air, and incubated for 21 days in a water bath maintained at 28° C.

Resting cells were obtained by centrifuging 18-h cultures at 27,000*g* in a continuous flow centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant was discarded and the cells washed twice in 0·2 M hydrochloric acid–*tris*(hydroxymethyl)aminomethane (*tris*) buffer, *p*H 7·2. Tests for the presence of hydroxylamine, nitrite and nitrate in the final wash solution were negative. The cells (35–40 mg dry weight) were rinsed out of the centrifuge tubes with 10 ml. of a substrate consisting of the *tris* buffer and 0·01 M $(NH_4)_2HPO_4$, 50 per cent v/v, *p*H 7·2. This cell-substrate suspension was transferred into duplicate 25 × 150-mm test-tubes, and vigorously aerated. The gases from each reaction tube were exhausted through a separate trap containing 10 ml. distilled, de-ionized water, or 10 ml. freshly prepared Griess-Ilosvay reagent[12]. The latter served as an indicator for the formation of any gaseous nitrogen oxide in that a red colour was produced proportional to the nitrous acid concentration. Oxides of nitrogen formed in the reaction tubes were also absorbed in separate traps containing 40 ml. of absorbing solution (35 ml. of 5 per cent potassium hydroxide solution and 5 ml. of 3 per cent hydrogen peroxide). The hydrogen peroxide ensured that all the oxides were in the nitrate form. Reagent tubes and traps were incubated up to 36 h in a water bath at 28° C. Uninoculated controls were maintained for each treatment. All glassware was acid washed.

Analyses for ammonia were made colorimetrically[13]. Protein was measured with Folin phenol reagent[14]. Hydroxylamine was determined by iodine oxidation[15]. Nitrite was estimated by the α-naphthylamine sulphanilic acid procedure. Nitrate was measured by the phenol–disulphonic acid method or by the Middleton technique[16].

The results shown in Table 1 are typical of those obtained with actively growing cultures.

Table 1. PRODUCTION OF HYDROXYLAMINE, NITRITE AND NITRATE BY GROWING CULTURES OF *Arthrobacter globiformis**

Product	μg N/ml.
Hydroxylamine	0·20
Nitrite	0 20
Nitrate	4 50

* Culture sampled after 5 days' incubation.

Results obtained with resting cells are shown in Table 2.

Table 2. PRODUCTION OF HYDROXYLAMINE, NITRITE AND NITRATE BY RESTING CELLS OF *Arthrobacter globiformis* after 24 H

Product	μg N/ml.
Hydroxylamine	0·05
Nitrite	1·50
Nitrate	1·00

At the termination of experiments with resting cells the products of ammonia oxidation together with the residual ammonia generally accounted for only about 80 per cent of the initial substrate. Analysis of the cells showed no increase in protein, suggesting that gaseous oxides of nitrogen might have been produced and lost through volatilization. In such an event it was anticipated that at least traces of such gas, or gases, would be swept over by means of the vacuum line used to aerate the system from the reaction tube into the trapping tube. Analyses of both the distilled water and the Griess-Ilosvay reagent in respective traps showed amounts of NO_2^- present in the order of 0·15 μg per ml. An attempt was made to identify the gas produced by treating the Griess-Ilosvay reagent with pure nitrous oxide and pure nitric oxide (The Matheson Co., Joliet, Ill.). Only nitric oxide gave a colour reaction corresponding to that obtained experimentally. Analysis for nitrate in the potassium hydroxide–hydrogen peroxide solution, however, gave a somewhat higher result, 0·5 μg NO_3^- per ml., suggesting that the production of some other gaseous nitrogen oxide could not be ruled out.

Of immediate interest, however, is the evidence suggesting that the genus *Arthrobacter* may be implicated in nitrification; moreover, it may be included among those few select genera reported capable of oxidizing nitrogen to nitrate. It is also of interest that in the oxidation of ammonia by *A. globiformis* gaseous nitrogen oxides are produced. The formation of these compounds suggests that nitrogen losses may occur through oxidative as well as reductive processes. In all these instances the relatively small experimental recovery may none the less be of considerable ecological and practical significance because of the large numbers of *Arthrobacter* in the soil[17].

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GENETICS

Inheritance of Serum Transferrins in Rhesus Monkeys

AN extensive polymorphism of transferrin, the iron binding protein of plasma, has been detected among rhesus monkeys and other macaques[1-4]. In previous work[5], 19 phenotypes due to different combinations of nine molecular forms of transferrin could be distinguished by starch-gel electrophoresis. Additional transferrin variations have now been discovered (Goodman, M., unpublished data), and there are at least 28 phenotypes and 11 molecular forms of transferrin present in the genus *Macaca*, the rhesus group alone showing 21 of these phenotypes and 9 of these transferrins.

The macaque transferrin of slowest electrophoretic mobility has been labelled[6] A and that of fastest mobility, H. (H^1 migrates between G and H, the newly discovered F^1 between E and F, and E' between D and E.) Typically a sample of macaque serum contains either one or two molecular forms of transferrin. Thus we can suppose that the observed transferrin variation is due to an allelic series of genes and that phenotypes such as CC consisting of a single transferrin are controlled by homozygous combinations of genes, whereas those such as CD consisting of two transferrins are controlled by heterozygous combinations.

In order to investigate the inheritance of macaque transferrins, we determined by comparative one-dimensional starch-gel electrophoresis in the *tris* discontinuous buffer system[6] the transferrin phenotypes of rhesus monkeys in the breeding colony of the Primate Laboratory of the University of Wisconsin. The results are presented in Table 1. It can be seen that the four offspring of the $CC \times CC$ matings were all phenotype CC. (In the breeding colony, as in the rhesus monkeys from the Nepal border of India[5], the most frequently occurring transferrin type was C.) There were 6 offspring of $CC \times CG$ matings; 4 were phenotype CC and 2 were CG. The 2 offspring of $CG \times CG$ were CG and GG respectively. The 1 offspring of $CD \times CD$ was DD. The 2 offspring of $CF \times CC$ were CC and CF respectively. Finally, 1 offspring of $CD \times CG$ was DG and another offspring originally attributed to the same mating combination was CH. Thus with the exception of monkey 62 (the offspring with CH phenotype) each offspring always had one transferrin identical in type to one of the mother's and one transferrin identical in type to one of the father's. A re-check of the records of 270, the mother of 62, raised doubt that R–16 (a male with CG phenotype) was the sire of 62 in that the date of access of R–16 to 270 could not account for the pregnancy of the latter. Nor did the records show which male could be implicated.

Table 1. TRANSFERRIN PHENOTYPES OF PARENTS AND OFFSPRING IN A BREEDING COLONY OF RHESUS MONKEYS

Designation of animal			Transferrin phenotype		
Mother	Father	Offspring (sex)	Mother	Father	Offspring
R–3	R–6	32 F	CC	CC	CC
299	R–2	81 F	CC	CC	CC
69	R–2	75 F	CC	CC	CC
69	R–2	100 F	CC	CC	CC
248	R–16	49 F	CC	CG	CC
R–15	R–16	61 F	CC	CG	CC
R–11	R–16	71 M	CC	CG	CC
296	R–16	43 M	CC	CG	CC
R–55	R–6	83 M	CG	CC	CG
302	R–2	A–25 F	CG	CC	CG
292	R–16	35 F	CG	CG	GG
302	R–16	57 M	CG	CG	CG
281	R–64	A–75 F	CD	CD	DD
310	R–6	33 F	CF	CC	CC
310	R–6	82 F	CF	CC	CF
270	R–16	30 M	CD	CG	DG
270	(R–16?)	62 F	CD	(CG?)	CH

Discounting the transferrin results for 62, our results support the hypothesis that an allelic series of genes at a single locus controls the variation of transferrin types in rhesus monkeys, with each gene responsible for a particular transferrin and with no gene in the allelic series having dominance over another. However, the earlier anomalous finding[4,5] of a rhesus monkey serum with three molecular transferrin types (BDG) suggests that a more elaborate genetic hypothesis may be needed to account for the full complexity of the transferrin polymorphism of rhesus monkeys.

We thank Dr. Gabriel W. Lasker for his advice on the genetic significance of the data.

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Size in Relation to Development-time and Egg-density in *Drosophila melanogaster*

A RECENT report by Wattiaux[1] emphasizes the importance of speed of development in relation to the expression of a quantitative character in *Drosophila melanogaster*, namely bristle number. Using another such character, size of thorax, I investigated the ubiquity of the disclosed relationship. However, different sets of environmental conditions, bearing on temperature and egg density (or larval crowding), were introduced in order to test its eventual variation.

A given number of flies of a wild-strain, Abeele (Belgium), aged 7 days, and hatched after an equal duration of development, were allowed to lay during a 4-h period, at 25° C, on an agar–acetic acid medium. The eggs were redistributed by lots of 30 or 240 per standard tube, and placed in three controlled-temperature chambers, at 18°, 25° and 29° C (± 0·1° C). At each temperature, 24 replicates were made for the density 30 and 6 for the density 240. Flies emerging at 29° and 25° C were scored and sexed twice, and those at 18° C once, daily. In each consecutive 12- or 24-h hatching group the totality or a sample (maximal 30 flies) of females and males was measured for thorax-length following the usual technique[2]. Figs. 1 and 2 show the results of this series of measurements. Table 1 gives the correlation coefficients development time-size (Spearman's correlation), the mean size and the viability of each temperature-density combination considered.

Table 1. SIZE-DEVELOPMENT TIME CORRELATION, SIZE, VIABILITY PERCENTAGE IN THE DIFFERENT TEMPERATURE–EGG DENSITY COMBINATIONS USED. A THEORETICAL 1 : 1 SEX-RATIO IS SUPPOSED IN THE VIABILITY CALCULATIONS FOR MALES AND FEMALES

	30 eggs		240 eggs	
	♀♀	♂♂	♀♀	♂♂
	Spearman's correlation coefficient			
29° C	—1·00*	—1·00*	—0·64	—0·30
25° C	—0·94*	—1·00*	0·23	0·05
18° C	—0·98*	—0·95*	—0·60†	—0·24
	Size			
29° C	38·7 ± 0·17	34·2 ± 0·13	33·4 ± 0·14	30·1 ± 0·15
25° C	40·4 ± 0·16	36 0 ± 0·15	34·3 ± 0·12	30·3 ± 0·11
18° C	41·2 ± 0·13	37·1 ± 0·14	37·7 ± 0·11	33·9 ± 0·10
	Viability			
29° C	60·3	52·8	47·3	44·0
25° C	76 1	73·6	70·0	57·9
18° C	81·1	81·7	61·9	56·9

* $P < 0·01$
† $0·01 < P < 0·05$

The main results are as follows:

(1) With an original density of 30 eggs a tube (Fig. 1), size, expressed as mean size for each 12- or 24-h group, decreases regularly as development time increases; the correlation coefficients are highly significant (P always $< 0·01$). The percentage decrease from the first to the last day of emergence is quite different for both sexes, varying between 7·1 and 9·5 per cent for males and 9·6 and 13·3 per cent for females. Temperature does not seem to influence this decrease.

(2) With an original density of 240 eggs a tube (Fig. 2) there is no relationship between prolongation of the development time and size, except for 18° C females showing a decrease of 5·4 per cent between the extremes.

(3) There is a relatively slight difference in viability between the two population densities. Of course, viability is always inferior at 240, the difference varying from ± 11 per cent at 29° and 25° to ± 22 per cent at 18° C. Supposing a 1:1 primary sex-ratio, the mortality rate is always higher for males (Table 1).

Three different hypotheses may be invoked on to explain the results:

(*A*) *Environmental modification of identical genotypes*. This hypothesis is implicit in Gordon and Sang's examination[3] of the modifications of a staling medium, and was considered by Wattiaux[1] as an explanation for the variation of bristle numbers in relation to speed of development. This hypothesis can be discarded, for it does not account for the size constancy observed in the high population density, where the influence of a staling medium, the duration of emergence being longer, should be acting at a maximum.

(*B*) *Differential selection*. Although the slight differences in viability between temperature and density groups render a hypothesis based on differential survival very questionable, it can be imagined that, especially and only at low densities, genotypes for high developmental rate and large size would survive. Since up to this moment the crucial evidence concerning the heritability of the different size-rate combinations is lacking, it seems useless to discuss the possibilities offered by classical polygenic models, based on pleiotropy[4], or on linkage for both characters[5], or on differential selection for heterozygotes versus homozygotes in given environmental sets[6].

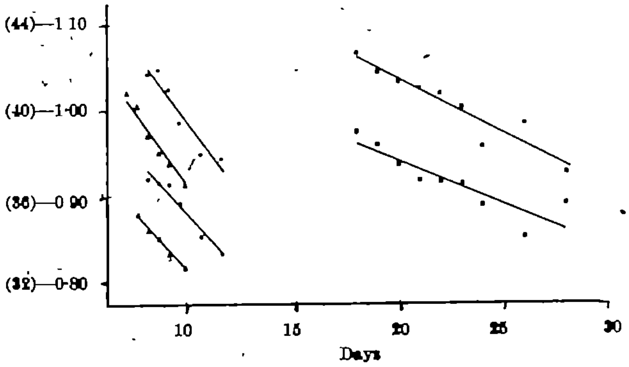

Fig. 1. Relation between size and duration of development at three temperatures and an original density of 30 eggs per tube. Duration of development is expressed in days from the egg-laying; size in arbitrary units (between brackets) and mm. 29°, ▲, ♀♀, △, ♂♂, 25°, ● ♀♀, ○, ♂♂; 18°, ■, ♀♀, □, ♂♂

(*C*) *Differential environmental effects on different genotypes*. Although this hypothesis requires the same crucial heritability demonstration, it seems *a priori* the most economical and simple one. It would mean that, in all experimental conditions, a population of the same average genotypical constitution for rate and size survives. However, only at low densities do different genotypes determine jointly the complete array of phenotypes from large-sized, rapidly developing flies to small-sized, slowly developing ones. It would be expected for nutritional reasons that at high densities the large body size, normally accompanying rapid development, does not reach expression. The relatively low expression of large body size at low densities and high temperatures can be explained on a similar basis.

As a partial and indirect confirmation of the last hypothesis, it must be remembered that it was possible to show repeatedly that the plus or minus sign and the significance of the size-development time correlations depend on the environment (temperature, density, culture medium)[7,8] and on the genetic nature of the strain[9]. Using the same wild-strain and an explanation based on this hypothesis, it was also possible to account for the failure of size ranges to coincide according to diverse temperature-density sets[9].

Whatever results are obtained in future experiments with respect to the morphogenetic relationship between size and time for development, and to the nature of the possible population genotypic variance with respect to

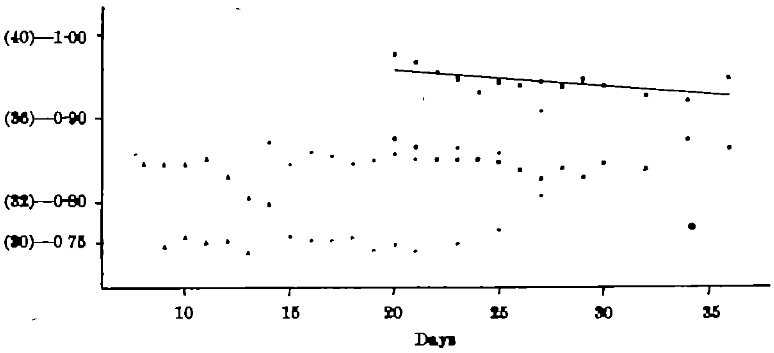

Fig. 2. As in Fig. 1 for an original density of 240 eggs a tube. Symbols same as Fig. 1

that relationship, present results emphasize the necessity of introducing into experiments concerning quantitative characters an array of controlled environmental conditions. Wattiaux's experiments and the present ones call, on the other hand, for a more thorough study of such a quantitative trait as rate of development, which possibly determines, directly or indirectly, and in a way depending on environmental conditions, many others; they should be considered as a first step, that of establishing the exact relationship between quantitative characters and development time, before scrutinizing the relationship of parental duration of development and the characters of the offspring.

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Effect of Grafting on the Incompatibility Response of *Oenothera organensis*

POLLEN-STYLE compatibility in *Oenothera organensis* is determined by a series of allelic genes designated S_1S_2, S_3S_4, etc.[1]. S_1 or S_2 pollen on an S_1S_2 stigma germinates but penetrates the style only a few millimetres. S_3, S_4 . . . S_n pollen on S_1S_2 grows through the entire length of the style (about 150 mm), reaching the ovary in a 12-h period. (Plants derived from the original Emerson[1] collections have retained their high degree of self-incompatibility. These collections were made in 1937 at a time when the population of *O. organensis* in this type location (Dripping Springs, Organ Mountains, New Mexico) was estimated to be more than 100 plants. A subsequent collection made in 1959 by Drs. S. Emerson and R. S. Bandurski has proved to have a much higher degree of self-fertility. At the time this later collection was made, the number of individuals in the type locality had been reduced to about 8 plants. It thus appears that the population became at least partially self-fertile as the plant neared extinction.)

Many lines of evidence indicate that pollen-style incompatibility is caused by an immune response of the type commonly associated with higher animals. For example, by labelling of the sexual partners with phosphorus-32 and carbon-14, followed by isolation of proteinaceous compounds formed during inhibited growth, Linskens[2,3] demonstrated that a "glycoproteid" was formed by both participants in incompatible matings which differed from that normally present. Serological tests with extracts from pollen and stylar tissue of *Oenothera organensis*[4] and *Petunia* hybrids[5] show that the homologous reaction is stronger than the heterologous one.

Demonstration that incompatibility in *Petunia* can be eliminated by ionizing radiation[6] is also in keeping with the suggestion of an immune response and further supporting evidence is provided by the observation of protoplasmic clumping following micro-injection of *Neurospora crassa* hyphae with cytoplasm from incompatible strains[7].

In this communication we report an effect of grafting on the incompatibility response of *Oenothera organensis* in which a partial tolerance to normally incompatible pollen is temporarily established. Several previous reports of lowered incompatibility in plants following grafting have appeared[8-10], and a recent report shows a temporary loss of tissue transplant immunity in parabiotic birds[11].

Plants of the same or differing allelic constitution grown from cuttings of non-grafted stocks were approach-grafted and kept in a greenhouse at 17° C night and varying day temperature. The plants were flowering at the time grafts were made. A photoperiod of at least 13 h was maintained. Graft unions were formed in a week and well established in two to three weeks. An adaptation of the technique of Emerson[1] was used to determine pollen-style compatibility. Flower buds were detached just prior to opening, emasculated and heavily pollinated. After a 24-h growth period at 25° C and 80 per cent relative humidity the styles were fixed and stained in an alcoholic solution of iodine in potassium iodide (iodine, 1 g; potassium iodide, 15 g; 30 per cent alcohol, 100 ml.). Pollen tube penetration can then be measured under the microscope in segments of the crushed stylar tissue. Results obtained with detached flowers were found to be the same as corresponding pollinations of flowers left attached to the plants. Five experiments of at least two pairs of plants of each graft-combination were performed.

Pollen-style incompatibility was determined at weekly intervals during the period zero to ten weeks following grafting. Results of the average of two typical experiments are presented in Fig. 1. As can be seen the ungrafted control S_1S_2 is self-sterile and pollen tubes do not penetrate the style when the plant is self-pollinated. The cross, S_1S_2/S_3S_4, is, of course, fertile and pollen tubes penetrate the entire stylar length. A further control is provided by an S_1S_2 plant grafted to an S_1S_2 plant. This graft has no effect on pollen tube growth. When, however, an S_1S_2 plant is grafted to S_3S_4 then, within 2–3 weeks following grafting, S_1S_2 pollen from an ungrafted plant will penetrate the S_1S_2 stylar tissue of the grafted parent. This result shows that grafting affects the immune response of the stylar tissue. The effect of grafting on S_1S_2 plant to S_3S_4 and then self-pollinating is greater than that seen when the pollen was from an ungrafted plant, suggesting that grafting also diminishes the immune response of the pollen. That this is the case is shown by the experiment of Table 1. In this experiment, again the average of two experiments, it can be seen that pollen from an S_1S_2 flower of an S_1S_2 plant grafted to S_3S_4 penetrated the style of an ungrafted S_1S_2 plant. Thus the effect of grafting on the pollen response is demonstrated. This effect is, however, delayed by 2–3 weeks and is one-third to one-half as strong as evidenced by an S_1S_2 plant grafted to S_3S_4 and self-pollinated.

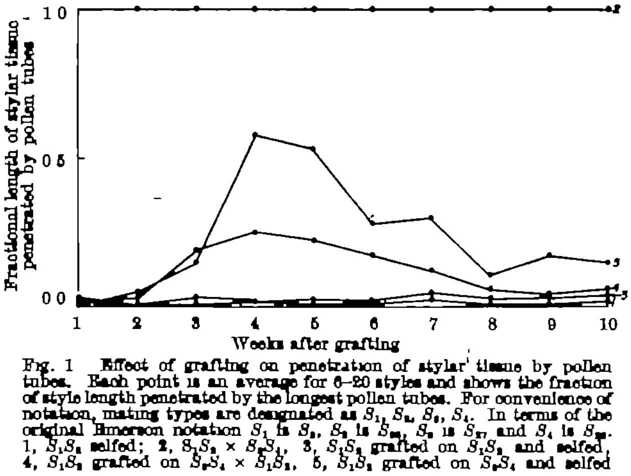

Fig. 1 Effect of grafting on penetration of stylar tissue by pollen tubes. Each point is an average for 6–20 styles and shows the fraction of style length penetrated by the longest pollen tubes. For convenience of notation, mating types are designated as S_1, S_2, S_3, S_4. In terms of the original Emerson notation S_1 is S_3, S_2 is S[illegible], S_3 is S_{17} and S_4 is S[illegible]. 1, S_1S_2 selfed; 2, $S_1S_2 \times S_3S_4$; 3, S_1S_2 grafted on S_1S_2 and selfed, 4, S_1S_2 grafted on $S_3S_4 \times S_1S_2$; 5, S_1S_2 grafted on S_3S_4 and selfed

Several conclusions may be drawn from data here reported. It is apparent that the incompatibility response involves both stylar and pollen factors since heterologous grafting of either the pollen or stylar parent diminishes the incompatibility response. In addition, since seed is not set following self-pollination of heterologously grafted plants (although pollen tubes have been observed to grow to the carpel) it may be concluded that there is a second barrier to self-fertilization. These conclusions are con-

Table 1. FRACTIONAL LENGTH OF STYLAR TISSUE PENETRATED BY POLLEN TUBES*

Graft-combinations pollinated	Weeks after grafting 1	2	3	4	5	6	7	8	9	10
1 S_1S_2 grafted on S_1S_2 and selfed	0	0	0	0	0	0	0·1	0	0	0
2 S_1S_2 grafted on S_3S_4 and an S_1S_2 flower selfed	0	0·1	0·1	0·6	0·6	0·3	0·2	0·2	0·2	0
3 S_1S_2 pollinated with S_1S_2 pollen from S_1S_2 grafted to S_3S_4	0	0	0	0·1	0·1	0·2	0·3	0·2	0·2	0·1

* Replication, notation and experimental conditions as for Fig. 1.

sistent with those of Linskens utilizing data from X-irradiated incompatible *Petunia*[5].

This work was supported in part by the Michigan Agricultural Experiment Station and by the U.S. National Science Foundation. We thank Dr. Adolf Hecht for cuttings of the mating types used and Drs. S. Emerson and R. S. Bandurski for advice and aid in making collections.

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VIROLOGY

Isolation from Man in Slovenia of a Virus belonging to the California Complex of Arthropod-borne Viruses

FOLLOWING observations[1] that in some areas of Slovenia, Yugoslavia, inapparent infections with Central European tick-borne encephalitis virus are common, attempts were made to isolate this agent from clinically inapparent but serologically detectable infections. In the course of this work two strains of another arthropod-borne virus were obtained. Designated Trojica Nos. 1 and 2 after the village where they occurred, these strains have now been identified as belonging to the California complex[2] of arthropod-borne viruses.

The isolations were made from two sera of about 5,000 collected from healthy human beings during the spring and summer of 1960 and 1961 in different localities in Slovenia known as endemic foci of Central European tick-borne encephalitis.

Serum collections were stored for 1–5 weeks at −20° C before being used for virus isolations. About half the specimens were tested individually, the remainder in pools of two or three. Sera were filtered through Seitz *EK* pads and centrifuged at 40,000 r.p.m. for 1 h. Sediments were resuspended in a volume of borate buffer, *p*H 7·2, between 1:10 and 1:20 of the original volume of the serum and inoculated into human embryo kidney cell cultures, chorioallantoic membranes of 12-day-old chick embryos and new-born mice.

Two sera that were pathogenic only for new-born mice yielded agents that could be serially propagated. Re-isolation from the original material kept at −20° C was successful in both cases.

The two strains had similar properties. By intracerebral or intraperitoneal inoculation they were pathogenic for new-born but not adult mice. They were sensitive to ethyl ether and sodium deoxycholate and were not neutralized by immune sera for several agents belonging in groups *A* and *B* of the arthropod-borne animal viruses.

The strains were also poor hæmagglutinin producers. A titre of 1:64 at *p*H 6·0 was obtained on one occasion with a new-born mouse brain antigen from strain No. 1, but this observation could not be repeated. After seven consecutive daily intracerebral passages in new-born mice, antigens with a titre of 1:10 at *p*H 6·0 and 6·1 were obtained. Both strains could be propagated in HeLa cell cultures, and by concentration of the fluid phase with an *LKB 6300A* ultrafilter[3], strain No. 1 yielded a low-titre hæmagglutinating antigen.

The strains were identified as belonging to the California complex in a complement-fixation test. Immune sera against both were prepared in adult mice given four intraperitoneal injections of a 10 per cent suspension of live virus. Antigens were prepared by acetone precipitation of a suspension of infected new-born mouse brain tissue in isotonic sucrose.

Neither serum reacted with complement-fixing antigens for the following viruses: eastern and western encephalitis, Semliki forest and Sindbis (group *A*); dengue type 2, Ntaya, Murray Valley encephalitis, Japanese *B* encephalitis, Central European tick-borne encephalitis, Russian spring–summer encephalitis, West Nile, yellow fever, St. Louis encephalitis and Zika (group *B*); and Guaroa, Simbu, Germiston, Bwamba, Chittoor and Neapolitan and Sicilian sandfly fevers. Both sera, however, had antibodies against California encephalitis and Tahyna antigens (Table 1).

Table 1. TITRES OF COMPLEMENT-FIXING ANTIBODIES IN IMMUNE SERA FOR TROJICA STRAINS NOS. 1 AND 2

Antigen	Serum Trojica No. 1	Trojica No. 2
California	16/8*	32/8
Tahyna	8/16	16/8
Trojica No. 1	16/16	32/8
Trojica No. 2	16/8	16/8

* Reciprocal of serum titre/reciprocal of antigen titre

The isolation of the Trojica strains is of interest, because viruses of the California complex have thus far been isolated from mosquitoes in several parts of the world but not from man. Thus, California encephalitis virus was obtained from *Aedes dorsalis* and *Culex tarsalis* in the United States[4], Lumbo from *Aedes pembaensis* in Mozambique[5], Tahyna from *A. vexans* and *A. caspius*[6,7] in Czechoslovakia, Trivittatus from *A. trivittatus* in the United States[4] and Melao from *A. scapularis* in Trinidad and Brazil[8]. Isolation of strains of this complex from man was not unexpected, however, since Bardos and Sevcoviceva[9] have reported the presence of Tahyna neutralizing antibodies in 8 per cent of 50 sera investigated from Slovenia.

Whether the two Trojica strains represent a new virus of this complex or a strain of one of the members already described—perhaps Tahyna—cannot be stated at the moment.

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Bacterial Origin of Certain Viruses: Identity of the Eaton Agent with *Streptococcus MG*

In a recent communication Pease and Bisset[1] have given evidence in support of a hypothesis for the origin of myxoviruses from mucous-membrane parasitic bacteria, among other things, from the circumstances attending virus pneumonia (*PAP*). This condition, although ostensibly caused by a virus (or mycoplasma[2]), is accompanied by the development of serum agglutinins against a bacterium, *Streptococcus MG*, in a significant number of patients. Although the *Streptococcus* can sometimes be isolated from cases of the disease, attempts to demonstrate an antigenic relationship with the Eaton agent have previously failed[3].

I have now shown that antigenically active surface material is produced by the Eaton agent but is lost by diffusion into the medium, as is known to occur with bacterial *L*-forms[4], and this material, either *in situ* in a gel-diffusion test, or in the form of an extract, gives a strong positive precipitin reaction with *Streptococcus MG* antiserum. The material is apparently similar to the *M* protein of *Streptococci*.

Thus, the development of agglutinins in virus pneumonia patients, and the failure of previous efforts to establish an antigenic link, capable of accounting for the phenomenon, are simultaneously explained. At the same time, this observation provides an illustration of a pathogenic, virus-like agent that gives every evidence of being, in fact, the *L*-form of a bacterium. Indeed, here a strain of the Eaton agent has actually dissociated to give a bacterium resembling *Streptococcus MG*.

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SOIL SCIENCE

Paper Electrochromatography of Clay Minerals

A major handicap in the study of clay minerals from soil systems is the lack of adequate methods for separating and quantitatively estimating individual minerals from clay mineral mixtures. Present success in the application of paper electrophoresis and paper chromatography to the study of complex organic mixtures suggests that such techniques also might prove promising in clay mineral studies. Although appearing theoretically promising, past electrophoresis studies of clay mineral suspensions have seemed to possess rather limited applicability[1-4]. Difficulties in obtaining and maintaining clear-cut separations of mineral mixtures have continually hampered clay mineralogists using such techniques. In an attempt to provide an improved technique for the separation and characterization of clay minerals from mineral mixtures, preliminary studies were undertaken on behaviour of the minerals under varying conditions of paper electrochromatography.

Minerals were located on dried filter paper curtains through the use of a 0·004 per cent solution of malachite green in nitrobenzene[5]. The stain was sensitive to as little as 1 μg of each mineral when used in the virtual absence of residual glycerol. Minerals were obvious as blue areas once the background of the surrounding paper had faded to its original white colour.

Conditions found during the initial series of experiments which permitted differential migration of clay minerals included the use of a Schleicher and Schuell 470-*A* paper curtain, with an electrolyte of 0·01 per cent sodium carbonate in a 20 per cent glycerol aqueous media (pH 9·6). A temperature of 8° C was maintained through the use of a water-cooled fractionation surface on which the curtain rested (Karler-Misco Electrochromatography Apparatus, Microchemical Specialties Company, Berkeley, California). Suspensions of less than 2μ reference minerals (0·01 per cent with respect to each mineral) were fed on to the curtain from a motor-driven syringe at the rate of 25 μg of each mineral per h.

Under the foregoing conditions, and with a potential gradient of 20 V/cm, bentonite was separated from a mixture with kaolinite and commercial vermiculite-like material in 48 h. The bentonite migrated diagonally toward the drip points on the anode side of the curtain (following the migration path previously demonstrated for reference montmorillonite): the kaolinite and vermiculitic material descended almost vertically with chromatographic buffer flow toward the drip points directly beneath the point of application (approximately 2 in. from the cathode side of the curtain).

Identity and integrity of minerals that had been subjected to electrochromatographic forces for a period of 72 h were checked by recovering sufficient quantities of the eluted minerals for X-ray analysis. Fractions from each of the 22 drip points were concentrated, the flocculent (glycerol saturated) spotted on glass slides, and the samples analysed with an X-ray diffractometer. Characteristic peaks for montmorillonite (18–19 Å), vermiculite (14 Å), and kaolinite (7 Å) were obtained in residues from drip points where migration paths of the previous experiments indicated these minerals would appear. The vermiculitic material appeared concentrated on the anode side of the kaolinite–vermiculite band, but apparently its migration was not sufficient to separate it from the non-migrating kaolinite. The separation into two bands was sufficiently clear-cut, however, to lend considerable support to the premise that electrochromatography or similar approaches may ultimately provide the means for the separation of at least simple clay mineral mixtures into their individual mineral components.

Adsorption of sample particles on supporting media, such as filter paper, has often been a major limitation in the application of paper electrophoresis to colloidal organic particles. In this series of experiments, strong adsorption of clay minerals on the paper resulted in slow migration speeds, tailing from the origin, and considerable broadening of the migration bands through diffusion. Such equipment difficulties might be resolved in the future by using less adsorptive supporting media or higher potential gradients.

More efficient apparatus, supporting media, and experimental conditions than were available for the present tests can undoubtedly be found. A modified Svensson and Brattsen apparatus, referred to by Mackenzie and Farquharson[6], may be more readily adaptable to macroscale mineralogical separations. (Some success with such an apparatus has been indicated by Mackenzie, personal communication, September 1961.) It is hoped that interest in this area will be stimulated such that efficient equipment will be developed and operating conditions defined for rapid separation and quantitative estimation of clay minerals by electrochromatographic techniques.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, March 18

Medical Research Council (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 4.30 p.m.—Prof. S. Spiegelman (University of Illinois): "The Use of DNA-RNA Hybridisation as an Analytical Tool for the Solution of Biological Problems".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Gilbert White (Chicago University): "The Contribution of Geographical Analysis to the Planning of River Development".

Society of Chemical Industry (joint meeting of the Pesticides, Fine Chemicals and Microbiology Groups, at 14 Belgrave Square, London, S.W.1), at 5 p.m.—Prof. B. Lythgoe and Dr. A. Rhodes: "The Chemical and Biological Properties of Venturicidin".

Institution of Electrical Engineers, Science and General Division P.G.S3 and Power Division P.G.P7 (at Savoy Place, London, W.C.2), at .30 p.m.—Dr. M. P. Reece: "Circuit Interruption in Vacuum".

Institution of Electrical Engineers (joint meeting with the London Graduate and Student Section, at Savoy Place, London, W.C.2), at p.m.—Discussion on "Merits of Using the Metric System".

Institute of Metal Finishing, London Branch (at the Borough Polytechnic, Borough High Street, London, S.E.1), at 6.15 p.m.—Mr. R. J. Brown: "Electroplating and the Use of Plastics and Corrosion Resistant Materials".

Tuesday, March 19

Institution of Electrical Engineers, and Royal Aeronautical Society Joint Group (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Semiconductors and their Application to Airborne Equipment" opened by Dr. C. S. Hudson.

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Unsteady Gas Dynamics of Internal Combustion Engines".

Wednesday, March 20

University of London (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. B. Lennox: "Tumours of Hair Follicles and Sweat Glands".*

East Malling Research Station (at The Laboratories, The Research Station, Maidstone, Kent), at 2.30 p.m.—Mr. W. R. Jones: "The Role of Advisory Services in a Changing World" (The Amos Memorial Lecture).

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. W. S. Hall: "The Rise and Fall of an Isolated Thermal in Wind Shear"; Mr. B. J. Mason and Mr. R. Emig: "Calculations of the Ascent of a Saturated Buoyant Parcel with Mixing"; Mr. B. J. Mason and Mr. C. W. Chien: "Cloud-Droplet Growth by Condensation in Cumulus".

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. Greatorex: "Thermal Treatment of Light Residual Fuel Oils to Minimise Wax Separation Problems".

Institution of Electrical Engineers, Power Division (at Savoy Place London, W.C.2), at 5.30 p.m.—Mr. P. Bowles, Mr. H. Hadley, Mr. M. J. Marchbanks and Mr. J. J. Wilkins: "Magnet Power Supply for the 7 GeV Proton Synchrotron Nimrod: Part 1—General"; Mr. J. A. Fox, Mr. D. G. Taylor and Mr. R. Wilson: "Magnet Power Supply for the 7 GeV Proton Synchrotron Nimrod: Part 2—Rotating Machines"; Mr. K. Rollig and Mr. H. Hodle: "Magnet Power Supply for the 7 GeV Proton Synchrotron Nimrod: Part 3—Mercury-Arc Converters".

British Institution of Radio Engineers, Computer Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Multi-aperture Ferrite Devices".

Institution of Electrical Engineers, Electronics Division P.G.E5 (joint meeting with the British Institution of Radio Engineers Medical Electronics Group at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Instrumentation for Applied Psychology".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. A. L. G. Lindley and Mr. R. E. D. Bishop: "Some Recent Research on the Balancing of Large Flexible Rotors" (James Clayton Paper).

Royal Microscopical Society (at Tavistock House South, Tavistock Square, London, W.C.1), at 6 p.m.—Prof. S. Tolansky, F.R.S.: "Surface Microtopography Studies using Interferometry".

Royal Institute of Chemistry, London Section (at the Northern Polytechnic, Holloway, London, N.7), at 7 p.m.—Prof. C. C. Addison: "Recent Applications of Non-aqueous Inorganic Solvents".

Thursday, March 21

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Meeting for the Election of Fellows. 4.30 p.m.—Prof. Neal E. Miller: "Certain Recent Developments in Experimental Psychology" (Special Review Lecture).

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. J. T. M. Taylor: "Research on Ground Control and Rockbursts on the Kolar Gold Field, India"; Mr. G. J. Brittingham: "Study of the Possible Application of Mineral Dressing Techniques for the Recovery of Uranium from the Conglomerates of the Serra de Jacobina, Bahia, Brazil".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Isabella Gordon: "The Role of Sound in Marine Biology"; Dr. P. J. Newbould: "Conservation and Biological Production".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. P. Erdos: "Problems and Results in Set Theory".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, W.C.1), at 6 p.m.—Mr. C. Hobbs: "Concrete Additives in the Building Industry from the Viewpoint of the Large Contractor".

Television Society (at 164 Shaftesbury Avenue, London, W.C.2), at 7 p.m.—Mr. Donald Wilson: "Writing for Television".*

Royal Society of Tropical Medicine and Hygiene (at the Royal Army Medical College, Millbank, London, S.W.1), at 7.30 p.m.—Laboratory Meeting.

Thursday, March 21—Friday, March 22

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1)—Conference on "The Education and Training of Technicians".

Friday, March 22

Royal Astronomical Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Discussion of Papers.

Royal Aeronautical Society, Man Powered Aircraft Group (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. B. C. Clear-Hill and Mr. J. C. Wimpenny: "The Problems of Man Powered Flight".

Society of Chemical Industry, Plastics and Polymer Group (joint meeting with the South Wales Section of the S.C.I., at University College, Cathays Park, Cardiff), at 7 p.m.—Dr. I. Goodman: "Newer Knowledge of the Chemistry of Terylene and Related Polyesters".

Society for Visiting Scientists (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Evolution in Society". Chairman: Prof. C. D. Darlington, F.R.S. Speakers: Mr. T. Burns, Prof. V. C. Wynne-Edwards and Prof. Marc Klein.

Monday, March 25

Royal Society of Medicine, Library (Scientific Research) Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Mr. H. Palmer: "The Pharmaceutical Industry—What it is and What it Does".

Royal Geographical Society (at Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. John D. Woods and Mr. Brian B. Matthews: "Archæology Under the Sea".

Monday, March 25—Wednesday, March 27

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2)—Convention on "H.F. Communication".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer (preferably with some interest in the thermodynamics of co-ordination compounds) in Inorganic Chemistry—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (March 22).

Lecturer (with special interests and qualifications in mineralogy and petrology) in Geology in the Faculty of Science—The Registrar, The University, Manchester 13 (March 23).

Senior Lecturer or Lecturer in Statistics; and a Lecturer in Pure Mathematics—The Secretary, The University, Northcote House, The Queen's Drive, Exeter, Devon (March 23).

Reader (with considerable research experience and the capacity to supervise postgraduate research students) in Botany—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (March 25).

Assistant in Genetics—The Secretary of University Court, The University, Glasgow (March 30).

Lecturer in the Department of Metallurgy—The Registrar, The University, Liverpool, quoting Ref. CV/270/N (March 30).

Lecturer, Senior Lecturer or Associate Professor in Applied Genetics at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 31).

Lecturers (2) in Computing—The Deputy Registrar, The University, Birmingham 15 (March 31).

Senior Lecturer (with special qualifications in analysis, preferably functional analysis) in Pure Mathematics—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (April 1).

Lecturer (with experience of cultivation and processing of tropical crops, and of field experimentation) in Crop Husbandry in the Department of Agriculture, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 4).

Chair of Preventive Medicine at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 7).

Lecturer (with qualifications in pure mathematics or in mathematical statistics) in Mathematics—The Registrar and Secretary, The University, 38 North Bailey, Durham (April 8).

Chair of Mathematics at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 8).

Lecturer (with a registrable medical qualification) in Physiology—The Registrar, Trinity College, Dublin 2, Republic of Ireland (April 8).

Second Chair of Mathematics at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 8).

Senior Lecturer/Lecturer in Anatomy; a Senior Lecturer in Physiology; Chair of Pharmaceutics; and a Chair of Pharmaceutical Chemistry at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

Lecturer or Assistant Lecturer (Probationary) (man or woman graduate) in Statistics in the Departments of Mathematics and Psychology—The Secretary, Bedford College, Regent's Park, London, N.W.1 (April 16).

Lecturer in the Department of Physiology—The Secretary, The University, Edinburgh (April 17).

CHAIR OF EDUCATION—The Secretary, The University, Aberdeen (April 24).

READER IN NUMERICAL ANALYSIS AND MACHINE COMPUTATION at Queen Mary College—The Academic Registrar, The University of London, Senate House, London, W.C.1 (April 24).

CHAIR OF NUCLEAR PHYSICS at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and the Registrar, University of Cape Town, Private Bag, Rondebosch, South Africa (April 29).

LECTURER (with research or industrial experience in automatic control and/or engineering systems design) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, Queen Mary College, Mile End Road, London, E.1 (April 30).

POSTDOCTORAL FELLOW (Organic Chemist), to participate in a study on the biosynthesis of natural products—The Registrar, University College of Swansea, Singleton Park, Swansea (May 1).

CHAIR OF EDUCATION at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, May 13).

A.E.I. RESEARCH STUDENT (graduate) IN THE PHYSICS DEPARTMENT to carry out research in either experimental or theoretical physics—Prof. R. W. Ditchburn, F.R.S., J. J. Thompson Physical Laboratory, The University, Whiteknights Park, Reading (May 20).

ASSISTANT PROFESSOR IN ANATOMY, with teaching duties in gross anatomy—The Chairman, Department of Anatomy, University of Saskatchewan, Saskatoon, Sask., Canada.

BIOCHEMIST (Senior grade) IN CHARGE OF THE GROUP BIOCHEMISTRY LABORATORY—Hospital Secretary, Altrincham General Hospital, Altrincham.

LIBRARIAN (with previous experience of working in a scientific library)—The Director, Research Station, Fruit and Vegetable Canning and Quick Freezing Research Association, Chipping Campden, Glos.

MASTER TO TEACH BIOLOGY throughout the School to University Scholarship standard—The Headmaster, The Perse School, Hills Road, Cambridge.

MASTER TO TEACH MATHEMATICS with emphasis on sixth form work—The Head Master, Cheltenham Grammar School, Cheltenham.

RESEARCH ASSISTANT (graduate, Graduate Member of the Royal Institute of Chemistry, or with equivalent qualifications) IN ORGANIC CHEMISTRY, to work under the supervision of Dr. G. Shaw on some aspects of the chemistry of nucleic acids and their derivatives—The Registrar, Dept. J/23, Bradford Institute of Technology, Bradford 7.

RESEARCH FELLOW, to work on catalytic reactions in molecular sieves—Prof. R. M. Barrer, Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

SCIENTIFIC INFORMATION OFFICER (Abstractor) (with a degree in agriculture or natural science, and able to translate from German and/or Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

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REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Memoirs of the Royal Astronomical Society. Vol. 68, Part 5: The Revised 3C Catalogue of Radio Sources. By A. S. Bennett. Pp. 163–172. (London: Royal Astronomical Society, 1962) 6s.; 1 dollar. [281

Ciba (A.R.L.), Ltd. Technical Notes, No. 240 (December, 1962): Araldite Road Surface for Tower Bridge. (Duxford: Ciba (A.R.L.), Ltd., 1962.) [281

Southern Regional Council for Further Education. Fifteenth Annual Report, October 1962. Pp. 38. (Reading: Southern Regional Council for Further Education, 1962.) [281

Falkland Islands Dependencies Survey. Scientific Reports, No. 33: The Dove Prion, *Pachyptila desolata* Gmelin. By W. L. N. Tickell. Pp. 55+8 plates. (London: H.M. Stationery Office, 1962.) 18s. net. [281

Bulletin of the British Museum (Natural History). Entomology. Vol. 13, No. 3: On the Genera of Indo-Pakistan Cleoninae and Hylobiinae (Coleoptera: Curculionidae). By Nasir Ahman Aslam. Pp. 45–66. (London: British Museum (Natural History), 1963.) 8s. [281

Metabolic Integrations. By P. G. Watson and D. E. S. Truman. Pp. 18. (Cambridge: W. Heffer and Sons, Ltd., 1962.) [281

Royal Institute of Chemistry. Lecture Series 1962. No. 3: Design and Construction of Laboratories: an Exercise in Professional Collaboration. By R. R. Young and P. J. Harrington. Pp. 14+6 plates. 4s. No. 4: Stereochemical Correlations. By Prof. W. Klyne. Pp. 23. 4s. 6d. No. 5: Peptide Synthesis. By Dr. H. N. Rydon. Pp. 29. 5s. (London: Royal Institute of Chemistry, 1962.) [281

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 61-24: Fernie Map-Area, East Half, Alberta and British Columbia. By R. A. Price. Pp. iv+65. 50 cents. Paper 62-15: Middle Devonian and Older Palaeozoic Formations of Southern District of Mackenzie and Adjacent Areas. By Helen R. Belyea and A. W. Norris. Pp. iv+82. 50 cents. (Ottawa: Queen's Printer, 1962.) [281

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Report of the Director on the Scientific and Technical Activities of the Office International des Epizooties, May 1961–May 1962. Pp. 94. (Paris: Office International des Epizooties, 1962.) [281

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United States Department of Commerce: Weather Bureau. Technical Paper No. 43: Rainfall-Frequency Atlas of the Hawaiian Islands for Areas to 200 Square Miles, Durations to 24 hours, and Return Periods from 1 to 100 Years. Pp. iv+60. (Washington, D.C.: Government Printing Office, 1962.) 40 cents. [281

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Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 128, No. 2: Eight New Species of *Xarifia* (Copepoda, Cyclopoida), Parasites of Corals in Madagascar. By Arthur G. Humes. Pp. 35–64+18 plates. Vol. 128, No. 3: Studies on Amphisbaenids (Amphisbaenia, Reptilia). 2: On the Amphisbaenids of the Antilles. By Carl Gans and A. Allan Alexander. Pp. 65–158+12 plates. Vol. 128, No. 4: New and Little Known Batoid Fishes from the Western Atlantic. By Henry B. Bigelow and William C. Schroeder. Pp. 159–244+1 plate. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [12

Museum of Comparative Zoology at Harvard College. Breviora. No. 170 (November 16, 1962): The Brain of the Emu (*Dromaeus novaehollandiae*, Lath). 1: Gross Anatomy of the Brain and Pineal Body. By Stanley Cobb and Tilly Edinger. Pp. 18. No. 171 (December 14, 1962): Notes on Amphisbaenids (Amphisbaenia: Reptilia). 6: Redescription and Range Extension of *Amphisbaena spurrelli* Boulenger. By Carl Gans. Pp. 11. No. 172 (December 14, 1962): A New Species of the Rodent *Pipestoneomys* from the Oligocene of Nebraska. By Raymond Alf. Pp. 7. No. 173 (December 24, 1962): New Species of Land Mollusks from the Republica Dominicana. By William J. Clench. Pp. 5+1 plate. No. 174 (December 24, 1962): A New Arctocyonid from the Paleocene of Wyoming. By Bryan Patterson and Paul O. McGrew. Pp. 10. No. 175 (December 24, 1962): A Picrodontid Insectivore (?) from the Paleocene of Wyoming. By Paul O. McGrew. Pp. 9. No. 176 (December 27, 1962): On the Races of *Kinixys belliana* Gray. By R. F. Laurent. Pp. 6. No. 177 (December 27, 1962): Rhipidistian Classification in Relation to the Origin of the Tetrapoda. By Keith S. Thomson. Pp. 12. No. 178 (December 27, 1962): On a New Species of the Earthworm Genus *Trigaster* Benham 1886 (Octochaetidae). By G. E. Gates. Pp. 4. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [12

Proceedings of the United States National Museum, Smithsonian Institution Vol. 114, No. 3467: Scarab Beetles of the Genus *Onthophagus* Latreille North of Mexico (Coleoptera: Scarabaeidae). By Henry F. Howden and Oscar L. Cartwright. Pp. 1–137+9 plates. (Washington, D.C.: Government Printing Office, 1963.) [12

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 87: Flora of Pennsylvanian Pictou Group of New Brunswick. By W. A. Bell. Pp. ix+72+56 plates. 3.50 dollars. Bulletin No. 92: Contributions to Canadian Palaeontology. Stromatoporoid Fauna of the Waterways Formation (Devonian) of North-eastern Alberta. By C. W. Stearn. *Columnaria pax* (Smith) and the Silurian *Columnaria columbia* n. sp. from British Columbia. By B. S. Norford. Pp. ix+80+12 plates. 1.50 dollars. Paper 62-12: Surficial Geology of Canterbury, Woodstock, Florenceville, and Andover Map-Areas; York Carleton, and Victoria Counties, New Brunswick. By Hulbert A. Lee. Pp. iv+8+4 maps. 50 cents. Paper 63-1: Summary of Research: Field 1962. Compiled by S. E. Jenness. Pp. iv+73. 25 cents. (Ottawa: Queen's Printer, 1962 and 1963.) [12

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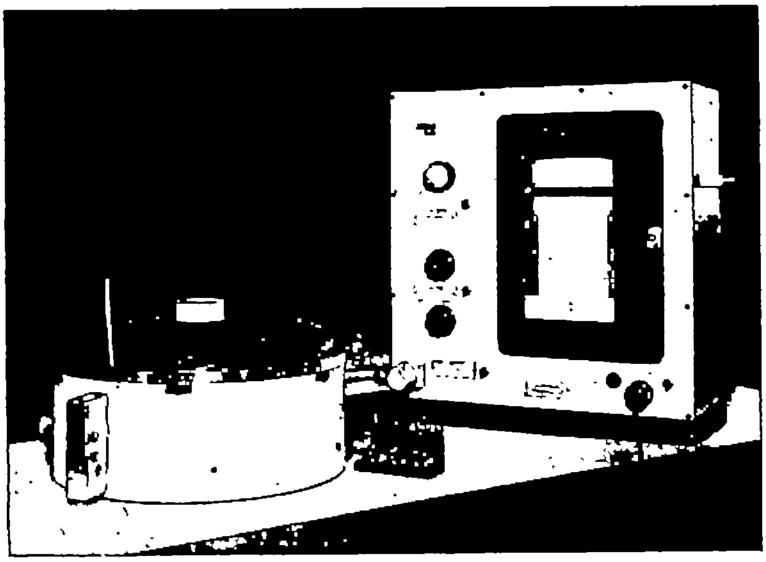

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Applications (three copies), should be lodged, not later than April 22, 1963, with the undersigned, from whom particulars may be obtained.

ROBT. T. HUTCHESON,
Secretary of University Court.

SCHIZOPHRENIA RESEARCH FELLOWSHIP

The Schizophrenia Research Fund, which has recently been formed, proposes to establish a long-term Fellowship, to be known as the Rothschild Fellowship; tenable for five to seven years, for research into schizophrenia. The grant will be made in the form of a Fellowship either to the individual who is to be wholly engaged on the work or to the head of a department who wishes to include such a post in his department and to associate himself with the work. The holder of the post should have research experience but need not be medically qualified. In certain circumstances the conditions of the Fellowship might be alterable. The salary will be in the range of £2,000 to £2,500 depending on qualifications and experience. Superannuation provision will be similar to the F S.S.U. scheme.

Applications should give details of the projected research and of the qualifications and experience of the proposed holder of the post and the names of three referees; and, unless coming from the head of a department, should state where the applicant proposes to do the work, what facilities and co-operation would be required from the institution concerned and whether these have yet been assured.

Applications should be sent to The Secretary, The Schizophrenia Research Fund, City Gate House, Finsbury Square, London, E C.2, to arrive not later than July 1, 1963.

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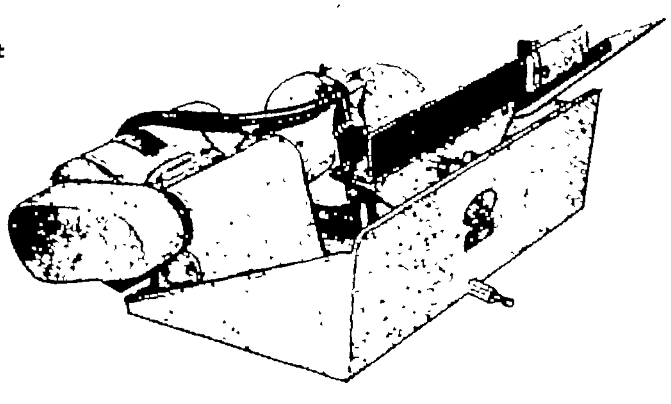

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SCIENCE AND TECHNOLOGY FOR THE BENEFIT OF THE LESS-DEVELOPED AREAS

MR. J. K. GALBRAITH'S little book, *Economic Development in Perspective*[*], is the outcome of five lectures which the United States Ambassador to India delivered in the summer and autumn of 1961 in five major Indian educational communities. It is not accordingly, as the title might suggest, a commentary on the economic situation of this country and the measures being adopted to meet that situation. It is rather a penetrating discussion of the root problems with which the recent United Nations conference on the "Application of Science and Technology for the Benefit of the Less-Developed Areas", held in Geneva during February 4–20, was concerned. Some at least of Mr. Galbraith's conclusions, too, are remarkably in line with those reached at the most recent Pugwash Conference. They are outlined with wit as well as cogency, and it is encouraging to find that they were reflected in some of the utterances at the Geneva conference.

Mr. Galbraith begins by insisting that the problem of economic development must be considered and handled as a whole, and he even challenges the rigid differentiation of countries into developed and under-developed. We have often failed to inquire whether measures which can contribute to economic development are, in fact, being utilized in a situation favourable to development. Furthermore, capital and technical knowledge may not be the only missing elements. In many of the newer African States and in parts of Latin America the first task is to build competent organs of public administration. Good development plans cannot be created or executed without good government and neither technical assistance nor trained technicians achieve much, or are even much needed, where administration is indifferent or bad.

If the best agricultural scientist can make little headway as adviser to a non-existent ministry, there are circumstances in which popular education must have priority over the dams, factories and other equipment of capital development and in which attention must be focused on the shortcomings of the social order. The most eloquent agricultural extension expert will not convince the peasant of the advantage of growing two blades of grass where one flourished before if the peasant knows full well that both will go inevitably to his landlord. The best-considered forms of agricultural investment or the most sophisticated techniques of agricultural expansion may be worthless unless the cultivator has some assurance that some at least of the gains will accrue to him. Effective government, education and social justice are critically important, and in many countries it is lack of them that is the barrier to advance: until that barrier is removed little will come from capital investment and technical assistance.

Next, Mr. Galbraith challenges the common assumption that, since capital and technically trained manpower are the limiting factors in India and Pakistan, they are the limiting factors everywhere. We can no longer have, he maintains, one diagnosis of the causes of under-development. Rather, we must have the particular diagnosis which fits the particular country. We cannot even assume that if competent planning is possible in India and Pakistan it is possible everywhere. It would, indeed, be a serious mistake to imagine that such planning is essential for nations in all stages of development. In earlier stages it may be neither necessary nor possible.

This point is further elaborated in the second chapter, in which Mr. Galbraith even more explicitly deprecates rigid distinction between the developed and the under-developed countries. He urges that provision of aid is itself a co-operative process in which all countries have something to contribute, and that discrimination is vital in the use of technology if scarce resources are not to be wasted and development itself handicapped. Again, in his third lecture, discussing the theory of development planning, he points out that a good plan must provide a strategy for economic advance: it must not be allowed to become a list of desirable projects. Taking account of both the visible and the invisible dimensions of industrial achievement, its proposals must have regard to a theory of consumption which takes due account of the needs of the particular consumption for the benefit of which in the last analysis the plan is designed. Abundant and efficiently produced food, clothing and shelter are requirements that come before the introduction of the last techniques, and fundamentally Mr. Galbraith is here doing no more than insist on the old but often disregarded adage that the tool should be appropriate to the job.

In his fourth chapter, Mr. Galbraith deals more specifically with education, pointing out that education is of high importance, both as an object of immediate consumption and as a form of investment for future consumption. Neither aspect can safely be disregarded, and Mr. Galbraith does not hesitate to point out some of the implications when education is viewed as an investment or the dangers which attend some experiments with democratic direction in Latin American universities. A developing country must consider its educational system as purposefully as any other form of capital outlay and in the light of the peculiar requirements of development. It cannot simply adapt from the older models, for adaptation is as demanding in its own way as innovation.

Some of Mr. Galbraith's comments in this chapter are particularly interesting in the light of the concern that has been recently expressed over the working of the Commonwealth Scholarships Scheme, especially in respect of the return of scholars to their own country on the expiration of their scholarships. If this aspect appears to have received comparatively little attention at the Geneva conference, many of his other points were well taken. Prof. P. M. S. Blackett, for example, once again stressed the vital importance of considering what known and available technology had to offer to meet the specific needs of a particular country and what research might be required in relation to local conditions. While there might be some new technologies particularly well designed to meet certain specific needs, generally speaking it was the older technologies that were most likely to prove appropriate. It might, for example, be wiser to eschew recent advances for the time being and concentrate on earlier technology of wider potentialities. In agriculture, for example, while many things are useful, mechanization is not the big thing. Water, fertilizers and improved seed can revolutionize agriculture, but to ignore the assistance and potentialities of abundant labour at low cost will only impede development and increase unemployment.

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[*] *Economic Development in Perspective*. By John Kenneth Galbraith. Pp. vi+76. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1962.) 10s. 6d. net.

This was equally recognized in a discussion on mechanization in the Middle East, South-East Asia and Africa which was concerned particularly with the appropriate type of tractor that was needed. It was suggested that a tractor much more like those in use on English and American farms forty years ago and much less complicated than the modern tractor designed to save labour was really required and should be specially designed with the labour situation in mind. All this, of course, is not new, and is in line with what Prof. Blackett and others, notably at the Cardiff meeting of the British Association, have long emphasized. It is encouraging, however, to find these ideas gaining acceptance at such a representative conference, even if the developed countries were present about four times more numerously than the under-developed countries.

In his final chapter, Mr. Galbraith dealt with a rather more specialized topic—the use of the corporation or company as an instrument of production. Once again he delineates the conditions of success with a clarity that makes his conclusions as relevant to productivity in an advanced as to an under-developed country. A corporation, he points out, is only effective if it has liberty to pursue specified goals. In making decisions every day its autonomy must be nearly absolute, including the right to make mistakes, and must include, subject to safeguards against abuse, the freedom to engage and dismiss its staff. The concomitant of freedom from intrusion on its decisions from outside is, however, that the goals expected of the corporation must be clearly and explicitly defined by society, and while tolerant of errors within the framework of success, society should be wholly intolerant of failure to achieve the specified goals.

In this widely different context, Mr. Galbraith thus gives a definition of public accountability which is highly relevant to any fresh consideration of the nationalized industries or the efficiency of the public corporation. By and large, however, accountability by result could be described as the dominant principle of the book, and it is a principle that found ample expression in the debates at the Geneva conference. It could equally be traced in a contribution from the Natural Sciences Department of the United Nations Educational, Scientific and Cultural Organization on the role of science in the development of natural resources, and in one by R. Dumont on accelerating African agricultural development, both of which recently appeared in *Impact* (12, No. 4; 1962). The latter, moreover, like Mr. Galbraith, stresses particularly the critical importance of an appropriate educational policy.

It is far too early to judge what will come out of the proceedings of a conference at which a hundred countries were represented and some 1,800 papers presented at a cost of some two million dollars, nor was the conference permitted under its terms of reference to pass resolutions for specific action. Nevertheless, it is not to be regarded simply as an exercise in communications, and, if it attested further the place and value of such schemes as the American Peace Corps and Britain's Voluntary Service Overseas in providing help at the sub-expert level, there was some very practical discussion as to how scientific and technical help could be given more effectively while Governments themselves are dealing with the larger strategy of development. The president of the conference, Prof. M. S. Thacker, in his opening address suggested the organization of a world committee, or "brains trust" as he called it, with a continually changing membership, representing various scientific disciplines, as well as nationalities, to evaluate international development plans and assist in their preparation. The director of the Food and Agriculture Organization, Mr. B. R. Sen, suggested a travelling team of scientists who would visit developing areas and keep the international committee in touch with those in the field.

Something of this idea may be seen in the mission to the Indus appointed in 1961 by Dr. J. B. Wiesner, President Kennedy's special assistant for science and technology. The aim of this mission was to study the problem of waterlogging and salinity in West Pakistan, but, as Dr. R. Revelle's paper shows, it found itself drawn into an interdisciplinary analysis and formulation of a development plan calling for unprecedented effort not only in underground water development and drainage but also in fertilizer plants, pest control facilities, education and research establishments and training programmes for technicians, administrators and field workers. There could, in fact, scarcely have been a more impressive demonstration of what is involved if science and technology are to render really effective assistance to the developing countries without waste of precious and scarce resources of trained manpower, capital and equipment.

Perhaps even more important than the demonstration of the immense possibilities was the contribution of the conference to the wide public understanding of the conditions of success and of what is required of Governments and peoples, including the scientist and technologist. The conference itself indicated some of the requirements for fresh thinking about the character and scope of research and teaching if use is to be made of the limited resources of able scientists and technologists to the best advantage. This is as important—perhaps even more important—for such a country as Britain as for less-developed countries, and the conference indicated how large are some of the special opportunities open. There are, moreover, some major problems in organization and administration, both in relation to the use of interdisciplinary teams and in regard to co-operation between existing organizations and institutions which call for forethought and careful analysis. There are priorities in research and development which may have to be re-appraised. However, whether or not new institutions are required, neither Mr. Galbraith in his book nor the Geneva conference leaves any doubt as to the immense amount of goodwill, of knowledge, and of technical and administrative experience already available to serve the developing nations—given the understanding and determination to use it resolutely.

CLOTTING OF BLOOD

Prothrombin

By Prof. Walter H. Seegers. Pp. xxvi + 728. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1962. Published for the Commonwealth Fund.) 120s.

THIS is a remarkable book, and one should not be misled into supposing that it deals only with 'prothrombin' as defined by the majority of coagulation workers. The author's views bring almost the whole field of research on blood clotting within this term. On the factual side he presents the results of many years' work at the Wayne State University, Detroit. Here he has formed an active school of research deeply impressed with the stamp of his personality and ideas. Its numerous publications have been rather widely scattered, and because of their divergence from what has seemed to be the main stream of research they have not always received adequate appraisal in reviews or text-books. It is of the greatest value, therefore, to the student of the history and practice of clotting research to have this authoritative survey.

The first four chapters deal with the definition, purification and properties of prothrombin. The great contribution here is first the separation and definition of a protein in terms of molecular weight, shape and other physical properties, and amino-acid composition, and secondly, the demonstration that it can undergo a series of changes, apparently spontaneously, including the appearance of thrombin activity. This activity is shown by the specific enzymatic action on fibrinogen, and esterase action on synthetic substrates such as TAME. The transformation of these prothrombin preparations can take place under various conditions but most strikingly in the presence of 25 per cent sodium citrate without addition of any other organic substance. This is the key observation, and proves beyond reasonable doubt that molecules with thrombin activity can be wholly derived from the protein molecules defined as prothrombin.

The following seven chapters deal with the activation of prothrombin in the presence of biological factors. It is here that divergencies from generally accepted views become apparent. Prothrombin activation in shed blood occurs in a few minutes following contact with a foreign surface, and in a few seconds in the presence of tissue 'thromboplastins', whereas citrate activation takes many hours. What is the explanation of this rapid conversion? The usual view is that a number of 'factors' interact in sequence to produce a specific prothrombin activator. These reactions are thought to be probably enzymatic, the product of each enzyme-substrate reaction being itself an enzyme which activates the next stage. While Prof. Seegers recognizes the existence of these factors, he seems to consider that they function simultaneously, as non-specific accelerators of the inherent tendency for prothrombin to form thrombin, and to deny their necessary part in sequential reactions preceding this change. He states that "in my perspective the whole order of development is concerned with prothrombin. It is ready to activate. It is 'encouraged' (procoagulants) and 'discouraged' (anticoagulants) by hundreds of substances and millions of combinations of them. My view advocates the existence of factors by the dozens". It is probable that the concept of multiple sequential reactions is a gross over-simplification, and too rigid a picture of events leading up to prothrombin activation; but Dr. Seegers draws a veil over any explanation of the essential function of these factors, including the disastrous effect of their deficiency in the hæmorrhagic states, and the beneficial effect of replacing them.

Prof. Seegers's views on the nature of prothrombin itself are stimulating. He considers that the molecule can undergo a series of transformations, yielding not only thrombin but also other derivatives with different activities in the clotting mechanism. These active derivatives he has named autoprothrombin I, II and *C*, and they probably correspond to factors VII, IX and X of the *International Nomenclature*. Prof. Seegers is wary of synonyms, however, and prefers to use the term "associated word symbol". Terminological wrangles apart, the idea of a multi-potent protein molecule capable of giving rise to different enzymes or activities at different stages of its degradation is intriguing, and might explain the fact that prothrombin and factors VII, IX and X have curious similarities, including their physical properties and their dependence on vitamin K for normal synthesis in the body.

The remaining chapters deal with the inactivation of thrombin, the preparation and properties of fibrinogen and thrombin and their interaction, with clot retraction, anticoagulants, and the hæmorrhagic states, and there is a brief excursion into the immuno-chemistry of prothrombin and thrombin. These contain a mass of useful observations and speculations, lucidly presented.

In general, the book is very well produced, and the style is clear and readable. The author has an enviable knowledge of the classical literature of his subject, and gives many obscure, but valuable, historical references. If he has less respect for much contemporary work he is at least tolerant of views which conflict with his own. In fact, the whole book is coloured by his philosophical attitude to scientific research as an individual experience. Whereas most workers either ignore, or attempt to avoid, subjectivity in experimental work, Prof. Seegers recognizes, and even appears to welcome, it, considering that it should be put to good use in forming a picture of Nature which may lead to new experience, no matter how individual this picture may be. Perhaps one of his quotations (from John Burroughs) aptly expresses this: "The eye is informed and sharpened by the thought. My boy sees ducks on the river where and when I cannot, because at certain seasons he thinks ducks and dreams ducks".

R. G. MACFARLANE

THE CLOUDS

Clouds, Rain and Rainmaking

By Prof. B. J. Mason. Pp. vii + 145 + 20 plates. (Cambridge: At the University Press, 1962). 22*s*. 6*d*. net; 4.50 dollars.

EXPERIMENTAL cloud physics is a new subject: it sprang from the work of Schaefer and Langmuir, carried out in the late 1940's, on the artificial modification of clouds. Their very first results were so impressive as to suggest that control of weather was almost within reach, and a prize so dazzling naturally attracted a great many workers. Many of them, unfortunately, were in too great a hurry, and either designed experiments badly or published results which had little statistical significance. Moreover, professional rainmakers were soon in business, presenting highly coloured advertising material in the guise of research.

Prof. Mason's earlier book *The Physics of Clouds* was the first comprehensive account of the new subject, and much of it was of necessity taken up by a thorough discussion of the heterogeneous mass of experimental data which had accumulated in the previous ten years. Now, by omitting all this detailed criticism, and by some simplification of the treatment, he has produced an up-to-date account of the same subject in about a third of the space.

The author has not changed the arrangement of his material greatly. There is a brief preliminary chapter dealing with the forms and visual appearance of clouds: it would perhaps have been better, in a book which aims to "enhance the enjoyment of those who watch the sky", if the treatment could have been a little fuller. The next four chapters are the heart of the book, and deal in masterly fashion with the processes of condensation and the growth of raindrops, snowflakes and hailstones. The last two chapters are concerned with rain-making experiments and the electrification of thunderclouds.

Most chapters have a kind of appendix in which experiments that the student may carry out for himself are described—an interesting innovation in a book of this sort, though the equipment required is sometimes rather elaborate for the sixth-form or undergraduate amateur of meteorology for whom the book seems to be intended. There are no references to original papers, and only one or two to text-books.

Mathematical proofs are kept few and short, and, as already hinted, historical experiments are not treated in great detail. As a rule all that is given is a short description in general terms, just enough to give the reader an idea of the observational basis of the subject: though a few important and recent experiments are a little more fully treated. Freed from such digressions, the story develops in a natural and logical fashion, and makes remarkably easy reading. The style is good, plain scientific English, which aims only at clarity and conciseness and relies on the subject-matter for its interest. Not even the chapter on rainmaking tempts the author to a lighter treatment, although few episodes in the history of science can be so rich in absurdity and chicanery.

The book is well illustrated with 29 very clear line drawings and 20 photographic plates. The latter include a short but well-chosen "cloud atlas" illustrating nine important cloud types, Prof. Mason's own remarkable photographs of artificially grown snow crystals, and some photographs of early cloud seeding trials which have not since been equalled for dramatic effect. This is a beautiful collection and adds greatly to the interest of the book.

There is a very adequate index for a book of its size. Printing and binding are excellent, in the usual style of the Cambridge University Press, and no misprints were noticed.

B. C. V. ODDIE

STATISTICS FOR STUDENTS OF THE NATURAL AND SOCIAL SCIENCES

Introduction to Probability and Statistics

By Henry L. Alder and Edward B. Roessler. (Series of Undergraduate Books in Mathematics.) Pp. xii + 289. (San Francisco and London: W. H. Freeman and Company, 1962.) 32*s*.

THIS is the second edition of a text-book first published in 1960. The main change is the addition of two chapters, on the *F* distribution and analysis of variance. The book is written to meet the needs of students in the natural and social sciences for a short course in probability and statistics at an elementary mathematical level; only arithmetic and simple algebra are required. The topics covered are standard and unenterprising, namely: the organization and analysis of data; elementary probability; standard probability distributions (binomial, normal); sampling theory including *t*, chi-square and *F* distributions; regression and correlation; analysis of variance; index numbers; time series. A brief and unsatisfactory discussion of multiple-range tests, the one slight deviation from orthodoxy in contents, is given without much confidence in Chapter 17.

The subject of statistics is taught, nowadays, to students with such varied qualifications and needs that there is scope for text-books dealing with the same principles and techniques from different angles and levels of sophistication. The re-issue of the book reviewed indicates that it has been popular. It is clearly and carefully written with well-presented tables and diagrams and also excellently printed and produced. Nevertheless, I think the method of exposition is wrongly conceived.

The development of the theory is formal by means of definitions and elementary algebraic proofs. Inevitably however, because of the restriction on the mathematics, the definitions have to be partial and many important results accepted without proof. Thus sampling is everywhere taken to be from a finite population and the expectation of a variable is treated only for the number of occurrences of an event in *n* trials; this leaves a gap in the logic of the relation between sampling and probability distributions which seems unnecessary. After all, ideas as abstract as those of geometry are taught at school. Minor algebraic results are derived for the probability-levels of *F* although the validity of this distribution for analysing particular aspects of the variability of data is stated but not discussed. Many practical examples are given with the arithmetic worked through in detail but they are nearly always very artificial. Either they deal with such subjects as coin tossing, balls in boxes, seating at table, etc., or, where framed in terms of observations made for a scientific purpose, the results have been constructed only as a numerical counterpart of the theory.

I do not think a natural or social scientist would obtain much of value from the algebraic proofs in this book. He could find from the text how to make estimates and apply tests of statistical significance given measurements suitable for such procedures. There would be little guidance on how he should determine what methods were useful and valid for analysing the data from a scientific investigation. He would spend his time more fruitfully studying a text which attempted to link an intuitive presentation of the principles of statistical theory with empirical studies of actual observations, using much arithmetic. There are a number of books of this kind in the tradition of Sir A. Bradford Hill's classic *Principles of Medical Statistics*. Only thus can the student hope to obtain some insight into the uses of the elegant mathematical methods in the interpretation of crude, unaccommodating, untidy observations.

W. BRASS

MICROBIOLOGICAL STANDARDIZATION

Proceedings of the 7th, International Congress for Microbiological Standardization

London, August 28 to September 1, 1961. Edited by A. F. B. Standfast, D. G. Evans, and B. G. F. Weitz. (International Association of Microbiological Societies: Permanent Section for Microbiological Standardization.) Pp. vii + 556. (Edinburgh and London: E. and S. Livingstone, Ltd., 1962.) 130*s*. net.

THE bulk of this heavy 'paper-back' comprises some seventy papers grouped into fourteen sections under such headings as "Adventitious Agents in Vaccines Prepared from Living Tissues" and "Helminth Vaccines". The sections include from three papers under "The Application of Biological Standards and Reference Preparations in International and National Requirements for Biological Products" to eight under "BCG Vaccine" and "Staphylococcal Antigens and Antibodies". Fifteen of the papers are in French (without English summaries) Several are less than two pages in length and, in view of recent criticisms about 'over-production of scientific literature', some papers could well have been contracted or even omitted altogether, especially when "the details of these experiments have been published previously" as one contributor admits.

As a reference volume the task of locating a specific paper should be easy. In fact, there is no subject-index, and the page headings give only the contributor's name opposite an abbreviation of the section heading. Nowhere are the titles of the papers listed and the "Contents" could most profitably have been expanded to include these under their appropriate section headings. The short "Reports of Sessions" at the end are a partial remedy.

The papers emanate from fourteen different countries (including U.S.S.R., Yugoslavia, Hungary and Czechoslovakia) enabling a comparison of standardization procedures in different countries to be made. This is a truly international volume, but rather expensive.

L. B. QUESNEL

UNIVERSITIES AND THE COMMUNITY IN SOUTH AFRICA*

By Dr. T. E. W. SCHUMANN

Deputy Chairman of the South African Atomic Energy Board

It is of course the duty of a university to keep pace to the best of its ability with the needs of the community it serves. I should say that the University of South Africa is fulfilling this mission in a very special sense. For, amidst the constantly changing conditions of the modern world, it is striving to create facilities for advanced education which the ordinary residential universities of our country are scarcely able to provide. Whether a university which uses postal courses as its main medium of instruction can in all respects be styled an ideal academic institution is of course a moot point. But that is not relevant here. No one can deny that the University of South Africa meets a very real need, and my impression is that it accomplishes its task very efficiently. I know of at least one very gratifying example—that of a student who came from Canada, first to Durban and then to Pretoria, for the specific purpose of completing his degree in mathematics under the professor of his choice.

The University can be felicitated on having on its staff men of such calibre that they are able to draw persons from overseas for academic training. I quote this case, because for those of us who, as members of an older generation, were virtually compelled to proceed to other countries for our advanced education, it is particularly gratifying to observe that in recent years there have been tangible signs that this process is no longer completely one-sided. I am convinced that in the future this University and other South African universities will attract students from beyond our borders in ever-increasing numbers. I have said that a university must always keep pace with the specific needs of the community it serves, but we find that in this technological twentieth century, changes and revolutions are occurring at such a bewildering tempo that adaptation to constantly changing conditions, also by our universities, has become a stupendous task.

No one will deny that we are to-day living in a crucial period in the history of mankind. Last year, I delivered a series of six radio talks entitled "Five Revolutions of the Twentieth Century" on the confused world in which we are living. The central theme of these talks, which included not only the technological revolution but also views on the revolutions caused by the advent of nuclear weapons, of Communism, and of the spiritual capitulation of the White man, was that within the space of a single lifetime we have experienced no fewer than five revolutions, any one of which would have been sufficient to disturb the spiritual equilibrium of any normal person. Every thinking person the world over is therefore faced with the task of a constant reconciliation with new ideas and new conditions. Without a doubt, for South Africa, with her great diversity of races, the task of adaptation in these tempestuous times is infinitely more difficult; and our universities must display a greater measure of flexibility to keep in step with constantly changing needs and fresh challenges.

In her struggle to maintain a measure of equilibrium and stability in these troublous times, South Africa is constantly subjected to bitter criticism, and our universities, too, come in for more than their fair share. Every inhabitant of our country who takes an intelligent interest in present-day developments knows, for example, that the present chaos in large parts of the African continent is by no means, as some optimistic but naïve overseas politicians would have us believe, very nearly a thing of the past, but merely the first stirrings of the turbulent times that lie ahead, and that far greater chaos will yet prevail before conditions change for the better. Knowing full well the shape of things to come, we shall strain every nerve to escape the chaotic developments in other African territories. This is in itself no easy task; and it is greatly exacerbated by constant interference from outside. With more than half of Europe divested of every semblance of democratic freedom, our friends overseas might do well to allow their sudden missionary ardour for suppressed nations to cool down somewhere outside Africa, and to adopt as the war-cry for their modern crusade a slogan such as the 'Liberation of Europe'. Or if this sounds too revolutionary, perhaps they could at least be pragmatic like a prominent British industrialist with vested commercial interests here which he is thinking of expanding, who stated in Pretoria recently: "I deplore your apartheid policy but I would not invest a cent in this country if you did not have it".

But to return to the subject of the development of our universities: a very strange attitude was recently manifested by the most widely read British scientific journal—that unique . . . journal *Nature*, which is to be found in every university library and in hosts of other libraries the world over. Within the space of two months this widely read periodical carried two very scathing leading articles on the universities and learned societies in the Republic. These are articles which call for comment.

The leading article of October 20, 1962 [1], concerns a speech delivered the previous April by a well-known British scientist, Sir Eric Ashby, at the University of the Witwatersrand. His principal theme was the condemnation of separate universities for Bantu in South Africa. I shall presently express a few opinions on this subject. Prof. C. H. Rautenbach, rector of the University of Pretoria, has already replied to Sir Eric. Prof. Rautenbach said, *inter alia*, that what Sir Eric had said in his eloquent peroration on the need for the Africanization of university institutions in Africa lay at the very root of our policy of separation. Experienced British officials from the Colonial period in Africa and elsewhere had already learnt what Sir Eric is now proclaiming, a long time back . . . but this was the first time that he had heard from the lips of an eminent British academician eloquent concurrence with that trend of thought.

It was progressing.

Already there was an implied recognition that there may be as many types of universities as there were communities. . . . Prof. Rautenbach added the rather cutting comment that when, in earlier years, a storm raged over the English Channel, the English newspapers carried banner headlines: "Europe isolated from the World".

The second leading article in *Nature*, of December 29, 1962 [2], discusses "Apartheid in South African Learned Societies". The following quotation will give an indication of the general slant of this article: "Dr. Verwoerd and his colleagues seem determined to leave nothing undone which could engender bitterness and inflame the racialism which is to-day such a grave and growing threat to the future of Africa".

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* Substance of an address delivered on February 21 at the opening of the academic year of the University of South Africa.

Whether it is mere chance that such conspicuous use should be made of a leading British scientific journal to add its voice to the torrent of preponderantly uninformed attacks on South Africa, and whether one should detect a suggestion of 'inspiration' in this curious coincidence, are matters on which I shall not dilate. I propose however to add a few desultory remarks to Prof. Rautenbach's well-reasoned and very interesting exposition.

First: what psychological explanation underlies *Nature's* touching concern for South African academic institutions when conditions much nearer home should give cause for far graver concern? My experience has been that not so very far from *Nature's* office, behind the Iron Curtain, very little academic freedom remains. At an international scientific conference which I attended some years ago, I was called upon to act as chairman of a small sub-committee which included a Russian and a delegate from one of the satellite countries. On an occasion when we found ourselves alone together, the latter said in rather broken English: "Dr. Schumann, I don't speak at our meetings for reasons which you may guess". To-day, still, at all international gatherings the delegates from the satellite countries, patently and with clumsy efforts at hiding their subordination, subject themselves to the dictates of the Kremlin to an extent that is often painful to behold.

Let us give our imagination free reign and contemplate the well-nigh inconceivable; let us imagine that the University of Moscow were to extend an invitation to Sir Eric Ashby to deliver a public address there: and let us imagine the equally fantastic—that he were to have the courage to advocate in that address the removal of the wire barrier which at present cuts Europe into two from North to South, because of its obvious curtailment not only of academic freedom but also of freedom in the more general sense. Finally, let us imagine that Sir Eric were to return unscathed from such an adventure in Moscow and deliver another address in Johannesburg. Would not his words then carry infinitely more weight, and would we not be far more inclined to give serious thought to his admonitions?

It would perhaps be even more exciting if the University of the Witwatersrand were to succeed in having as its guest speaker at its following triennial 'Chancellor's Address' a scholar from behind the Iron Curtain. If such an erudite guest, say from Prague, which can boast of one of the oldest universities in the world, were to tell us how shamefully we fall short of the lofty ideal of academic freedom as realized in Communist countries, we would accept it without reserve. This suggestion should not be dismissed as a frivolous joke: for, would it really be asking too much of the University of the Witwatersrand seriously to consider extending such an invitation to a scientist of repute from behind the wire barrier? At any rate his visit would be an event of international importance, and it would be difficult to find a hall in Johannesburg large enough to accommodate the audience: and no objection could be raised if after that *Nature* were to write a leading article in support of the Czechoslovakian or Russian pundit's exhortations and admonitions. We would then decidedly be prepared to listen with all due deference and rapt attention, and, if need be, to do penance for our many shortcomings.

Meanwhile the Editor of *Nature* might be prepared to bethink himself on the significant utterances of Sir Eric that universities in Africa should not be mere replicas of European universities, but should adapt themselves to the specific needs of the Negro and the Bantu. I am in favour of co-educational schools, but the fact remains that, like Britain, the Republic has many separate schools for boys and girls, and possibly there are very good educational reasons for this state of affairs. Likewise there may well be many more compelling and convincing proofs that separate educational institutions for different races are preferable to, and give better results than, integrated institutions, and as a scientifically trained person, the editor of *Nature* would surely never reject such a possibility *a priori*. On the other hand, it might be better for his own peace of mind if he were to refrain from delving too deeply into the problem on which he has written with such fluency and ardour. He runs the very real risk of finding himself in the same dilemma as Sir Eric, who, on one hand, advocates the Africanization of the universities of this continent and in the same breath expresses his indignation at the fact that South Africa in all sincerity tries to implement his own recommendation. But then, of course, confused thought-processes often land one in such dilemmas.

One final comment on the triumphant note on which the first article in *Nature* ends: "Sir Eric remarks trenchantly that the only convincing evidence for apartheid existing in a university would be the inferiority of black men to white".

This sentence, obviously intended as a knock-out blow, is so pitifully erroneous as to merit closer analysis. Intellectual superiority or inferiority can never be the primary ground for the need for separate universities. The primary ground was mentioned by Sir Eric himself, who spoke of the necessity for the adaptation of universities to the specific background and way of life of the Bantu and the Negro; in other words, the development and growth of universities on the continent to meet the requirements of the different races. For, after all, one could never suspect Sir Eric of thinking only of the needs of the black races, nor yet of advocating that the whites be swallowed up by the black majority!

But the same sentence contains by implication another erroneous statement, namely, that there is no difference whatsoever in the intellectual potential from race to race. Here is a statement of Communistic origin which is nowadays proclaimed by the liberalists as a sacred dogma throughout the world—and with such success that to-day it demands exceptional courage even to inquire after the scientific proof upon which this doctrine rests. Indeed, everywhere one finds the staggering phenomenon that no one is as intolerant as the liberal when his basic tenet is subjected to analysis. It calls for a high degree of moral discipline to accept the intolerance which has become the most salient feature of our modern liberalist.

Most probably the liberalist thinks that no proof is necessary. Curiously enough, I am not without a hint of sympathy for that attitude, for I must confess that I, too, believe certain things on the strength of personal observation and without any scientific proof. So, for example, I believe that some nations have more musical talent than others. I believe that there is a considerable difference in the intellectual capacity of the Japanese and that of the Pygmies of the Congo, in the intellectual capacity of the Jews and that of the Bushman of the Kalahari, or of the British and of the Australian aborigines. Obviously, however, these beliefs are purely subjective and of no significance to other people, for to-day scientific validation is rightly considered to be indispensable.

Very extensive research on this problem was carried out in the United States of America. During the two World Wars tests were applied to millions of recruits, and always the results were the same—there was a significant difference between the intellectual potential of the American Negro and that of his White compatriot.

Perhaps the Editor of *Nature* could quote research results in refutation of these findings; but I venture to suggest that he will have an exceedingly difficult task.

We find, therefore, that the statements in *Nature* proceed from such poorly grounded premises and such extremely assailable axioms that to discuss its necessarily erroneous conclusions with any logic would be attempting the impossible. If one were merely bent on winning debating issues, one could for example point out that Basutoland, within the boundaries of the Republic but for more than a century under British rule, is very effectively safeguarded against criticism of the type levelled at the Republic. The reason, of course, is that, as a result of the general backwardness of the territory and the high percentage of illiteracy among its inhabitants, there is no university or academic association there.

I shall however not take this any further: my object was merely to lay bare the blind prejudice with which our universities have to contend when even such a prominent and influential journal as *Nature* makes no effort at least to be slightly better informed than the ordinary daily press before launching an attack on our university institutions.

There are so many interesting problems of topical interest that I might have discussed. I might have debated the need in South Africa for what the Germans call a *Technische Hochschule*; I might have spoken of the world-wide demand for outstanding natural scientists and engineers, the results of which are also felt in South Africa. But all I have done is to dwell on an already threadbare theme.

The University of South Africa offers advanced tuition to all racial groups in Southern Africa and even far beyond its borders, and I trust that it will pursue this task peacefully and steadfastly, without excessive complacency, but also without undue concern at the all too capricious so-called 'world-opinion'. I, for my part, am convinced that when in a few years' time the "wind of change" over Africa has spent itself and the world can no longer deny its disastrous aftermath, South Africa will receive due recognition for her efforts at finding a peaceful *modus vivendi* in a country where different racial groups are destined to work out their own salvation: and I hope that when that time comes, it will not be necessary for me to bore you with an address on the eternally recurrent subject of race relations.

[1] *Nature*, 196, 201 (1962).

[2] *Nature*, 196, 1241 (1962).

" SCIENCE AND APARTHEID " *

[By Dr. R. HOFFENBERG

Department of General Medicine, University of Cape Town]

To the Editor, The Argus.

SIR,—Dr. T. E. W. Schumann's outburst (*The Argus*, February 22) against the highly esteemed scientific journal *Nature* for publishing criticism of apartheid in learned South African societies is reminiscent of Germany in the 1930's, when Nazi 'scientists' strove to defend their racialistic principles against the censure of the civilized world. [Readers who are interested in this point may wish to read the article entitled: "The Pragmatic and the Dogmatic Spirit in Physics", by Prof. J. Stark, published in *Nature* of April 30, 1938.—Editor, *Nature*.]

Speaking as a scientist, I must admit that factual evidence for the equality of all men may be lacking—this happens to be a field in which precise measurements cannot easily be made.

Dr. Schumann knows this, too, which makes his disturbing statements about the racial inequality so much more irresponsible. [The subject of intelligence tests of Negroes and Whites was forcefully challenged by D. R. Hughes, of the Duckworth Laboratory of Physical Anthropology, University of Cambridge, in *The Times* (London) of February 26.—Editor, *Nature*.]

Conflict between prejudice and scientific fact is all too common and discipline is indeed necessary to resolve this dichotomy.

When an unorthodox or isolated view is held, however, the onus is on the holder to prove his point; he must not ask, as Dr. Schumann does, that the rest of the world should prove that it is in step.

Remember Lysenko

I am astounded at the 'double-think' which allows Dr. Schumann to claim that his bigoted attitude requires a 'high degree of moral discipline'.

Discipline, certainly, but surely not moral, since it seeks to suppress the free flow of scientific inquiry!

As deputy chairman of a body engaged in sponsoring research, Dr. Schumann should know better.

If he persists in broadcasting his views, he will only bring to the field of scientific endeavour in this country the sort of opprobium that engulfed Russian scientific achievement after the biased Lysenko papers. (Readers will recall this Soviet agronomist's disastrous efforts to inject Communist doctrine into methods of wheat-production.)

Separate Bodies

I, personally, would like to congratulate the Editor of *Nature* on his two editorials.

The academic world should be kept aware of governmental inroads into education in this country.

The separation of scientific and professional organizations on a racial basis alone, to which the Editor of *Nature* objects, cannot easily be condoned on moral or any other grounds.

To quote this journal (December 29, 1962) it can only 'endanger further the advance of science and learning and the conditions in which they flourish'.

In conclusion I would like to assure the Editor of *Nature* that there is still in this country a substantial body of academicians who deplore this apartheid policy as strongly as he does.

R. HOFFENBERG, M.D., M.R.C.P.

Kenilworth.

With reference to the article in *Nature* on "Apartheid in South African Learned Societies", the Secretary-General of the Commission for Technical Co-operation in Africa: Scientific Council for Africa (Lagos, Nigeria), writes to the Editor of *Nature*: ". . . at the 17th Session of the Commission for Technical Co-operation in Africa, held in Abidjan in February 1962, South Africa was expelled from the Commission because its political policies were most unacceptable to African Member Governments. . . . This latest move on the part of the South African Government to introduce the practice of apartheid into the realm of science is greatly to be deplored by all who are concerned with science itself and

* From *The Cape Argus* (Cape Town) of February 2: reproduced by kind permission of the Editor and of Dr. Hoffenberg.

its application to the needs of newly emergent independent nations".

In an article describing his recent tour of Africa and reporting on his first-hand examination of the practical results of apartheid in South Africa, Mr. Frank Giles, the *Sunday Times* (London) foreign editor, has written (*Sunday Times*, March 9):

"In Johannesburg I talked with many politically-minded Africans, some of them the leaders or representatives of the banned African parties—the African National Congress and the Pan-African Congress—some of them prosperous lawyers, doctors or business men. Taken as a whole, they represented an average of intelligence and capacity far higher than can be met with in most of the newly-independent African States in the rest of the continent, a fact due principally to the higher education of many Africans in South Africa's excellent and hitherto multi-racial universities.

"From now on, however, the African will have to go to the newly-established 'tribal colleges' for Bantu only, where the educational effort is concentrated on what the Government conceives to be suitable subjects for Bantu study. None of the Africans I met had anything but the profoundest scorn for these 'bush universities', designed, so they maintained, to ensure that the African never rises above a certain level: 'We are not interested in a return to tribalisation but in advances that Western civilisation can bring'."

On March 8, the chancellor of the University of Natal, Dr. Denis Shepstone, unveiled a plaque erected by the students at the main buildings of the University in Durban. The plaque is intended to commemorate the death of academic freedom through the imposition of racial segregation in South African universities. It bears the inscription: "The right of this university to determine who shall be admitted as students was taken away in May 1959, and restored . . .". Dr. Shepstone, in his speech, referred to the curtailment by legislation of the autonomy and academic freedoms of South African universities. In thus prohibiting non-White students the Government had curtailed one of the basic freedoms of university autonomy, namely: "Who may be taught". He also appealed for "dedication to the idea of open universities". The ceremony was attended by members of the University Council, the Senate, Convocation, the teaching staff and many students.

NEW PHYSICS BUILDING AT QUEEN MARY COLLEGE, UNIVERSITY OF LONDON

By Prof. G. O. JONES

Professor of Physics and Head of Department

On January 23, the new building for the Department of Physics at Queen Mary College was formally opened by Sir Harrie Massey, in the presence of nearly 200 senior members of universities, Government and industrial offices and laboratories, local schools and other organizations. Deputizing at short notice for Sir Nevill Mott, who was prevented by illness from attending, Sir Harrie—describing himself as the "wrong half of Mott and Massey"—gave a racy exposition of some of the realities of modern university physics. The great virtues of practising science in university departments were that teaching and research were then united, that there was continuous rejuvenation by the flow of young people through the university and that one's choice of research was free. But the enormous growth and change in physics since the War posed serious difficulties for modern departments. The growth of physics made satisfactory teaching in three or four years an increasing problem, since physics was a basic professional training, called for by technologies of all kinds. The changes in the techniques of physical research and the increase in team-work added problems which demanded that the University physicist should have adequate financial, technical and administrative support.

Reasonable amenities were essential for the large staffs who would work in modern departments of physics, and the design of buildings could contribute greatly. In declaring the new building open, Sir Harrie expressed the opinion that it was both workmanlike and attractive and would provide opportunities for "fun at a high cultural level".

The new building (Fig. 1) is designed for an annual student intake in the special honours course of 60. There will be 40–50 full-time postgraduate research students working for the Ph.D. degree, an academic staff of about 20, 6–10 postdoctoral research Fellows or research assistants and about 40 technical, secretarial and other staff. The building was erected in two stages. The first stage, a large eight-storey block, consisting mainly of teaching laboratories and lecture rooms, was completed in 1960. The final stage, a three-storey wing consisting mainly of research rooms, was completed in 1962.

In order to reduce traffic through the building, the main lecture theatre has been placed where it can be entered directly from the entrance hall. It seats about 140 in a steeply raked terrace and is furnished in beech and mahogany with upholstered tip-up seats. An unusual feature is a set of double-glazed wooden louvres covering most of one side of the theatre. To the audience looking towards the lecturer only the woodwork can be seen. Walkers in the corridor outside can, however, look directly into the theatre, and the lecturer can look outside. Two very large revolving chalk-boards are power-operated and cleaned. There are full facilities for showing slides and films from a projectionist's console and service points for demonstration experiments.

A preparation room for lecture demonstrations is adjacent to the main lecture theatre. It connects also with two smaller lecture rooms, seating about 60 and 80 respectively, one directly, and the other by service lift.

The five upper floors of the building house the teaching laboratories, nearly the whole of one floor normally being allocated to the needs of a given group of students. The laboratories have their own preparation rooms, optics rooms, photographic dark rooms, seminar rooms and small libraries for private study, but are very different from each other in detail. The third-year laboratory has also a large students' workshop and glass-blowing room, and a computing room.

The main research area of the building is concentrated in the first floor. There is, however, a vertical column of staff rooms at one corner of the building, designed primarily for convenience in supervising teaching laboratories. and many of these are combined with research laboratories so that there is, in fact, research in progress on every floor above the ground-floor.

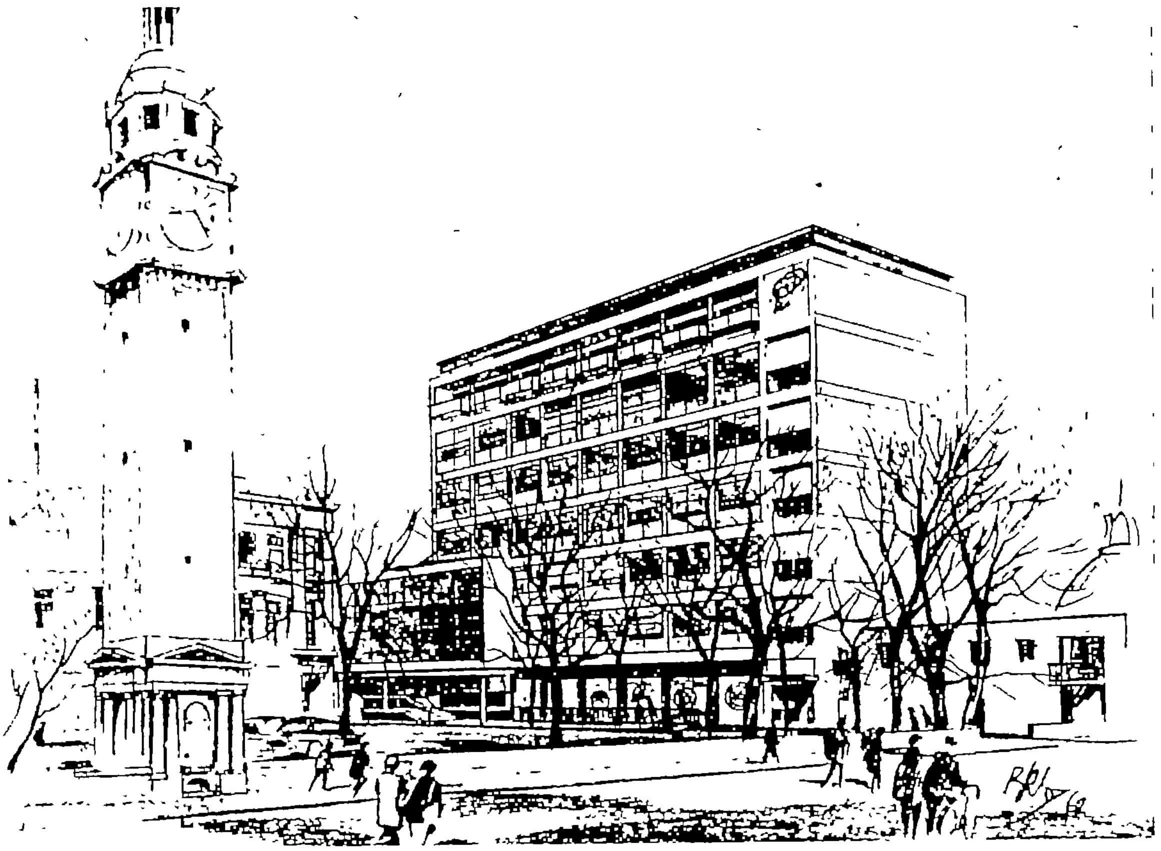

Fig. 1 Architects' sketch of the new physics building, Queen Mary College (University of London) (Architects Playne and Lacey and Partners)

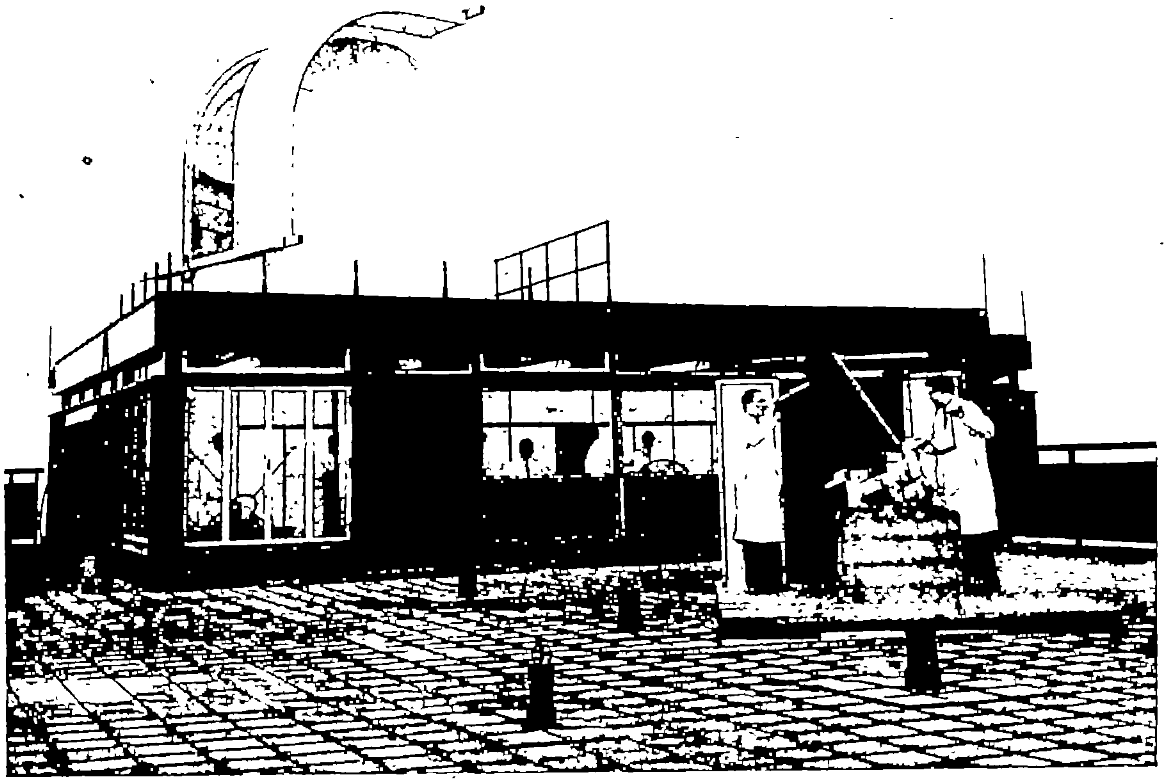

Fig. 2. The astrophysics laboratory on the roof of the physics building (by courtesy of *The Times*)

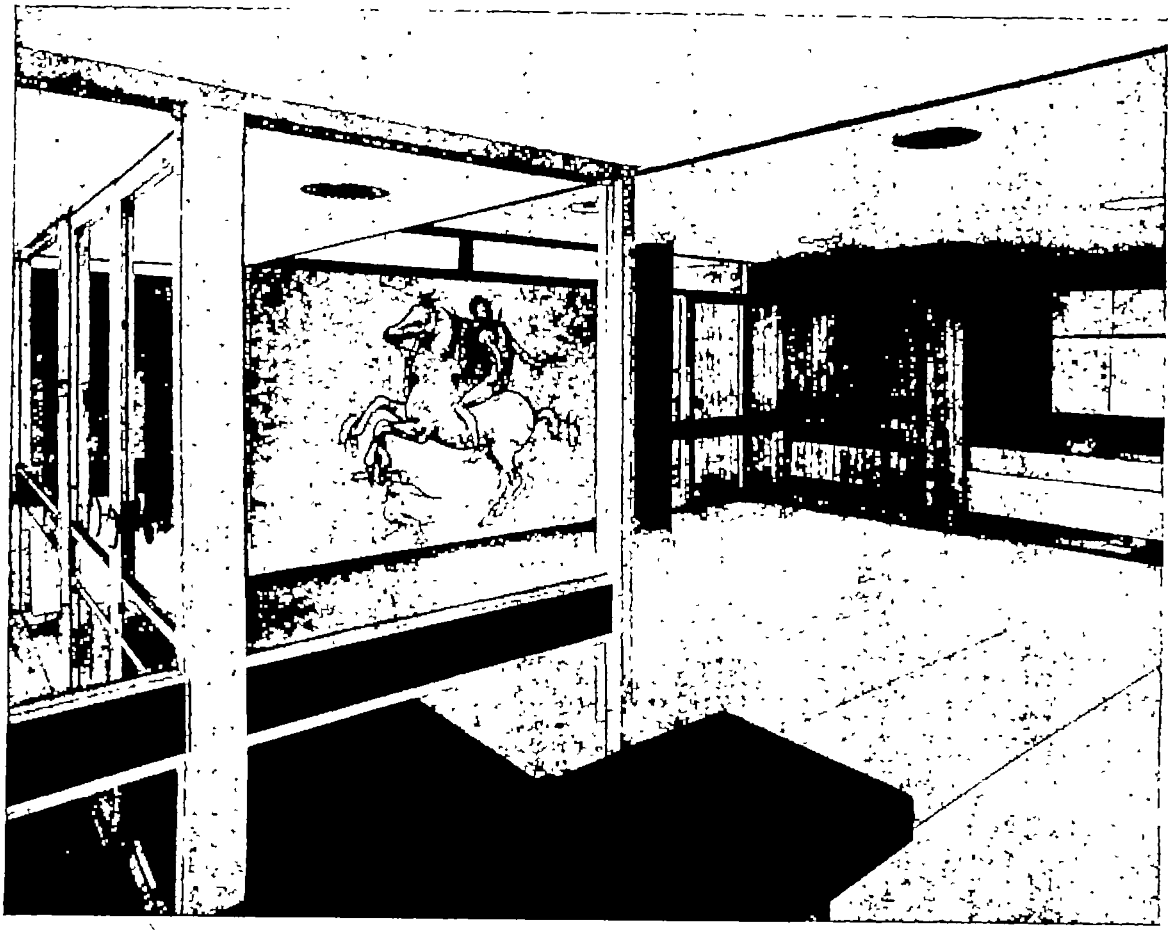

Fig. 3. A view of the entrance hall, showing a photographic enlargement of a Leonardo drawing

The research rooms have been made of many shapes and sizes in order to provide flexibility. There is at present a wide range of research carried on in the Department, the main studies being in low-temperature physics, sub-millimetre spectroscopy, nuclear magnetic resonance, high-energy nuclear physics, astrophysics at sub-millimetre wave-lengths, architectural acoustics and in other branches of solid-state physics, including the study of ferroelectrics and X-ray crystallography. Many of the theoretical studies in progress are related to the experimental programme. Students working in theoretical physics have a suite of small rooms, each accommodating two workers, near the main experimental research area.

There are a departmental library open to third-year undergraduate students and research workers, and a room for research seminars.

The main departmental offices are adjacent to the head of department's room and near those of senior members of the department, and the laboratory steward has a room and clerk's office adjacent to the main entrance hall.

The main service rooms are centrally situated, near the main research area of the building. They include a large main store, a suite of dark-rooms and rooms for specimen preparation, instrument repair, drawing, computing and a first-aid room. There are a large main workshop with an office for the technician-in-charge and subsidiary workshops for research students and for brazing. There are a large room for compressors and liquefiers which houses two Philips air liquefiers and an A. D. Little helium liquefier, and an office for the technician-in-charge. At semi-basement level there is a further set of service rooms which include two radioactive laboratories and a chemical laboratory.

Rooms and laboratories throughout the building share the same general design features. Throughout the building the built-in furniture is either of beech and sapele, or of beech and afromosia, matching the movable furniture in use. Wherever experiments are to be done, there are aluminium slotted strips and strips of soft wood attached to the wall at several fixed heights. Window walls are kept quite clear of services so that desks and tables may be used at the windows. Walls opposite windows, leading into the corridors, are provided with as many built-in cupboards as can be fitted.

Piped services are brought to service shelves which project from the walls at bench height. Among the unusual piped services are return lines for helium gas, waste lines for gases to be discharged at the top of the building, and lines for special high-pressure and low-pressure gas supplies (such as hydrogen and oxygen) and for high-pressure water.

To avoid flooding, all laboratories have drains at floor-level. A feature of many research rooms is that the doors, like those of railway train compartments, have large glass windows which can be lowered.

Electrical supplies are brought to service panels immediately above the service shelves. A feature of these supplies is that a very large number of 240-V a.c. points are supplied for 13-amp fused plugs, and relatively few points for other supplies, which include 240-V d.c., 12-V a.c., 1 kc/s a.c., 415-V 3-phase a.c., and spare lines. Considerable care has been taken to avoid electrical interference. All the steelwork of the frame of the building was carefully bonded during construction. Filters are provided on all the electrical supply systems—particularly at joints—in order to reduce pick-up from outside and to prevent interference generated within the building from

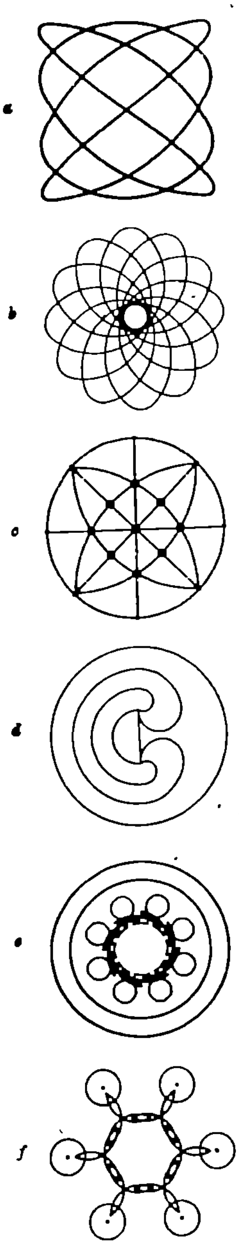

Fig. 4. Designs used for the tile patterns
a, a Lissajous figure; *b*, path of a particle in a precessing orbit; *c*, the stereographic projection of a cubic close-packed structure; *d*, spreading of dislocations from a Frank–Read source; *e*, a cavity magnetron; *f*, the electron-orbital scheme of benzene

being distributed. Special care has been taken to shield the supply cables. A limited access special earth is provided in selected laboratories quite separate from the ordinary a.c. earth. No commutator motors or other devices producing sparks are allowed in the building. All heavy electric motors and devices requiring large currents (such as workshop machinery) are supplied separately direct from the sub-station. The laboratory a.c. outlets are fed via a voltage stabilizer. Some laboratories have tungsten lighting, although fluorescent lighting is generally used.

One room extending over two floors is designed for high-voltage apparatus and completely screened, with a shuttered opening leading to an adjacent research room at the upper level.

On the roof of the building there is a laboratory (Fig. 2) designed for astrophysical experiments at millimetre wave-lengths. The roof of this laboratory carries a revolving dome housing a 60-in. reflecting telescope.

Where there is heavy vibration, as in the main workshop and in the liquefier and compressor room, the whole floor is mounted separately from the rest of the building. Two first-floor rooms have especially strengthened floors for heavy equipment, and one, in which a *Varian* 3-ton magnet is installed, has a removable wall and window so that heavy equipment may be hoisted directly into position from outside the building.

A feature of nearly all rooms is that the pipes and cables are brought in behind panels or above the false ceilings and cannot be seen. Moreover, alterations and additions to services can be made without defacing the building. The delivery area for the building is a yard with entrance at lorry tail-board height to the goods lift which serves all floors. Gas cylinders are stored in the yard with underground connexions to the main distribution points inside the building. There is also an underground store here for acids and solvents. All the air supplied to the building is cleaned by filtering.

A great deal of thought has been devoted to the problem of making the building comfortable and pleasant as well as efficient. Among the decorative features are a photographic reproduction of a drawing by Leonardo da Vinci in the entrance hall (Fig. 3), a mural painting by Feliks Topolski in the research wing, a sculpture by Mr. T. B. Huxley Jones on the exterior of the building, and a series of tile patterns decorating the lower part of the exterior, based on physical ideas derived from several branches of physics (Fig. 4).

Technicians working in the department have their own common room and locker rooms with showers. A pleasant amenity is the departmental museum, situated in the middle of the research area on the first floor, which is used as a meeting place and tea-room for all permanent occupants of the building. The museum houses a scientific exhibition and is also used as an art gallery, open to the public and advertised in the artistic press as Gallery 273. The changing exhibitions in this Gallery provide an interesting background for those who work in the building.

CARCINOGENESIS AND CANCER PREVENTION

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THE most important application of theories of carcinogenesis is to the problems of cancer prevention and therapy. It is generally believed[1] that a viral ætiology should open up the possibility of prophylaxis through immunization. By contrast it is thought[2] that somatic mutation hypotheses are 'pessimistic' because, to quote Burnet[3]: " . . . Everything suggests that, somatic cells being what they are, the impact of the environment must inexorably lead to an accumulation of mutant cells, some of which will have malignant descendants if life persists long enough".

Unfortunately, evidence analysed in previous articles[4,5] makes it reasonably certain that most human malignancies have a genetic basis. It is highly probable that somatic mutation (of genes or chromosomes) is a causal factor in all human cancers, while inherited mutant genes or chromosomes certainly feature in the ætiologies of many[4]. Although oncogenic viruses may often play a causal part in malignant development in the young (through 'horizontal' transmission) their involvement in adult malignancies is probably rather rare.

One very important and obvious implication follows from the conclusion[4,6] that many cancers are 'prezygotic'[6]—involving one, and very occasionally two, inherited mutant genes, or an inherited mutant chromosome[7]. If carriers of inherited 'carcinogenic mutations' could be identified before the reproductive age is attained, and if they could then be dissuaded from procreation, a great reduction would result in the incidence of prezygotic malignancies in succeeding generations. It is estimated[4] that the majority of malignancies are of this kind, and hence the importance of developing tests to identify carriers can scarcely be over-stressed. Although the detection of carriers of the Ch^1 chromosome[7] is a relatively simple matter, the task of diagnosing 'point'-type gene mutations is likely to prove much more difficult. Nevertheless, a solution to this scientific problem may be confidently anticipated; the public acceptance of eugenic practices is more problematical, but even in this domain, the widespread apprehension concerning cancer could overcome entrenched attitudes.

Eugenic practices would, however, have no effect on the incidence of postzygotic tumours—those devoid of an inherited "carcinogenic mutation". This article is concerned with: (1) the roles of gene mutation, viruses, hormones and immune reactions in malignant transformation; (2) the theoretical possibility of limiting the incidence of postzygotic cancer.

Mechanisms of Carcinogenesis

A broad outline of the proposed overall mechanism of human carcinogenesis is given in Table 1; it resembles earlier proposals[4,8].

Table 1. POSSIBLE GENERAL OUTLINE OF THE STAGES OF HUMAN CARCINOGENESIS

INITIATION[a,b] (MULTI-STAGE) ↓	Random accumulation of mutant nuclear genes, or occasionally gross chromosomal defects in a generative somatic cell (ref. 4). In many malignancies, especially in the young, one, and occasionally two, mutations will be inherited (refs. 4, 5).
CARCINOGENIC 'NUCLEOTYPE' ↓	The initiation stage culminates in the emergence of a cell in which particular genes associated with the control of differentiation and mitosis have undergone mutation (or occasionally, deletion). Normally, except in childhood and adolescence, four specific mutations will be present in the carcinogenic 'nucleotype'—a cell the nuclear constitution of which confers malignant potentiality.
CARCINOGENIC CELL ↓	When the cytoplasmic constitution reflects the nuclear changes, the resulting cell is described as the carcinogenic cell. It is still not neoplastic.
LATENT PERIOD[b] ↓	There is usually a long interval—up to many years—before the carcinogenic cell is converted into a malignant cell. This phase probably corresponds to the 'promotion' stage discerned in experimental chemical carcinogenesis. A hypothesis of the latent period is described in this article.
CANCER CELL[c]	

(a) Phase, or stage, principally affected by ionizing radiations.
(b) Oncogenic viruses may affect either or both of these phases.
(c) Cancer formation is often preceded by a premalignant condition, in which there is a partial loss of control of normal mitosis and differentiation. In this stage, it is probably cells containing three out of the four carcinogenic mutations that exhibit hyperplasia.

The theory of the initiation-stage is based principally on an analysis of age-specific mortality statistics, epidemiological evidence, and inheritance relations for spontaneous human leukæmias. However, the dose-response and age-dependence relations for radiogenic malignancies are quantitatively consistent[4,9,10] with the view that ionizing radiation can induce somatic mutations characteristic of the initiation-stage. A highly critical test of the theory of the initiation-stage is given by the values of the mutagenic efficiency of radiation in somatic cells deduced from several investigations, ranging from the effects of small doses of obstetric X-rays on the fœtus, to those of large doses of nuclear-bomb radiation on the survivors at Hiroshima[4,9,10]. The values found span the same kind of range as that observed for the radiogenic induction of mutations in mouse germ cells[11] (at 7 recessive loci) and in somatic cells[12] (at 4 of the same loci).

An understanding of the overall carcinogenic process requires an interpretation of the phenomenon of the latent period. Among the Hiroshima and Nagasaki survivors, the onset of radiogenic leukæmia between the years 1947–58 followed on approximately Gaussian-type distribution in time with a median latent period of about 6–7 years[9,13,14]. It is significant that the latent period is not noticeably dependent on age; furthermore, the mean latent period for the onset of chronic myeloid leukæmia at Hiroshima was about 6 years, whereas that for acute myeloid leukæmia was about 8 years. This effectively disposes of the view that the variability in time of onset simply reflects differences in the growth rate of malignancies. The facts suggest rather that the growth-phase—from one, or a few cells, to symptoms and signs of leukæmia—is relatively short, and that one or more random events intervene between the formation of the carcinogenic nucleotype and the malignant transformation[8]. Provided these events are not further somatic gene mutations (if they are, they should be inducible at the time of irradiation) then it no longer follows that somatic mutation will necessarily lead to cancer development, for these malignant-transforming processes may be preventable. This possibility will be discussed later.

Initiation Stage

Detailed conclusions regarding the initiating requirements for leukæmias have been described elsewhere[4,9,10]. The key derivation is that in the absence of stress (for example, bacterial infection, viral, or hormonal factors), a malignancy generally develops from a cell with four specific mutations, one of which, in spontaneous carcinogenesis is frequently inherited. Evidence discussed in a preceding article[5] indicates that two of the carcinogenic mutations affect both homologous genes at a particular locus, or possibly, at one of a limited number of loci. In the case of leukæmias and genetically associated cancers, cytogenetic and pedigree evidence imply that

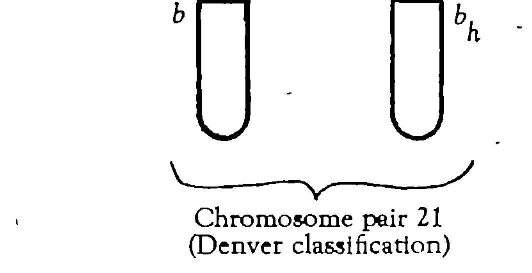

Fig. 1. Proposed approximate location of genes involved in the initiation of leukæmias and genetically associated cancers. The 'a' locus genes are assumed to have a regulator function, and the 'b' locus genes a combined operator–structural function, although the reverse attribution is equally likely

this locus is on chromosome 21; the same kind of observations suggest that a second distinctive locus on chromosome 21 is also implicated. (This 'second locus' could conceivably be one of several alternatives.) It will be assumed that the other two 'carcinogenic mutations' affect both homologous genes at this second locus. Although the symmetry of the situation (Fig. 1) makes this an æsthetically attractive assumption, it will be shown that it is also consistent with the evidence.

Consider now the functional properties of these two loci—are they structural, regulator, or operator[15]? Apparently, recessive inheritance at one locus (arising from a consanguineous marriage, for example) can result in childhood malignancies[8]. It will be shown that this locus is unlikely to have a structural function; on the other hand, the immunological evidence indicates that at least one of the 'carcinogenic loci' is of the structural kind. A fuller insight can be obtained by considering the general characteristics of the latent period and of malignant cells.

Latent Period and Malignant Cells: Some Characteristics

The following phenomena must be accommodated in any adequate theory of the carcinogenic process:

(1) The existence of a long and variable interval between the completion of the initiation-stage (the formation of the 'carcinogenic cell'[4]) and the onset of malignant growth. In experimental carcinogenesis, it appears that this interval can be shortened by co-carcinogens.

(2) The distribution of the latent period, as already mentioned, is approximately Gaussian in form. This phenomenon is also observed in experimental animals[16].

(3) When the malignant transformation occurs, it is generally irreversible. There are, however, hormone- and virus-dependent tumours in experimental animals, and the same may be true of man.

(4) Human malignancies are often, but not always, preceded by a pre-malignant or hyperplastic condition[17].

(5) Some evidence (see later) indicates that the malignant cell has lost immunological specificity.

(6) In contrast to (5), other evidence points to the existence of cancer-specific antigens.

(7) The malignant cell tends to be de-differentiated, and there is a connexion between the acuteness of a leukæmia, for example, and the extent of de-differentiation. Nevertheless, wide variations in tumour cell differentiation are found.

(8) It is now apparent that malignant growth is usually characterized not by an increase in the mitotic-rate of generating cells, but by the fact that differentiation, and loss or death of cells is arrested[18]. Furthermore, both daughter cells emerging from a mitosis must sometimes become generating, or mitotically competent cells. This latter property may represent one of the most important distinctions between benign and malignant neoplasms. In the former, it will be very rare for both daughter cells from a mitosis to become generating cells, and growth of the neoplasm will be either very slow or non-existent. The rate of growth of an established primary cancer, or metastasis, will be determined by the relative frequency of 'doubling-divisions'.

(9) An outstanding property of the cancer cell is a surface change, resulting in lowered adhesion[19].

The following hypothesis represents an attempt to provide a unified interpretation of the foregoing phenomena, and to relate them to the conclusions regarding the initiation-stage.

Hypothesis of Latent Period

It is proposed: (1) that two autosomal loci are generally implicated in carcinogenesis (as illustrated in Fig. 1) and that in carcinogenic and malignant cells in human adults, all genes, a, a_h, b and b_h (homologous at both loci) are generally mutant; (2) one locus, 'a', has a regulator function; (3) the other locus, 'b' has a structural function; (4) for human leukæmias, and genetically associated cancers, these loci are situated on chromosome-pair 21; (5) genetically associated malignancies involve at least one common allele or at least one common locus.

Recent observations[20] suggest that the concepts of Jacob and Monod[15] regarding the regulation of protein synthesis in bacteria may well be applicable (at least in essence) to mammalian cells. However, in the latter, regulator genes do not, apparently[21], control the synthesis of metabolically sequential enzymes as they do in bacteria.

Mutant structural genes will determine the synthesis of 'mutant' protein via 'mutant' messenger RNA (*m*-RNA). A polypeptide of the altered protein will exhibit an anomalous amino-acid sequence (frequently associated with a change in molecular folding) and, in general, this should produce a 'not-self' steric pattern[22]; the anomalous protein should therefore be autoantigenic (that is, capable of reacting immunologically with one or more specific antibodies). In the absence of viral or hormonal factors (see later), it is suggested[4,8], following Green[23], that the malignant transformation of the carcinogenic cell depends on a specific immune reaction between complementary-pattern antibody and the autoantigenic protein. According to Green[23], the autoantigen is probably a lipo-protein, and this attribution is supported by the findings of Vogt[24] and of Björklund[25] *et al.* On this view, the long duration, and the Gaussian distribution of the latent period, will arise from the small (and random) probability of specific antibody reacting with the exceedingly low concentration of 'homologous' autoantigen (borne by only one, or at the most, a few, carcinogenic cells).

It is now necessary to consider how this immune reaction could effect the malignant transformation of the carcinogenic cell. It is suggested that the lipo-protein, a component of the cell membrane and endoplasmic reticulum, is normally the 'source' of one or more factors (cf. inducer and repressor metabolites in bacteria) that combine with or affect repressor molecules to determine the differentiation state of the cell, and its overall metabolic pattern. Dumonde *et al.*[26] have shown that antibody, present only on the surface of mouse ascites tumour cells, and in the absence of complement, can exert profound changes on intracytoplasmic particles and enzyme concentrations. It is axiomatic that "Cancer is clearly the result of a defect of the homeostatic mechanisms which maintain the balance among cells in an organism"[27], and the problem is to relate the immune reaction to an irreversible change in a control system that involves two loci, one of which is structural and the other regulator in function.

It is proposed that the essential change produced by the immune reaction is the removal, inhibition, or alteration of a 'differentiating-factor', *DF*. The altered factor, or condition, will be symbolized by *NDF*—which could well correspond to the absence of *DF*. It is suggested that *NDF*, in conjunction with 'mutant' repressor from the mutant regulator locus 'a', causes the inhibition of *m*-RNA synthesis at the homologous mutant structural genes at locus 'b'. The consequent inhibition of 'control' lipo-protein synthesis and the disorganization of the endoplasmic reticulum now removes the source of *DF*. Hence, an immensely important theoretical requirement—an irreversible change—has been established. Normal differentiation in the affected cell, and its daughters, can no longer proceed, because the source of *DF*, the control lipo-protein, cannot be re-established. The change-over from *DF* to *NDF* will affect *m*-RNA synthesis at many structural loci in addition to 'b' (by inhibition or release), and the balance of enzyme concentrations, and therefore of metabolic pathways in malignant cells will be different from that in normal cells. (This is, of course, a well-known phenomenon.) The anaplastic malignant cell will tend to be geared to mitotic, rather than normal differentiation functions (as in embryonic life), and the disorganization of the endoplasmic reticulum will produce a morphological deterioration.

In bacteria, repression of enzyme synthesis can be very variable—from less than 0·1 per cent to rather more than 10 per cent of the unrepressed value[13,14], and it must be supposed that a rather wide variation of control lipo-protein synthesis can exist in neoplastic mammalian cells. The degree of repression will probably depend on the extent of complementarity between the mutations at the regulator and structural loci. (It is envisaged that the operator locus, which in bacteria is very closely linked to a structural locus[15], actually forms a part of the structural locus, 'b'.) When the 'b' locus is poorly repressed, there may be sufficient lipo-protein synthesis in some cells to yield a concentration of *DF* adequate for more or less normal differentiation. On this view, chronic leukaemias, for example, with a slow growth-rate and little de-differentiation, result from a rather 'leaky' inhibition of the 'b' locus.

This suggested control system is outlined in Fig. 2 where, for simplicity, only one chromosome of a homologous pair is shown.

Although the role of *DF* in cellular differentiation is conjectural, some form of cytoplasmic-nuclear interaction is essential, according to Mather[15], to account for the allied phenomenon of paramutation (Brink)[16]. The present hypothesis has the merit of satisfying the following stringent conditions: (1) 'dormancy' of the carcinogenic cell from the time of its formation to neoplastic conversion; (2) the long latent period (resulting from very low concentrations of autoantigen and specific antibody) and its Gaussian distribution; (3) the irreversibility of the malignant transformation (because of diminished concentration of *DF*); (4) the loss of antigenic specificity in the malignant cell—as a consequence of the absence of a normal lipo-protein; (5) the gain of a cancer-specific antigen (not necessarily the same in all cancers) due to the residual synthesis of an autoantigenic lipo-protein; (6) the variability in the 'acuteness' and degree of differentiation of a cancer—depending on the extent of repression of *m*-RNA synthesis at the 'b' locus, and the concentration of *DF* in the cytoplasm; (7) compatibility with the conclusions regarding the initiation stage; (8) a change in the surface properties of the cancer cell; this should follow from the inhibition of synthesis of a lipo-protein component.

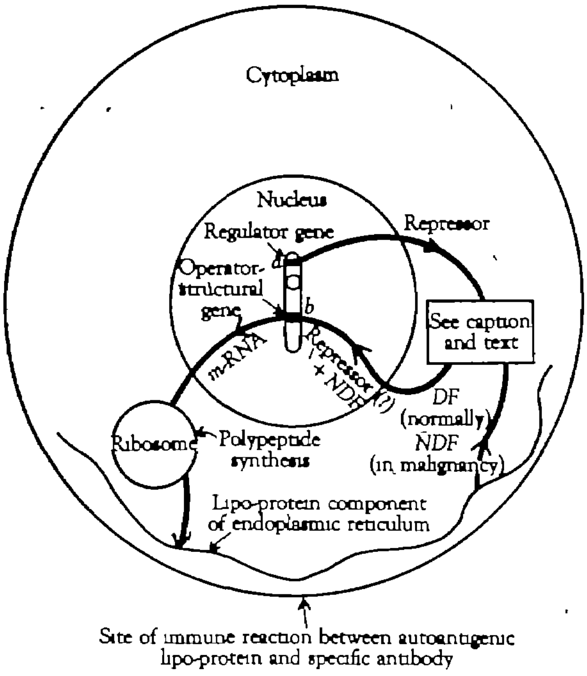

Fig. 2. Proposed metabolic pathways in malignant transformation. For clarity, only one chromosome of the autosome pair 21 is illustrated. The operator–structural gene 'b' determines the amino-acid sequence of a polypeptide in the 'control' lipo-protein, forming a part of the endoplasmic reticulum, and cell membrane. When 'b' is mutant, the lipo-protein is autoantigenic. An immune reaction between autoantigenic lipo-protein and antibody of the appropriate complementary steric specificity affects the nature of differentiation–mitosis metabolites in the cytoplasm. (Other environmental factors such as contact with neighbouring cells, hormones, etc., can also affect these metabolites.) Normally, the presence of *DF*, and repressor molecules from non-mutant regulator loci produce a pattern of inhibition and release of structural loci appropriate to ordinary differentiation. However, when mutations at the 'a' and 'b' loci are complementary, the replacement of *DF* by *NDF* through antibody attack causes the 'mutant' repressor plus *NDF* to inhibit *m*-RNA synthesis at the mutant 'b' locus. The concentration of autoantigenic lipo-protein is now greatly reduced, and the source of *DF* is irreversibly diminished. Normal differentiation is no longer possible even in the absence of antibody, and the pattern of structural locus activity is that of the malignant cell

The common phenomenon of pre-malignant hyperplasia[17] remains to be accounted for. From the analysis of the initiation-stage, it was concluded that this probably involves a proliferative advantage, or hyperplastic growth of cells containing three out of four carcinogenic mutations. This will be accompanied by a changed differentiation. It is suggested that in hyperplastic cells, homologous regulator genes (a and a_h) are mutant, but that only one (heterozygous) structural gene, b or b_h, is mutant. For, if homologous structural genes become mutant when only one or neither of the a, a_h genes is mutant, antibody attack (probably in conjunction with complement) is likely to prove cytotoxic because the cell with a heterozygous mutant regulator gene will be unable to repress the synthesis of autoantigenic lipo-protein. However, with a and a_h mutant, and b or b_h mutant, antibody attachment to lipo-protein, a part of which is autoantigenic, may reduce the concentration of *DF* sufficiently so that occasionally a cell will undergo mitosis instead of normal differentiation. By the same token, a hormone with mitotic-stimulating properties ('stimulation' may entail the neutralization of mitotic-inhibition) could well give rise to a hormone-dependent neoplasm where cells have homozygous mutant regulator genes and a heterozygous mutant structural gene. Removal of the hormone will permit *DF* to re-establish a high degree of normal differentiation, and the hormone-dependent tumour will then regress. The occurrence of the fourth carcinogenic mutation in a cell in a hormone-dependent tumour would, of course, establish autonomous growth, and it is well known that tumours in experimental animals that are initially hormone-dependent often become autonomous. (It is interesting in this context that human growth hormone resembles α- and β-globulins[21].)

Some Implications

Certain implications of the preceding hypothesis may now be considered. Suppose an individual inherits homozygous mutant structural genes (as the result, for example, of a consanguineous marriage). According to the Burnett[22]–Lederberg[23] approach to immunology, and a recent development of it[24], the presence of 'antigen' in the organism since fertilization should result in the development of immune tolerance to that 'antigen', which is indistinguishable from 'normal self'. Consequently, the carrier of homozygous mutant structural genes should develop immune tolerance to the protein the structure of which is determined by them. Such a carrier should, therefore, be no more prone to cancer than ordinary non-carriers. (Only further mutations at that locus will produce autoantigenic lipo-protein.) Nevertheless, in a mating between a homozygous carrier and a non-carrier, every offspring will be heterozygous for the structural gene. Although the 'penetrance' during a life-span of 70 years of a heterozygous 'carcinogenic mutation' (in the phenotypic form of any malignancy) is likely[8] to be in the region of 0·5, very occasionally, even large families should be observed in which neither parent is cancerous (one being a homozygous carrier of mutant structural genes), but where every (heterozygous) offspring develops cancer. A remarkable family, effectively answering to this description, has been mentioned by Lentz[34]. In this family, neither parent developed cancer, although all four sons died of gastric carcinoma, and three out of the four daughters died of carcinoma of the uterus; the remaining, fourth daughter, although not herself a victim of cancer, was very probably a genetic carrier, because her daughter died of carcinoma of the pancreas. Such families, however, will occasionally arise where either or both parents are heterozygous carriers (of either structural or regulator genes), and very extensive studies will be necessary to elucidate completely the genetics of cancer. Obviously, the same allele is involved in various carcinomas, and the importance of detailed and systematic pedigree evidence is immense. If a particular allele at the structural locus is common to many carcinomas, immunological tests should reveal a common lipo-protein antigen in the malignant cells of human carcinomas. Precisely this claim has been made by Björklund *et al.*[35], who found a similar, heat-labile, lipo-protein antigen in each of 60 individually tested human carcinomas. Even more astonishing is the "suggestive evidence" of McKenna and Blakemore[36] for a common antigen, transcending species barriers, in various human and murine (cultured) malignant cells. The antigen is absent from cultures of non-malignant tissue.

Antigenic differences between malignant and normal cells should not be confined to cytoplasmic constituents. It is postulated that the substitution of *NDF* for *DF* in the malignant cell introduces a changed pattern of gene regulation and therefore of *m*-RNA synthesis. There are several indications[36,37] that histones are associated in some way with the suppression of *m*-RNA synthesis at structural loci. Although an RNA repressor molecule complementary to the DNA of the structural gene seems a better candidate for the actual agent of repression, histones could presumably conjugate with the RNA–DNA complex. Whether histones are the cause or the effect of repression, antigenic differences between the deoxyribonucleoproteins of malignant and normal cells may be anticipated. Such differences have indeed been demonstrated by Messineo[38]; antigens found in the deoxyribonucleoprotein fraction from leucocytes of normal cells differ from those found in the cells of leukæmic patients. Byers *et al.*[39] report specific antigens in the nuclear fraction of human carcinomas that were destroyed by DNase and by pepsin (in addition, specific antigens in the microsome fraction of a sarcoma were destroyed by RNase and pepsin).

Numerous reports of circulating antibody in cancer patients may be directly attributable to residual autoantigenic control lipo-protein; but antigens may well be released from necrotic tissue, and it is also conceivable that the chromosomal defects that commonly arise in malignant cells may sometimes be associated with the synthesis of autoantigenic proteins.

Viral Carcinogenesis

Within the context of the present hypothesis, the following potential ætiological roles for oncogenic viruses can be distinguished. The list does not pretend to be exhaustive, and more complex virus–genome interactions can be imagined.

(1) *Virus as co-carcinogen.* A virus of the appropriate antigenic specificity will cause proliferation of particular immunologically competent cell lines. Should the host defence system fail to eliminate or inactivate the virus, a dependent neoplasm could arise. In this ætiology it is envisaged that the viral antigen plays a part equivalent to that performed by antibody in 'normal' carcinogenesis. The induction or latent period should be short. In the new-born immunologically immature mouse, for example, such viruses would be ineffectual; should they persist, acquired immune tolerance to the virus could well develop. However, subsequent mutation of the virus producing an antigenic change could initiate dependent neoplastic growth in an immunologically competent cell line of the appropriate specificity.

Neoplasms induced by this ætiology will tend to be diffuse in origin; the virus could contain RNA or DNA.

(2) Proliferation of a virus in the cytoplasm could affect the synthesis of *DF*. If through competitive or other means the concentration of *DF* were drastically diminished, a dependent neoplasm could be initiated. (A carcinogenic cell would be rapidly transformed into an autonomous malignant neoplasm.) The introduction of this type of virus into the new-born mouse could induce a dependent neoplasm of diffuse origin. The origin in adult mice would also be diffuse. Such a virus should contain RNA.

(3*a*) Proliferation of an RNA virus in the cytoplasm could interfere with the synthesis of control lipo-protein. The consequent diminution in the concentration of *DF* would lead to a dependent neoplasm (or to malignant transformation of a carcinogenic cell). Induction would again be rapid. This type of virus would be carcinogenic in the new-born mouse.

(3*b*) Exactly similar results to those in 3*a* would follow if a virus interfered with the synthesis of *m*-RNA at the control structural locus. Tumours induced by both of these ætiologies should be diffuse in origin.

(4) A DNA virus might transduce or mutate a regulator gene, or affect it by a 'lysogenic-type' action. A neoplasm would result only when the control structural genes are also mutant. This mechanism would be effective in mice of all ages, although it would be characterized by a long latent period. An immune or hormonal attack would be required to convert the carcinogenic into a malignant cell. Except for large doses of virus, the origin of tumours for this and the next mechanism will tend to be uni-focal.

(5) A DNA virus might affect operator–structural control genes by mechanisms similar to those in (4). Neoplastic development would require mutant regulator genes. Again, conversion from the carcinogenic to the malignant cell would require an immune or hormonal attack, and the latent period would be long.

In all the foregoing examples, the outcome of introducing viruses into an organism will depend on the competition between the rate of the carcinogenic process and the rate of elimination of viruses by the defence mechanism of the host. The significant reduction in the yield of thymic lymphomas in the irradiated mouse when a part (other than the thymus) of the animal is shielded[40] could well be due to the preservation of immunologically competent cells from the cytotoxic action of the irradiation, and their subsequent neutralization of the lymphoma-inducing virus.

In general, there is insufficient evidence to decide whether any actual oncogenic virus belongs to any one of the hypothetical types enumerated here, although it is interesting that all of them would appear to have some parallels in known animal oncogenic viruses (see a review by Kaplan[41]). The *in vitro* studies of Dulbecco[42] on the RNA-containing Rous sarcoma virus indicate that this is probably a type 2 or 3*a* virus. Thymectomy in mice greatly reduces the incidence of virus-induced lymphocytic leukæmias and lymphomas. Miller and others[43] have shown that the normal maturation of the thymus in the immediate neonatal period is essential to the development of full immunological competence, and it is possible that thymectomy suppresses a malignant-transforming immune reaction. If this is the case, a type 4 or 5 oncogenic virus may be implicated. Alternatively, thymectomy may simply remove the source of cells that would otherwise become malignant. However, Miller[44] has shown that when thymic tissue from a donor is subcutaneously grafted to a thymectomized mouse, the thymic lymphosarcoma induced by the Gross virus generally consists of host, and not graft cells. This favours the view that thymectomy suppresses a malignant-transforming immune reaction, and this interpretation is further supported by the observation that the effect of a grafted thymus is in part genetically determined.

If, as suggested by the present analysis of the initiation stage, viruses may often play an important part in human childhood malignancies[4], this is probably the consequence of relative immunological immaturity during the early years. The repeated failure to demonstrate virus involvement in non-leukæmic malignancies in adult humans may be due to the genuine absence of such an ætiological factor. The immunological competence of adult man may well be responsible for this, potential oncogenic viruses being neutralized by an adequate concentration of specific antibodies (probably cell-borne). On the Burnet[22]–Lederberg[23] view, the number and variety of specific antibody-producing cells should depend not only on the size of the animal but also on its growth-rate, and the (absolute) time available for the development of immunologically competent cells. Small short-lived animals are at a severe disadvantage in these respects, and it is suggested that this is the principal cause of their vulnerability to oncogenic viruses. Should the present hypothesis of carcinogenesis be applicable to such animals, non-genetic factors (in addition to genetic ones) are essential to account for an appreciable incidence of a malignant condition in them. The probability of a cell in an organ of the mouse acquiring spontaneously even 2 specific somatic-mutations within the normal life-span of the mouse will not be high at normal mutation-rates.

Cancer and Immunization

Attempts to immunize against cancer have been based on two assumptions: (*a*) cancer-specific antigens exist; (*b*) passive immunization with the appropriate antisera, or a suitable reinforcement of the host's immune system by active immunization, will reject the autoantogenic tumour.

Although several authors[25,35,36,45] claim to have detected antigens specific to spontaneous human malignancies (and see reviews by Milgrom, Rapport and Graf[46], and Old *et al.*[47]), others[48] find that tumour cells are deficient in certain proteins, and exhibit a concomitant loss of immunological specificity. The latter type of finding constitutes the basis of the 'deletion theory' of carcinogenesis.

According to the present hypothesis, both schools should be correct, and the cancer cell should be characterized by the simultaneous loss of one specific 'antigen' and the gain of another. In addition, the general balance of enzyme activity will be altered. The acquired autoantigen should normally be present, however, only at a low (but variable) concentration, and accordingly the outlook for orthodox cancer immunization appears to be poor. Experiments with animals give little encouragement. Some success in this direction has been obtained by Levi *et al.*[49], but in their experiments the test system was an Ehrlich ascites tumour inoculated into *C3H* mice. Despite an overall antigenic compatibility between the ascites tumour and the normal inbred *C3H* mice, small antigenic differences could account for the fact that anti-tumour serum obtained from inoculated mice produced some degree of passive immunization. This type of experiment with non-autochthonous tumour inocula is, however, largely irrelevant to the problem of immunization against spontaneous tumours. Comments of a similar nature have been made by Kaliss[50].

Several workers[47,51] have shown that " . . . sarcomas induced in rats and mice by the polycyclic aromatic hydrocarbons are antigenic in the animal, and in the inbred strain of origin[52]". Furthermore, some degree of immunization against this form of chemically induced tumour can often be obtained. On the other hand, and significantly, the immunization procedures used have no detectable effect on the incidence of spontaneous sarcomas[52]. This evidence indicates that these chemical carcinogens affect not only the differentiation–mitosis control system but that they also form, or induce, cellular antigenic constituents that are not associated with the control lipo-protein. These antigens will be absent from spontaneous tumours, and immunization therefore has no effect on the latter.

In an attempt to immunize against spontaneous tumours, Hirsch and Iversen[53] pre-treated inbred or back-cross *C3H* mice (free from the mammary tumour agent) with injections from strain-specific or strain-related mammary tumours of recent spontaneous origin. There was a significant reduction in the age at which spontaneous mammary tumours appeared in the pre-treated mice. If the present view is correct—that spontaneous tumours retain a low concentration of auto-antigenic control lipo-protein, then, pre-treatment should if anything slightly sensitize the host, thus producing a condition that is more likely to enhance, than to diminish, the malignant-transforming immune reaction between antibody and autoantigenic control lipo-protein in the carcinogenic cell.

It can be claimed that theory is in excellent accord with the evidence from cancer immunology.

Does this mean, therefore, that at the outset, attempts at cancer immunization are doomed to failure? The orthodox approach has so far proved unrewarding, but there remains an untried, fundamentally opposite procedure—the induction of acquired immune tolerance, or of immunological paralysis.

Acquired Immune Tolerance, Immunological Paralysis and Cancer Prophylaxis

According to Green[13], and to the present hypothesis, the malignant transformation of the carcinogenic cell frequently depends on an immune reaction between specific antibody (probably cell-borne[22]) and autoantigenic lipo-protein. It follows, therefore, that if this reaction could be prevented, the carcinogenic cell would remain 'dormant' and, viral or hormonal intervention apart, the latent period would be extended indefinitely. In other words, the tumour would never develop. In principle, this condition of non-reactivity could be induced through the phenomena of acquired immune tolerance or immunological paralysis[22,54]. The mechanism of immunological paralysis is obscure, and the practical difficulty of administering—and perhaps maintaining—a high concentration of cancer specific antigens would seem to be formidable. Equally, the induction of acquired immune tolerance may involve insuperable difficulties, and the following procedures would be involved:

(1) Identification and isolation of cancer-specific antigens. The major theoretical difficulty here is that if mutations in the structural control gene are random, a wide range of alleles should result, with the consequent possibility that control lipo-proteins, of slightly different immunological specificities, will be synthesized in the carcinogenic cells of different individuals. Distinctive tissues may be expected to synthesize tissue-specific control proteins, although pedigree and immunological evidence already quoted indicates that closely similar antigens may be common to many types of tumour.

(2) Assuming that the problems of identification and isolation of cancer-specific antigens can be overcome, and that supplies of effective antigens can be made available, then to induce tolerance, their presence will be necessary at an adequate concentration in the immunologically developing organism. In human beings (though perhaps not in mice) this would almost certainly entail the formidable difficulty of introducing the antigens into the embryo or fœtus via the maternal circulation and the placenta. The presence of antigen should prevent the formation of clones of antibody-producing cells, that, in the normal course of events, would react with the autoantigenic control lipo-proteins in the immunologically mature organism[22].

(3) To maintain tolerance, antigens would need to be injected at regular intervals.

The foregoing procedures could be very readily tested in mice, although infection with types 1, 2 or 3 virus, or hormonal transformation of the carcinogenic cell, would defeat their purpose; unfortunately the relevance of such procedures to man would appear to be rather remote. Nevertheless, the carcinogenic- (as opposed to the malignant-) cell with its (theoretically) high concentration of autoantigen, probably represents the stage of carcinogenesis most vulnerable to prophylactic measures. If the induction of acquired immune tolerance or immunological paralysis should prove to be impracticable, some method of blocking the immune attack on the carcinogenic cell (by pseudo-antibody ?) or alternatively, of rendering it cytotoxic, might still be devised. A better understanding of the action of the complement system might assist the latter objective.

The possible therapeutic measure of artificially supplying and maintaining a sufficient concentration of *DF* to every malignant cell in a tumour and its metastases should be considered. However, the hypothetical *DF*, or the '*DF* system', would first need to be identified, and superficially, this form of therapy would appear to offer little promise. Theoretically, prevention would seem to be more practicable than cure.

This article should not be concluded without some reference to the possible biological consequences of cancer elimination. According to the hypothesis[4,5] of the initiation-stage of carcinogenesis, the equilibrium-level of mortality from pre-zygotic tumours is determined by the mutation frequency in germ cells (including certain chromosomal aberrations incurred at meiosis) and the selective mortality rate in genetic carriers before, and during, the reproductive phase. In the absence of any exploitation of eugenic practices, the elimination of cancers in early life would eventually lead to a great increase in the number of carriers of (normally) 'carcinogenic mutations'—unless some additional unforeseen checks should arise. Provided anti-cancer measures were scrupulously maintained, this might be of little concern, but there is a risk that these mutant genes may cause some other, as yet unappreciated, detrimental effects[55]. Although it is not easy to imagine sequelæ as devastating as cancer, it should not be assumed automatically that its elimination would be wholly beneficial. But this perhaps is an anxiety we can bequeath to our doubtless more knowledgeable successors.

A part of this work was performed during a one-year leave of absence at the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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OBITUARIES

Prof. T. S. Wheeler

ALL who had ever met Thomas S. Wheeler, professor of chemistry in University College, Dublin, will have been deeply grieved to hear of his sudden and unexpected death on December 13 within a few hours of having attended a meeting at the College of Pharmacy. His untimely passing at the age of sixty-three is a tragic loss to the University College where he was not only an outstanding scientist and administrator but also a personal friend respected by everyone who, in whatever capacity, worked or studied there.

Although his work for the College over nearly eighteen years must remain unfinished, Prof. Wheeler has by his energy, his passion for progress, and his genius for inspiring others, much more than fulfilled his own modest ambition to leave the department better than he found it. It is much to be regretted that he has not lived to see the completion of the new chemistry department, now under construction, to the planning of which he had devoted much time and thought. His work for chemistry, for scientific education in Ireland and in other fields will long be recalled. Not least will he be missed by the Irish Government, which frequently sought his advice on practical scientific problems, advice which he was uniquely able to give owing to his remarkably wide interests in science, combining as he did extensive knowledge and research interests in all branches of chemistry and even in physics and mathematics. Prof. Wheeler endeared himself to all by his understanding, his humour, and his felicity of phrase.

Prof. Wheeler was born in Dublin in 1899. He was educated at O'Connell Schools and received a scholarship which enabled him in 1916 to proceed to the Royal College of Science for Ireland, where he gained the associateship in chemistry in 1920. He became for one year a demonstrator and research worker at the Royal Technical College in Glasgow, where his work gained him the external Ph.D. degree of the University of London. In 1923 he was appointed a research chemist at Woolwich Arsenal, and during 1928–31 was a senior chemist at the Winnington Research Laboratories of Imperial Chemical Industries, Ltd.

Prof. Wheeler left industry in 1931 to become for seven years principal and professor of organic chemistry at the Royal Institute of Science, Bombay, where he was active in building up flourishing schools of teaching and research and starting a postgraduate Department of Chemical Technology. He returned to Ireland in 1938 as State chemist in charge of the State Laboratories, and was appointed to the chair of chemistry in University College, Dublin, in 1945. Prof. Wheeler was the author, or joint author, of some two hundred original papers, patents, articles, and books, on an extraordinarily wide range of topics, the largest single group being on the organic chemistry of flavonoids and related compounds. Prof. Wheeler gave freely of his time in the service of many important committees and his loss will be keenly felt not only in Ireland and Great Britain but also in India and the United States, where he had many warm personal contacts. He is survived by his wife, whom he married in 1926, a son and two daughters. W. BAKER

Dr. J. S. Greenhow

THE death of Dr. J. S. Greenhow on November 3 at the early age of thirty-four has brought to an untimely end the career of a leading exponent of the study of the upper atmosphere by radar methods. In the span of twelve years he published nearly forty important research papers, and for most of these he was either the sole author or the leader of the research.

A graduate of the University of Manchester, he began his research work at Jodrell Bank in the early years of meteor observations by radar methods. He became interested in the characteristics of the meteoric ionization and the factors controlling the movement, diffusion and decay of the meteor trails. Observation of the motion of meteor trail echoes proved to be the best method for studying winds in these regions of the upper atmosphere. In this way, Dr. Greenhow obtained detailed information of wind speeds and directions, diurnal variations, turbulence and so on. Further knowledge of the conditions in the upper atmosphere came from the rates of decay of meteor echoes, and these were used to derive the atmospheric densities and temperatures. The initial stages of trail formation then attracted his attention, and he also studied how echo properties vary with the radar wave-length.

In 1960, Dr. Greenhow joined the Royal Radar Establishment at Malvern. Here he made the first meteor observations obtained at a wave-length as short as 25 cm. The results strikingly confirmed the view he had expressed previously, that a meteor trail has a surprisingly large initial radius, of the order of a metre. At the short wave-lengths, the trail also generally appears as only a short column of ionization close to the meteor. More recently, he has led the first British experiments to investigate upper atmosphere ionization by the important method, discovered in the United States, of measuring the radio scattering by free electrons at the short wave-lengths which penetrate through the ionosphere. He completed his last paper, "The Electron Scattering Cross Section in Incoherent Backscatter", just before his last brief illness. He had given no hint to his colleagues that for more than a year previously he had known that he might not have long to live.

All Dr. Greenhow's work was characterized by directness of approach, elegance of experimental techniques, and clarity of analysis, so that valuable and interesting results followed from every investigation. He was extremely capable in all stages of a research, from designing and constructing apparatus to the writing of the final report. He had a purposeful and stimulating enthusiasm which made him an inspiring team leader. At the same time, he was modest and friendly, and he is greatly missed by his colleagues. He leaves a widow and a daughter.

J. S. HEY

Mr. Herbert Womersley

HERBERT WOMERSLEY, a distinguished student of the taxonomy of the Acarina and primitive insects, died in Adelaide on October 14, 1962, at the age of seventy-three.

He was born in Warrington, Lancashire, on April 10, 1889, the son of amateur lepidopterist, Fred Womersley. He trained as an industrial chemist in soap and chemical manufacturing, specializing in fuel economy and water softeners. In his youth he became an amateur entomologist with a leaning to microscopy, getting training from Abraham Flatters in Manchester, and publishing an early paper in Flatters' journal, *The Micrologist*, on the use of terpineol. In 1914 he joined the Royal Army Medical Corps, and later transferred to the Chemical Corps, Royal Engineers, in one of the British Gas Companies, seeing service on the Western Front. In 1917 he was transferred back to Great Britain in explosives manufacture. In 1920 he moved from Warrington to Bristol as a departmental manager of Christopher Thomas and

Bros. soapworks. In Bristol he identified himself with local naturalists' groups, serving as president of Bristol Naturalists Society. In his evenings and week-ends he studied the apterygote fauna of the south-west of England. In several years he established himself as an authority on the Collembola and Protura, initially under the guidance of J. M. Brown.

He worked with great industry and speed—habits that never left him—and soon collections of foreign material were sent to him for working up. He was elected as associate (*hon. causa*) to the Linnean Society. In 1930 he was able to transfer to entomology in a professional capacity, as entomologist, Pasture and Field Pests Section, Council for Scientific and Industrial Research (now C.S.I.R.O.), stationed in Western Australia, his work having attracted the notice of Tillyard, then chief of the Division of Economic Entomology. Womersley's task was to study cultural, chemical and biological methods of controlling the lucerne flea (Collembola: *Sminthurus viridis* (L.)) and the red-legged earth mite (*Halotydeus destructor* (Tucker)), which were causing considerable damage. This work was done in collaboration with the Department of Agriculture, Western Australia. In the biological control field it was found that the bdellid mite *Biscirus lapidarius* (Kramer) was an important predator on *Sminthurus*, and the results were considered promising enough for lots of this mite to be transhipped by air to various Australian localities in an effort to establish them locally.

In 1933 Womersley was appointed to the South Australian Museum as entomologist, on the death of Arthur M. Lea. Here he found his most important role, that of a descriptive taxonomist, with a virtually unworked continent before him. Initially he continued his earlier studies on the Apterygota, these culminating in his monograph, *The Primitive Insects of South Australia* (1939), which, in fact, monographed the whole of the Australian fauna.

Afterwards, he gradually changed to the Acarina, and within a few years was virtually pre-occupied by the vast number of new mites requiring classification and description. His *magnum opus* was his monograph of the Trombiculidae of the Oriental and Australian regions (1952), which ran to 673 pages. These scrub-typhus and scrub-itch mites had assumed importance during the Second World War, and immense collections of them were referred to him during the war years and for years afterwards, as teams of workers concentrated on this subject in Malaya, New Guinea, India, China and Japan.

Womersley retired as entomologist in the South Australian Museum in 1954, but was immediately appointed acarologist, a salaried position created for him. The reference collection of mites he built up there is one of the great reference collections of the world. He retired from this position at the age of seventy, in 1959, but continued his work with zest, although by then dogged by increasing ill-health. However, his enthusiasm never waned, and he was working on the mites until a fortnight before his death.

He identified himself with naturalists' organizations both in England and Australia, taking an important part in wild-life conservation. A former president of the Royal Society of South Australia, he was awarded the Verco Medal for his achievements in biology in 1943, and in 1962 was made an Honorary Fellow.

R. V. SOUTHCOTT

NEWS and VIEWS

Biology in the University of East Anglia:
Dr. J. A. Kitching, O.B.E., F.R.S.

DR. J. A. KITCHING, whose appointment to a chair of biology in the University of East Anglia has just been announced, has been reader in zoology at Bristol since 1949, and was first appointed there as lecturer in 1938. From 1939 until 1945 he went first as a Research Fellow to Princeton and, with the outbreak of War, to Canada, where he worked on protective clothing for aircrew and served as secretary to the National Research Council's Committee on Aviation Medicine. A graduate of Trinity College, Cambridge, he is best known for his classical work on the osmoregulatory function of the contractile vacuole in Protozoa; he has also contributed to investigations on ciliary movement and on the physiology of Suctoria, where his interest in the experimental effects of high hydrostatic pressure has played a significant part. He has also made substantial contributions to marine ecology, recently as leader of an annual group of scientists and students investigating the hydrography, fauna and flora of Lough Ine, in south-west Ireland. In Bristol he played a leading part in the establishment of the Joint School of Biological Chemistry under the late Prof. W. E. Garner, and served as the secretary of its first Board of Studies. With Profs. T. A. Bennet-Clark and J. Dainty, the University of East Anglia has thus established an outstanding team to head its new School of Biological Sciences.

Chair of Transport at Imperial College of Science and Technology:
Prof. C. D. Buchanan

AT the outbreak of the Second World War, the part-time Maybury chair of highway engineering lapsed with the retirement of Prof. R. G. Clements. The small post-graduate school started by him has continued studying problems mainly allied to the needs of lesser-developed countries. The increasing national need for traffic engineering and long-term planning in urban and highway developments demands detailed examination and research. The College's new civil engineering building will enable the Department to undertake this work and to direct proper attention to the wider study of the complex social and planning problems of modern transportation. Mr. C. D. Buchanan, at present urban road traffic adviser to the Ministry of Transport, has been appointed to a newly established chair of transport tenable at the College as from October 1. Mr. Buchanan, an old student of the College, has held many distinguished posts in the Ministry of Transport and of Housing and Local Government. The appointment of Mr. Buchanan, who has a recognized professional reputation in this field, with qualifications in town planning and architecture as well as civil engineering, will enable detailed attention to be paid to long-term planning in urban and highway development.

The Institute for Operational Research

THE Operational Research Society and the Tavistock Institute of Human Relations have concluded negotiations for the establishment of an Institute for Operational Research as a non-profit-making body. This new Institute, of which Mr. W. N. Jessop will be the director, will be set up within the framework of the Tavistock Institute. The Operational Research Society is a scientific body which does not itself engage in project work. The Tavistock Institute consists of a linked group of active research units—so far, the range has covered sociology, social psychology and medical psychology, and it will now be extended to include operational research. Each unit has a high degree of autonomy and the new Institute will be free to develop its own programme and associations, both within and outside the Tavistock Institute.

The Operational Research Society is associated with the new Institute through representation on the Tavistock Council and through a special Advisory Panel with Sir Charles Goodeve as chairman. The aims of the new Institute are to extend the application of operational research to other fields as well as industry, to bring it into closer relationship with the social sciences and to carry out fundamental research. It will encourage the development of operational research in the universities, but because operational research is essentially concerned with action, its own connexion with the world of affairs will remain close. It hopes in time to include in its programme a study of the broad problems of social and economic policy where decisions in one area affect those in another. The Institute will keep closely in touch with existing operational research groups in industry. Mr. Jessop, who is a member of the Council of the Operational Research Society, is a graduate of the Imperial College of Science and Technology, a mathematician and statistician, and has been concerned with industrial operational research since 1948. He is at present in charge of operational research in Courtaulds, Ltd., and takes up his new appointment on May 1.

The Whiting and Industrial Powders Research Council

An Industrial Powders Division has been added to the Research Council of the British Whiting Federation, now re-named the Whiting and Industrial Powders Research Council, with the view of helping manufacturers and users having powder technology problems. Valuable experience of grinding, classifying, handling, mixing and particle size analysis of powders had been gained during work on improving the manufacture and quality of whiting. (Whiting is ground, classified chalk used as filler, abrasive or chemical in some forty industries.) This work gave the Research Council a good start in extending its activities to powders in general. Members of the new Division will decide the scope of future research, but sampling, grinding, classification, flow and packing properties of powders and particle size analysis are already under investigation. A bibliography of particle size analysis, an assisted translation scheme, and the creation of a 'powder bank' of powders of genuinely known characteristics are projects that the Division will undertake. The advantages of membership include access to all the results of jointly financed research supported by Department of Scientific and Industrial Research grants, the availability of experts for consultation, and participation in an established library and information service. Further information can be obtained from the Director of Research, The Hall, Welwyn, Herts.

Survey of Audio-visual Aids in Teaching and Research

In a written answer in the House of Commons on March 1, the Financial Secretary to the Treasury, Mr. A. Barber, stated that the University Grants Committee, the Ministry of Education and the Scottish Education Department, with the approval of the Treasury, had set up a joint committee, with Dr. Brynmor Jones as chairman, to survey the present-day use of audio-visual aids in teaching and research in the pure and applied sciences in Britain. This would take into account their use in similar fields in institutions of higher education in selected countries overseas, to assess their potential usefulness and possible lines of development in Great Britain. Here 'applied science' includes medicine, dentistry, agriculture and veterinary science. The other members of the committee are: Mr. G. Barclay, Prof. W. M. Humphrey Davis, Prof. A. L. d'Abreu, Mr. D. J. Johnston, Prof. H. Lipson, Prof. E. W. Parkes, Mr. C. W. Tonkin and Prof. C. M. Yonge.

Nuclear Power for Peaceful Purposes

In written answers in the House of Commons on February 26, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that the advanced gas-cooled reactor was now exporting electricity to the grid at the design net power output of 28 MW (E), and the present view of the Atomic Energy Authority as to the economic projects of this system was that given in their eighth annual report. The problems of running the Dounreay fast reactor at power levels up to 30 MW were overcome last year and the reactor had been operating at this level since October 1962. The stability of the reactor during operation, in particular, had been shown to be satisfactory, but much work remained to be done on developing large power-producing fast reactors, particularly in the field of fuel elements.

In a written answer in the House of Commons on March 5, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that hitherto the Atomic Energy Authority development of thermal reactors had been concentrated on the gas-cooled graphite moderated reactors, and power stations built in Britain were of this type—the advanced gas-cooled reactor at Windscale being an advanced version of the same system. For some time the Minister for Science and the Atomic Energy Authority had been concerned about the narrow technical base on which Britain's reactor development rested, and close touch had been kept with developments abroad on various water-moderated reactors. For some five years the Authority had intensively studied such reactors to evolve a design offering good neutron economy and low capital costs and the steam-generating heavy-water type reactor was the outcome of these studies. This reactor, with boiling light-water coolant and a potential capacity for superheating the steam, utilized pressure tubes as distinct from the pressure vessels of the gas-cooled graphite reactors. For the past two years a design and research and development programme had been undertaken to ensure that feasibility and design problems could be solved. Mr. Freeth was confident that this type showed promise as an advanced water reactor and would sufficiently broaden the basis of reactor technology in Britain. There had been close collaboration and agreement with the nuclear consortia in the assessment and design of this reactor and the capital cost of the authorized prototype was £16 million, including the cost of built-in experimental facilities and a contingency against uncertainties in estimating.

Besides its joint programme with the Belgonucléaire and other Belgian firms for the *Vulcain* nuclear marine reactor (see *Nature*, 197, 650; 1963)—the costs of which, shared equally, were confidential—the Atomic Energy Authority was participating with the European Nuclear Energy Authority in the *Dragon* high-temperature gas-cooled reactors in the United Kingdom (current annual expenditure, £4·9 million) and the Maldon boiling-water reactor in Norway (current annual expenditure, £550,000). The total cost to December 21, 1962, of these projects was £10·2 million and £2·25 million, respectively; about £4·5 million of the former figure was from the United Kingdom. Construction of the *Dragon* reactor was well advanced and fuel should be loaded within the next twelve months. The international team had already carried out extensive research and development work, the results of which would be available to the participating countries. In a written answer on February 27 regarding research into protective shielding of nuclear-powered ships, Mr. Freeth stated that work was being done on the most economic form of structural shielding to prevent damage to the reactor in the event of collision, and these collision barriers, which absorbed the energy of the striking ship, were the subject of experimental work at the Naval Construction Research Establishment. Designs were also being prepared to ensure the reactor did not suffer damage if the ship grounded. Nuclear shielding against ionizing radiation followed the principles and methods adopted for land-based stations, but a special study had been commissioned to adapt these to the ship.

The Overseas Service Aid Scheme

In a written answer in the House of Commons on March 5, the Secretary for Technical Co-operation, Mr. D. Vosper, gave the total number of teachers and other educational staff subsidized under the Overseas Service Aid Scheme at December 1, 1962, as 1,607, compared with 1,025 in June 1960, mostly in East and Central Africa. Of returns to the National Council for the Supply of Teachers Overseas from United Kingdom, employers for the school year 1961–62 notified 659 British teachers (121 officially seconded) as leaving for posts in Commonwealth countries and territories excluding Canada, Australia and New Zealand, in which a further 468 took up posts. On March 1, the Secretary of State for Scotland, Mr. M. A. C. Noble, stated that returns for the two years to August 31, 1962, showed that 130 Scottish teachers went to posts in developing countries in the Commonwealth, 8 on secondment, and 15 others were seconded to posts which did not come within the official arrangements. According to a written answer from the Minister of Education, Sir Edward Boyle, on March 7, 1,232 Commonwealth teachers from overseas are receiving teacher training in the United Kingdom.

Support for Medical Research in Britain

In a written answer in the House of Commons on February 26, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that the Medical Research Council had entered into an agreement with the National Research Development Corporation and three pharmaceutical firms for a programme of collaborative research on the production of a common cold vaccine. Recent work at the Common Cold Research Unit maintained by the Council and the Ministry of Health had shown that antibody production could be stimulated by the injection of particular cold viruses, but further work would be required to discover whether such injections protected against colds. The work formed part of a broader programme of virological research supported by the Council and progress depended on an adequate supply of volunteers to test the efficiency of the vaccine. In a further written answer, Mr. Freeth stated that a generous response had been received to an appeal made by Mr. W. J. Dempster, reader in experimental surgery at Hammersmith Hospital Postgraduate Medical School, for funds for work in connexion with the development of kidney grafting. Mr. Dempster's work had received continuous support from the Medical Research Council since 1950, but when, in accordance with arrangements between the Research Councils and the University Grants Committee, the Council wrote to all universities in November 1960 inquiring whether they would be willing to include in their estimates for the 1962–67 quinquennium provision for research projects hitherto financed by the Council this project was not accorded sufficient priority by the Medical School to qualify for support within the funds available. The Postgraduate Medical School, however, had already been informed by the Council that if they received insufficient funds for the 1962–67 quinquennium to maintain all the useful projects which they wished to take over, the Council would be pleased to discuss any such projects with them. In accordance with the usual practice, a reminder had been sent to Mr. Dempster three months before the expiry of his grant inviting him, if he so wished, to submit a report in application for a renewal of grant, and Mr. Dempster made his appeal the day after the Council's reminder was posted. He has since replied that he does not require further support from the Council.

New Technique for preparing Lasers

Semiconductor lasers have been produced during the past few months by a number of research organizations, including Services Electronics Research Laboratories, Baldock, and Standard Telecommunication Laboratories, Ltd., Harlow, in the United Kingdom. The method used by Standard Telecommunication Laboratories, Ltd., Harlow, for making gallium arsenide lasers is based on a technique using cleavage, and leads to the manufacture of lasers with unusually good optical properties. A flat *p-n* junction is produced by diffusion in a carefully prepared slice of very homogeneous material, and this slice is broken into laser dice by applying mechanical pressure along accurately aligned axes with a hard stylus. If the original slice has been properly prepared, the gallium arsenide can be made to cleave along optically nearly perfect, parallel faces without difficulty. The laser is completed by adding suitable electrodes and mounting on a heat sink. The new process results in good and well-finished surfaces without the usual lapping and polishing operations, and the laser dice are extremely thin, and have, therefore, excellent thermal conduction properties. Lasers, with the high standard of perfection of their optical flats produced in this way, have been found to emit light having excellent spatial and polarization coherence.

The American Museum of Natural History

With funds which, based on British standards, are large, the American Museum of Natural History is the envy of many in the museum world. The ninety-third annual report (1961–62) contains abundant proof of the forward look in museum presentation with exhibits of *Man in Space* and *Dating the Past with Atoms* (Pp. 95. New York: American Museum of Natural History, 1962). Combined, these exhibits cover a vast range of time and indicate the rapid pace of progress in science. Although the Museum is best known for exhibits inside the building the institution goes out in many ways to the public. The Museum programme "Journey into Nature", now in its third year, is broadcast every Sunday morning and its long-established extension service to schools, colleges, libraries, hospitals and children's museums makes heavy demands on the Department of Education for circulating exhibits.

The National Art Gallery and Dominion Museum, Wellington

The annual report of the National Art Gallery and Dominion Museum in Wellington, New Zealand, for the year ended March 1962 records many bequests and gifts (Pp. 24. H. 21. Wellington: Government Printer, 1962). Particular mention should be made of a valuable cash bequest by the late Mrs. Mary McLauchlan to provide a fund to help young New Zealand artists. Loans of pictures have been made to other galleries, and the Prime Minister's suite in Parliament buildings. There has been a recent upsurge of interest in systematics of New Zealand organisms and it is pleasing to note that there is a growing tendency for workers in natural history to donate type material of new species to the Museum.

The Coryndon Memorial Museum, Nairobi

The annual report for 1961–62 of the Trustees of the Coryndon Memorial Museum indicates that this institution continues to make progress under the energetic curatorship of Dr. L. S. B. Leakey (Pp. 34. Nairobi: Coryndon Memorial Museum, 1962). In order to devote himself to field work at Olduvai and elsewhere Dr. Leakey resigned the curatorship in June 1961 and was appointed honorary curator. During the study of the fossil Bovidae Dr. Leakey found that the living African Bovidae were in a great taxonomic muddle and, in consultation with living world authorities, started a re-examination of the African species. The study was based on skulls and skeletons and not only on the skins. A two-weeks safari to Bwamba Forest in Western Uganda added much valuable entomological material to the collections.

Gem Diamonds : Lecture Course and Certificate

A COURSE has been running this session at the College of Arts and Crafts, Birmingham, in the School of Jewellery and Silversmithing, on "Gem Diamonds". This course, the first of its kind to be established in Europe, is restricted to qualified Fellows of the Gemmological Association of Great Britain. Extending over thirty-five sessions, it is being given by Mr. N. Harper. The comprehensive syllabus covers crystallography, geological occurrence and genesis, mining and marketing, cutting, polishing, synthetics, etc. The course is both theoretical and practical, and those taking it will handle both rough diamonds and cut gem-stones. Organized by the Gemmological Association, those Fellows who complete the course are invited to take an examination and, if successful, will be awarded a 'Diamond certificate'. Prof. S. Tolansky, Royal Holloway College, London, has agreed to act as examiner and will set and mark the theoretical papers. The practical examination will be conducted by I. Balfour, of the London office of De Beers. The course is now in full swing and about a dozen Fellows of the Gemmological Association are expected to sit for the first certificate examination on June 5. Further information concerning the course can be obtained from the Gemmological Association of Great Britain, 2–4 Cary Lane, London, E.C.2.

Courses in Technology

FACILITIES for advanced courses in technology have greatly improved during recent years but these facilities are not generally as well known as they deserve. The publication of a *Bulletin of Special Courses in Higher Technology* by the London and Home Counties Regional Advisory Council for Technological Education is therefore welcome and meets a real need. The *Bulletin* is published in two parts: Part 1, issued in July, gives details of advanced courses held in the autumn term, and Part 2, published in December, contains information about similar courses held in the spring and summer terms (Pp. 97. London: London and Home Counties Regional Advisory Council for Technological Education. 3*s*. 6*d*., 1962). The current *Bulletin* provides particulars of more than 300 part-time (usually evening) courses and nearly a hundred full-time courses (of less than three-month duration), the subjects ranging from Boolean algebra and technical writing to photoelasticity and ultra-sonics. To illustrate the wide range of classes available to prospective students in the area, the following have been picked at random from the *Bulletin*: transistor theory, high-vacuum techniques, nuclear engineering, metrology, mathematical aids to management, the interpretation of air photographs and trade with Japan.

University News : Birmingham

DR. J. WALKER, at present reader in reactor physics, has been appointed to the newly established chair of applied nuclear science in the Department of Physics from April 1. Titles of reader have been conferred on Dr. V. G. Welsby (electrical engineering) and Dr. J. Hardwicke (experimental pathology). Dr. H. Thompson has been appointed senior lecturer in the Department of Pathology. The following appointments have also been announced: *Lecturer*, Dr. S. Garber (industrial metallurgy); Dr. I. J. Maddix (pure mathematics). *Research Fellow*, Dr. L. A. Biran (Imperial Chemical Industries Research Fellow in the Department of Medical Biochemistry and Pharmacology); H. G. Clarke (Senior Research Fellow in the Department of Neurosurgery); Dr. O. J. Fagbemi (Imperial Chemical Industries Research Fellow in the Department of Mechanical Engineering); Dr. J. Keilson (pure mathematics).

London

MR. A. V. BRADSHAW, reader in extraction metallurgy at the Imperial College of Science and Technology, has been appointed to the chair of applied metallurgy tenable at that College. Dr. R. E. Burge, reader in biophysics at King's College, has been appointed to the chair of physics tenable at Queen Elizabeth College. Dr. E. M. Crook, reader in biochemistry at University College, has been appointed to a chair of biochemistry tenable at St. Bartholomew's Hospital Medical College. Mr. B. R. Jones, senior lecturer at the Institute of Ophthalmology, has been appointed to the chair of clinical ophthalmology tenable at the Institute. Dr. E. S. Perkins, reader in ophthalmology at the Institute of Ophthalmology, has been appointed to the Semble chair of experimental ophthalmology tenable at the Institute. Dr. S. J. Wyard has been appointed to the readership in physics tenable at Guy's Hospital Medical School, and Mr. H. S. Smith, Fellow of Christ's College, Cambridge, has been appointed to the readership in Egyptian archæology tenable at University College. Titles of reader have been conferred on: Dr. R. Niedergerke (biophysics) in respect of his post at University College; Dr. B. R. Coles (physics) in respect of his post at the Imperial College of Science and Technology; Dr. J. S. Wilkie (history and philosophy of science) in respect of his post at University College.

Announcements

DR. B. R. Nijhawan, director, National Metallurgical Laboratory, Jamshedpur (India), has been elected an honorary vice-president of the Iron and Steel Institute, London, at the international symposium on "Iron and Steelmaking", recently held in Jamshedpur (India) and jointly organized by the National Metallurgical Laboratory and the Indian Institute of Metals in collaboration with the Iron and Steel Institute. Dr. Nijhawan has also been elected vice-president of the Mining, Geological and Metallurgical Institute of India.

THE following appointments to the Board of Bristol Siddeley Whittle Tools, Ltd., have been made: Sir Arnold Hall (chairman), Air Commodore Sir Frank Whittle (deputy chairman), Mr. H. Ballantyne, Mr. B. Davidson, Dr. S. G. Hooker and Mr. S. W. Mansell.

THE third international conference on "The Mössbauer Effect" will be held at the Laboratory of Atomic and Solid State Physics, Cornell University, during September 4–7. Further information can be obtained from A. J. Bearden, Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York.

A SYMPOSIUM on "Chemical Process Hazards with special reference to Plant Design", organized by the Institution of Chemical Engineers (North Western Branch), will be held at the Manchester College of Science and Technology during April 2–4. Further information can be obtained from A. V. Bailey, 2 Finney Drive, Wilmslow, Cheshire.

AN international conference on "Plant Tissue Culture", sponsored by the North Atlantic Treaty Organization and other agencies, will be held at the Pennsylvania State University during May 28–June 1. The programme will cover the problems of nutrition, metabolism, morphogenesis, cytology, genetics and pathology. Further information can be obtained from F. Wally Lester, Continuing Education Conference Center, Pennsylvania State University, University Park, Pennsylvania.

THE 1963 conference of the Institute of Metal Finishing will be held in Edinburgh during April 30–May 4. The programme will include the following technical sessions: aluminium coatings and anodized aluminium; internal stress in electrodeposits; nickel and chromium coatings; discussion forum on metallic coatings; organic coatings; discussion forum on organic coatings; miscellaneous electrolytic processes. Further information can be obtained from the Conference Secretary, Institute of Metal Finishing, 32 Great Ormond Street, London, W.C.1.

RADIOBIOLOGICAL INSTITUTE OF THE ORGANIZATION FOR HEALTH RESEARCH TNO, HOLLAND

ON October 23, 1962, the new laboratory building of the Radiobiological Institute at Rijswijk, Holland, was formally opened by the Minister of Social Affairs and Public Health, Dr. G. M. J. Veldkamp. This ceremony marked the completion of the new facilities of the Institute, which was founded in 1956. Besides the main laboratory building (Fig. 1) there are three animal pavilions for the mouse colony, for an *SPF*-rat colony and for a monkey colony and a separate building housing the workshop and a conference room.

The Institute, which is under the direction of Dr. D. W. van Bekkum, has a broad research programme in the field of somatic radiation effects and their modification. Radiation damage to the living cell is investigated by means of biochemical and histological techniques, using not only tissues from animals exposed to radiation but also simpler systems such as mammalian cells cultured *in vitro*. For biochemical and biophysical investigation, cloning techniques as well as suspension cultures provide large quantities of cells. The reaction patterns of these cell populations in response to radiations of different ionization density give a clue to the fundamental processes involved in cellular radiation damage. In addition, these investigations are expected to provide a theoretical basis for a more rational X-ray therapy of tumours. In order to verify the patterns observed *in vitro* under various conditions *in vivo*, parallel investigations are carried out with transplantable tumours in experimental animals.

One of the functions of the Institute is to promote the clinical application of data obtained from experimental radiobiological research. Co-operation has been established with the Rotterdam Radiotherapeutical Institute and a radiologist is working under the joint auspices of the two Institutes on subjects of direct practical interest.

A second major field of study is the prevention and treatment of radiation disease in which bone marrow transplantation in monkeys at present plays an important part. In addition, various immunological problems are being examined which are directly related to phenomena observed after transplantation of bone marrow. These more fundamental investigations are usually carried out with rats and mice. Clinical work on these problems is not performed at the Institute, but one of the staff members has a formal affiliation with the University Hospital at Leyden to organize and direct clinical studies in the field of bone marrow transplantation.

A third group of investigations concerns the toxic effects of radioactive isotopes. In recent years, emphasis has been laid primarily on the late effects of strontium-90 contamination in mice and the evaluation of various methods of treatment. The present new facilities permit an appreciable expansion of this work, both with respect to an increase in the number of experimental animals and the inclusion of other isotopes.

For some time the Radiobiological Institute has also carried out low-level determinations of radioactive isotopes in various biological products, but whenever these

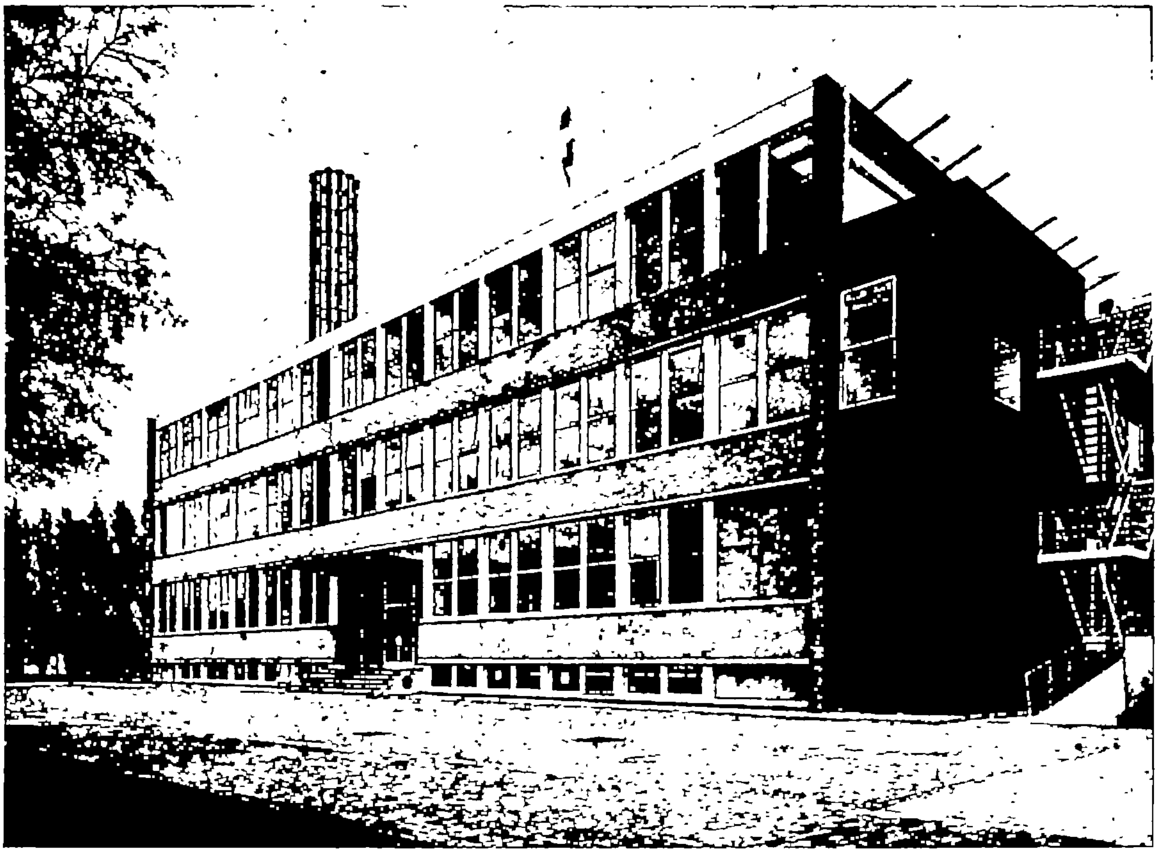

Fig. 1. Laboratory building of the Radiobiological Institute TNO, Rijswijk, Holland

determinations have become a routine they have been taken over by other groups.

The Institute is well equipped with a variety of modern instruments and apparatus for biochemical and biophysical work. In addition, the radiobiological investigations require two special kinds of facilities, namely, radiation sources and experimental animals of very high quality. The Radiobiological Institute has at its disposal, besides small α- and β-sources, a 200-c. cæsium-137 γ-source for prolonged radiation of experimental animals and a 250-kV 'Maxitron' X-ray unit with circular beam used for short-term radiation. The special tube with which this apparatus is equipped makes it possible to irradiate a number of large animals simultaneously.

Lastly, a neutron generator is in use for measurements in the field of neutron dosimetry, and with this machine small biological objects such as cultured cells or small numbers of mice can be irradiated with neutrons. Co-operation has been established with the Reactor Centre of the Netherlands at Petten, which will make it possible to expose larger experimental animals to fission neutrons.

The laboratory has four specially designed rooms for biological work with higher levels of radioactive isotopes with installations for the removal of airborne and fluid waste.

In the Institute an attempt is made to maintain unusually high standards of health in the experimental animals used for radiobiological research. This policy, in which a great deal of effort and resources is involved, is followed for two reasons. In the first place, in many experiments the animals are exposed to total-body irradiation which greatly reduces their resistance to infection. When pathogenic micro-organisms are present even without causing manifest disease symptoms, there is a risk that the animals will develop the disease after irradiation. The consequent mortality will often prevent the successful conclusion of experiments. In the second place, a very prolonged observation period is required for the investigation of the so-called late effects of radiation and of isotope intoxication. These long-term experiments usually extend over the entire life-time of the animal. In the past, the health of the ordinary laboratory animals (conventional animals) often was so poor that most of them died of specific diseases at 'middle-age' or earlier, even without being exposed to other injurious factors.

The opinion held at the Radiobiological Institute is that long-term experiments with insufficiently selected animals will result only in loss of time and money. A start was therefore made some years ago, in co-operation with the Medical Biological Laboratory RVO–TNO, on a programme of far-reaching sanitation of the animal colonies. The procedure was based on Cæsarean-delivered, hand-fed young animals raised under strict isolation. The hand-feeding has now been abandoned since gnotobiotic animals are available for foster-nursing. The experience obtained so far has led to a modified approach —which is at present being pursued—namely, a multiple-step system for the production of satisfactory animals. These steps range from heavily protected gnotobiotic animals, via specific pathogen-free stocks to lightly protected production units. In cases of infection or other disorders in the production colonies, supplementation and, if necessary, total replacement can be made with animals from the next higher step. This project receives financial support from Euratom, and one of its objectives is to determine what grade of sanitation is optimal for the various kinds of radiobiological work.

The staff of the Institute includes, in addition to two physicists and two biochemists, seven medical graduates with training in different fields.

L. M. VAN PUTTEN

THE CARNEGIE INSTITUTION OF WASHINGTON

YEAR BOOK No. 61* of the Carnegie Institution of Washington, covering the year ended June 30, 1962, and including the reports of the President, the Mount Wilson and Palomar Observatories, the Geophysical Laboratory, the Committee on Image Tubes for Microscopes, and the Departments of Terrestrial Magnetism, Plant Biology, Embryology, and of Genetics, marks the sixtieth anniversary of the Institution. The Institution's budget is now more than 2·8 million dollars, all of which is spent on six operating departments, whereas in 1905 the budget was less than 600,000 dollars, of which 302,000 dollars went to ten departments, about half of this being for the Solar Observatory on Mount Wilson, then under construction. Some 130,000 dollars were distributed in grants and nearly 30,000 dollars on subsidizing publications.

Experiments on maize had already begun, as had cytological research on *Drosophila*, and the Department of Genetics is still finding these lines of investigation productive. During the year, Dr. Barbara McClintock confirmed the hypothesis that there is a high probability that genic control systems in maize and bacteria act in similar fashion. In the meantime, the group working on the application of cytology to genetics found that direct chromosomal breakage occurs in *Tradescantia* root tips in presence of 5-bromodeoxyuridine, which acts by modifying the base sequences in nucleic acid rather than the phosphate–sugar helixes attacked by deoxyribonuclease, and that Golgi bodies, one of the types of cytoplasmic organelle, exhibit different forms in the progression of microsporogenesis in *Tradescantia*. The Department has also established the molecular weight of the deoxyribonucleic acid of a bacteriophage known as $T5$ by first establishing an ingenious pair of scales by analysing the deoxyribonucleic acid fragments of another phage ($T2$), and has found that the molecular characteristics of the deoxyribonucleic acid of phage lambda differ remarkably from those of other deoxyribonucleic acids, possessing a broad range of denaturation temperatures. The Department is now being terminated as a separate administrative unit and staff remaining in the Genetics Research Unit will co-operate with the new Laboratory of Quantitative Biology which is being organized.

In the Department of Plant Biology attention is still directed on the functions of chlorophyll and the accessory pigments in photosynthesis in which it has been interested since 1911. The persistence of the chemically unidentified material previously formed by illumination of chlorophyll *a* has been measured, and by measuring oxygen exchange of Swiss chard chloroplasts four separate effects of light, each with its characteristic action spectrum, have been distinguished. Using the new technique of electron paramagnetic resonance, two distinctly different light-induced resonances have been observed, and it is concluded that the rapid-decaying signal, seen only when cells are illuminated, is associated with chlorophyll and arises from the 'primary' act of photosynthesis. Any inhibition of oxygen evolution, as by 3-(3′ : 4′-dichlorophenyl)-1 : 1-dimethyl urea, or by limiting the manganese–ion concentration in the growing medium, produces an enhanced rapid-decaying signal. Moreover, besides the evidence that chlorophyll is the source of one type of free electrons in an intact photosynthetic organism and that plastoquinone is the site of another type, the two types of signal have been correlated with the evolution of photosynthetic oxygen. Some particularly interesting studies of the photosynthetic rates of six races of *Mimulus* originating in diverse climates and altitudes indicated that climatic races within the same species may show differential patterns of response linked with variations in internal physiology.

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* Carnegie Institution of Washington. *Year Book*, No. 61. July 1, 1961–June 30, 1962. Pp. xi+112+526+21 plates. (Washington, D.C.: Carnegie Institution of Washington, 1962.)

The Department of Terrestrial Magnetism, in which the emphasis of the programme has markedly changed from earlier years, apart from studies of the intensity of the charged particles in the Van Allen trapped-radiation belt (as recorded during the transits of satellite *Explorer VII* through the belt between 1959 and 1960), was concerned with the application of physical techniques to a wide range of geophysical and biological problems. Using a simple and effective new procedure, it has been demonstrated that the deoxyribonucleic acid-like ribonucleic acid comprises about 1 per cent of the total ribonucleic acid of bacterial cells, and that it has a half-life during active synthesis of about 2 min. If this ribonucleic acid is, in fact, the template for protein synthesis, a single molecule acts catalytically for the synthesis of many polypeptide chains. The method can also be used to exploit the specificity in the hybridization process, which depends on long regions of complementary nucleotide sequences in molecules of ribonucleic acid and deoxyribonucleic acid and it can also be used in studies of the transcription of genetic information and of differentiation.

With support of the National Science Foundation, the Department is establishing a new Carnegie Radio Astronomy Station in Argentina, and it is also constructing an interferometer array from parabolic disk antennæ to obtain precise positions of radio-noise sources in the sky. During the period covered by the report, the observational programme in radio astronomy continued and observations of the hydrogen gas content at the centre of our Galaxy confirmed that the motions of hydrogen close to the Galactic centre are complex and that the hydrogen gas is expanding as well as rotating. A major co-operative project of the Seismic Studies Group was an intensive study of the Earth's crust in Maine which demonstrated the very real limitations imposed on our ability to learn about crustal 'layers' by the customary methods of explosion seismology, and its findings postulate appreciably less granitic material than is customarily thought to be in a continental crust. The Group concerned with the radioactive dating of rocks participated in an exchange with the Geological and Mineralogical Institute of the University of Kyoto and has compiled a new map of age-distribution in crystalline basement rocks of North America. The Department is also associated with the work of the Committee on Image Tubes for Telescopes which has now reached a stage when a real gain in research work over the best photographic plates is just beginning to be realized by means of convenient (and permanent) sealed-off image tubes.

The Geophysical Laboratory focused much of its work in experimental petrology during the year on pyroxene minerals and the study of phase equilibria at higher pressures. Additional experimental data for constructing concepts of the Earth's mantle and crust are being obtained from an examination of the melting relations of silicates at high pressures. Much effort was expended on studies of the mineralogy of meteorites, and new investigations in organic geochemistry included analysis of Precambrian carbonaceous materials. Fatty acids were isolated from rocks as old as 500 million years, including myristic, palmitic and stearic acid, and the same acids have been isolated from recent sediments but with a marked difference in the relative proportions of acids. Much the longest of the departmental reports, that from the Geophysical Laboratory, includes summaries of more than fifty investigations.

At the Mount Wilson and Palomar Observatories an extensive study of the accumulated magnetograms to classify magnetic regions and correlate them with optical and radio phenomena showed that the unipolar magnetic regions of the Sun correlate in position with the calcium absorption phenomenon observed spectroscopically. Studies of the spectra of the major planets were commenced and two lines of the hydrogen molecule were found in the spectrum of Saturn. The analysis of high-dispersion spectra of Venus afforded evidence of large changes in the temperature of the atmosphere of Venus. Colour-magnitude diagrams have been constructed for many globular and galactic clusters and high-dispersion spectra used to make detailed quantitative chemical analyses of stellar atmospheres. New knowledge was obtained about differences in chemical composition among stars, the correlation of chemical composition and star movement, and fresh evidence on the expansion of the cosmos from studies of nearby galaxies by photoelectric scanning methods. From studies of the orbits around the nucleus of our Galaxy of a large number of dwarf stars, both normal and sub-dwarf types, the time of formation of the Galaxy out of the medium of the universe has been fixed at about 10 billion years ago.

The completion of the new building for the Departmen
of Embryology was a feature of the year in which th
Department's most striking progress was in continuatic
of its studies of the problems of muscle developmen
Investigation of the cytodifferentiation of embryon
skeletal muscle cells in dispersed cell culture using fresl
isolated embryonic muscle cells showed a *plating* efficie
of about 10 per cent, and in plates cultivated f
days about 1 in 10 colonies showed unmistaka
skeletal muscle cell differentiation. Further
that the medium is altered by the me
of cells cultured in it, and the resul
themselves, represent something of a
through in the task of cell culture.
tions include studies of the physiologi
embryo growth from the stage of th
the nature of the testicular antigen in
genesis, of the role of deoxyribonuc
metamorphosis of the tadpol
study of the developing hu
of the vascular pattern o
in situ.

VASCULAR A

A SYMPOSIUM on the "Vascul
Responses in Injury", organize
Pathology of the newly established Fac
of the University of New South Wales,
ogists from all parts of Australia, and
the presence of Sir Roy Cameron, of U

into a central zone containing corpuscles, and a peripheral zone composed of plasma. This 'plasma skimming' is increased by the addition of substances which increase the sedimentation rate of the blood, for example, high molecular weight polymers like dextran.

Next, Dr. W. E. Stehbens (Canberra) discussed the structure of vascular endothelium with particular reference to the existence of intercellular cement substance. Carbon, chylomicra and platelets were injected into the circulation but did not stick to the space between vascular endothelial cells on electron microscopic examination. Transparent glutinous masses present in the rabbit's ear chamber, and in the frog's web after the intravascular administration of carbon, were shown to be agglutinated masses of platelets with some fibrin, rather than cement substance. Accordingly, the evidence for the existence of capillary cement substance is inconclusive.

Dr. I. K. Buckley (Melbourne) described the structure of cellular and vascular reactions after injury. Injury was produced by a high-frequency electric current to ear chamber tissue. A progressively enlarging area showed vascular damage, and nearby granulocytes migrated towards this centre of injury. Thin-walled transparent globular structures, probably myelin figures, developed at the surface of heat-injured cells.

Dr. W. J. Cliff (Perth) described electron microscopic studies of the process of wound healing in the rabbit's ear chamber. Cell division occurred near, but not at, the tip of capillary sprouts. The tip of the cord consisted of a mass of endothelial cells, which gradually separated; the lumen was constituted by the intercellular space so formed. Basement membrane was probably produced by interaction between endothelium and the extravascular tissues, while both adventitial cells and smooth muscle cells probably developed from fibroblasts.

The session concluded with a report on the reaction of cells of peripheral and intermediate lymph to foreign
materials by Dr. J. G. Hall (Canberra), read in his absence
by Dr. B. Morris. Foreign materials were injected into
he hind limb of a sheep the popliteal lymph node of which
ad been cannulated on either the afferent or efferent
de. Radioactive isotope studies showed that a non-
rticulate antigen—human globulin—was not retained
the lymph node, whereas a particulate antigen, the
an red cell, was. About 50 h after the initial injection
cell population of the efferent lymph from the node
change: initially, large immature cells poured
ph, followed later by lymphocytes and plasma
tput of RNA correlated quite closely with
l response.

sion was given over mainly to the
ated with inflammation. The session
Roy Cameron (London), who described
ellular events following injury.

ns to injury are chemically mediated,
e, aided in some species by 5-hydroxy-
inning of increased permeability is
antihistamine. The delayed
ned vasodilatation and in-
more complex. These
lasma factors—kinins
and antagonized by
ugmented hist-
ve loss of

dense the fraction the smaller the lipoprotein particle. The smaller molecules passed across the membrane more readily than the larger. After heat injury in hyperlipaemia, there was an increase in the lipids in the lymph, which may become milky. It is postulated that materials pass through the capillary wall in vesicles or otherwise and that the polysaccharides in the basement membrane act as a sieve. After injury, in hyperlipaemic animals, lipoproteins also passed across the endothelium in arteries, and were deposited below the internal elastic lamina.

Vascular permeability changes in ultra-violet injury were described by Dr. G. Logan and Prof. D. L. Wilhelm (Sydney), using the technique of 'blueing'. In ultraviolet, as in other types of injury, the inflammatory reaction is characterized by three vascular responses—vasodilatation, increased permeability and migration of leucocytes. In this case, however, they develop relatively slowly and can be dissected apart. Erythema and increased permeability each occur in two phases: the leucocytosis rises and falls 3–4 h in front of the late phase of increased permeability. The early phase of increased permeability is mediated by histamine in guinea pigs and rabbits, by 5-hydroxytryptamine in rats. The mediator of the late phase is unknown.

Dr. J. Margolis (Sydney) gave an account of his quantitative work on plasma kinins. These factors may be released from plasma by kallikrein, trypsin or the glandular 'kallikreins' from saliva and urine. The normal release of these factors is dependent on three factors: component *A*, or kallikreininogen; Hageman factor, which is also involved in blood clotting; and compound *B*. a protein easily adsorbed on to glass. Hageman factor is easily activated by contact with a negatively charged surface. Trypsin and the glandular kallikreins are independent of the Hageman factor.

The plasma kinins are bradykinin, a nonapeptide, and kallidin, a decapeptide. These may be loosely attached to plasma globulins as kininogens; plasma kallikrein may act on one substrate (globulin plus nonapeptide) corresponding to the nonapeptide precursor compound *B*. The others act on both substrates (globulin plus nonapeptide, and globulin plus decapeptide); trypsin produces bradykinin, and glandular kallikrein produces both the nona- and deca-peptides. The decapeptides are attacked by a plasma peptidase to produce a nonapeptide which is probably bradykinin.

The last paper of the day was given by Dr. B. Morris (Canberra). He described techniques for producing permanent lymphatic fistulæ in sheep. The blood vascular and lymphatic flow in an area are both altered by exercise. Thus, exercise induces a cessation of flow of intestinal lymph and a fall in hepatic lymph flow, but an increase in the flow of leg lymph. In the exercised lactating sheep mammary flow increases. Exercise also increases the rate of turnover of radioactive protein between the intravascular and extravascular compartments.

The main topic of the third session (November 3) was the response of cells in inflammation. Dr. J. V. Hurley (Melbourne) demonstrated the existence of an active principle which induces leucocytic migration. This substance may be derived from burn extracts, from polymorphs and from activated serum; it is non-dialysable, is destroyed
ut not by ethylenediamine tetraacetic acid
rmeability inhibitors.

tion of how cells recognize one another
viour is controlled by other cells was
. V. Boyden (Canberra). The chemo-
he first part of the recognition of foreign
vestigated. Cells were observed while
gh a millipore collodion membrane
material on the other side; the more
rial, the greater the tendency for the
rate towards it. In the case of cellulose
gration was much more marked in the

presence of serum. It was concluded that the power of recognition of the foreign material lay not in the cells but in the serum.

Dr. V. J. McGovern (Sydney) reported on work carried out in collaboration with Dr. D. Bloomfield. The ability of leucocytes to stick to vascular endothelium was investigated using *en face* preparations. Saline was injected into the adventitia of arteries, and 1·5–3 h later leucocytes stuck to the uninjured endothelial surface. These penetrated the endothelium and continued to do so until about 18 h after the original trauma. If in making the injection the endothelium was injured to the point of thrombosis, or nearly so, leucocytes stuck to the endothelium much more rapidly. The worse the injury, the sooner did they stick.

The structure of macrophages was the subject of an account by Dr. I. Carr (Sydney). The common feature in granulomata induced by introduction of foreign materials was the presence of spherical masses of phagocytic cells arranged around the foreign material. It was suggested that lipid was phagocytosed in these circumstances by a process which involved the membrane disappearing and the material passing into the cell free of membrane. The hypothesis was put forward that the cell membrane may dissolve in the extracellular lipid. The giant cells in these lesions probably are formed largely by cell fusion.

The last paper was that of Dr. E. G. Cleary (Sydney). Dr. Cleary described his analysis of the content of collagen and elastin in samples of human arterial intima and media. More elastin was present proximally and more collagen distally, but the sum of these was constant. The elastin content was probably increased in the new-born; collagen decreased with age. Even muscular arteries were found to contain as much as 52 per cent collagen by chemical analysis.

In his summing up, Sir Roy Cameron commented on the wide range of interest of the symposium, which indicated the emergence of a distinctive Australian approach to experimental pathology.

I. Carr

GELATINE AND GLUE RESEARCH

The twenty-fourth research panel meeting of the British Gelatine and Glue Research Association was held at the Strand Palace Hotel, London, W.C.2, on November 7. The chairman of the Association, Mr. S. G. Hudson (Richard Hodgson and Sons, Beverley), presided and directed attention to the place of the first paper on wool structure in the research panel series, which has lately included papers on other complex proteins such as collagen[1] and elastin[2].

Dr. A. Robson (Wool Industries Research Association, 'Torridon', Leeds) contributed a paper on "The Sulphur Balance in Wool". In the past it has often been noted that total sulphur analyses for wool are significantly larger than the sum of the individual sulphur-containing compounds estimated by other methods. Most of the sulphur in wool is in cystine, and colorimetric and chromatographic assays of cystine in wool hydrolysates differ by 10 per cent. This fact caused the problem of the sulphur balance to be re-examined. Hydrolysates of wool labelled with sulphur-35 were chromatographed and found to contain several unidentified sulphur compounds, of which one became very abundant when hydrolysis was prolonged. It was identified as *bis* (β-amino-β-carboxyethyl) trisulphide, that is, the trisulphide analogue of cystine[3]. Thus some of the cystine decomposes during wool hydrolysis and from careful analyses it is concluded that wool contains sulphur only in the form of cystine, cysteine and methionine, thus bringing it into line with other proteins and removing the discrepancy in the sulphur balance. The discussion centred around the mechanism by which the trisulphide artefact might be formed.

In the second paper, Mr. L. J. H. Slack (English Abrasives Corporation, Ltd., Tottenham, London, N.17) discussed "Comparisons of Natural and Synthetic Glues in the Coated Abrasives Industry". The industry is a key one in relation to many others of much greater size, for example, metal working. There is a considerable variety of coated abrasives and the glues used include hide glue and synthetics based on alkyds, urea, melamine and phenols. The merits and demerits of hide glue were discussed, and it was concluded that there was need for improvement of the final film properties to enable the natural material to maintain its competitive position.

The director of research, Dr. D. A. Sutton, reported on "Some Recent Impressions of the American Gelatine and Glue Industry". Emphasis was laid on the speed and hygienic control which are characteristic of the production of American pigskin gelatine; these factors are reflected in the quality of the final product. The plants and processes used were briefly outlined and the other types of gelatine produced were commented on. Attention was directed to the extent to which hide and bone glue production are mechanized in the United States and this factor enables natural glue to be sold at a low price in spite of very high labour rates. There is no central research organization parallel to the British Gelatine and Glue Research Association, but many large companies have large well-equipped laboratories covering all their products and including facilities for gelatine and glue.

D. A. Sutton

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THE BRITISH LEATHER MANUFACTURERS' RESEARCH ASSOCIATION

An outstanding feature of the past year was the strengthening of contacts between the British Leather Manufacturers' Research Association and the footwear industry. The great value of these contacts lies in showing the Research Association how it can adjust its research programme to help the leather industry to meet the requirements of its main customer and they ensure that the Association's work is relevant to the present needs of the footwear industry. The demands made on leather arising from the heat and steam lasting of shoe uppers, for example, are being met and these new methods, which have great advantages, have been established and are likely to increase. A great deal of work has been done on the assessment of different leathers and finishes for these new treatments.

Investigations of skin proteins have continued at the Association's research laboratories, Milton Park, Egham, Surrey, but new emphasis is being given to seasonal variations in lambskins. The methods developed in this long-continued study of the constituents of skin have also been applied to the examination of deteriorated leathers under a research project sponsored by the U.S. Department of Agriculture. New work has been the application to collagen and certain tanned collagens of principles and techniques developed by workers in investigations of rubber-like elasticity and the cross-linking of large molecules. Determinations have been made of the number of cross-links (per unit weight) and the relative stabilities of cross-links in collagen tendons tanned with certain aldehydes. It is gratifying to record this beginning of an experimental study of a fundamental question in the theory of tanning and note that glutaraldehyde, first advocated as a tanning agent by the Department of Agriculture, Eastern Regional Laboratory, has been found very effective in producing stable cross-links. Work on curing, enzyme de-woolling and pigment finishing continues.

For many years the Research Association has given forewarnings of the problems facing the industry from effluent disposal. Recent legislation has justified these warnings to the full. Throughout Great Britain, members are steadily adopting the Association's advice to "reduce, replace and re-use". Conditions vary from tannery to tannery so that no one method is likely to be generally applicable, but amounts of lime and sulphide can sometimes be reduced without loss in quality, a proportion of the sodium sulphide normally used can be replaced by caustic soda, and the re-use of lime and chrome is practised on a wider scale. The acute problem of destroying sulphide in effluent has been tackled on a broad front and the costs of various processes for the removal of sulphide have been compared. In addition, the work of the British Leather Manufacturers' Research Association on the behaviour of tannery effluents in biological treatments is proving of immense help in negotiations with local authorities.

Income for the year 1961–62 was £88,698, an increase of £9,400 over the previous year; expenditure was £88,816.

POLARIZATION OF RADIO SOURCES AND FARADAY ROTATION EFFECTS IN THE GALAXY

By F. F. GARDNER and J. B. WHITEOAK

C.S.I.R.O. Division of Radiophysics, University Grounds, Sydney

IN two previous communications[1,2] observations of linear polarization in the radio emission from a number of extra-galactic sources were reported. The first, by us, gave details of the detection of linear polarization of the radio emission from seven out of nine sources observed at 20 cm. The result is strong support for the hypothesis that the emission originates in synchrotron radiation. A number of these sources were known to be doubles, and in several cases the E-vector of the 20-cm radiation was aligned with the direction of elongation of the source. However, it was pointed out that, owing to the possibility of Faraday rotation, the position angle of the E-vector at a single wave-length cannot be used to infer the intrinsic polarization of the source, which on the synchrotron hypothesis[3] is approximately perpendicular to the magnetic field.

The second note, by Cooper and Price[2], was directly concerned with Faraday rotation. It was found that for three selected points in the radio source Centaurus A the received polarization progressively rotated with increasing wave-length at a rate proportional to the wave-length squared. This is in agreement with Faraday rotation, for which the rotation θ in radians at a wave-length λ metres by a medium of electron density N per cm^3 and longitudinal magnetic field B_L in gauss is given by:

$$\theta = 8{\cdot}1 \times 10^5\ \lambda^2 \int N B_L \, dL$$

where L is measured in parsecs. We shall call the value of θ/λ^2 in radians/m^2 the rotation measure for the source. It measures the integrated value of the product of electron density and longitudinal field along the path from the

Table 1. (a) POLARIZATION DIRECTIONS FOR SOURCES MEASURED AT THREE OR MORE WAVE-LENGTHS BETWEEN 10 AND 30 CM

Source	Galactic Co-ords. l^{II}	b^{II}	Position angle of polarization (wave-length cm) 30	24	21	19	15	10	Intrinsic polarization angle	Rotation measure radians/m^2
3C-33	130	−49	33 ± 5		60 ± 2		81 ± 5	55 – 134	94	−12
Fornax A (a)*	240	−57	52 ± 3	64 ± 3	61 ± 3	68 ± 5	67 ± 3	56 ± 4	66	−1·9
Fornax A (b)			94 ± 3	91 ± 5	101 ± 3	105 ± 5	98 ± 5	98 ± 10	103	−1·2
Pictor A	251	−34	169 ± 3		50 ± 5		172 ± 5	117 ± 5	110	+45
Taurus	185	−06	10 ± 4		91 ± 3		120 ± 3	143 ± 2	157	−27
3C-161	215	−08	70 ± 5		26 ± 5			140 ± 3	142	+23
3C-270	282	+67	131 ± 4		118 ± 3	118 ± 10		108 ± 8	105	+3·7
3C-273	290	+64			142 ± 10		157 ± 9	156 ± 3	164	−8·2
Centaurus A (a)†	310	+20	180 ± 5	138 ± 4	175 ± 3	20 ± 10	70 ± 3	115 ± 3	147	−60
Centaurus A (b)			49 ± 3	15 ± 3	60 ± 5	98 ± 7	150 ± 3	20 ± 20	56	−68
13S6A	310	+02			172 ± 3	122 ± 15		53 ± 5	20	+59
Hercules A	23	+29	173 ± 15		60 ± 4			25 ± 4	04	+24
3C-327a	12	+38	40 ± 9		02 ± 5			165 ± 10	156	+11
3C-353	21	+19	123 ± 12	24 ± 4	171 ± 5	160 ± 4	137 ± 3	105 ± 2	80	+39
21-64	331	−41	02 ± 5		108 ± 5			45 ± 2	28	+30
23-64	314	−55	128 ± 3		67 ± 3	49 ± 8	47 ± 9	19 ± 13	11	+23

* Fornax A (a) is the larger component at 03h20·5, −37° 18′; (b) is the smaller one at 03h22·7, −37° 28′. At 50 and 75 cm both components are in the aerial beam.

† Centaurus A (a) is the central component at 13h23·2, −42° 48′; (b) is in the extended region at 13h24·0, −42° 30′.

Table 1. (b) MEASUREMENTS NOT INCLUDED IN TABLE 1 (a)

Source	λ = 75 cm P.A.	%	λ = 50 cm P.A.	%	λ = 30 cm P.A.	%	λ = 21 cm P.A.	%	Intrinsic polarization angle	Rotation measure radians/m^2
Fornax A (a)	0 ± 10	2·0 ± 1·0	25 ± 5	3·5 ± 1·0						
3C – 78					135 ± 10	8·1 ± 1·5	124 ± 7	2·6 ± 1	110	+3·7
3C – 98					82 ± 5	3·5 ± 0·5	74 ± 4	6·0 ± 0·5	65	+2·5
3C – 227					124 ± 5	9·3 ± 3	136 ± 7	5·6 ± 0·5	148	−3·7
3C – 279					0 ± 5	8·4 ± 1	153 ± 3	4·3 ± 1	126	+10

and sparse phenocrysts (plagioclase, quartz, magnetite and orthopyroxene) set in a matrix of glass and mineral dust. The dust is mainly cryptocrystalline and the pumice vesicles are coated with an unidentified birefringent material. Within the lenticles various pumiceous textures, ranging from fibrous to microvesicular, are seen. Textures characterized by globular and distorted vesicles are seen in some thin sections where fibrous pumice lenticles are cut obliquely or transversely. These textures are the same as those illustrated in photomicrographs by Steiner[5] and by Hjelmquist[10]. They can be seen in pumice of non-ignimbritic origin also.

Examination of crushed specimens in oils shows conclusively that the so-called 'globules' interpreted by Steiner as evidence of immiscibility in the Waikino ignimbrite are not a separate glass phase but merely drawn out or distorted gas vesicles within the pumice lenticles. In specimens from different parts of an outcrop the refractive index of the glass matrix may differ from 1·500 ± 0·002 to 1·503 ± 0·002, and individual lenticles may differ from others by as much as 0·002. No truly contrasting glasses corresponding to Steiner's mesostasis, base, or globules could be found, however, by us. Wherever strong Becke lines were observed, either in thin section or crushed fragments; they were between glass and rounded vesicles filled with oil or mounting media.

Similar results were found in glassy specimens from 20 different ignimbrites in a current study of the Taupo Volcanic Zone[8]. Only one multiglass ignimbrite has been recognized:

The Murupara Gorge Ignimbrite of the Meseta Central Type. An exceptional rock composed of intermingled acid and intermediate glass shards and pumice is exposed at a proposed dam site seven miles south of Murupara, New Zealand. This deposit, here called the Murupara Gorge ignimbrite, is similar to the multiglass welded tuffs of Costa Rica described by Howel Williams. It is known only at this type locality, overlying the extensive Waitaha Ignimbrite Group[9]. It is 70 ft. thick and consists of buff to grey pumice tuff, uncommonly glassy throughout. The top and base of the sheet are non-welded; the central part is lightly welded.

Pumice fragments up to 2 in. long comprise about 35 per cent of the rock. They are slightly flattened and lie sub-horizontally in the welded zone. Black and white pumice fragments are nearly equal in quantity. The white pumice appears to have resisted flattening more than the black. Their indices of refraction are 1·498 ± 0·002 and 1·538 ± 0·002 respectively.

The tuff is a compressed sinter of colourless and brown glass shards corresponding to the white and black pumice. The ratio of colourless to brown shards is about 2 : 1; their refractive indices are 1·498 ± 0·002 and 1·530 ± 0·003 respectively. Shards are mainly one colour or the other, but approximately 5 per cent are composite, consisting of both colourless and brown glass with no distinct boundary between. Many of intermediate colour and refractive index also occur.

Phenocrysts comprise 7 per cent by volume, in the following proportions: quartz 2·5 per cent, plagioclase 87 per cent, orthopyroxene 6·5 per cent, and magnetite 4 per cent.

Although a few recent papers have tended to emphasize the alleged existence of separate glass phases in ignimbrites or the possibility of immiscibility in igneous lavas, it is cautioned that the presence of more than one glass in these rocks is very rare. In the case of the Murupara Gorge ignimbrite, which appears to have been formed by simultaneous pyroclastic eruption of acid and intermediate magmas, the presence of individual multicoloured shards, and those of intermediate refractive index, refute any suggestions of immiscibility between these parent magmas.

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INHIBITION OF THE BIOLOGICAL ACTION OF THYROID HORMONES BY ACTINOMYCIN *D* AND PUROMYCIN

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THE stimulation of basal metabolic rate induced by thyroid hormones has often been attributed to an alteration of the efficiency of oxidative phosphorylation by direct interaction between the hormone and mitochondrion[1]. It has recently been shown by us that, when administered in small amounts, thyroid hormones cause an early and substantial increase in the rate of protein synthesis by both microsomes and mitochondria, without any of the direct effects on mitochondrial function described earlier[2,3]. We suggested, therefore, that both the calorigenic and growth-promoting actions of thyroid hormones (and probably other multiple biological actions, as well) are secondary manifestations of a primary action at the cellular level which influences the rate of protein synthesis. I have recently obtained further evidence which supports this suggestion.

It has been shown that actinomycin *D* inhibits protein synthesis by suppressing the formation of messenger RNA (ref. 4) whereas puromycin inhibition occurs at the stage of assembly of new protein at the ribosome[5]. Starvation suppresses protein synthesis by depriving the synthetic machinery of essential amino-acids as well as by lowering the level of RNA in the cell[6]. The effect of each of these inhibitory agents on the physiological response to the thyroid hormones, L-thyroxine and 3,5,3′-triiodo-L-thyronine, has been measured. The results indicate that stimulation of protein synthesis is an essential requirement for the full physiological response to thyroid hormone, and that direct action of hormone on mitochondrial respiration and phosphorylation is not an adequate explanation of the increased basal metabolic rate observed after administration of the hormones. The effect of actinomycin *D* in particular also suggests that thyroid hormones may act by controlling the availability of messenger RNA rather than by direct stimulation of protein synthesis.

Fig. 1 shows the effect of a small dose (26 μg/100 g body-weight) of triiodothyronine on basal metabolic rate and on growth-rate of thyroidectomized rats, either in the presence or in the absence of actinomycin *D*. In the absence of this inhibitor of protein synthesis, the hormone caused a 30–40 per cent increase in basal metabolic rate over the control values and a substantial acceleration of growth rate. Actinomycin *D* alone (8 μg/100 g) caused an immediate fall of 10–20 per cent in the basal metabolic rate and a corresponding drop in body weight. The loss in body-weight is due, at least in part, to a substantial reduction in food intake. When triiodothyronine and actinomycin were given together, the calorigenic action of the hormone was entirely abolished and there was, likewise, no hormonal effect on body-weight. The growth and basal metabolic rate depressant effects of the antibiotic, in the doses used, lasted for only 4 or 5 days and the animals also developed a tolerance to further or more toxic amounts. It was therefore not practical to study the effect of pretreatment with actinomycin *D* on the biological action of a single dose of thyroid hormones.

Results similar to those shown in Fig. 1 were obtained when thyroxine replaced triiodothyronine or by using intact rats of either the Wistar or hooded strains. The differences lay essentially in the steepness of the curves of changes in growth or basal metabolic rate as a function of time. If deoxyguanosine, which complexes strongly with actinomycin *D* (ref. 7) was administered in the molar ratio of 2,500 : 1, 3 h after the antibiotic, the inhibitory effect was diminished to the extent of 20–30 per cent. Actinomycin *D* did not affect the stimulation of basal metabolic rate caused by administration of 2,4-dinitrophenol. This suggests that it acts specifically by preventing the stimulation of protein synthesis which is the normal response to small amounts of thyroxine or triiodothyronine. So far as is known, at the extremely low doses of actinomycin *D* we have used, it can only do this by inhibiting the synthesis of messenger RNA (ref. 4). The possibility therefore arises that thyroid hormones stimulate protein synthesis by regulating somehow the level of messenger RNA at the site of protein synthesis.

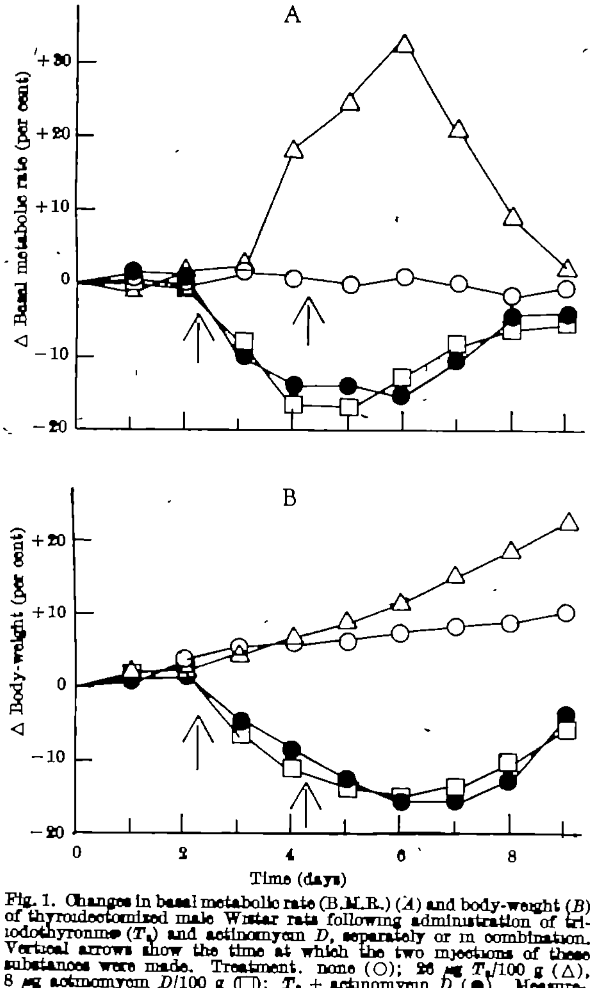

Fig. 1. Changes in basal metabolic rate (B.M.R.) (*A*) and body-weight (*B*) of thyroidectomized male Wistar rats following administration of triiodothyronine (T_3) and actinomycin *D*, separately or in combination. Vertical arrows show the time at which the two injections of these substances were made. Treatment. none (○); 26 μg T_3/100 g (△), 8 μg actinomycin *D*/100 g (□); T_3 + actinomycin *D* (●). Measurements were made individually on four animals per group. Values are expressed as change from the averages for each group on day 0, when B.M.R. = 0·54 ± 0·04 ml. oxygen/g/h and body-weight = 130 ± 4 g (compared with a B.M.R. of 0·72 ± 0·06 ml. and body-weight of 250 ± 12 g for intact controls). T_3 was injected subcutaneously, while actinomycin *D* was given intraperitoneally

Table 1. Effect of Actinomycin *D*, Puromycin and Starvation on the Triiodothyronine (T_3)-stimulated Basal Metabolic Rate (B.M.R.) and Body-weight in Thyroidectomized Rats

Treatment*	Dose of antibiotic (μg/100 g)	Change after 72 h† (per cent) B.M.R.	Body-wt.	Inhibition of T_3 effect (per cent)
None	—	± 1·5	+ 5·4	—
34 μg T_3/100 g	—	+35·5	+ 7·8	—
Actinomycin *D*	8	−14·0	−13·2	—
Actinomycin *D* + 34 μg T_3	8	−13·8	−14·1	100
Puromycin	12,000	+ 1·0	− 7·7	—
Puromycin + 34 μg T_3	12,000	+ 9·8	− 6·0	69
Starvation	—	+ 2·1	−16·8	—
Starvation + 34 μg T_3	—	+ 5·9	−19·0	84

* Injections were made on day 0 and again 48 h later; T_3 was injected subcutaneously and the antibiotics intraperitoneally.

† The values are based on the differences in B.M.R. and body-weight just before treatment was begun and 72 h after treatment. Actual initial values were: B.M.R. = 0·52 ± 0·04 ml. oxygen/g/h; body-weight = 125 ± 6 g.

As shown in Table 1, administration of puromycin or starvation both suppressed the calorigenic action of triiodothyronine to a considerable extent. The drop in body-weight during 72 h of starvation was 2–3 times that caused by the administration of either antibiotic. Unlike actinomycin *D*, neither puromycin treatment nor starvation alone lowered the basal metabolic rate below the levels observed in thyroidectomized animals. Over a thousand times more puromycin than actinomycin *D* was necessary to produce a comparable inhibition of thyroid hormonal activity. Such a dose relationship is compatible with the different mechanisms of inhibition of protein synthesis by these two antibiotics[4,5]. It is also significant that thyroid hormone-dependent stimulation of amino-acid incorporation into protein as well as the increased activities of some enzymes were abolished in starved animals[1,6].

In conclusion, a normal functioning of protein synthetic mechanism is essential for thyroid hormones to elicit their calorigenic and growth-promoting actions. Such a requirement may also apply to other hormones such as insulin, growth hormone and testosterone[9]; actinomycin *D* has also been shown to block some actions of cortisone[10]. In any event, the foregoing results corroborate our suggestion made elsewhere that the stimulation of total-body oxygen consumption by thyroid hormone is likely to result from an increased synthesis of some mitochondrial respiratory constituents[1,2]. A direct or indirect regulation of the synthesis or turnover of certain cellular constituents may also underlie the multiple biological actions of thyroid hormones, provided that there is a high degree of specificity with respect to the particular nucleic acids or proteins the turnover of which is being affected. An important question arising from the present investigation is to determine to what extent the inhibition of thyroid hormone action by actinomycin *D* reflects a primary hormonal regulation of the genetic informational mechanism or a less specific effect of a disturbed pattern of protein synthesis, regardless of the level at which the disturbance may take place. A study of isolated nuclei and other cell-free preparations, similar to those we have initiated for the physiological action of thyroid hormones at the cell level[1,2], may provide an answer.

I thank Prof. E. Boyland for a generous gift of actinomycin *D* and Mr. A. J. Bell for assistance.

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PERIODIC NATURE OF SPONTANEOUS PERIPHERAL NERVOUS SYSTEM ACTIVITY

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RHYTHMIC central nervous system activity and spontaneous autonomic nervous system activity are well documented[1,2]. The literature relating the central and the autonomic nervous systems function is also quite extensive (cf. review by Dell and Bonvallet[3]). Yet, little is known of rhythmic activity in peripheral nervous system functions (cyclic fluctuations in variables such as heart rate or skin temperature are usually specified on the order of hours rather than minutes or seconds). It was therefore predicted that relatively high-frequency rhythmic activity could be discerned in measures mediated by peripheral physiological systems.

This hypothesis was tested by means of a variance (or power) spectrum analysis of resting-levels of the psychogalvanic response, finger blood volume, heart rate and electromyograms. The power spectrum is the integrated Fourier transform of the auto-correlation function of a time series[4,5], and allows an estimate of the variance at each of a series of frequency intervals. 6 min of continuous data were scored at every second for each of the four measures on each of 40 subjects. Power spectra of each 360-sec series were then derived on a CDC 1604 computer, following the formulæ of Blackman and Tukey[6]. Sixty computational lags were used allowing the examination for periodicity at each of 60 frequency-intervals. The frequencies studied ranged from 0·5 c/s to 0·008 c/s. Their reciprocals, or periods, range from 2 sec to 120 sec. Periodicity, when present, was indicated by a hump in the spectrum when plotted as the proportion of the total variance per frequency interval (Fig. 1). The degrees of freedom appropriate to evaluating the significance of any such hump was 12, and, based on a χ^2 distribution and a Gaussian assumption, the value at the top of the hump had to exceed that of the bottom of the valley by a factor of 4, to differ at the 95 per cent level of confidence. Humps greater than valleys by a factor of 2 would differ at the 80 per cent level of confidence.

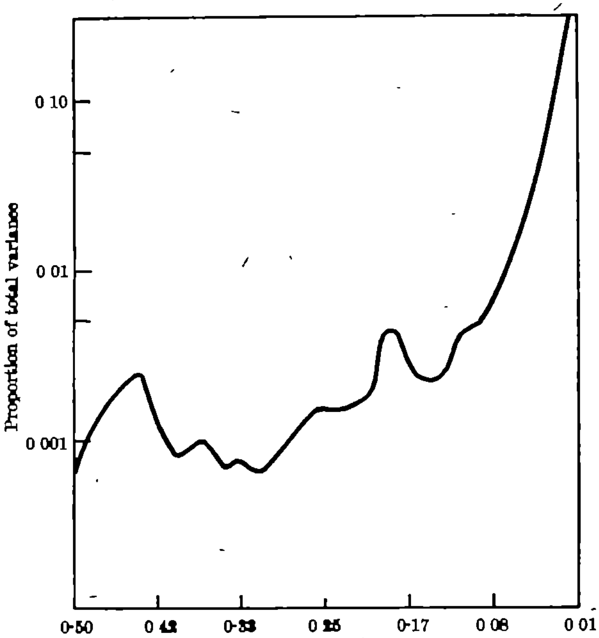

Fig. 1. Average power spectrum of finger blood volume. No. = 40

The 6 min of each variable scored were taken after a 15-min period in which the subject had been asked to relax with his eyes closed, while alone in a sound, temperature and humidity regulated test room. Heart rate was scored to the nearest beat per minute; finger blood volume, taken from the forefinger of the left hand, to the nearest 0·1 c.c.; psychogalvanic response, taken at the palm of the left hand, was accurate to 1 per cent of actual ohms resistance, and the frontalis electromyogram to the nearest 5 mV. Subjects were taken from a larger group and were composed of 20 psychiatric in-patients and 20 non-patient volunteers, balanced for sex, in an age-range of from 15 to 34 years. Subjects under heavy drug doses, or exhibiting any organic involvement, were excluded from the analyses.

Most spectra showed an overall upward trend of variance as the length of the period increased—indicating an adaptation effect over the 6-min period from which the spectra were derived. Cyclical changes over and above the long-term changes were of primary interest, however, so each spectrum was examined separately for the presence of peaks more than twice the variance of the base-line. One subject of the 40 did not exhibit any periodicity on any of the four physiological measures. Thirty-eight of the 40 subjects exhibited periodicity on heart rate; 33 of these in a 2–7 sec range were cyclical fluctuations which correspond with respiration. It is likely that this observed arrhythmia is a reflex regulation of heart rate initiated at least in part by pulmonary receptors in the chest wall stimulated by expansion[7]. As such they would not reflect entirely spontaneous, centrally mediated rhythms, so such periodicities were not included in the following determination. (The ability of the power spectrum programme to pick up the well-known sinus arrhythmia effect is, however, an indicator of its efficacy.) Other than heart rate periodicity in the range excluded, four subjects were not periodic in any of the other three variables examined, bringing to a total of five the subjects who were not periodic in any variable. Therefore, 87·5 per cent of all subjects tested were periodic in one or another of the physiological variables recorded. The periodicity of 20 of these 35 subjects was of a magnitude four times the base-line.

The five subjects whose spectra did not peak on any of the variables sampled were possibly periodic at periods longer than those the power spectrum could discern. This notion is supported by the fact that the greatest portion of the variance was often at 120-sec periods, the longest the programme could examine, and the variance at this point was greater than that for the infinite period.

Eighteen of the forty subjects were periodic on the electromyogram spectra; the period at which humps occurred ranged from 2 sec to 60 sec with an approximately even distribution across this range.

Twenty-three of the total number were periodic on finger blood volume; the range was again from 2 to 60 sec periods. Most of the periodicity was at high frequencies, however, only three subjects having periods greater than 10 sec (10, 13 and 40 sec).

Eight subjects were periodic on psychogalvanic response—the longest period was one of 15 sec.

As indicated previously, there were five subjects who were periodic on heart rate at a frequency which could not be accounted for by respiration rate (for example, periods of 45 sec or 2 sec).

The differences between individuals, with respect to the variables on which they were periodic, is consistent with the growing body of literature dealing with individual 'stereotypy'—the tendency for a given individual to respond to a number of stimulus situations with a characteristic autonomic variable[8].

The presence or absence of periodicity did not bear any consistent relationship to other physiological scores such as mean level or variance. The presence of periodicity in electromyograms was associated with higher mean electromyogram-levels. However, subjects exhibiting finger blood volume periodicity had generally higher mean volumes than did subjects non-periodic on the measures—contrary to what might be expected if periodic function was concomitant with arousal. Mean heart rate and psychogalvanic response differences between subjects periodic and non-periodic on each of these measures were not significant. Analyses by psychiatric and control groups and various psychological measures in relation to frequency of periodicity will be reported later.

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EVIDENCE FOR MULTIPLE CALCIUM POOLS IN THE BLOOD OF DOGS

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IN the course of investigating thyroparathyroidectomized dogs after a single injection of calcium-45, a considerable difference in the specific activity of blood and urine was observed. While most of the results obtained will be reported elsewhere, the specific activity ratio for blood and urine are discussed in this report. Likins and Craven previously reported an apparent difference in the renal clearance rates for calcium-45 and calcium-40. They studied fasting and fed rats[1]. Dow and Stanbury reported examination of human beings, utilizing a single injection of calcium-45, during which they noted specific activity ratios of blood to urine which were approximately 1·5 (ref. 2). They compared the specific activity of serum collected at time *t* with that of urine collected over an interval from time *t* to some later time t_1. The results presented here concern observations made on a single thyroparathyroidectomized dog and suggest that the various forms of calcium present in blood equilibrate with calcium-45 at different rates. Similar, but less-significant, differences were observed in normal dogs.

A thyroparathyroidectomized male dog, weighing 35 lb. and maintained on 0·1 mg of thyroxine per day, was used. The dog was injected intravenously with 17·5 μc. calcium-45 (specific activity of 478 μc./mg calcium-40). 36 h later oral intake was limited to water. The dog was anaesthetized with 'Nembutal' 48 h after administration of calcium-45. A catheter was inserted into the bladder for urine collection and an arterial needle was placed in the femoral artery. An infusion of an isosmotic solution containing creatinine and mannitol was started in order to produce an osmotic diuresis and to allow for calculation of the glomerular filtration rate. About 30 min after the start of the infusion, a 60-min urine collection was initiated. Arterial blood was collected over heparin at the mid-point of this collection. At the end of the first urine collection, calcium chloride labelled with calcium-45 was added to the infusion (0·13 μc. calcium-45 and 15 mg calcium-40/min). Three subsequent hourly urine collections with appropriate arterial blood samples were obtained.

Chemical calcium analyses were performed on urine, plasma, plasma ultrafiltrates and on trichloroacetic acid (TCA) filtrates of plasma by a modification of the method of Collip and Clark[3]. Plasma ultrafiltration was performed by Toribara's method[4]. The error for these determinations was 2 per cent (at least 3 aliquots of each specimen were analysed). Creatinine was determined in plasma and urine by the method of Bonsnes and Taussky[5]; clearance studies were conducted as described by Steiner *et al.*[6]. Calcium-45 counting was performed using a gas-flow counter as described by Goldsmith (unpublished results). Plasma calcium values used to calculate specific activity were obtained from analysis of the TCA filtrate since calcium-45 counting was performed on TCA filtrates of plasma. Phosphorus determinations were performed according to Fiske and Subbarow[7] to establish a basis for calculation of the tubular re-absorptive capacity for phosphorus.

These investigations were conducted on one of a series of animals similarly operated. These animals separate into two groups soon after surgery, one demonstrating normal and one abnormal blood calcium and phosphorus concentrations[8]. This particular animal was one with normal blood mineral values from the second postoperative week. Hypoparathyroidism was probable, despite normal chemistries, since subsequent re-exploration of the neck area did not disclose (grossly or by serial sections of paratracheal tissue removed) parathyroid tissue and since measurement of phosphorus clearance and re-absorption gave values expected in hypoparathyroidism. As seen in Table 1, not only was the initial value for the tubular reabsorption of phosphorus high, it failed to increase as the serum calcium concentration rose with the infusion of calcium 40. This has been considered evidence of hypoparathyroidism in man[9]. The ratio of the phosphorus clearance to the creatinine clearance increased during the period of calcium infusion (in 2 of 3 collections). This, too, is suggestive of the absence of parathyroid hormone secretion[9]. Table 2 contains the chemical calcium concentrations of each specimen. The plasma ultrafilterable calcium concentration increased with the increase in total calcium, but the partitioning remained relatively constant. Table 3 contains the radioactivity clearance data. The glomerular filtration rate decreased as the plasma calcium concentration increased, as reported elsewhere[10]. Before labelled calcium was added to the infusion the bladder urine had a specific activity only 55 per cent that of the simultaneously obtained plasma ultrafiltrate. The same general relationship was maintained throughout the infusion but the urine specific activity increased to 71 per cent of that of the simultaneously obtained plasma ultrafiltrate after 3 h. The ratio of total plasma/plasma ultrafiltrate specific activity was less than one at the outset of the infusion of labelled calcium but approached one during infusion. The ultrafiltrate specific activity (considered synonymous with the glomerular filtrate specific activity) increased as expected during the constant infusion of labelled ionic calcium.

Table 1. PHOSPHORUS CLEARANCE DATA

Time (min)	0–60	60–120	120–180	180–240
Plasma creatinine*	0·308	0·355	0·405	0·450
Urine creatinine†	770	753	718	714
Plasma inorg. phos.*	0·040	0·034	0 040	0·046
Urine inorg. phos.†	8	7	3	9
C_P/C_{Cr}	0 076	0·101	0·048	0·121
TRP (per cent)	93	90	95	88

* mg per ml.
† mg. per 60 min.

Table 2. BLOOD CALCIUM AND PHOSPHORUS VALUES DURING INFUSION

Time (min)	Plasma Ca (mg per cent)	
	Total	Ultrafiltrate
30	8·7	5·6
90	9·6	6·4
150	11·3	7·4
210	13·4	9·2

Table 3. CLEARANCE VALUES DURING INFUSION

Time (min)	GFR c.c./min.	Specific activity of calcium (n.c./m/mg)			
		Total plasma	Plasma ultra-filtrate*	Tubular resorbate†	Bladder urine
0–60	41·7	1,180	1,312	1,332	722
60–120	35 4	2,049	2,625	2,634	2,202
120–180	29·5	4,090	4,331	4,388	3,218
180–240	26·4	5,125	5,555	5,658	3,968

* Plasma ultrafiltrate is assumed identical to glomerular filtrate.
† Derived values.
GFR, Glomerular filtration rate.
n.c./m/mg, net counts per minute per mg calcium-40.

This investigation has shown that 48 h after the injection of calcium-45 the specific activities of total blood calcium (protein bound plus complexed plus ionic forms) and of its ultrafilterable fraction (complexed plus ionic forms) were dissimilar. Either the filtering membrane handled the two isotopes differently or the various species of blood calcium were labelled heterogeneously. The latter possibly is by far the more likely. Plasma ultrafiltrate is probably identical with glomerular filtrate[11] and its specific activity should be the same as that of the bladder urine if all blood calcium fractions are labelled homogeneously. The investigation has also demonstrated that, at the same 48-h interval, the specific activity of the filtered calcium (complexed plus ionic forms) was greater than that of the excreted calcium (complexed plus ionic forms). The most reasonable explanation of this is that, under the conditions of this study, complexed and ionic forms of calcium have different specific activities and are handled differently by the renal tubule. The calcium-45 appears to label ionic and protein-bound calcium preferentially and complexed calcium only secondarily. Therefore, if the renal tubule selectively re-absorbs ionic calcium, the present results can be explained. In the fifth column of Table 3 the specific activity values are calculated for the calcium re-absorbed. At all times, re-absorbed calcium had a specific activity about 2 per cent greater than did filtered calcium. Since most filtered calcium is reabsorbed, the difference between the specific activities of these two fractions need only be slight to account for a large difference between specific activities of filtered and excreted calcium. This entire explanation is in agreement with present-day physiological concepts concerning renal handling of calcium[10,12,13] and with the data referred to in the introduction[1,2].

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A FLUX-CARRIER HYPOTHESIS OF DRUG ACTION

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SEVERAL general theories of drug action have been proposed, most of them based on the concept of drug receptors. It has been assumed that when a tissue is exposed to a drug the receptors in the tissue react with the drug to form drug-receptor complexes. The ensuing reaction produces a physiological or biochemical response, often by some unspecified mechanism. The original receptor theory of Clark[1] was modified by Ariens[2] and afterwards by Stephenson[3] to account for the existence of partial agonists. Those modifications apply only to the steady state and assume that the response is related to the number of drug-receptor complexes. More recently, Paton[4] has proposed a 'rate' theory of drug action according to which the response is related not to the number of drug-receptor complexes but to their rate of formation.

The actions of drugs on the neuromuscular junction and on ganglia appear to be too complex to be easily explained by a 'steady state' receptor theory. Compounds which act by blocking transmission across these synapses can be divided into two main classes. The first class is composed of drugs which produce an initial response by depolarizing the membrane of the effector cell. If the drug and tissue remain in contact the initial depolarization may disappear, the post-synaptic membrane repolarizing. However, transmission across the synapse may still be reduced or blocked, this residual block being of the competitive type. Such drugs are said to have a 'dual' type of action[5,6]. Members of the second class of drugs do not produce an initial depolarization and so there is no response by the effector cell. The block which they produce is of the 'competitive' type.

It appeared to me that the competitive type of block could be adequately explained by the receptor theory of Clark[1]. The initial depolarization and subsequent repolarization of the post-synaptic membrane, on the other hand, could be explained by the 'potential' theory of Straub[7] (see also Clark[1]). A complete explanation of the mechanism of action of agents which block synaptic transmission at ganglia and at the neuromuscular junction should therefore contain some of the features of both theories. The flux-carrier hypothesis is an attempt to achieve such a combination.

It is first assumed that the net influx of a drug across a cell membrane may cause the membrane to depolarize. A second assumption is that the flux of drug may be facilitated by mobile carriers or by highly specific fixed sites in the membrane (for example, Wilbrandt and Rosenberg[8], Bowyer and Widdas[9], and Fatt[17]). The possible consequences of these assumptions will now be explored.

There are two main possibilities depending on whether or not the drug, or its carrier complex, can penetrate through the post-synaptic membrane. These possibilities are indicated in Fig. 1*a* and 1*b* respectively. When the drug, *A*, can penetrate the membrane it may act as an agonist, according to the first assumption of the hypothesis. Since it is not known whether carriers are mobile or fixed the results to be expected will be treated qualitatively. The symbol *S* will be used for the site or carrier with which the drug A reacts, and SA represents the drug-carrier complex. The reaction may be written as:

$$S + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} SA \qquad (1)$$

The sites or carriers will have a certain affinity for the drug, characterized by the affinity constant:

$$K_A = \frac{k_1}{k_2} = \frac{(SA)}{(S)(A)} \qquad (2)$$

The parentheses indicate concentrations of the enclosed molecular species. However, on such a submicroscopic scale concentrations probably have little meaning, and should perhaps be regarded as representing probabilities. The constant K_A might also be considered to represent the distribution coefficient of the drug between the aqueous phases and the membrane surfaces. The net influx, J, of the drug from α to β is given by an equation of the form:

$$J = \frac{D_A}{\delta}\left\{(SA)_\alpha - (SA)_\beta\right\} \qquad (3)$$

if A diffuses in some uncharged form. D_A is the diffusion coefficient of the molecular form in which A penetrates the membrane, and δ is the membrane thickness. The subscripts α and β indicate the positions at which the various concentrations apply. Provided that the diffusion process within the membrane is not too rapid, the equilibrium distribution of the drug between the aqueous phases and the membrane surfaces will be approximately maintained. Equation 2 may then be substituted into equation 3. The net flux through the membrane will then be a function of D_A, K_A, $(A)_\alpha$ and $(A)_\beta$, given by:

$$J = \frac{D_A K_A S_T}{\delta}\left\{\frac{(A)_\alpha}{1 + K_A(A)_\alpha} - \frac{(A)_\beta}{1 + K_A(A)_\beta}\right\} \qquad (4)$$

where:

$$S_T = (S)_\alpha + (SA)_\alpha = (S)_\beta + (SA)_\beta \qquad (5)$$

The initial flux, when $(A)_\beta$ is zero, is:

$$J = \frac{D_A(SA)_\alpha}{\delta} = \frac{D_A S_T}{\delta}\left\{\frac{K_A(A)_\alpha}{1 + K_A(A)_\alpha}\right\} \qquad (6)$$

The maximum initial flux of the drug, obtainable at very high values of $(A)_\alpha$, is then:

$$J_{\max} = \frac{D_A S_T}{\delta} \qquad (7)$$

Since the response is assumed to be related to the flux, it follows that each member of a group of drugs, all of which utilize the same carrier, may produce a different maximal response depending on its D_A value. The diffusion coefficient D_A of a drug may therefore be closely related to its 'intrinsic activity', as defined by Ariens[2].

The initial flux, after a short period of time required for the attainment of a steady state within the membrane, is given by equation (6). As the drug accumulates within the cell the net influx decreases (see equation (5)), until, according to the oversimplified model considered here, it becomes zero when $(A)_\beta$ is equal to $(A)_\alpha$. However, in practice the intracellular and extracellular concentrations of drug may differ even when a new steady state has been attained, because of active processes occurring within the cell.

The rapid initial influx of drug is postulated to cause depolarization of the post-synaptic membrane and thereby produce a response. As the net influx of drug decreases with time the cell will ultimately be able to repolarize the membrane, even though the drug is still present in the intracellular and extracellular fluids. The normal processes involved in the maintenance of the membrane potential of the cell are considered to be in opposition to the effects of the net influx of drug, the influx being a function of time. The drug can be washed out of the extracellular fluid more rapidly than it can be removed from the interior of the cell. If the extracellular fluid is freed of drug in this way and a second dose of drug is then administered, equal to the first, the second response may be less than that obtained initially. This is because the maximum influx during the second dose is less than that during the first, due to accumulation of drug within the cell. The reduced influx results in a reduced response to the second dose. This may be the explanation of tachyphylaxis. Similar ideas have already been proposed by Taylor[10,11]. Previously the idea that entry of depolarizers into cells might be associated with their depolarizing properties was suggested by Burns and Paton[12]. This idea has now been abandoned by Paton[13].

It should also be mentioned that if the drug penetrates the membrane as a cation then the membrane potential may initially be a more powerful driving force than the trans-membrane concentration gradient. If the flux arising from the concentration gradient within the membrane is small compared with that due to the potential gradient, then the initial influx of drug can be shown to be approximately:

$$J = \left[-\frac{Z_A F\,\Delta\varphi}{RT\delta}\right] D_A(SA)_\alpha \qquad (8)$$

$$= \left[\frac{-Z_A F\,\Delta\varphi}{RT\delta}\right] D_A S_T\left[\frac{K_A(A)_\alpha}{1 + K_A(A)_\alpha}\right] \qquad (9)$$

$[-\Delta\varphi]$ is the membrane potential, F the Faraday and Z_A the charge on the species diffusing within the membrane, R is the gas constant and T the absolute temperature. Equation (9) is similar in form to equation (6). In both cases it is assumed that reactions at the membrane surfaces are not rate controlling.

When the drug is unable to penetrate the post-synaptic membrane the situation corresponds to that in Fig. 1b. Since there is no influx of drug there is no depolarization of the membrane. However, the drug I is able to combine with carrier sites at the surface of the membrane. When agonist A and inhibitor I are present together they will compete for carrier. The concentration of carrier-agonist complexes at position α will then be reduced to:

$$(SA)_\alpha = \frac{K_A(A)_\alpha . S_T}{1 + K_A(A)_\alpha + K_I(I)_\alpha} \qquad (10)$$

where K_I is the equilibrium constant for the formation of carrier-inhibitor complex and:

$$S_T = (S)_\alpha + (SA)_\alpha + (SI)_\alpha$$

Substitution of equation (10) into equations (6) and (8) gives the initial flux of agonist in the presence of inhibitor, under concentration or potential gradients. These reduced fluxes are respectively:

$$J = \frac{D_A S_T}{\delta}\left[\frac{K_A(A)_\alpha}{1 + K_A(A)_\alpha + K_I(I)_\alpha}\right] \qquad (11)$$

and:

$$J = \left[\frac{Z_A F}{RT}\ \frac{\Delta\varphi}{\delta}\right] D_A S_T\left[\frac{K_A(A)_\alpha}{1 + K_A(A)_\alpha + K_I(I)_\alpha}\right] \qquad (12)$$

The reduced fluxes would be expected to produce smaller responses. It will be seen that equations (6) and (9), and equations (11) and (12), are closely related to equations (3) and (7) in the paper by Ariens[2].

On the other hand, suppose that an agonist A_1 is added to the extracellular fluid and left in contact with the tissue until the net flux across the membrane has fallen to such a value that the membrane potential has been re-established. If a second agonist A_2 is now added this drug too may penetrate the membrane and cause depolarization. However, in order to do so it must compete with the first agonist A_1 for carrier molecules or fixed sites. The influx of A_2 will therefore be less in the presence of A_1 than it would be in its absence. Thus the agonist A_1 has residual competitive properties even when its depolarizing effect has disappeared.

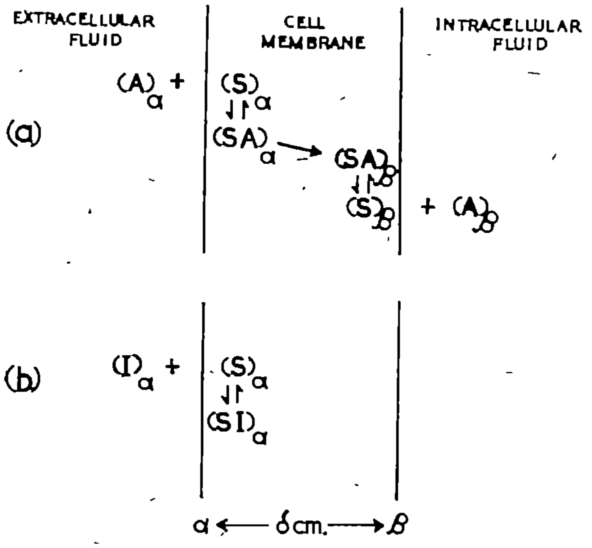

Fig. 1

If A_1 is considered to represent an artificial depolarizing agent, A_2 to represent acetylcholine, either injected or released by stimulation of pre-synaptic nerves, and I to represent 'competitive' blocking agents then consideration of the previous examples will show that the flux-carrier hypothesis is capable of explaining the existence of tachyphylaxis, partial agonists, 'competitive' and 'dual' block at synapses, as well as drug specificity.

The penetration of the post-synaptic membrane by depolarizers might explain the function of the cholinesterase which is present within the effector cell[14] since destruction of acetylcholine penetrating the membrane will reduce the tendency for tachyphylaxis to develop on repeated stimulation of the pre-synaptic nerve.

The suggestion that depolarizers, including acetylcholine, may depolarize the post-synaptic membrane by penetrating it, is contrary to the commonly held belief that acetylcholine acts on the membrane surface. However, this is not the first time that such a possibility has been considered[10,11,12]. The poor intestinal absorption of quaternary nitrogen bases tends to weigh heavily against a ready acceptance of such an idea. However, it should be remembered that synapses are highly specialized structures, and that many polar or charged molecules do in fact penetrate into cells. There is also a limited amount of experimental evidence suggesting that some quaternary nitrogen bases may penetrate cell membranes. Waser[15] has investigated the distribution of curarine, decamethonium and muscarone (labelled with carbon-14) in mouse diaphragm. Whereas with the competitive blocking agent curarine there was a clear-cut localization of the drug on the neuromuscular junction, the depolarizing agents produced more diffuse patterns. Waser states that the latter compounds may be penetrating the cells. A second paper of interest in this connexion is that of Creese, Taylor and Tilton[16], in which they showed that a 'dual'-type quaternary blocking agent, labelled with iodine-131, was concentrated in various tissues. This concentrative uptake of labelled drug was reduced by curarine, a result readily understandable on the basis of the flux-carrier hypothesis.

Several other problems have been deliberately avoided for the present. For example, the depolarization produced by the influx of drug may either be a graded or an all-or-none effect. This also applies to the relation between depolarization and response. There is also the problem of how the influx of a drug could depolarize the membrane. One interesting possibility, suggested by Fatt[17], is that a net influx of base may constitute a net influx of positive charge sufficient to cause depolarization of a small region of the post-synaptic membrane. The local depolarization produced by this flux, which must presumably exceed some critical value, might then act as a trigger to depolarize the membrane of the effector cell.

Although the flux-carrier hypothesis has been designed primarily to explain the actions of drugs on ganglia and on the neuromuscular junction, it may be of wider application. The actions of agents which block synaptic transmission are sufficiently well explained by the hypothesis for it to merit further examination.

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GLOMERULONEPHRITIS INDUCED IN MONKEYS BY INJECTIONS OF HETEROLOGOUS GLOMERULAR BASEMENT MEMBRANE AND FREUND'S ADJUVANT

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I HAVE previously demonstrated that sheep injected every two weeks with heterologous glomerular basement membrane and Freund's adjuvant uniformly develop fatal, fulminating glomerulonephritis within 27–90 days after the first injection[1]. This new, previously unknown nephritis in sheep presumably occurred by an auto-immune mechanism.

I was interested in applying this experimental technique to other species to test the applicability of this method and to utilize any advantages that study of other species may possess. I was particularly interested in testing this experimental model in monkeys because of their close phylogenetic relationship to man.

This article presents evidence for the successful production of a chronic glomerulonephritis in monkeys by injections of heterologous glomerular basement membrane and Freund's adjuvant.

Glomerular basement membrane (GBM) was prepared from the isolated glomeruli of normal human, rabbit, rat and dog kidneys by a method previously described by me[2].

Table 1. Course of Nephritis induced in Monkeys by Injections of Heterologous Glomerular Basement Membrane (GBM) and Freund's Adjuvant

No. of monkey	Antigen	No. of injections*	Total amount of basement membrane (mg)	Interval during which proteinuria started (days)	Day of killing	Blood urea nitrogen (BUN) at death (mg%)	Renal histology
936	Rat GBM	24	1,200	78–87 (trace)	485	20	Mild diffuse proliferative glomerulonephritis
940	Rabbit GBM	24	1,200	21–32	485	17	Mild diffuse proliferative glomerulonephritis
937	Human GBM	5	250	22–36	102	99 (19 on day 61)	Chronic exudative and proliferative glomerulonephritis
956	Human GBM	3	150	21–32	45 (moribund)	455	ditto
938	Dog GBM	18	900	21–32	313	116 (48 on day 44)	ditto
5	Freund's adjuvant only	20	0	none	330	Normal	Normal limits
994	ditto	16	0	none	240	Normal	Normal limits

* The monkeys were injected with 2 c.c. of emulsion containing 50 mg GBM (wet weight) by multiple portals every 2 weeks. Injections stopped on day 30 because of severe ulcerations at injection sites and were resumed 56 days later.

The GBM was homogenized in isotonic saline with a sonic oscillator (Raytheon sonic oscillator, model *S*102*A*, 50 W, 9 kc, Raytheon Co., Waltham, Massachusetts) for 3–5 mm and made up to a concentration of 38–50 mg (wet weight) per ml. Merthiolate was added to give a final concentration of 1 : 10,000. The homogenized suspension was slowly added to Freund's complete adjuvant (85 ml. 'Bayol *F*', 15 ml. 'Arlacel *A*' and 200 mg *Mycobacterium butyricum* (Difco) to make a 1 : 1 water-in-oil emulsion. The final concentration of GBM in the emulsion varied from 19 to 25 mg/ml.

Seven rhesus (*Macaca mulatta*) monkeys were obtained from a local monkey colony. The monkeys were free of overt disease and had normal urine and blood urea nitrogen. There were 1 male and 6 female monkeys and they ranged in weight from 5 to 10 lb. Each monkey was housed in a separate metabolism cage and fed 'Purina' monkey chow (Purina-Ralston Co., St. Louis, Missouri).

Five monkeys were injected every 2 weeks with 2 ml. of emulsion containing 38–50 mg of GBM. Each monkey received GBM from a single species. The injections were given by intramuscular, subcutaneous and intradermal routes at each administration. For example, 2 ml. of emulsion was divided as follows: 1 ml. intramuscularly, 0·8 ml. subcutaneously in 2 locations, and 0·1 ml. intradermally. Two monkeys were injected with Freund's adjuvant alone as controls. Injections in the 5 monkeys given heterologous GBM were stopped after the 30th day for an interval of 56 days because of ulceration at the injection sites. Periodic urinalyses and blood urea nitrogen determinations were performed on all the monkeys during the course of immunization. The urine protein was determined by the heat and acetic acid method. Blood urea nitrogen, serum albumin, globulin and cholesterol were determined prior to injection and at death in most of the monkeys. Several serial wedge biopsies were taken by surgical exposure of the kidneys on all the monkeys.

The monkeys were killed when judged to have severe nephritis or at termination of the experiment.

At death complete autopsies were performed, bladder urine analysed and the kidneys examined grossly and microscopically. The tissues were fixed in ice cold 10 per cent formalin and stained with hæmatoxylin and eosin, periodic acid–Schiff reagent with Alcian blue and Masson's trichrome stain.

All monkeys injected with heterologous GBM and Freund's adjuvant developed some degree of renal damage as demonstrated by two or more of the following three criteria: proteinuria, azotæmia and glomerular lesions in the kidney. However, the clinical course and histopathology of the nephritis varied considerably in the different monkeys. Because of the small number of monkeys used, it was not possible to ascertain the experimental variables which were responsible for the variations in disease produced. Two monkeys given 16 injections of Freund's adjuvant alone did not develop any evidence of disease at 240 and 330 days. The experimental results are summarized in Table 1.

Proteinuria. The onset of proteinuria is the end of the latent period or incubation period. Proteinuria began between 21–36 days after the first injection for 4 monkeys. This proteinuria was persistent during the entire period of observation and varied between one to four plus. Two monkeys, No. 994 and monkey No. 5, were injected with Freund's adjuvant alone as controls and did not develop proteinuria or nitrogen retention during the entire experimental period of 240 and 330 days for each monkey.

Course of the disease. The 5 treated monkeys may be conveniently divided into 2 groups clinically on the basis of their blood urea nitrogen. Two monkeys, No. 936 and No. 940, had a normal BUN throughout the period of observation and when killed 485 days after the first injection. They had persistent but scant proteinuria during this entire period. They did not appear sick.

On the other hand, the other 3 monkeys all developed azotæmia. They did so at different rates of speed. Monkey No. 956 had a BUN of 455 mg per cent on day 45. This monkey appeared feeble, subdued and anorexic for several days and was killed when in a moribund state. The disease in this monkey was a fatal, fulminating glomerulonephritis. Monkey No. 937 developed azotæmia between 61 and 102 days after the first injection. The BUN was 99 mg per cent at death. This monkey had a more slowly progressing disease. Monkey No. 938 had a BUN of 48 mg per cent on day 44, 113 mg per cent on day 198 and 116 mg per cent when killed on day 313. The course of disease in this animal was relatively stabilized and chronic. He looked thin and listless for 12 weeks before death and developed œdema of hands and feet several days prior to being killed. The total serum protein was 4·8 gm per cent and the cholesterol was 115 mg per cent at time of death. He had moderate serous drainage from ulcerated injection sites for several months. The œdema was felt to be due to hypoproteinæmia secondary to protein loss from the ulcers and in the urine. None of the other monkeys was œdematous in life or at autopsy.

Histopathology. The renal histological changes could be nicely correlated for the 2 clinical groups.

The two monkeys without azotæmia had a homogeneous histological picture. The renal architecture was relatively well preserved. There were casts present in the lumen of some tubules, but the tubule cells were normal. The pathological changes appeared to be confined to the glomeruli. The glomeruli were enlarged and lobulation was increased. Lobular stalk thickening was present and due to hypercellularity probably arising from endothelial (or mesangial cell) proliferation. The endothelial nuclei were small, dark and mature. At least one lobule of almost every glomerulus seemed involved, but there was considerable variation within a glomerulus. In monkey No. 940 there seemed to be increased eosinophilic material in some lobular stalks which might represent early hyalinization. This monkey also had occasional adhesions and focal thickening of the glomerular basement membrane. These 2 monkeys had a mild diffuse proliferative glomerulonephritis.

On the other hand, the 3 monkeys with azotæmia presented a different homogeneous group. In all 3 monkeys the cortical architecture was destroyed. No glomerulus appeared to be completely normal. Some glomeruli were severely distorted due to advanced disease. Lobular stalk thickening was common due to endothelial cell proliferation and hyalinization. The histological variations within this group correlated well with the clinical course. Monkey No. 956 had severe glomerular damage. The glomeruli were partially hyalinized and contained adhesions and occasional fresh cellular crescents. Some glomerular tufts contained areas of necrosis, infiltrates of polymorphonuclear cells and seemed to be occasionally lined with large epithelial cells of varying sizes and shape and which sometimes were found free in Bowman's space. Occasionally a multi-nucleated cell was found fused with a glomerular tuft or free in Bowman's space. Polymorphonuclear and red cells and casts were frequently present in tubules. Marked dilation but little atrophy of the tubules was seen. There was some interstitial fibrosis and interstitial infiltrates of mononuclear cells. Like the clinical course, the histopathological picture was that of a rapidly fulminating injury with signs of acute and chronic damage.

The second and third monkey had much less evidence of acute damage. The glomeruli in monkey No. 938 were severely diseased. They showed endothelial proliferation and marked hyalinization (or scarring) of the glomeruli. There were adhesions present in many glomeruli and occasional old crescents. The histological appearance was compatible with a long-standing process. There was moderate interstitial fibrosis with scant mononuclear cell infiltration. These interstitial cells had small dark nuclei. There was marked dilation and atrophy of tubules with cast formation. The microscopic characteristics of the kidney of monkey No. 937 were intermediate between the last two. Many glomeruli were hypercellular. Lobular stalks were thickened by endothelial cell proliferation and hyalinization. Some glomeruli contained eosinophilic-staining material which sometimes seemed to be in glomerular tufts and sometimes in Bowman's space. Large epithelial cells lined the glomerular tufts or were free in Bowman's space. An occasional multinucleated cell was seen in Bowman's space. A frequent distinctive lesion was a peculiar fusion of loosely proliferating spindle-shaped cells between part of a glomerulus and the pericapsular connective tissue. This loose proliferation of cells seemed to obliterate Bowman's capsule at the place of fusion. Some tubules contained hyaline droplets and some tubular lumens contained red cells. There was less interstitial fibrosis than in monkey No. 938 and more interstitial cell infiltration. Sometimes these cells with small dark nuclei seemed to be invading tubules. These 3 monkeys with azotæmia may be said to have severe chronic mixed exudative and proliferative glomerulonephritis.

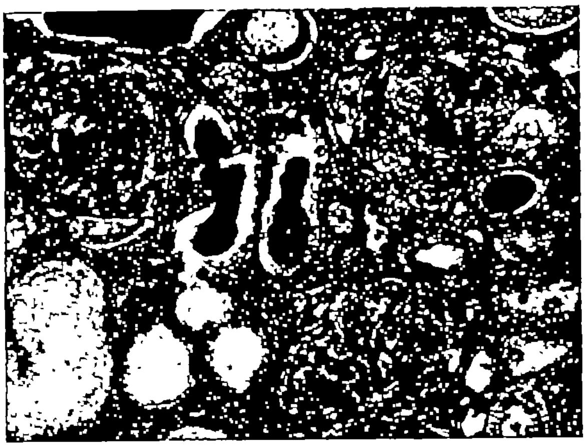

Fig. 1. Kidney section taken from monkey No. 956, 45 days after the first of 5 injections of a total of 250 mg of human GBM. The BUN was 455 mg per cent. The glomeruli are severely damaged. They are partially hyalinized and hypercellular. Extensive fibro-epithelial proliferation obliterates Bowman's space. There is wide variation in size and shape of the epithelial cells. Hyaline droplet degeneration is present in proximal tubules. Most tubules are dilated and contain casts. There is some interstitial fibrosis and cellular infiltration. (Periodic acid–Schiff × 170)

Focal areas of interstitial fibrosis, focal areas of inflammatory cells and pericapsular fibrosis were occasionally found in normal monkey kidneys and the control monkey kidneys given Freund's adjuvant alone. No importance therefore was attached to these occasional histological findings. The glomeruli of the monkeys given Freund's adjuvant alone appeared normal.

Skin tests for hypersensitivity. The monkeys were tested for delayed hypersensitivity to the antigens used for immunization. 0·1 ml. homogenized GBM (concentration 10 mg/ml.) was injected intradermally on the abdomen of each monkey. The reactions consisted of a firm swelling which seemed to start about 3–6 h after injection and reached a maximum at 24–48 h. Sometimes a red-brown area could be seen in the centre of the swelling and occasional oozing from this coloured area was observed. Each of the 5 monkeys when injected with the GBM used for immunization of that monkey showed a delayed skin reaction as judged by gross appearance. The two control monkeys did not have a similar swelling at site of injection with any of the GBM antigens.

I have previously observed that sheep injected every 2 weeks with heterologous GBM and Freund's adjuvant regularly and uniformly develop a fatal, fulminating glomerulonephritis within 90 days after the first injection[1]. On the contrary, even with the small number of monkeys used, there is a wide variation in the course of the disease. Two monkeys did not even develop azotæmia 485 days after the first injection. Histologically these 2 monkeys had a mild diffuse proliferative glomerulonephritis that seems to have stabilized as judged by serial biopsies.

Even the 3 monkeys which became azotæmic had somewhat different clinical and histological courses although they could be readily grouped together as distinctive from the non-azotæmic pair of monkeys. Monkey No. 956 had a fatal fulminating course clinically. This monkey was moribund on day 45. Its kidney revealed severe acute and chronic glomerular damage. The changes in the tubules and interstitial tissue were secondary to the glomerular changes and had not had time to develop far advanced chronic stigmata. Monkey No. 937 had more advanced interstitial fibrosis and tubular changes with less evidence of acute changes. Monkey No. 938 had relatively stabilized elevated azotæmia for about 9 months. Its kidney revealed the most advanced interstitial fibrosis and long-standing tubular changes. The glomeruli appeared to have progressively hyalinized. These 3 monkeys may be said to have severe chronic mixed exudative and proliferative glomerulonephritis. An unusual feature of this monkey nephritis was the occasional presence of multi-nucleated giant cells in Bowman's space.

The histological variations might be a function of the kidney antigen of the species injected. Since one monkey was used for each antigen (two in the case of human GBM), nothing can be said about whether this variation was due to the antigen used or was a random distribution among the monkeys to essentially the same kind of stimulus (Table 1).

It may be that the histological features of the kidney damage are more characteristic of the species injected. The histological appearance of the nephritis in the sheep seemed quite different from that induced in the monkeys. Further, the rat lesion described in the auto-immune rat nephrosis by Heymann[3] is said to be a membranous glomerulonephritis and is different from either the sheep or monkey lesions.

In conclusion, the injection of heterologous GBM and Freund's complete adjuvant into 5 monkeys produced glomerulonephritis as judged by renal histology and proteinuria in two monkeys; in addition, azotæmia was present in the three other monkeys. The glomerular lesion presumably occurs by an auto-immune mechanism. The 2 monkeys without azotæmia had similar mild diffuse proliferative glomerulonephritis histologically and a similar clinical course. The 3 monkeys with azotæmia had severe diffuse glomerular disease and may be said to have chronic mixed exudative and proliferative glomerulonephritis.

All 5 monkeys demonstrated delayed hypersensitivity to the antigens used for injection as judged by the gross appearance of the swellings which resulted from intradermal injections of the antigen.

The two control monkeys injected with Freund's adjuvant alone had normal urine and kidneys. They did not have delayed skin reactions to GBM antigens.

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EFFECT OF HYPERTONIC SALINE INJECTIONS ON THE SECRETION OF THYROID HORMONES IN RABBITS AND GUINEA PIGS

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THERE have been some contradictory observations about whether the activity of the thyroid gland is affected directly by administration of vasopressin or whether vasopressin acts indirectly by increasing the plasma thyrotropic hormonal level. Several authors observed a change in thyroid function in experimental animals and also in human beings following the injection of antidiuretic hormone[1-5]; others did not find any such change[6,7]. Recently, Garcia *et al.*[8] found that vasopressin exerted a direct action on the excretion of thyroid hormones.

Verney[9] has shown that the urine volume is controlled by a mechanism sensitive to changes in the osmotic pressure of the blood. If during water diuresis in normal animals a hypertonic solution of salt, dextrose or sucrose was injected into the carotid artery, or intravenously, antidiuretic hormone was released and a prompt diminution in the rate of water excretion was seen. Using his technique this work is an attempt to elucidate further the question of whether the appropriate stimulation of the discharge of endogenous vasopressin is associated with an alteration of thyroid function.

Female chinchilla rabbits, body-weight 2–3·5 kg, were injected subcutaneously with 100 μc. carrier-free iodine-131. After two days, when nearly all the inorganic iodine has been excreted and only iodine-131-labelled thyroid hormones were present in the gland and in the circulation, the secretion of endogenous thyrotropic hormone was blocked by subcutaneous injection of 60 μg thyroxine. The thyroxine injection was repeated every day for a week at the end of each day's experiments.

The rabbits were conscious and unrestrained throughout the whole experiment; between 9 a.m. and 5 p.m. the food and water consumption were restricted. The injections of salt solutions were carried out either subcutaneously or intravenously. 10–50 ml. 0·9, 0·6 and 10 per cent solutions were administered within 10–20 min in 10-ml. portions, or infused over a period of 2 h. During infusion the animals were placed on a hot plate at a temperature of 27°–30° C. The left marginal ear vein was cannulated to obtain blood samples, and the solutions were infused into the right marginal ear vein.

Every 2 h blood samples were taken; about 2 ml. were collected into tubes containing 2–3 drops of heparin and the radioactivity of 1·0 ml. blood was determined.

Fig. 1 shows a representative experiment. It is seen from this figure that the iodine-131 hormones decayed in the rabbits' blood throughout the whole experiment. The only increase in the radioactive hormone-level was observed when 50 ml. of 10 per cent sodium chloride was infused; the injection of 50 ml. 0·9 or 0·6 per cent sodium chloride had no effect as did the placing of the animal on the hot plate. The increase of radioactivity was proportional to the amount of injected hypertonic saline solution. 40 ml. 10 per cent sodium chloride caused 130 per cent, 20 ml. 75 per cent, 10 ml. 35 per cent increase of the hormonal iodine-131 level of the blood.

The plasma was separated from the blood sample, the protein-bound iodine precipitated and the radioactivity assayed. 82–97 per cent of the radioactivity of the plasma was found in the precipitate. This result showed that the rise of radioactivity in the blood was due to protein bound iodine-131. This response was similar to that observed after direct vasopressin administration[8].

Similar results were obtained with 8 rabbits. Each week groups of 4 rabbits were used and permutation of

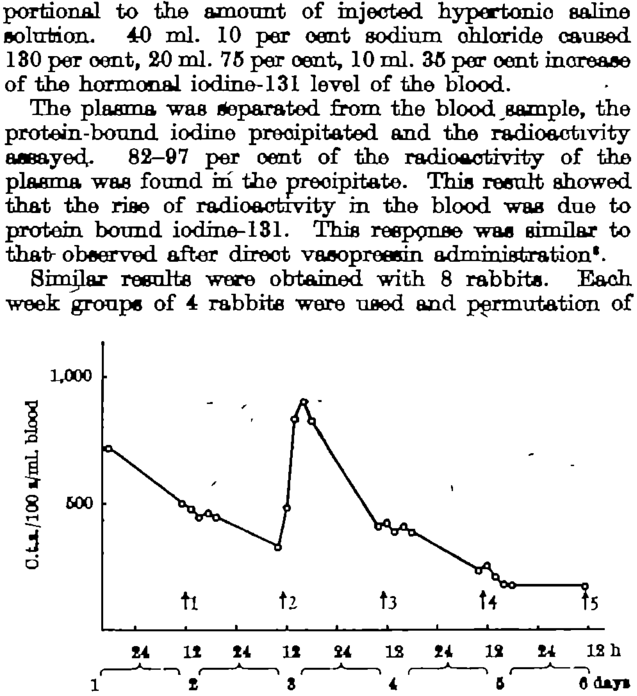

Fig. 1. Effect of different salt solutions and water deprivation on the release of thyroid hormones in the rabbit The curve represents the blood-level of iodine-131 thyroid hormones (c.t.s./100 s/ml. blood) during the 6 days experiment. Blood samples were taken every 2 h between 9 a.m. and 6 p.m. and counts were taken on an Ekco scintillation counter. At the time marked with arrow 1, 50 ml. 0·9 per cent sodium chloride, at the arrow 2, 50 ml. 10 per cent sodium chloride, and at the arrow 3, 40 ml. 0·6 per cent sodium chloride were injected over a period of 20 min, the animal being placed on a hot plate at 28° C for 2 h; at arrow 4 the rabbit was placed for 2 h on the hot plate. At arrow 5 the animal was killed

infusions with the different solutions during subsequent days was carried out.

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Subcutaneous injection of 5–10 ml. hypertonic sodium chloride solutions caused an increased concentration of labelled thyroid hormones in the blood of female guinea pigs, body-weight 500–700 g. In these cases 60 µc. carrier-free iodine-131 was injected subcutaneously and after two days the pituitary activity was inhibited by 50 µg thyroxine. The blood samples were collected from the ear vein.

The classic work of Verney[9] demonstrated that hypertonic saline solutions exerted an action on the posterior pituitary and stimulated the release of stored vasopressin from the gland. Axelrod *et al.* found that prolonged administration of sodium chloride affected the function and the morphology of the thyroid gland[10–12].

Our experiments give evidence that the acute stimulation of the neural lobe by the administration of hypertonic sodium chloride solution, which resulted in a relative deprivation of water in normal animals, led to a rise in the output of the thyroid hormones into the circulation reaching a maximum between 2–6 h depending on the amount injected. The change in endogenous thyrotropic hormone was blocked by a rather large amount of thyroxine so the increase in thyroid activity was not affected by anterior pituitary activity. Evidently the secretion of antidiuretic hormone seems to be responsible for the activation of the thyroid gland, or it is possible that the liberated vasopressin by the effect of hypertonic saline solution could overcome the thyroxine block of the pituitary and release endogenous thyrotropic hormone. Although these results are not conclusive (some further experiments with anterior and posterior lobectomized animals and study of the effect of hypertonic saline solution on isolated thyroid lobes would be necessary) it is supposed that as well as the anterior pituitary, the posterior lobe also plays some physiological part in the regulation of the thyroid function.

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ENCEPHALOMYOCARDITIS VIRUS AND ITS RIBONUCLEIC ACID: SEDIMENTATION CHARACTERISTICS

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Sedimentation Coefficients of Encephalomyocarditis Virus and its Ribonucleic Acid

INFECTIOUS ribonucleic acid (RNA) has been obtained from several animal viruses or from cells infected with animal viruses[1]. Little is known of the physical properties of this RNA, mainly through difficulty in preparing sufficient quantities of pure material; sedimentation coefficients of infective RNA have been estimated for foot-and-mouth disease and mouse encephalitis viruses[2,3]. This article describes the determination of sedimentation coefficients by a modification of the procedure of Yphantis and Waugh[4] (using a cell with a fixed rather than movable partition) which does not require pure material. The material is centrifuged in a separation cell and the amount left above the partition at the end of the run (*Cu*) is compared with that originally present (*Co*). A specific quantitative test for the material under investigation is needed; infectivity, measured in plaque-forming units, and hæmagglutinating ability, measured in hæmagglutinating units, were both so used to determine the sedimentation coefficient of encephalomyocarditis (EMC) virus; infectivity, measured under different conditions, was also used for a similar determination with EMC virus RNA.

EMC virus was grown *in vitro* in continuously agitated suspensions of Krebs-2 mouse ascites carcinoma cells[5]. When more than 80 per cent of the ascites cells were dead, the cell debris was removed by centrifuging at 3,000*g*/5 min followed by 9,000*g*/15 min. The tissue culture supernatant obtained was not purified further before determination of the sedimentation coefficient of the virus, or extraction of its RNA. The number of hæmagglutinating units and plaque-forming units was determined by the method of Sanders, Huppert and Hoskins[5].

Infective RNA was prepared from the crude virus by phenol extraction in the presence of bentonite[6] as described by Montagnier and Sanders[7]. The precipitated RNA was stored at 4° in 67 per cent ethanol until required for use when it was redissolved in 0·01 M phosphate buffer *p*H 6·8. Infectivity of the RNA was measured by plaque titration[8]; the ability of RNase to destroy infectivity was tested for all RNA preparations.

The sedimentation coefficient of EMC virus was determined, using plaque-forming and hæmagglutinating units as markers at 20° and at speeds of 20,410 and 29,500 r.p.m. in a Spinco model *E* ultracentrifuge. Schlieren optics was used to check the alignment of the centrifuge cell and the level of the meniscus. Several runs were made for different periods of time (10–30 min) to give a range of *Cu*/*Co* values of 1–50 per cent. The results from the virus infectivity (Table 1) give a mean value for the sedimentation coefficient of 161*s* with a standard error of ± 3·3. The sedimentation coefficient from the hæmagglutinin activity was 164*s* with a standard error of ± 4·5; typical results obtained are shown in Table 2. On some occasions the contents of the top compartment of the fixed partition cell were used for measurements of both hæmagglutinating and plaque-forming units and the values for sedimentation coefficients obtained were very similar; compare, for example, samples 364–1, –2 and –3 (Tables 1 and 2).

Table 1. SEDIMENTATION COEFFICIENT OF EMC VIRUS USING INFECTIVITY AS MARKER

Sample	Speed (r.p.m.)	*Cu*/*Co*	S_{20}
325–2	29,500	7·8	154·8
364–1	29,500	12·5	165·1
364–2	29,500	8·9	152·5
364–3	29,500	10·7	176·6
377–1	20,410	24·6	169·7
377–1	20,410	25·0	164·0
377–2	20,410	2·8	158·5
377–3	20,410	2·6	146·4

Mean value S_{20} 161 ± 3·3 (*S.E.* 8 determinations)

Table 2. Sedimentation Coefficient of EMC Virus using Haemagglutinin as a Marker

Sample	Speed (r.p.m.)	Cu/Co	S_{20}
322–1	29,500	3·1	148·5
361–1	20,410	6·3	178·8
361–2	20,410	12·5	176·6
364–1	29,500	12·5	165·1
364–2	29,500	6·3	157·8
364–3	29,500	12·5	172·4
377–3	20,410	1·6	147·8

Mean value S_{20} 163·9 ± 4·5 (*S.E.* 14 paired determinations)

Our value of 161–164*s* for the sedimentation coefficient of virus infectivity and hæmagglutinin is close to that of 156–160*s* quoted by Faulkner, Martin, Sved, Valentine and Work[9] obtained by the sedimentation velocity analysis of crystalline virus. The similarity of the figure shows that the method of determining sedimentation coefficients described in this present report gives results close to that obtained by more conventional methods. Since the S_{20} value obtained for virus hæmagglutinin was almost identical with that of virus infectivity it would appear that both these virus properties reside in the same particle. One of the disadvantages of the present method of determining sedimentation coefficients is that if two or more particles with a common property which is used as a specific marker, but with different S_{20} values, are present, then the value obtained will only be an average sedimentation coefficient, the exact value of which will depend on how much of each component is present. Since a similar figure was obtained for virus infectivity and hæmagglutinin (cf Tables 1 and 2), this suggests that other particles having either of these properties (for example, free hæmagglutinin) in crude virus preparations, must be present in small amounts only, if at all.

Table 3. Sedimentation Coefficient of EMC Virus RNA (Plaque-forming Units) using Infectivity as a Marker

Sample	Speed (r.p.m.)	Cu/Co	S_{20}
363–4	44,770	35·9	38·8
384–1	44,770	4·3	36·2
384–1	44,770	4·8	35·1
384–3	44,770	1·1	36·1
383–1	44,770	1·6	36·4
383–1	44,770	2·1	38·1

Mean S_{20} 37 ± 0·5 (*S.E.* 6 determinations)

Virus RNA was run at a speed of 44,770 r.p.m. for 10–30 min giving values of Cu/Co of 1–40 per cent (Table 3). This gives a mean sedimentation coefficient of 37*s* with a standard error of ± 0·5, which fits in well with the results of Montagnier and Sanders[7]. They mixed virus RNA labelled with phosphorus-32 with RNA from uninfected Krebs cells and centrifuged the mixture on a sucrose gradient; the RNA infectivity and radioactivity was found to sediment faster than the largest cellular RNA component, which has an S_{20} value of about 30*s*. A value of 37*s* was also obtained for the RNA from foot-and-mouth disease virus by Strohmaier and Mussgay[2] using a deuterium oxide–water density gradient.

Values of 31–32*s* have been suggested for RNA from mengo, West Nile encephalitis and poliomyelitis viruses[10] and from eastern equine encephalitis virus[11]. These conclusions are based on the fact that the infectious components present were of high molecular weight and that preparations from infected cells when examined in the analytical ultracentrifuge had no component detectable by optical methods with a sedimentation coefficient greater than 31–32*s*. Similarly, RNA from EMC infected cells does not show any component with an S_{20} value greater than 30*s* when examined in the analytical ultracentrifuge[12] using either schlieren or ultra-violet optics. Since the S_{20} value for EMC virus RNA appears to be about 37*s*, this probably means that there is so little virus RNA present in infected cells that it cannot be detected in the analytical ultracentrifuge using conventional methods. Moreover, analysis by sucrose gradient centrifugation[7] shows that the bulk of the infectious RNA recoverable from infected cells, as detected by plating for infectivity, behaves like the RNA extracted from purified virus, for example, sediments appreciably faster than the 30*s* cell component. The values of 31–32*s* for the virus RNA itself from mengo, West Nile, poliomyelitis and EEE viruses are thus open to doubt[10,11]. This is particularly so since evidence that poliomyelitis virus RNA is larger than cellular RNA is suggested by the work of Kubinski and Koch[13], who showed viral RNA was eluted after cellular RNA when RNA from infected cells was chromatographed on a methylated serum albumin–'Celite' column; this system is thought to separate nucleic acids according to chain-length[14], and also by that of Fenwick[15], who found that the newly labelled RNA in poliomyelitis virus-infected cells sedimented slightly faster than the heaviest cell RNA and at the same rate as RNA from pure virus.

Gierer[16] has shown that the molecular weight (M) and sedimentation coefficient of TMV RNA may be equated as follows:

$$M = 1100\, S^{2\cdot 2}$$

If it is assumed that the RNA from EMC virus has similar physical properties to TMV RNA, then an S_{20} value of 37*s* would give a molecular weight for EMC virus RNA of $3\cdot 1 \times 10^6$. EMC virus contains 30 per cent RNA and an S_{20} value of 160*s* for the virus would give a particle of a molecular weight of about 1×10^7 (ref. 9). This would mean the RNA present in the virus particle would have a molecular weight of the order of 3×10^6. Since this agrees with the figure obtained from the S_{20} value of EMC RNA it suggests that there is one RNA molecule of molecular weight—3×10^6 per EMC virus particle and that this can be extracted as a single piece with phenol.

We thank R. Hay and G. Warriner for assistance.

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Sedimentation Properties of Infective Ribonucleic Acid extracted from Encephalomyocarditis Virus

EMC virus itself, and Krebs-2 cells infected *in vitro* with this virus, yield infective ribonucleic acid (RNA) when extracted with phenol[1,2]. Before the precise mode of replication of viral RNA can be investigated, answers must be obtained to the following questions: Is the whole RNA content of one virus particle required to carry the genetic information for virus replication: that is, does the size of the smallest infective unit of RNA correspond to the RNA content of a single virus particle? Infective RNA, in general, has only a fraction of the infectivity of the virus from which it can be extracted[3,4]. Is this due to the relative insensitivity of the system used to assay RNA infectivity, or does the infectivity recorded belong only to a small fraction of the extracted molecules, most of them having been broken up by the extraction method ?

To answer either of these questions requires knowledge of the physical properties of EMC virus RNA; until now it has been impossible to examine these directly, mainly owing to the technical difficulty of obtaining sufficient quantities of pure material. However, we have now been able to obtain tentative answers to the above questions by studying the sedimentation behaviour of a small quantity of EMC viral RNA labelled with phosphorus-32 in a sucrose gradient, assaying both radioactivity and RNA infectivity, and using non-radioactive Krebs-2 cell RNA with known sedimentation values as a velocity marker. At the end of centrifugation, fractions were collected and analysed for three properties: (1) radioactivity; and (2) infectivity, indicating the position of viral RNA; (3) ultra-violet absorbance at 260 mμ locating the cellular RNA. Knowing the sedimentation coefficients of the cellular RNA components, an approximate value could be assigned to the viral RNA. Moreover, a comparison of the respective positions of the radioactive and infective bands would indicate whether the majority of infective molecules differ in size from the bulk of the molecules extracted by the procedure used.

EMC virus, having its RNA heavily labelled with phosphorus-32, was obtained by growing the virus *in vitro* in continuously agitated suspensions of washed Krebs-2 ascites cells (37° C; multiplicity of infection × 3). The medium consisted of Earle's saline buffered with citrate instead of phosphate and containing 100 μc. of carrier-free phosphorus-32 per 10^7 cells. The label was added 1 h before infection. At 5 h after infection, a time at which all infective RNA had been synthesized in the cells[5], the culture was diluted with fresh medium containing the normal concentration of unlabelled phosphate, and incubation continued until 16–18 h after infection. Labelled virus in the supernatant was purified by the method of Faulkner *et al.*[6], using trypsination, chromatography on two successive calcium phosphate (brushite) columns and centrifugation. Virus eluted from the first column was treated with 1 per cent sodium deoxycholate in order to lyse cellular endoplasmic membranes, which electron microscopy showed occasionally to contaminate virus preparations when this step was omitted. To identify the radioactive final product as a single viral component, a portion was centrifuged to equilibrium in a solution of rubidium chloride with an initial density of 1·33 using the method described by Crawford[7]. After equilibrium had been reached (40 h; 34,000 r.p.m.; *SW* 39 head) analysis of fractions collected from the centrifuge tube showed that both radioactivity and virus hæmagglutinating ability banded together in a single peak at a density of 1·33 characteristic of EMC virus.

RNA was extracted from such virus preparations in acetate buffer 0·01 M, ethylenediamine tetraacetic acid (EDTA) 0·025 M, *p*H 7, containing 0·05 per cent of bentonite, a powerful absorbent of ribonuclease[8]. Two alternative methods of deproteinization were used: in the first, the virus suspension was shaken twice for 10 min at room temperature by hand with an equal volume of redistilled analytical reagent phenol; in the second method, the virus suspension was shaken with 0·25 per cent sodium dodecylsulphate for 10 min and then 2 or 3 times with the Sevag mixture (chloroform/octanol[9]). The subsequent stages of preparation were identical for both methods. The aqueous layer containing RNA was freed from bentonite by high-speed centrifugation (35,000 r.p.m.; 15 min) and the RNA precipitated by ethanol without addition of a carrier; to absorb the last traces of protein (particularly ribonuclease), the RNA pellet was again dissolved in a suspension of bentonite (0·05 per cent), and the bentonite removed by centrifugation. The final RNA extracted by both methods contains from 60–75 per cent of the radioactivity originally present in the virus preparation; the remainder could be found in the reagents used for extraction. The infectivity of such preparations was remarkably stable (several months at 4° C) provided that the storage medium contained both EDTA and bentonite. Both methods of extraction yielded specific infectivities of about 1 × 10^3 infective units per μg of RNA, as recorded by plating on agar layers of Krebs-2 cells[10]. Krebs-2 cellular RNA was also extracted by the phenol method (using reagents containing bentonite and EDTA as described here.

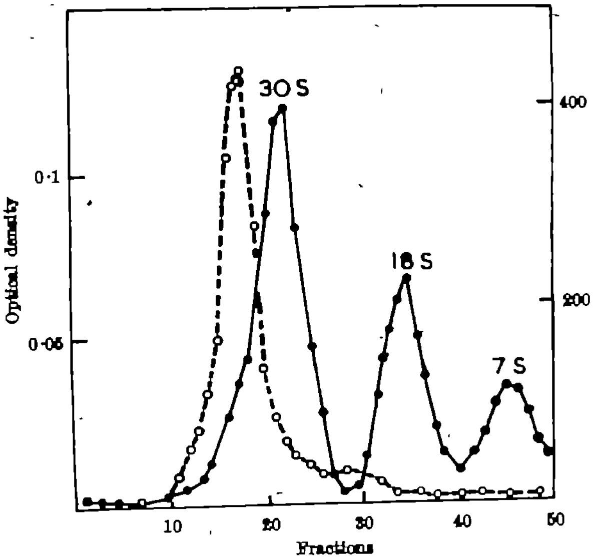

Fig. 1. Sedimentation of a mixture of RNA from Krebs-2 cells and ^{32}P-labelled EMC virus RNA, both extracted by phenol, in a sucrose gradient 10–20 per cent containing *tris*–HCl 0·005 M, EDTA 0·02 M, *p*H 7·2 (25,000 r.p.m., 12 h + 4° C, *SW* 25 rotor). After centrifugation, the tube was pierced and fractions of approximately 0·5 ml. were collected. Fraction No. 1 is the first fraction collected from the bottom of the tube. Continuous line, optical density at 260 mμ; dotted line, radioactivity

By centrifugation in the Spinco model *E* analytical centrifuge, using either schlieren or ultra-violet optics, cellular RNA can be resolved into 3 components having sedimentation values of 30*s*, 18*s* and 5–7*s* at neutral *p*H and moderate ionic strength (sodium phosphate buffer 0·02 M). The same 3 components could be identified when these cellular RNA preparations were centrifuged on a sucrose gradient (Fig. 1). The two rapidly sedimenting components presumably come from ribosomes; the

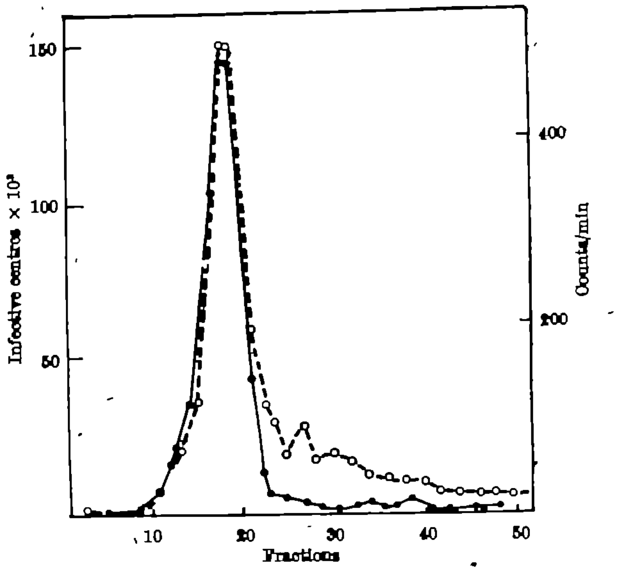

Fig. 2. Sedimentation in a sucrose gradient of ^{32}P-labelled EMC virus RNA extracted by sodium dodecylsulphate. No cellular RNA added. Conditions of centrifugation as in Fig. 1
Continuous line, infectivity; dotted line, radioactivity

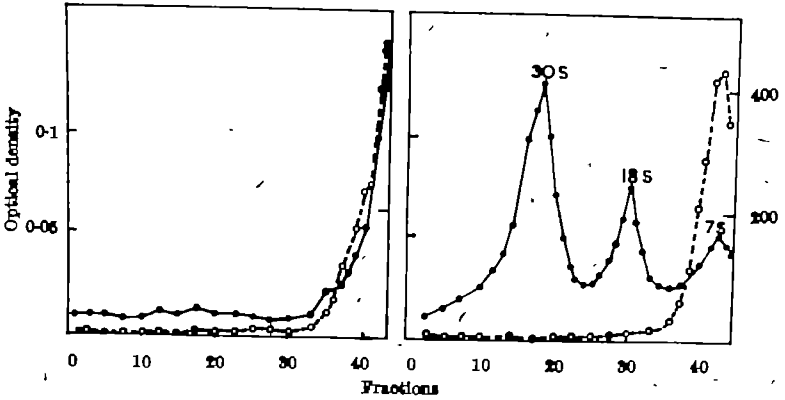

Fig. 3. Effect of ribonuclease. (a) Sedimentation in a sucrose gradient of a mixture of Krebs-2 cellular RNA and ^{32}P-labelled EMC virus RNA, both treated with ribonuclease (1 μg, 30 min, 10° C). Conditions of centrifugation as in Fig. 1. (b) Sedimentation of a mixture of untreated Krebs-2 cellular RNA and ^{32}P-labelled EMC virus RNA, treated with ribonuclease (0·5 μg, 1 h, 10° C), and then freed of enzyme by the addition of 0·1 per cent of bentonite (latter removed by centrifuging 20 min at 36,000 r.p.m.). Medium of centrifugation as in Fig. 1. Run, 18 h. 25,000 r.p.m. Continuous line, optical density at 260 mμ; dotted line, radioactivity

slowest fraction (4–7s) probably includes the 'transfer RNA' fraction which is soluble in 1 M sodium chloride.

When EMC virus RNA labelled with phosphorus-32 is mixed with cellular RNA and centrifuged on a sucrose gradient, the former, as detected by its radioactivity, sediments as a single peak travelling faster than the 30s component of cellular RNA (Fig. 1). When EMC RNA was centrifuged in the absence of marker RNA a similar sedimentation profile was obtained, showing that the sedimentation of viral RNA was not affected by the presence of cellular RNA, provided that the concentration of the latter was not too high ($\leqslant$ 500 μg/ml.) and that it too had been extracted in the presence of a chelating agent. It was also found necessary for EDTA to be present in the sucrose gradient itself in order to sharpen separation between the different components. Control with the analytical centrifuge showed that sodium versenate at the same ionic strength as that used in the gradient did not affect, or only slightly diminished, sedimentation coefficients of the components of cellular RNA.

Viral RNA infectivity, as tested by plating on Krebs-2 cells, sedimented at the same rate as radioactivity (Fig. 2). Comparison between the radioactive curve and the curve for infectivity, however, indicated that there is a tail of lighter radioactive material the specific infectivity of which is too low to be significant. These results indicate quite clearly that the major fraction of the RNA consists of molecules of a given size, an unknown proportion of which is both infective and radioactive, and that only a small part of the RNA is broken by the method of extraction. This portion loses its infectivity but remains radioactive. Similar results were obtained whatever method of extraction of viral RNA was used, and this makes it unlikely that the peak of sedimenting material observed is an artificial product of the method of extraction. There is, moreover, no doubt that the material studied is RNA. All the sedimenting peaks are sensitive to extremely small amounts of ribonuclease (0·5 μg/ml., 1 h at 4° C) which reduces the RNA to small pieces sedimenting at low velocities (Fig. 3).

Evaluation of the molecular weight of infective EMC RNA. Cellular ribosomal RNA consists of two components sedimenting at 18 and 30s respectively, and having molecular weights of 7 × 10^5 and 1·5 × 10^6 (refs. 11 and 12). Assuming that the viral RNA has the same fundamental structure as ribosomal RNA, our results suggest that EMC virus RNA has a molecular weight > 2 × 10^6. This is confirmed by an independent determination of the sedimentation velocity of EMC viral RNA in the Spinco analytical ultracentrifuge. RNA from a large batch of purified virus was sedimented in the analytical centrifuge and its sedimentation examined by ultra-violet optics. The value obtained was 37s, which is in good agreement with data obtained by Burness *et al.* (preceding communication), using an independent method. They found a sedimentation value for EMC RNA of 37s and calculated a molecular weight of 3 × 10^6. On the basis of the analytical results of Faulkner *et al.*[9], this would suggest that the infective RNA molecule extracted from purified EMC virus corresponds to the entire RNA content of a single virus particle. Investigation of the change in ultra-violet absorbance which follows heating suggests that EMC RNA exhibits in solutions of moderate ionic strength a similar degree of intramolecular hydrogen bonding to the cellular RNA components. Moreover, sedimentation values of EMC RNA, like those of both 18s and 30s ribosomal RNA, are strongly dependent on the ionic strength of the medium of centrifugation, and also on the presence of divalent cations. Fig. 4 shows the result of an experiment in which a mixture of EMC virus RNA labelled with phosphorus-32 and Krebs-2 cellular ribosomal RNA was centrifuged at very low ionic strength (0·001 M EDTA). Under these conditions ribosomal RNA molecules show a greater degree of extension than at higher ionic strengths, owing to rupture of intramolecular hydrogen bonds, and consequently have lower sedimentation coefficients[13]. In media of low ionic strength the sedimentation constant of EMC viral RNA is also diminished, but to a much greater degree than that of the cellular component; consequently the radioactive peak in 0·001 M EDTA now lies between the peaks of '30s' and '18s' cellular RNA, and not in front of the former. Such variations, however, are fully reversed by increasing the ionic strength, and infectivity always remains associated with the radioactive viral peak. A reasonable assumption based on such results is that EMC virus RNA in solution, like ribosomal RNA, is a single-stranded chain.

Recently we have also investigated the sedimentation properties of infective RNA synthesized by cells in response to infection. Such an RNA is synthesized before the appearance, within the cells, of mature virus particles.

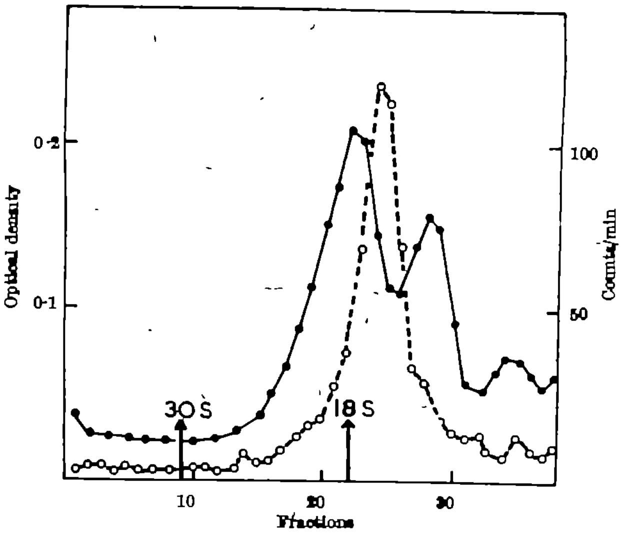

Fig. 4. Sedimentation of a mixture of phenol-extracted Krebs-2 cellular, and ^{32}P-labelled EMC virus RNA, in a sucrose gradient 10–20 per cent containing *tris*–HCl 0·001 M, EDTA 0·001 M, pH 7·2 (25,000 r.p.m., 15 h, 4° C, *SW* 25 rotor)
Continuous line, optical density at 260 mμ; dotted line, radioactivity. The positions occupied by the 18s and 30s components in a high ionic strength medium as in Fig. 1 are indicated by the arrows

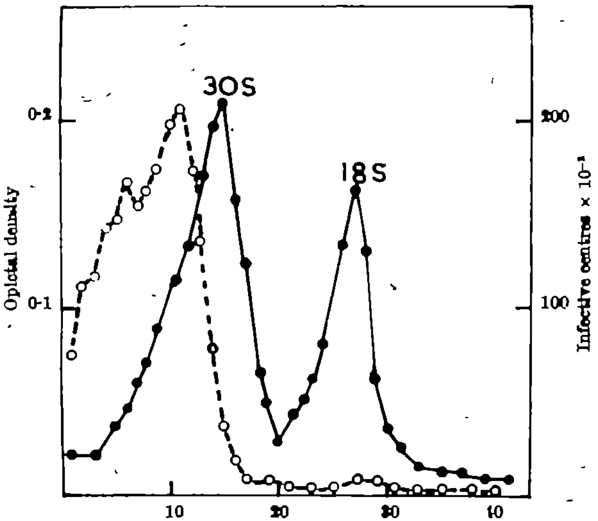

Fig. 5. Sedimentation in a sucrose gradient of RNA extracted by phenol from Krebs-2 cells incubated for 3 h at 37° C after infection by EMC virus at multiplicity 5 (fraction insoluble in 1 M NaCl). Medium of centrifugation as in Fig. 1—14 h, 25,000 r.p.m. Continuous line, optical density at 260 mμ; dotted line, infectivity

Fig. 5 shows the sedimentation of infectivity when RNA extracted from infected cells is spun on a sucrose gradient. The bulk of the infective molecules sediments faster than the 30s ribosomal component but the peak found is broader than when virus RNA is centrifuged as a mixture with cellular RNA. Experiments are in progress to decide the relevance of these differences to the process of RNA replication.

Our results at present, however, provide a clear answer to the two questions proposed at the beginning of this article. When tested by its ability to perform all the duties of a virus particle, that is, to produce a plaque on an agar suspension of susceptible cells, infective RNA appears to consist of molecules each of which corresponds in size to the total RNA content of one virus particle. Phenol extraction of virus releases the bulk of the RNA in the form of molecules of this size. The relative lack of infectivity of RNA preparations compared with the virus from which they have been derived must therefore be ascribed to inefficiencies in the assay system used rather than to breakage of RNA occurring during extraction, although, conceivably, the extraction procedures might inactivate most of the RNA without breaking it, or else break it near one end with a negligible effect on sedimentation rate. However, recent improvements in the assay system have now achieved RNA infectivities of the order of 0·1–1·0 per cent of the infectivity of the purified virus from which these preparations were made, suggesting that it is the sensitivity of assay rather than the quality of the RNA which is at fault.

We thank Mrs. B. Burford and Miss M. Wall for assistance.

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SELECTION IN NATURAL POPULATIONS: *MERYCHIPPUS PRIMUS*, A FOSSIL HORSE

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THE distribution of intensities of phenotypic selection in natural populations has never been adequately investigated. There are a rather larger number of instances (to be reviewed elsewhere) in which phenotypic selection in natural populations has been demonstrated and from which estimates of intensity can be made[1]; but most of these serve mainly to show that natural selection does in fact occur. The reason for this is that there is an unknown degree of bias toward high intensity in these cases in that for the most part only significant results are published. Cases in which natural selection is not demonstrated are presumably often left unpublished.

Many groups of organisms are unsuitable for the kind of investigation to be reported here. The two main requirements are that a method of establishing individual relative age at least approximately must be available, and that a character must be available which does not change ontogenetically between these age-groups. The characters to be reported here (and in the press, *Nature*) are chosen without respect to the likelihood of selection acting on them, but only for the technical reasons given in this paragraph, as well as availability.

Statistical parameters, most commonly mean and variance, are estimated for each age-group. If the age-groups differ in one or more statistics, this difference is due either to natural selection or to a difference in the environments in which the age-groups developed[2]. The latter alternative can be controlled by sampling several generations or by sampling both dead and surviving individuals. Whether or not a significant difference between age groups is found, the apparent selection intensity is then calculated for each statistic.

The intensity I_p of natural (or artificial) selection by means of differential viability, relative to statistical parameter or set of parameters p, may be defined as the minimum reduction of the original population that would yield a population with specified values of the parameter or set of parameters. This is an extension of Haldane's definition[1], and can itself be readily extended to include selection on fertility. (Both definitions actually refer to amounts, rather than intensities of selection; division by the number of generations, usually 1, converts them to intensities.) The total intensity I is the minimum reduction required to produce the total multivariate distribution for all characters and parameters after selection. I can never exceed the proportion of the original population that is lost, but can be less than this value. The derivation of quantitative estimates of selection intensity and their tabulation will be presented elsewhere.

A bias of estimation, in the direction of high intensity, exists when the difference of the means (or correlations) of the age-groups is low in relation to its standard error.

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This is true because the difference is taken to be positive whether an increase or a decrease in mean size has occurred, and even if no actual change took place some difference would almost inevitably be produced as a result of sampling error. The estimation of this bias is complex and the correction for it has not yet been tabulated, but in no case will the correction be as large as the estimate of intensity derived from the probable error of the difference between the age-groups. The symbol Q may be applied to the ratio of the observed difference between two age-groups, to the difference expected to occur 50 per cent of the time by chance. Q is dependent on both the power of the significance test in a given case and the level of significance reached.

If only decrease in variance is recognized (or if an increase is not recognized unless its significance-level reaches such a value as 0·01, the value I use), the foregoing bias is scarcely applicable to the variance. The ratio of the variances of the age-groups should, however, be divided by the appropriate 50 per cent F ratio to eliminate a small bias caused by different sample sizes; this produces a ratio with the same meaning as Q.

Since the age-groups available may not cover the entire time the structure under consideration was open to selection, a correction for this may be made by multiplying the observed selection intensity by the total time the structure exists before the mean reproductive age, and dividing this product by the difference between the means of the age classes. It is possible that selection may occur for the same biological reasons even when the structure under consideration is absent; but this is another matter and is perhaps more or less offset by the possibility of changes in selection intensity with age. A further correction must be introduced if the age determination of the sample is not entirely accurate.

To estimate the combined intensity for more than two age-classes, the intensity over the interval between each successive class should first be calculated. Then the first intensity should be subtracted from 1; this difference should be multiplied by the second intensity, and the product should be added to the first intensity. The resulting intensity should, of course, be used as the first one for any further accretions.

To combine estimates of intensity on two characters together, it should be noted that the square of the correlation (in the unselected population) between the characters, multiplied by the higher intensity, gives the intensity expected on the second character from the correlation only. This intensity should be subtracted from the lower intensity and the signed difference added to the higher intensity. Symbolically:

$$I_{\text{combined}} = I_1 + (1 - r^2)I_2$$

Partial correlations can be used to combine the estimates for more than two characters if desired.

As with life-tables[3], the estimate of selection intensity depends somewhat on whether the sample is of a living population (or of an entire population killed at once by a catastrophe) or of individuals that died in the normal course of the history of the population. In the latter case, but not in the former, each age-group should be added cumulatively to the next earlier one to provide the best approach to a random sample of the individuals living at the earlier age. This need not be done for the determination of the significance of differences between the age-groups, however.

The sample used here is of a Middle Miocene horse, ***Merychippus primus***, from Stonehouse Draw, Nebraska, in the collection of the American Museum of Natural History. The characteristics of the sample will be described in detail elsewhere; it is sufficient for present purposes to know that deciduous teeth can be unambiguously aged to year-groups and that, as indicated by the preponderance of young individuals and the arguments given by Kurtén[3] for this and similar samples, the specimens are probably a more-or-less random sample of a living population rather than the result of the normal attrition of mortality. Measurements of width were taken for the deciduous teeth DP^{2-4}_{2-4} and the first premolar P^2, and of lingual length for DP^2 and P^2 only. All measurements were taken at the base of the tooth and are unaffected by wear, including wear between adjacent teeth in the same jaw. The distribution in age-group I of no character differed significantly from normality, as determined by χ^2 tests against normal distributions with the same variances as the samples.

Table 1. STATISTICS FOR AGE GROUPS OF *M. primus*. UNUSUAL SYMBOLS ARE EXPLAINED IN THE TEXT

l Refers to length and w to width

	N	$\bar{Y}$	Q	$P(m_1 = m_2)$	s^2	$\frac{F}{F(50\%)}$	$P(s_1^2 < s_2^2)$
Age-group I							
l DP^2	21	17·93 ± 0·23			1 076		
w DP^2	23	16·71 ± 0·23			1·265		
w DP^3	21	17·42 ± 0·26			1·370		
w DP^4	18	18·14 ± 0·32			1·840		
w DP_2	57	8·12 ± 0 08			0·409		
w DP_3	68	10·68 ± 0·08			0 439		
w DP_4	64	10·74 ± 0·09			0·566		
Age-group II							
l DP^2	9	17·57 ± 0 33	1·31	0·30–0·40	1·003	1·02	0·49
w DP^2	10	16·08 ± 0·27	2·59	0·05–0·10	0·725	1·68	0·10–0·25
w DP^3	13	16·85 ± 0·20	2·59	0·05–0·10	0·505	2 66	0·02–0·05
w DP^4	15	17·39 ± 0·17	3·06	0 02–0·05	0·413	4·41	0·001–0 005
w DP_2	28	8 32 ± 0 08	2·61	0 10	0·260	1·57	0·05–0·10
w DP_3	44	10·81 ± 0·08	1·61	0·20–0·30	0 261	1·66	0·02–0 05
w DP_4	52	10·93 ± 0·07	2·10	0·10–0·12	0·278	2 04	0·005
Age-group III*							
w DP_4	5	10 74 ± 0·11	2·07	0 15	0 063	3·74	0 05–0 10
Younger half P^2							
w	45	17·69 ± 0 15			1·007		
l	41	17·76 ± 0·16			1·069		
Older half P^2							
w	44	17·95 ± 0·16	1·74	0 20–0 30	1 108	0 95	0·50–0 60
l	32	18 06 ± 0 20	1·67	0·20–0 30	1·346	0·88	0·60–0·70

* Comparison is with age-group II.

In Table 2 the means and variances of the age-groups are compared. Although in only one case (DP^4) is the significance of the difference of the means less than the 0·05 level, it is of interest that in no case does the P value exceed 0·4, which it would be expected to do in three-fifths of the cases without selection. In the upper dentition the trend is uniformly towards smaller size, while in the lower dentition the trend between the first and second years is towards larger size and between the second and third years towards smaller size. Except for the last instance, this situation is reminiscent of that found by Kurtén[4] at a cave bear site. It is of interest that this lineage of horses was increasing in size, so the selection for smaller upper teeth indicates a temporary or local reversal of this trend. Such temporary or local reversals may well be a common phenomenon.

The intensities of selection (Table 2) required to produce the observed changes of means, as well as of variances, are remarkably high. The correlation between the widths of DP^3 and DP^4 in age-group I is $r = 0·846$. For DP^3 and DP^4 the joint intensity required to produce the observed shift of mean is $I_m = 0·44$. This means that two-fifths of the individuals in age-group I must have died simply for there to be this difference (the bias is in this case small). Similarly, combining the intensities on the mean for DP_4 over the two year-periods, the combined intensity is between 0·2 and 0·3, with a net

Table 2. INTENSITIES OF SELECTION REQUIRED TO PRODUCE THE OBSERVED CHANGES IN SAMPLE MEANS AND VARIANCES, AND SPURIOUS INTENSITIES CALCULATED FROM THE PROBABLE ERRORS OF THE DIFFERENCES OF THE SAMPLE MEANS

l refers to length and w to width

	I_σ	I_m	I_m (50 % chance)
l DP^2	0 002	0·20	0·14
w DP^2	0 12	0·34	0·11
w DP^3	0 20	0 29	0·09
w DP^4	0 22	0 34	0 09
w DP_2	0·08	0·17	0·05
w DP_3	0 09	0·10	0·05
w DP_4	0·14	0·14	0·05
w DP_4*	0 27	0·20	0 08
w P^2	−0 005	0·14	0·07
l P^2	−0 021	0·16	0 08

* Age-group III compared with age-group II.

Negative intensities strictly are meaningless; but they are useful in an unbiased estimation of the distribution of intensities.

result of no change in mean. These are minimal unbiased estimates of the frequency of deaths; without a sharp cut-off point the number would be somewhat higher.

P^3 is different from the deciduous premolars in that age-groups cannot be unambiguously recognized from it. (The other upper permanent teeth are excluded because they cannot be identified unambiguously at all stages of wear.) The P^3's were divided into two groups by the amount of wear. It is of interest that the amounts of selection on P^3 are ostensibly lower than those on DP^3, despite the longer interval in years between the midpoints of the age groups of P^3. Regressions of width and length on mesostyle height (a good measure of degree of tooth wear) gave values of $- 0·013 \pm 0·022$ and $- 0·036 \pm 0·032$ respectively, with Q for the regressions being 0·83 and 1·67 respectively. There is thus no evidence of selection on either dimension of P^3.

Table 3. CORRELATION COEFFICIENTS FOR AGE GROUPS OF A SAMPLE OF *M. primus*

The ratio Q is explained in the text. l refers to length and w to width

	N	r	z	Q	$P(r_1 = r_2)$
Age-group I					
w-l DP^3	21	+0·85	1·29 ± 0·24		
w DP^{3-4}	16	+0·96	1·98 ± 0·28		
w DP_{2-3}	51	+0·92	1·68 ± 0·14		
w DP_{3-4}	47	+0·85	1·24 ± 0·15		
w DP_{2-4}	62	+0·91	1·49 ± 0·13		
w-l P^3	38	+0·68	0·84 ± 0·17		
Age-group II					
w-l DP^3	9	+0·66	0·80 ± 0·41	1·53	0·30–0·40
w DP^{3-4}	12	+0·91	1·54 ± 0·33	1·48	0·30–0·40
w DP_{2-3}	28	+0·79	1·06 ± 0·20	3·14	0·02–0·05
w DP_{3-4}	26	+0·67	0·81 ± 0·21	2·48	0·09
w DP_{2-4}	41	+0·83	1·19 ± 0·16	2·11	0·10–0·20
w-l P^3	32	+0·75	0·98 ± 0·19	0·86	0·50–0·60

Correlations are given in Table 3. The estimation of selection intensities for correlations is complex and not yet fully worked out; but again Q is greater than one except for P^3, indicating that correlations have probably changed as a result of selection.

The estimated intensities of selection on individual characters can be compared with the estimated mortality rate between age-groups I and II. There are 154 specimens of age-group I and 105 of age-group II, giving an ostensible mortality rate of 0·32 in this interval. This is lower (although not significantly so) than the combined estimate of I_m for DP^3 and DP^4, and is surely lower than the total I for all characters and parameters. This discrepancy can be accounted for in at least four ways. First, the distributions in age-group I may really have differed from normality, even though this could not be proved. Secondly, sampling error in the estimation of either mortality rate, selection intensities, or both, may have been in the direction to produce the anomalous effect. Thirdly, the age distribution may not have been stable; if the cohort at age II at death was more numerous when at age I than the cohort that died at age I, the real mortality rate would have been higher than that estimated. Fourthly, the differences between the first two age classes may have been due to environmental effects that differed between their years of birth, although the reduction in variance argues strongly against this. If the deaths were not catastrophic but attritional the anomalous effect would have been increased or unchanged. If a is the number in A, the first year-class; b the number in B, the second; and c the number of all older individuals C, for catastrophic death and a stable age-distribution I is estimated by comparing B with A and the first-year mortality rate by $\frac{a-b}{a}$. For attritional death and a stable age-distribution, I is estimated by comparing B with $(A + B)$, assuming B is a random sample of $(B + C)$, and will be larger than this estimate if the last assumption is false (unless the direction of selection is reversed). The first-year mortality rate is estimated by $\frac{b+c}{a+b+c}$. Comparison of these ratios shows that the first-year mortality rate is affected more than I by attritional death.

A theory of the estimation of the intensity of selection is presented and applied to teeth, mainly deciduous, of a Miocene horse. Estimates of the intensities on single characters range from zero to 34 per cent; the latter is comparable with an estimate of the mortality rate during this age interval. In older individuals the intensities appear to be lower. The intensities of selection required to produce the observed changes in means are similar to those required for the variances. Selection on the upper teeth for smaller size is opposite to the direction of phyletic change in this lineage.

I thank Mrs. Sheila Maynard Smith for helping to solve some integral equations, Mr. John Maynard Smith and Dr. Timothy Prout for discussions, and Mr. Carl Sorenson for information on the occurrence of the sample in the field. Some of the measurements were made during the tenure of a Boese postdoctoral fellowship from Columbia University with special calipers kindly loaned by Mr. Giles MacIntyre.

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LONGEVITY, PALATABILITY AND NATURAL SELECTION IN FIVE SPECIES OF NEW WORLD SATURNIID MOTH

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WILLIAMS and Williams[1], following Medawar[2], have suggested that natural selection should in the majority of cases prolong the reproductive lives of animals. They show, in addition, that the force of selection must fall throughout reproductive life, to reach zero when reproduction is over; for this reason, selection cannot be held to modify post-reproductive or non-reproductive life-spans except in certain special instances. Among them may be quoted the entire life-spans and indeed the morphological specializations of the non-reproductive castes of social insects, and the survival of old members in the societies of higher vertebrates, where it is supposed that their experience is of value to the population at large; in both cases it is necessary to postulate the action of group selection. The former authors also consider that selection could in principle favour characters which are individually disadvantageous but socially useful, but conclude that the actual likelihood of such an action occurring is remote.

A possible special case has already been proposed, in which the individual disadvantageous character is an abbreviated life-span, and in which group selection either lengthens or shortens the duration of post-reproductive survival[3]. Consider two species of insect with non-feeding adults, one cryptic and palatable, the other aposematic and distasteful, and both subject to predation.

(a) The longer that a cryptic insect survives after reproducing, the greater the chance that it will be found

Table 1

Sex	Species	Coloration	Protective devices*	Palatability		
				Wild summer tanager (*Piranga rubra*)	Marmosets (*Oedipomidas spix*)	Monkeys (*Cebus albifrons*)
Males	*Lonomia cynira* Cramer	Procryptic leaf-mimic	Immobility to disturbance	Accepted	Accepted	Accepted
	Automeris junonia Walker	Forewings: leaf mimicry Hindwings: eyespots	Eyespot display	,,	,,	,,
	*A. boucardi** Druce	,, ,,	,, ,,	,,	,,	,,
	Dirphia (*Dirphiopsis*) *eumedide* Stoll*	Aposematic with banded abdomen	Display of abdomen, with discharge of foul-smelling meconium	Ignored	Rejected	Accepted after removal of fur and meconium
	D. (*Periphoba*) *hircia* Cramer	Drab with hairy and banded abdomen	Display of abdomen with oriented discharge of foul meconium	,,	,,	,, ,,
Additional females	*Automeris tridens* H-S *A. rubrescens* Walker *A. vinosus* Conte *A. fouchari** Bouvier	Forewings cryptic Hindwings with eyespots	Eyespot displays	Accepted	Accepted	Accepted
	Hylesia 5 spp.†	Drab, with light hair on abdomen	Display of urticating hair on abdomen	Ignored	Rejected	Accepted after hair had been rubbed off abdomen
	Hyperchiria nausica Cramer	Cryptic forewings, brightly banded abdomen	Display of abdomen: scented scales and meconium	,,	,,	,,
	Dirphia (*Dirphia*) *avia* Stoll	Banded, hairy abdomen	Discharge of foul meconium	—	—	—

* Provisional identification only. † Not identified

by a predator. Birds, at least, must learn to find individual cryptic prey species[4], and the lesson must be periodically reinforced by success. Survival, therefore, prejudices the safety of other individuals, which may still be reproductive. Group selection should shorten post-reproductive survival, and direct selection should shorten the reproductive life-span should the ecology of the species permit it to do so.

(*b*) The converse of this argument applies to the aposematic insect. Birds also learn to avoid conspicuous but unpalatable prey, and they may even do so by single trial learning[5]. The post-reproductive survival of an aposematic insect, then, should favour the survival of its siblings, for it is available to train a predator to avoid them without prejudicing the reproductive potential of the population. Group selection should lengthen the post-reproductive survival of the aposematic species; direct selection may be expected to lengthen the overall life-span.

Even if this argument were correct, there are few natural situations which will permit it to be tested. An attempt has been made to do so by using hemileucine saturniid moths in seasonal tropical evergreen forest on Barro Colorado Island in the U.S. Panama Canal Zone. In order that the species should be comparable, the five chosen for the comparison of males: (*a*) are all of the same order of size; (*b*) have all been found in their early stages within 20 ft. of ground-level beneath the forest canopy. The rather scanty available evidence implies that temperatures in the canopy itself are both higher and subject to greater fluctuations than beneath it[6], and a broader comparison would be of doubtful validity; (*c*) all five species are of approximately the same order of abundance. They are listed, together with their palatabilities to three species of predator and their defensive behaviour (summary of unpublished data), in Table 1, which includes the additional genera used in the comparison of females.

The moths were captured at an ultra-violet light trap, which was watched throughout the night; individuals were removed as soon as they arrived at the light, and inspected immediately for damage. Immaculate moths were assumed to have hatched on the night of capture, while those with wing abrasions, damage to the fringe scales or torn wings were rejected as being more than 24 h old. This procedure prejudices the adult life-tables against the hypothesis, for aposematic species tend to have hard scaling and are consequently less susceptible to mechanical damage. The male insects were stored in small plastic boxes under continuous illumination to inhibit flight activity. Moths so stored remain quiescent until they are disturbed by tactile stimulation, or until the last few days of life, when they become hyperexcitable. The flight times of male hemileucines are very narrowly restricted, often to a period of no more than 15 min in each night, and immaculate males are assumed to be virgins. Females were stored in larger cages, and were not illumined at night. The storage conditions approximated to those recorded for forest shade on Barro Colorado at a height of some 20 ft. from the ground[6]. Adult saturniids have degenerate mouth-parts and do not feed.

The adult male life-tables are presented in Fig. 1. The hypothesis predicts that the life-spans should be ranked in the following order: *Lonomia* < *Automeris* < *Dirphia*; although the two species of *Automeris* are rather distantly related, as are the two species of *Dirphia*, no prediction can be made about their order of ranking, and the data were pooled within genera for analysis by Kendall's method[7], which yields $P < 0{\cdot}0001$ for the ordered distribution. The data from male moths, then, support the hypothesis, in so far as the action of direct selection is concerned.

The results from the females are more difficult to interpret. In the first place, only a small proportion of the moths trapped at light are females. For this reason, the data for all species of female, irrespective of size, have been used for comparing the length of post-reproductive survival between palatable and unpalatable species. Secondly, it is not possible to estimate the age of females with such certainty as that of males. Female hemileucines, apart from a few exceptional species, do not fly until they have mated; if it is assumed that mating usually occurs on the night of emergence, then females replete with eggs which are captured at light must be supposed to be 24 h old. Moths so caught wait a further 24 h before starting to lay eggs, and this delay is a consequence of the method of capture, for bred moths rarely delay more than 24 h between mating and oviposition. In view of these uncertainties, the only practicable comparison is

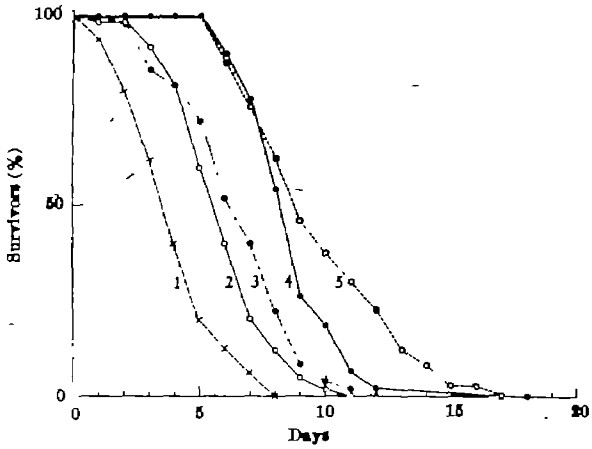

Fig. 1. Adult male life-tables for five species of hemileucine moth. Curves from left to right: 1, *Lonomia cynira* (50 males); 2, *Automeris junonia* (60), 3, *A. boucardi* (50), 4, *Dirphia eumedide* (60); 5, *D. hircia* (50)

that between survivals from the laying of the last egg to death. Such a comparison again biases the results against the hypothesis, for the trapped palatable females lay their eggs after capture and are seen to die shortly afterwards; the distasteful females are more usually captured after oviposition has been completed, and may be at any stage of their post-reproductive life-span, and such 'spent' females have been included in the data. Table 2 pools the data for each of the two classes of moth. The survivals again rank in the predicted order ($P < 0{\cdot}001$).

Table 2. POST-REPRODUCTIVE SURVIVAL OF FEMALES

Days	Palatable	Unpalatable
1	10	0
2	3	13
3	0	6
4	0	12
5	0	7
6	0	4
7	0	1
8	0	0
9	0	2
Total	13	45

The patterns of survival so far described are clearly 'idealized' and need bear no relation to the life-tables for feral moths. There are striking differences between the behaviour of the palatable and distasteful forms; the palatable *Lonomia*, *Automeris* and *Gamelia* spp. become increasingly excitable with age, and spend their last 12 h of life in unrestrained flight activity. Unpalatable forms (*Dirphia* (*Dirphia*), *D.* (*Dirphiopsis*), *D.* (*Periphoba*), *Hyperchiria* and *Hylesia* spp.) do not appear to undergo marked age-dependent changes in behaviour, and their excitability is relatively stable.

In the males of three species (*L. cynira*, *A. junonia* and *D. eumedide*) it has proved possible to measure these changes in behaviour with age in terms of the rocking response[8]. When hemileucine moths settle after any preceding activity they rock from side to side, and the strength of the response may be measured by the number of oscillations in a single train. For the Brazilian *Automeris aurantiaca* it has been shown that the strength of the response $y = a + bx$ where x is the duration of precurrent flight in minutes, and a and b are age-dependent constants[9]. b lies between 0 and 1·0, and relates inversely to the state of flight excitability of the individual moths. a cannot be determined for moths taken at light traps, for the previous flight history is unknown. b was measured by removing the wings and abdomens from moths of known age from capture, and subjecting them to forced tethered flight for successive 3-min periods. At the end of each period the strength of the response was measured six successive times at 1-min intervals, and the three highest values out of each group of six were used to compute b. The values of b differ slightly according to whether the moths walk or fail to walk before each settling response[9], but the difference is slight and can be ignored in the present context. Fig. 2 plots log b against age for each of the three species. By this criterion, the behaviour of the two cryptic species undergoes a continuous change with time, which is rather nicely adjusted to the adult life-span. Indeed, when $b < 0{\cdot}2$, it is virtually unmeasurable, in part because the moths have become too excitable to test, and also, arguing from the condition in *A. aurantiaca*, because a has so decreased that very long periods of flight would be needed to give real positive values to y. The age at which $b < 0{\cdot}1$ corresponds roughly to the point at which unrestrained terminal flight begins. *Dirphia eumedide*, on the other hand, behaves consistently throughout its life-span, and the increase in the excitability of flight with age is far less marked. Preliminary measurements on the aposematic species of *Hyperchiria* and *Hylesia* indicate that they share the stability of *Dirphia eumedide*.

These differences in age-dependent behaviour are likely to have certain consequences for moths in wild populations. Saturniid moths do not feed as adults, and are dependent on reserves of food which they contain at hatching. The rate at which these reserves are utilized must relate to the general excitability of the moths. High excitability associated with 'soft' scaling in the cryptic species must accelerate the obliteration of the colour patterns by mechanical damage to the wings, and simultaneously increase the vulnerability to predators. In the case of the behaviourally stable aposematic species with 'hard' scaling, the moths may be largely protected from an age-dependent increase in vulnerability. It is possible on these grounds that in Nature there is a greater disparity between the life-tables of cryptic and aposematic species than the present data would indicate; this, however, can only be determined by marking and recapture experiments.

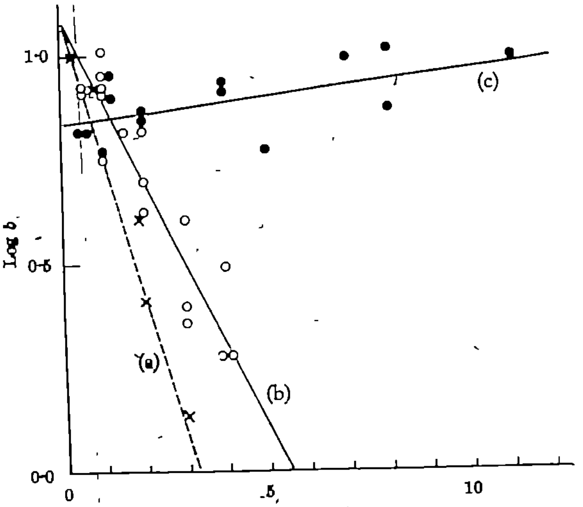

Fig. 2. Age-dependent changes in the values of b in the equation $y = a + bx$ for (*a*) *Lonomia cynira*, (*b*) *Automeris junonia*, and (*c*) *Dirphia eumedide*. Values of log b are plotted against the adult age in days

One feature of these results is of interest, for it bears on the general interpretation of ageing changes. Natural selection has been shown to have modified the adult longevities of the hemileucines studied; since the colour patterns and display behaviour of the aposematic species are the most highly elaborated in the group, and their larvæ are the most specialized, the direction of change has probably been to lengthen the life-spans from the condition found in *Automeris*. In principle, selection could modify an adult life-table of the type found for *Automeris junonia* in at least two ways: (1) The maximum age at death remains the same, but the age of onset of dying is progressively postponed. The life-table develops a plateau. The change resembles that found in the survival curves of a human population with increasing medical care[10]. For the present material, this would correspond to a hypothetical case in which the maximum adult longevity is determined by the food reserves available at hatching, which are not alterable by selection, but in which the moths are subject to lethal physiological 'accidents' with an age-dependent incidence which selection can postpone. (2) The age of onset of dying and the maximum age are shifted by an equal amount, and the rate of dying remains constant. The life-tables would resemble those found in *Drosophila* and mouse populations when breeding procedures have incidentally modified their life-spans[11].

The life-tables for the hemileucines unequivocally belong to the second category; apart from their displacement in time, they are remarkably similar. In contrast, the patterns of behavioural ageing are not homogeneous. It follows that the latter cannot be used as a fundamental measure of senescent change, whatever differential effect they may prove to exert on the mortality of wild populations. Instead, it seems that the programmed changes in the central nervous systems of the moths have been adjusted by selection to fit their overall ecology.

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BEHAVIOUR OF YOUNG *OCTOPUS BRIAREUS* ROBSON

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OCTOPUS briareus is of interest to students of cephalopods as it has no pelagic larval stage in its development: the young, which emerge from large eggs, are benthic from the moment of hatching. As they are relatively large, their behaviour can be usefully compared with that of an adult octopus. This species, described first by Robson[1,2], and later, more critically, by Pickford[3,4], is a shallow, warm-water form from the Caribbean, common along the Florida Keys[5]. This article reports observations made on living adult and young *O. briareus* at the Marine Laboratory of the University of Miami in April 1962.

A female, of mantle-length about 60 mm, and her clutch of eggs had been taken locally and installed in a tank with running sea-water. There were about 130 eggs, each with a long (10-mm) stalk: bunches of up to about 20 eggs were formed by the individual egg-stalks twisting around one another to form a long (18 mm) greenish strand, which attached the eggs to a rock. The bunches hung down in the water and they were closely guarded by the female, who refused to feed or leave the eggs during the period of observation. She continually agitated them by movements of the arms and body. The eggs were pearly, by reflexion, and semi-transparent, so that it was possible to see the chromatophores on the body of the unhatched animal: the empty egg-cases were white.

It has been estimated that *O. vulgaris* lays about 150,000 eggs[6], each about 2·5 mm long, which produce larvæ of about 1·5 mm[7]. It is interesting to note, therefore, that *O. briareus* produces far fewer eggs, the dimensions of which are relatively enormous. The empty egg-cases measure 12 mm × 6 mm, excluding the stalk; the newly hatched animals have an arm-span of almost 17 mm and a mantle-length of about 5·5 mm and are, in their bodily proportions, smaller versions of an adult octopus and quite unlike the planktonic larva of *O. vulgaris*. Hatching was observed in the laboratory and although it was probably premature, the behaviour observed confirms Voss's observations[5] that the animals never pass through a planktonic stage.

The young animals emerge backwards from the lower, blunt end of the pyriform egg and immediately swim or crawl downwards, often blowing ink at the same time. They have conspicuous eyes, often surrounded by a dark ring, and they change colour as rapidly as adult octopuses. There are fewer chromatophores per unit area than in the adult, so that the animals have a speckled appearance; there is a conspicuous double row of large chromatophores down each arm. Waves of chromatic activity can be elicited by sudden movements of the observer at distances of up to 2 ft., and rapid approach of a hand is followed by reflexion of the arms, paling and spreading of the interbrachial membrane. This 'dymantic response'[8] thus appears to be innate. It is conceivable however that it is acquired during the last stages of life in the transparent egg, although the usefulness of such a response then is not very obvious. It is noteworthy that in *Sepia*, which has an opaque (black) egg, no dymantic response has been observed in very young animals[9].

The newly hatched young seldom move, and usually sit, with head uppermost, on the side wall of the aquarium at the air-water interface, sometimes half out of the water. Commonly the arms are spread backwards and gathered around the body, so that the outline of the animal is roughly circular. When disturbed the animals walk away rapidly and jerkily; they may eject ink. Only rarely do young *O. briareus* swim. This might be because of their innate tendency to cling strongly to a surface, here usually vertical, a tendency which, together with their ability to change colour, must afford them considerable protection from enemies. Indeed, the very fact that *O. briareus* lays so few eggs suggests that the survival rate of the young is very high; large numbers of eggs are laid not only by *O. vulgaris* but also by other octopods producing planktonic larvæ[10]. Certainly the tendency to adhere to a surface is so marked in *O. briareus* that it was possible to lower the water-level in their tanks gradually without causing the animals to move down with it, even though this resulted in their being left completely high and dry.

The feeding behaviour, too, reflects this tendency to remain attached; the animals always seized the prey with a few arms and anchored themselves with the remainder. They never swept down on the prey like adult octopuses and indeed many feedings were the result, not of an attack by the octopus, but of the prey accidentally entangling itself with some of the arms. When this

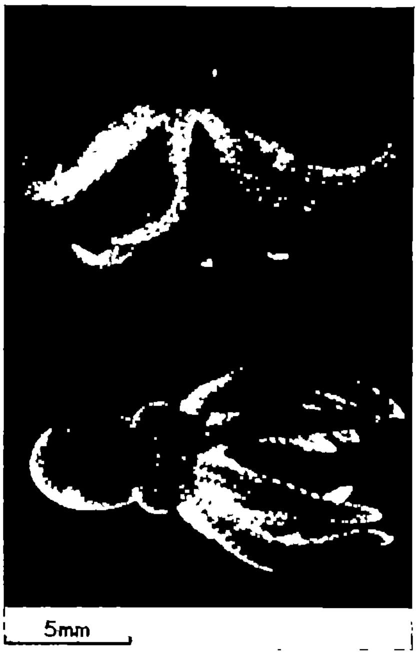

Fig. 1. Above, fixed specimen, 16 days old note the prominent eye, large funnel and conspicuous chromatophores; below, fixed specimen, 2 days old, showing the double row of large chromatophores down each arm and the well-developed suckers

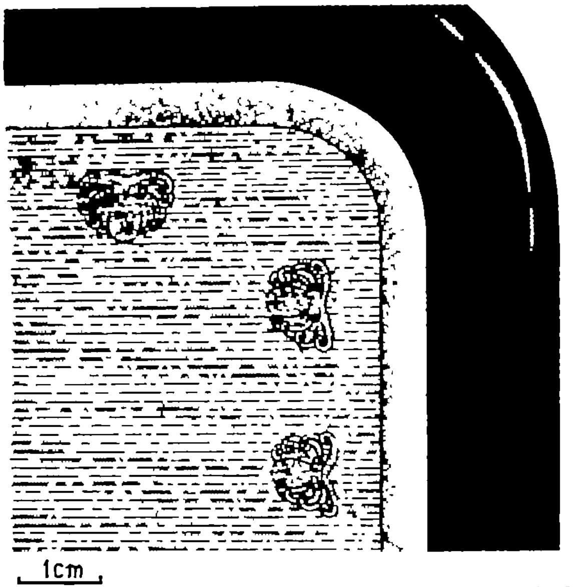

Fig. 2. Drawing, from Kodachrome transparencies, of living animals —6 days old—in a typical resting position

happened other arms would be brought round to the prey to pass it under the large interbrachial membrane in the usual way, in which process the octopus might let go of its substrate for a moment. Although the animals rarely attacked living prey, they could be relatively easily 'force fed'. In 'force feeding' a very small piece of freshly killed shrimp was offered to an arm with fine forceps or at the end of a fine thread. The arms and suckers explored the meat and the arm quickly passed it under the web to the mouth.

Live food comprised crustaceans, less than 10 mm in length, such as *Latreutes fucorum*, *Leander* sp., *Hippolyte* sp., and some unidentified amphipods; *Artemia* (young and adult) were also tried, but, like the other species, never elicited 'good' attacks. Nor did offering them result in any obvious visual fixation of the kind visible in *Sepia* when they are presented with mysids.

Adult *O. briareus* eat bottom-shrimps, bivalves, crabs, probably gastropods and occasionally polychaetes[11], but nothing is known about the diet of the young animals though this is of critical importance if rearing these animals in the laboratory is to be done or if behavioural investigations are contemplated. In this latter context it is unfortunate that the question of innateness of response by *Octopus* to crabs could not be clarified as it is of some importance[12]: no crabs small enough to present to these octopuses as food were found, however.

No yolk sac was visible at hatching (although the eggs hatched prematurely) so that we might expect the young to start feeding at once unless there was a sufficient supply of yolk internally to last for a day or two. No experimental evidence for this was forthcoming; indeed the animals seemed no more likely to attack the food offered after ten days than after two, or after several 'force feedings'.

That attacks were so rare may have been because of failure to offer the natural food; but it would be unwise to assume that the choice of prey is highly specific (cf. *Sepia*[13]). The animals might be scavengers and detritus feeders at this stage.

Generally young *O. briareus* are very inactive and only two other types of behaviour deserve mention. First, 'attention' movements[14]—raising and lowering the head several times in rapid succession—were seen on some occasions, generally coincident with movements of the observer. Secondly, two octopuses were sometimes seen to approach one another head on, and entangle the arms, which flailed wildly. Changes in colour occurred, but no consistent pattern of change was seen.

In Europe, the behaviour of adult *O. vulgaris* has been the subject of intensive analysis, but the larvæ, because they are planktonic and minute, are difficult to examine: it is therefore impossible to assess the relative importance of innate and acquired components in the behaviour of the adult, although this is clearly of great significance. This difficulty does not arise in *Octopus briareus*, and if more could be learnt about the food requirements, so that the young could be reared in the laboratory, this species might be an ideal one for workers investigating the innate components of behaviour in octopods.

This work was done while I was in the Department of Zoology, Yale University, New Haven, Conn. I thank the Fulbright Commission for providing a grant for travel to the United States, and Dr. Grace E. Pickford for her considerable help while I was at Yale. I also thank Dr. G. L. Voss and the Director and staff of the University of Miami Marine Laboratory for their kind help and hospitality, and Prof. J. Z. Young, and Mr. B. B. Boycott for their helpful comments on the manuscript.

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NEW FOSSIL HOMINOID MATERIAL FROM UGANDA

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UP to 1962, the published record of large hominoid fossils of Mid-Tertiary age was limited to ten specimens consisting of teeth and mandibular fragments found in Kenya. Six of these were designated *Proconsul major* by Le Gros Clark and Leakey[1], and the other four provisionally ascribed to *P. major* by Le Gros Clark[2]. Of these ten pieces unfortunately only two specimens, single left and right M^3, represent the upper dentition.

In Uganda a single right upper canine collected in 1958 from Napak (34° 17′ E., 2° 5′ N.) has been assigned recently to *P. major* by Leakey[3].

In August 1961 an expedition from the Department of Geology, University of Glasgow, visiting Karamoja District, Uganda, collected five further hominoid specimens at Napak and two specimens from a locality to the north of Moroto Mountain (34° 45′ E., 2° 32′ N.). The

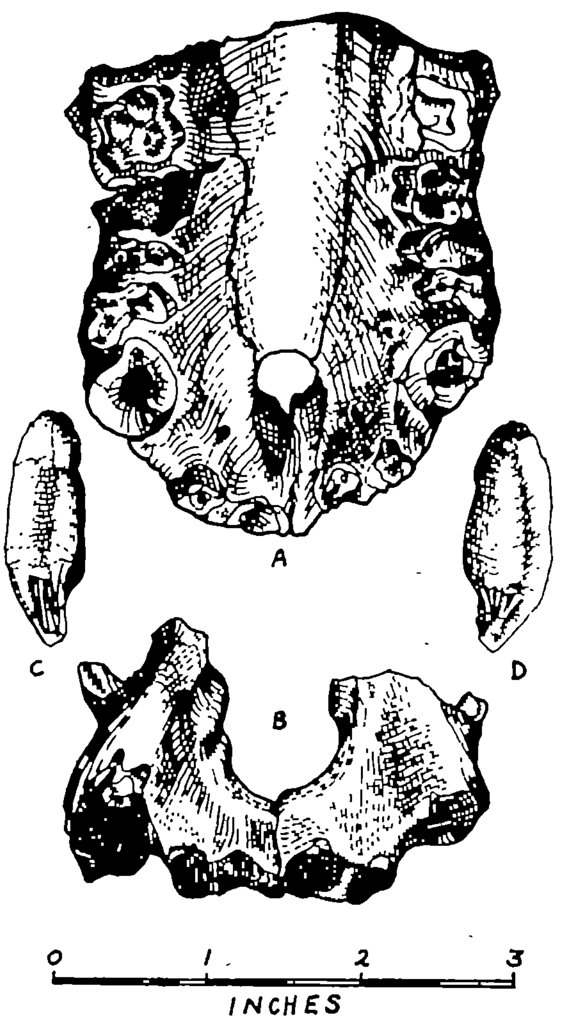

Fig. 1 Specimen *UM P*.62-11 in palatal view (*A*) and front view (*B*). Specimen *UM.P*.62-12 in labial view (*C*) and lingual view (*D*) (Drawings by Mr. W. Serumaga)

preliminary stratigraphical and palæontological results of the expedition and the location of the sites have already been described by Bishop and Whyte[4].

In December 1961 a party from the Department of Anatomy, Makerere University College, re-visited the Moroto site and recovered four additional fragments of the upper dentition of a large hominoid. Three of these pieces and one of the specimens recovered during the Glasgow expedition (all of which were found on the surface within a few feet of each other) proved to be contiguous left and right maxillæ and two other adjoining fragments from a single individual. The fragments have been joined together and registered as one specimen (*UM.P*.62-11).

In August 1962 a party of the Brathay Exploration Group visited Napak and found the broken crown of a large primate molar.

Thus, a total of ten specimens of large fossil hominoid, many of them representing the upper dentition, are now known from Uganda localities. They are registered in the collections at the Uganda Museum, Kampala (*UM.P*.62-03 *et seq*.). The purpose of this article is to give a preliminary description of this material, which comprises the reconstructed maxillæ together with two other pieces from Moroto and seven specimens from Napak.

The Glasgow expedition was financed by a Royal Society scientific grant-in-aid and the Makerere College work by a grant from the Wenner-Gren Foundation for Anthropological Research and the Wellcome Trust, to whom grateful acknowledgment is made. We are indebted to Sir Wilfred Le Gros Clark, Dr. L. S. B. Leakey and Mrs. S. Coryndon for helpful comments and criticism, and to members of the Anatomy Department, Makerere University College, in particular, Dr. E. C. B. Hall-Craggs for practical assistance.

(1) Specimen *UM.P*.62-11, Moroto II, August and December 1961

Osteology. The reconstructed specimen is undistorted and comprises most of the middle facial skeleton and a part of the upper dentition. The reconstruction is made up of two large fragments and two smaller fragments from either side. The specimen consists of the lower part of the nasal aperture, the incisor region, the maxillæ on both sides as far back as M^2, and the palate, excepting the median 1/3 caudal to the incisive canal (Fig. 1*A* and *B*).

There is an intermaxillary suture. The incisor bearing premaxilla is wide, relative to the bicanine width but is relatively short rostrocaudally. Diastemata are present between the medial and the lateral incisors and the latter and the canines. Sub-nasal prognathism is not marked. The presumed middle part of the nasal aperture is the widest. Its margin at the midline smoothly leads on to the external alveolar surface of the premaxilla. Only in the infero-lateral margin of the nasal aperture is there the beginning of an edge. Canine fossæ are just discernible, but the external aspect of the maxilla is rounded and filled out because of the massiveness of the canine roots. The external and medial face of the right premaxilla is roughened and the incisive fossa and prominance for the root of the medial incisor (obvious on the left) is not present. The maxillary sinuses extend rostrally to the level of P^4 and are still large at the anterior roots of M^2. The palate is shallow but the palatal processes are thick opposite P^4 (8 mm) and are still 4 mm thick at M^2. The inferior meatus is deep (average of 12 mm) and wide (27 mm at M^2-level). The incisive canal is large even though the caudal border on one side is incomplete. It averages 6·5 mm in diameter. The partial remains of the maxillary-premaxillary suture are visible. The width of the palate at the level of the canine teeth is 37 mm, at P^3 (the widest part) it is 39 mm, and the estimated width at M^2 is 35 mm.

Dentition. All the incisors and both canines are broken near the alveolar margin. The roots of the left incisors are stout, especially in the anterior–posterior direction and the alveolar spaces between them suggest that they had spatulate crowns. On the right the medial incisor root is small and fragmented. On the left, the apex of the root of the canine is exposed as a small disk embedded in dense maxillary bone. Both pre-molars are broken at the alveolar margin, the roots only of M^1 are present, the whole of M^2 is preserved and the anterior sockets of M^3 are present. On the right P^4 and M^1 are complete but show moderately advanced attrition, obscuring the details of the cusp pattern. M^2 is broken level with the alveolus and the anterior labial socket of M^3 is present. It is, therefore, possible to reconstruct tentatively the position of the upper dentition in which the sides of the arch are parallel or may even converge a little posteriorly.

The dimensions of the teeth are given in Table 1.

The premolars are narrow antero-posteriorly as they are in *Proconsul* maxillary specimens *R*.1948, 50 (*R*.106) and *CMH*.2. Right P^4 is stout though the roots are not so robust as those of P^3. The principal (labial) cone exceeds

Table 1. TEETH OF SPECIMEN *UM.P* 62-11

	Left			Right			
	A.P.	Tr.	Ind.		A.P.	Tr.	Ind.
I^M Cervix	10·0	9·0		Crushed			
I^L Cervix	10·0	7·2		Crushed			
C Cervix	19·5	16·0	(82)	Cervix	19·0	16·0	(84)
P^3 Cervix	10·2	15·7		Cervix labial palatal	9·0 7·0	14·5	
P^4 Cervix	6·3	15·8		Crown	7·5	14·0	187
M^1 —	—	—		Crown	11·0	12·0	109
M^2 Crown	12·9	13·8	107		—	—	—
M^3 Root socket est.	14·0	17·0			—	—	—

the worn deuterocone in height by 3·5 mm and is connected to it by a single ridge. An anterior and an external cingulum are present, though not very distinct. The dimensions are larger than P^4 in *Proconsul nyanzae* (*CMH.2*) though the proportions are similar.

Right M^1 is stout and worn. The worn concavity in the position of the protocone dominates the abraded surface of the crown and the cavity in the position of the hypocone is half its size. The metacone is relatively unworn and the paracone is low, flattened and unworn. The internal cingulum has been removed by attrition. The dimensions are greater than in the corresponding tooth of *CMH.2* (Table 3).

Right M^2 is broken at the cervix, and the medial roots are also absent. Left M^2 has a prominent crenated internal cingulum, an external cingulum, a large worn protocone, small unworn paracone, metacone conical and unworn and a large worn hypocone. The tooth is similar in morphology to M^2 in *CMH.2* of *P. nyanzae* though larger, but is smaller than *CMH.35* (*S*) and *S*.381'49 provisionally assigned to *P. major* by Le Gros Clark and Leakey[1,3] (Table 3).

*Discussion of UM.P.*62-11. By comparison with palatal and maxillary dimensions of modern gorilla and chimpanzee material, it is probable that the individual was of generally similar size to a full-grown female gorilla.

The freedom from post-mortem distortion of *UM.P.*62-11 is of particular importance. Unfortunately, both of the adult specimens of *P. africanus* (*R*.1948,50,*R*106) and *P. nyanzae* (*CMH.2*) which are relevant for comparison have suffered some post-mortem deformation. This raises doubt as to the original morphology of the face and palate. Such does not apply to the juvenile specimen (993.1947) of *P. nyanzae* described by Le Gros Clark and Leakey[1], or to the sub-adult maxilla of *P. africanus* (*Rs*.51/1499) described by Napier and Davies[5]. The slight malformation of the right premaxillary region in *UM.P.*62-11 does, however, result in some distortion of this side of the inferior nasal aperture.

With this caveat, comparison indicates the following skeletal and dental features in which the present specimen is similar to, or differs from, the *Proconsul* specimens so far described.

(1) Comparison of the dental arcade with the known adult forms of *Proconsul* is not valid because of the distortion of the latter. The arcade resembles that of the juvenile *P. africanus* and *P. nyanzae* in that the lines of the cheek teeth appear parallel or may even converge slightly posteriorly (*vide* Plate 2 of ref. 5). A similar-shaped arcade is found in modern cercopithecoid monkeys.

(2) The palate is shallow. It is deeper in the specimens of *Proconsul africanus* (*R*.1948,50,*R*106) and *P. nyanzae* (*CMH.2*) but these have suffered compression or shearing of the palatal bones.

(3) The nasal aperture is distinctly less cercopithecoid than the available adult *Proconsul* specimens. Although not like a modern cercopithecoid monkey neither is it typical of a modern adult pongid. It is most similar in form to that of a young chimpanzee, in which the morphology of the nasal aperture is not obscured by the need to buttress the stout, curved canine roots found in the adult.

(4) The external maxillary surfaces are smoothly curved to accommodate the straight and robust canine roots. There are barely discernable external depressions just lateral to the canine roots (the canine fossæ). In the *Proconsul* specimens both canine and incisive fossæ are present although in the sub-adult *Rs*.51/1499 described by Napier and Davies[5] they are shallow.

(5) Sub-nasal prognathism is slight. The skull of *P. africanus* from Rusinga was described as prognathous but this feature 'may be slightly exaggerated by warping' as noted by Le Gros Clark and Leakey[1] and discussed further by Robinson[6]. In the sub-adult *Rs*.51/1499, prognathism is less pronounced.

(6) The incisive canal is large and resembles that of the modern cercopithecoid monkeys. The deformed palate of the adult *Proconsul africanus* of *Rs*.51/1499 is cercopithecoid in appearance.

(7) Comparison of the dental characteristics with those in named *Proconsul* specimens shows marked similarity. The sections of the roots of the broken canines are narrowed in the labila-lingual direction and are similar to the canine tooth (*UMP*.62-03) ascribed to *P. major* by Leakey[3]. The proportions of the pre-molars are similar to those of *Proconsul*, showing antero-posterior narrowing and the cusps, though worn, are also characteristic. The cusp patterns of the molar teeth are like those ascribed to the genus. The dimensions of the cheek teeth are all greater than those of *P. nyanzae* (Tables 2 and 3).

Table 2. UPPER CANINES FROM MOROTO AND NAPAK

		A.P.	Tr.	Index	Length actual	Length reconst.	Crown height
M	*UM.P.*62-11	(19·0)	(16·0)	(84)	—	—	—
O	Right canine. Cervix						
R	*UM.P.*62-11	(19·5)	(16·0)	(82)	—	—	—
O	Left canine. Cervix						
T	*UM.P.*62-12	17·0	16·0	94	51	—	20
O	Left canine. Crown	(18·0)	(16·0)	(89)			
N	*UM.P.*62-05	19·0	16·0	84	50·7	55·5	22
A	Left canine. Crown	(20·0)	(17·0)	(85)			
P	*UM.P.*62-04	19·5	16·3	84	53·3	56·0	22
A	Right canine. Crown	(20·3)	(17·5)	(86)			
K	*UM.P.*62-03	16·5	13·5	80	46·0	52·0	20
	Right canine. Crown	(17·5)	(14·5)	(83)			
Proconsul nyanzae. Upper canine *CMH.2* Holotype		15·0	12·0	80	—	—	24
Proconsul africanus. Upper left canine *BM.M*14084 Holotype		11·3	9·1	80 5	—	—	15

Figures without brackets refer to measurements taken at the base of the crown while those inside brackets give dimensions approximately in the same plane, as the exposed fractured canines of *UM.P.*62-11.

Table 3. UPPER MOLARS FROM MOROTO AND NAPAK

	A.P.	Tr.	Index	Crown height
*UM.P.*62-11 Right M^1	11·0	12·0	109	7·0
*UM.P.*62-07 Right M^1	11·6	13·0	112	6·6
*UM.P.*62-11 Left M^2	12·9	13·8	107	7·0
UM.P 62-08 Left M^2	15·2	17·2	113	7·8
S.381'49 Left M^2	14·8	16·7	113	8·0
CMH 35(*S*) Right M^2?	12·7	15·3	120	—
Holotype of *Proconsul nyanzae* *CMH.2* Average M^1	9·0	11·5	128	6·0
Average M^2	12·0	13·0	108	6 0

The dental characters of *UM.P.*62-11 are compatible with the diagnosis of a large form of the genus *Proconsul*, while the bone morphology is cercopithecoid though still compatible with the genus *Proconsul*.

In view of the variability of the skeletal morphology in the modern anthropoid apes, it seems desirable to avoid setting up new genera for their fossil antecedents until there is clear comparative evidence that a new genus (or even species) is required. This is particularly true in view of the limited material available and until topo-typical maxillary specimens of *Proconsul major* are found from Songhor in Kenya.

With this proviso the specimen *UM.P.*62-11 is provisionally assigned to *Proconsul major*, which was defined by Le Gros Clark and Leakey as 'approximating in size to a gorilla' with the definitive feature being 'the great size of the teeth and mandible' by comparison with *Proconsul nyanzae*[1].

The qualifying statement of Le Gros Clark, concerning the difficulty of assigning to a particular taxon, isolated teeth and small fragments, might well be repeated here[2]. However, for similar reasons to those given above for *UM.P.*62-11, the following nine specimens are also provisionally assigned to the species *Proconsul major*.

(2) Specimen *UM.P.*62-10, Moroto II, August 1961

A fragment of massive right mandibular ramus. The dimensions of the specimen measured at the base of the ascending ramus are *AP*. 31·9 mm, *Tr*. 22·8 mm. The maximum depth of the broken mandible is 24·2 mm. M^2 and M^3 have been broken level with the alveolus. The maximum transverse width of the socket of M^2 is 12·8 mm and of the anterior socket of M^3 13 mm.

(3) Specimen *UM.P.*62-12, Moroto II, December 1961

A little worn left upper canine broken slightly at the tip of the root and having a length of 51 mm (Table 2). It is identical in morphology to the description given by Hopwood[7] in referring to the type canine of *Proconsul africanus* but is approximately one and a half times as large (Fig. 1*C* and *D*). Cross sections of *UM.P.*62-12 drawn in the same plane as the upper and lower fractures of the roots of the canines in *UM.P.*62-11 are similar in size and form to this specimen.

(4) Specimen *UM.P.*62-03, Napak I, June 1958

A right upper canine slightly broken at the distal and proximal ends and having an actual length of 46 mm. Reconstruction suggests that the original length was probably about 52 mm (Table 2). The morphology is similar to the Moroto II canine *UM.P.*62-12, but the tooth shows pronounced wear facets on the posterior and antero-lingual faces of the crown. This specimen has been attributed to *Proconsul major* by Leakey[3].

(5) Specimen *UM.P.*62-04, Napak V, August 1961

A right upper canine slightly broken at the tip of the crown having a reconstructed length of 56 mm (Table 2). Very similar in size and morphology to *UM.P.*62-03 and 62-12 but with a rather more robust root.

(6) Specimen *UM.P.*62-05, Napak V, August 1961

Left upper canine. The left counterpart of *UM.P.*62-04 and identical in morphology in every respect (Table 3). Cross-sections of these two canines at the appropriate levels are almost identical to those of the broken canines of *UM.P.*62-11 (Table 2).

(7) Specimen *UM.P.*62-06, Napak V, August 1961

A fragment which is tentatively ascribed to *P. major* consisting of the anterior left mandibular ramus of a juvenile, broken in the region of the symphysis. The specimen has been badly broken along the alveolar margin exposing the unerupted left I^2. The broken socket of the left canine is visible together with fragments of the broken roots of the deciduous cheek teeth. The unerupted germ of what is possibly M^1 can be observed deep in the mandible through a break in the bone. The maximum depth of the mandibular fragment is 28 mm and its total thickness 15 mm. The symphysial region shows the beginning of rostro-caudal thickening but there is no evidence of a simian shelf. The unerupted incisor has a crown height of 13 mm and is markedly triangular in A.P. section. The transverse width of the biting edge is 6·0 mm and the A.P. width at the base of the crown is 7·0 mm.

(8) Specimen *UM.P.*62-07, Napak V, August 1961

A right first upper molar exhibiting moderate wear of the cusps. The lingual root is present, although broken at the tip, and is widely splayed laterally. Both anterior and posterior pressure facets are present. Although the tooth is considerably less worn its cusp pattern and proportions are similar to the right M^1 of *UM.P.*62-11. In general morphology it recalls *CMH.*35, tentatively designated right M^2 (?) of *P. major* by Le Gros Clark and Leakey (Table 3) and it is considerably larger than M^1 in the holotype of *P. nyanzae*.

(9) Specimen *UM.P.*62-08, Napak V, August 1961

The crown of a left upper second molar showing moderate wear and with well-marked anterior and posterior pressure facets. The details of the morphology agree perfectly with the description of the left M^2 *S.*381′49 provisionally ascribed to *P. major* by Le Gros Clark[2]. The Napak tooth is rather larger but the indices are identical (Table 3). It is larger than M^2 of *UM.P.*62-11 and rather less worn, but again there is a pronounced similarity in the details of the tooth morphology and in the indices.

(10) Specimen *UM.P.*62-09, Napak I, August 1962

Fragment of the crown of an unerupted left molar (? M^2), comprising approximately the labial half of the crown. A pressure facet is present on the anterior aspect of the tooth, but the deeply crenated cusp enamel of the occlusal surface shows no sign of wear. The anterior-posterior measurement of the tooth is 15 mm and as the cusp pattern resembles that of *UM.P.*62-08, the fragment is also tentatively ascribed to *P. major*.

Distribution

P. major has been found at four sites. On Rusinga Island from a total of 124 specimens of *Proconsul* only two teeth are attributable to *P. major* while at Songhor the proportion is eight teeth or jaws of *P. major* from a total of 37 *Proconsul* specimens. At Napak the total is 7 finds of *P. major* from a total of only 10 *Proconsul* remains; at Moroto all three primate finds (or 6 pieces prior to reconstruction) are of the large species. At the last two sites the totals are small and are undoubtedly over-shadowed by the occurrence of several pieces representing one individual. However, the lack of many specimens of the large species at Rusinga, the rise to almost 25 per cent at Songhor and the dominance of *P. major* at the two Uganda localities may imply variations in the abundance of this creature possibly resulting from different ecological conditions.

Conclusions

Ten primate fossils found at Mid-Tertiary sites in Karamoja, Uganda, are provisionally assigned to *Proconsul major*.

The bones are thick and massive but the morphology has cercopithecoid features. The upper canine teeth are pongoid and are similar in form to those of *Proconsul africanus* although larger, having stout uncurved roots. The available upper canines of *P. nyanzae* appear to have narrower roots and rather higher crowns. The cheek teeth cusp patterns and crown proportions are precisely those of the genus *Proconsul* in every major particular. When allowance is made for sexual dimorphism and the fact that the two sites of Moroto and Napak are more than 50 miles apart and possibly do not represent precisely contemporaneous periods of fossilization, the material can be seen to constitute a remarkably homogeneous series of specimens.

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LETTERS TO THE EDITOR

PHYSICS

A Direct Terrestrial Test of the Second Postulate of Special Relativity

THE following is a preliminary report of an investigation performed to test directly, in a terrestrial experiment, the second postulate of special relativity, which states that the velocity of light is independent of the motion of the light source. A direct test means here that the velocity of the light from a moving source is measured by a time-of-flight technique and not by use of interference effects in a closed light path or a frequency measurement. Investigations of the last-mentioned type may lead to difficulties in the interpretation of the result and are therefore not very satisfactory, as pointed out by, for example, H. Dingle[1].

In testing the second postulate it may be of advantage to use γ-radiation instead of ordinary light. One should thereby avoid possible effects caused by interaction between the light and stationary material situated between the source and the detector, as discussed, for example, most recently by J. G. Fox[2]. According to this author it may be that older evidence for the second postulate obtained from ordinary light experiments as, for example, the well-known double star evidence, are impaired with systematical errors which would weaken their reliability.

The principle of the investigation here undertaken was to measure the velocity difference between γ-rays from recoiling nuclei and nuclei at rest.

The moving γ-ray source was ^{12*}C excited in the reaction ^{12}C$(\alpha,\alpha')^{12*}$C and the γ-ray source at rest was ^{16*}O from the reaction ^{16}O$(\alpha,\alpha')^{16*}$O. The 14-MeV α-beam and the time-of-flight equipment of the 80-cm cyclotron of the Nobel Institute of Physics were used for the measurement[3]. The targets were placed at 30 cm from each other in the beam direction and could be interchanged in a few seconds. Half the beam intensity was used for the ^{12}C target and half for the ^{16}O target. The time delay between the irradiation of the two targets was about 12 ns.

As the half-life of the 4·43-MeV level in ^{12}C is $6 \cdot 5 \times 10^{-14}$ sec (ref. 4), the γ-ray is emitted before the nucleus has been stopped. The half-life of the 6·13-MeV level in ^{16}O, on the other hand, is $1 \cdot 2 \times 10^{-11}$ sec (ref. 4), so that the nucleus is at rest when this γ-ray is emitted. Doppler shift measurements with a sodium iodide crystal showed that the mean forward velocity of the recoiling ^{12}C nucleus was $(1 \cdot 8 \pm 0 \cdot 2)10^{-2}$ c, where c is the velocity of light in vacuum. In the case of ^{16}O the Doppler shift measurements confirmed that the nucleus was stopped before emission of the 6·13-MeV γ-ray. A small amount (< 15 per cent) of 7-MeV γ-rays from ^{16}O was also present. The influence of these on the measurements could be corrected for, however.

In the time-of-flight experiment the γ-rays were detected by a liquid scintillator (5 in. × 2 in.) located at a mean distance of 5 m from the target arrangement and at an angle of 10° with respect to the α-beam. A small plastic scintillator, placed at 1 m from the target arrangement, was used in parallel with the large detector to give reference peaks in the time spectra.

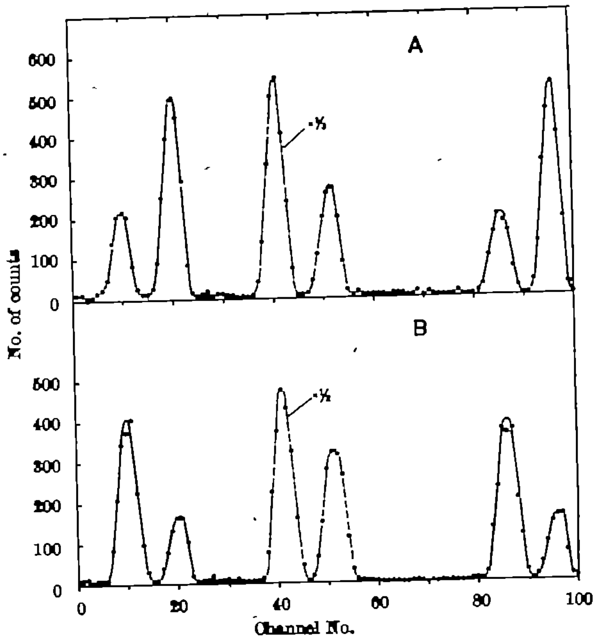

Fig. 1. Time-of-flight spectrum of the γ-rays detected in the small counter (dashed curve) and large counter. Channel width, 1·1 ns. *A* shows the case when target ^{12}C is first reached by the beam; *B* when the two targets have been interchanged

In Fig. 1*A* is shown an example of a time-of-flight spectrum when target ^{12}C is first reached by the beam. Fig. 1*B* shows the case when the targets have been interchanged. The small detector gives rise to the two peaks in the middle of the spectra while the large detector is responsible for the two other groups. The distance between these corresponds to one cyclotron period. If the distance between the centre of gravity of the two peaks in each group is τ_1 in Fig. 1*A* and τ_2 in Fig. 1*B* then $\tau_1 = \tau_2$ if the velocity of the γ-radiation is independent of the motion of the source. On the other hand, if the velocity of the γ-rays is $V = c + kV_s$, for example, then $\Delta\tau = \tau_1 - \tau_2 = 2S \times V_s \times k \times c^{-2}$, where k is a constant and V_s the source velocity. With $k = 1$ and $S = 4$ m (the distance between the two detectors), $\Delta\tau$ would be 0·5 ns.

From several different runs the following mean value has been found:

$$\Delta\tau = -0 \cdot 2 \pm 0 \cdot 2 \text{ ns}$$

A systematical deviation (in both detectors) of $\Delta\tau$ from zero, independent of the source to detector distance, was present. This systematical error causes the uncertainty of the measurements to be somewhat larger than that predicted from time resolution of the equipment and statistics alone. It is hoped that this systematical error will be eliminated in the continuation of these measurements of which a more detailed report will be given in *Arkiv Fysik*.

The result of this investigation is in agreement with the second postulate but does not agree with the result of a recent work by W. Kantor[5]. It would certainly be of interest to perform a test where the source velocity is larger than that used in the present measurement, as, for example, with a π^0-source as suggested by, among others, W. G. V. Rosser[6].

We thank Prof. O. Klein and Dr. K. G. Malmfors for valuable discussions.

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Change in Velocity of Light emitted by a Moving Source

SOME interest has been aroused recently by a paper by W. Kantor[1], describing an experiment to detect the change in the velocity of light emitted by a moving source. The author explains his results on the hypothesis that the velocity of light as it leaves the moving surface of a glass block through which it has been transmitted is given by the expression $c' = c + \rho v$, where c is the usual velocity of light, v the velocity of the glass surface in the direction of motion of the light beam, and ρ is some constant, equalling 1 on classical theory and 0 according to relativity. The experiment described then shows an effect which can be explained by assuming $\rho = 2/3$.

We have recently constructed an apparatus to test this hypothesis. White light is passed through a slit and is then collimated. It then passes through a semi-circular disk of glass which is rotated by a high-speed electric motor about an axis in its own plane, the axis of rotation being normal to the direction of motion of the light beam. It is then possible to show quite simply on the foregoing hypothesis that the wave front emerging from the apparatus will be moving more slowly on one side than on the other, and so should rotate as it is propagated.

The light was received in a telescope which could be placed at different distances from the apparatus, and the deviation of the light could then be detected by comparison with the image which had not passed through the disk. The angle of deviation is $\theta = \rho w x / c$, where w is the angular velocity of the glass disk, and x is the distance of the telescope from the disk.

Under the conditions of our experiment $w = 1{,}500$ ($= 14{,}300$ r.p.m.), x, the distance of the telescope from the disk $= 20$ m, and $c = 3 \times 10^{10}$ cm/sec, so that if $\rho = 1$, $\theta = 20$ sec of arc. This is of course easily detectable in a moderate-sized telescope, and in our case the minimum angle detectable was 0·5 sec, the limit being set by the size of the diffraction pattern seen in the telescope.

At no time was any deviation of the light observed and we conclude that ρ in this case is less than 0·025, if indeed it is different from zero.

Special relativity predicts a small effect of 0·0008 sec of arc due to Fresnel drag in the glass, but this deviation is too small to be detected by this technique.

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Sub-sieve Sizing by Sedimentation Balance

IN determining the size distribution of particles in samples of finely ground barytes, difficulties have been experienced in determining experimentally the ultimate balance reading corresponding to sedimentation equilibrium[1]. It takes an inordinately long time for equilibrium to be attained, and even then this does not necessarily represent complete sedimentation of all solid particles. Consequently, even at equilibrium the balance reading (S_∞) will be less than the theoretical maximum reading corresponding to the weight of solid introduced (S_m).

In these circumstances, sub-sieve sizing by sedimentation balance can become a protracted and inconvenient operation if the conventional gravimetric methods of determining S_∞ and S_m are adopted. However, some preliminary success has been achieved in these laboratories by using forms of exponential functions to determine S_∞, permitting termination of the experiment long before sedimentation equilibrium is attained and also obviating any need to evaporate liquid and weigh dry samples.

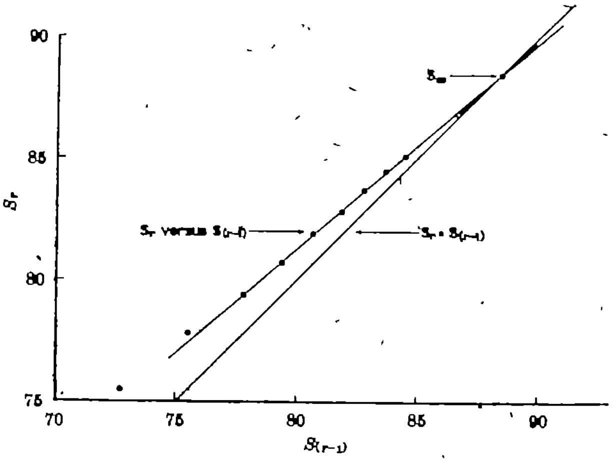

Fig. 1. Graphical determination of S_∞ ($\Delta = 5$ min)

It is our experience that in many cases the upper 10–20 per cent of the sedimentation curve can be described by the equation:

$$S = S_\infty (1 - e^{-bt}) \tag{1}$$

where t is time, S is the balance reading and b is a rate constant.

This equation may be represented by the pair of equations:

$$t_r = t_{(r-1)} + \Delta \tag{2}$$

and

$$S_r = S_{(r-1)} \cdot e^{-b\Delta} + S_\infty (1 - e^{-b\Delta}) \tag{3}$$

where r is an integer and $\Delta > 0$ and is constant[2].

A solution of equation (3) is $S_{(r-1)} = S_r = S_\infty$, and by plotting S_r against $S_{(r-1)}$, S_∞ is readily obtained; b is not of significance in this instance. The experimental results given in Table 1 are plotted in this manner in Fig. 1; the point of intersection of the experimental line, S_r versus $S_{(r-1)}$, with the theoretical line, $S_r = S_{(r-1)}$, is the value of S_∞.

In Table 1, the times from 5 min onwards advance in arithmetic progression in accordance with equation (2). The usual experimental technique is to take balance readings at times advancing in geometric progression; in this case, suitable values for plotting according to equation (3) are obtained by graphical interpolation. Additionally, interpolation has the advantage of smoothing instrument reading errors. Having determined S_∞, the size distribution is determined in the usual manner[3].

Table 1. SEDIMENTATION BALANCE READINGS FOR CLASSIFIER OVERFLOW PRODUCT (BARYTES)

Time (min)	Scale reading (S)	Time (min)	Scale reading (S)
0	0·8	30·0	75·5
1	4·8	35·0	77·8
2·5	14·8	40·0	79·4
5·0	31·2	45·0	80·7
7·5	43·7	50·0	81·9
10·0	52·2	55·0	82·8
15·0	63·3	60·0	83·7
20·0	68·3	65·0	84·5
25·0	72·7	70·0	85·1

($S_\infty = 88·5$ from Fig. 1)

This procedure has been used successfully in this Department to analyse a number of sedimentation balance curves and has also been used in other similar applications[4]. The curves were related to separations according to particle size in a sedimentation classifier, and it has been logical throughout to relate sizing analyses to that proportion of the solid in suspension which would settle; that is, to S_∞ and not S_m. Results obtained by other workers have been examined successfully by the same procedure.

In some instances the plot of S_r versus $S_{(r-1)}$ does not give a straight line, but the curve obtained would probably fit another function having an unknown asymptote. In the case illustrated, the plot is linear over the last 12 per cent of the sedimentation curve; but curvature becomes apparent if the relationship is plotted over a wider range of balance readings.

This point is at present being investigated and further results are being analysed.

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Very Precise Thickness Measurement of Thin Films

IT has already been pointed out[1] that the Smith type of interference microscope, using a Wollaston prism in the rear focal plane of the objective, is a suitable instrument for measuring thin films. The film is deposited, with a reasonably sharp edge, on a flat surface and covered with a reflecting metallic film, using, for example, silver or aluminium. The image of an illuminated slit, doubled by the Wollaston prism, is projected by the objective, via a prism type of vertical illuminator, normally on to the specimen. The reflected beams are polarized mutually at right angles and the path-difference between them is zero if both slit images fall either on to the substrate only or on to the film only. If one image falls on to the film and the other on to the substrate the path-difference is equal to twice the film thickness, and the problem becomes the polarimetric one of measuring this path-difference.

A suitable form of compensator for performing this measurement has been described[1], together with a visual half-shade device for making the settings and an optical device for translating the slit images across the specimen. However, as the visual method is found to be very fatiguing under the conditions imposed by the microscope, a photo-electric method was sought.

A satisfactory method has been developed using the electro-optical effect in ammonium dihydrogen phosphate[2]. The slit image formed in the image plane of the microscope passes through a plate of ammonium dihydrogen phosphate, cut normal to the optic axis and provided with transparent electrodes, then through an analyser and into a photo-multiplier. An a.c. voltage is applied between the electrodes. It can then be shown that the photo-multiplier output contains a component of frequency equal to that of the a.c. which is zero only when the path-difference between the two component beams is a whole number of half-wave-lengths. This fundamental component is detected by a phase-conscious rectifier which feeds a centre-zero meter. The setting operation then merely consists of operating the compensator until the meter reads zero.

By this means it has been found possible to make settings with a standard deviation corresponding to an uncertainty in film thickness of only 0·1 Å. The uncertainty is due largely to air currents, although the two beams are separated by only 0·2 mm.

This degree of accuracy is, of course, largely academic, as it is unlikely that a substrate as flat as this over 0·5 mm or so can be found. However, it is clear that settings to 1 Å unit can be made with certainty.

I thank Dr. T. E. Allibone, director of the Laboratory, for permission to publish this article.

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MINERALOGY

Paragonite from the Dalradian

Two muscovite-rich red phyllites, one of chemical composition (per cent): SiO_2 59·6, TiO_2 0·9, Al_2O_3 18·1, Fe_2O_3 3·4, FeO 4·6, MnO 0·1, MgO 3·4, CaO 0·2, Na_2O 0·9, K_2O 4·7, H_2O 3·4, P_2O_5 0·2, CO_2 nil, total 99·5, carry a quartz–paragonite–muscovite–chlorite–hæmatite–rutile–apatite mineral assemblage. They come from the Dunoon phyllite, a low-grade horizon of the Dalradian, ¼ and ¾ mile north along the coast from Dunoon, Argyllshire. This is the first recorded appearance of paragonite as a rock-forming mineral from the Scottish metamorphic rocks, and its appearance elsewhere in low-grade rocks without albite is considered likely.

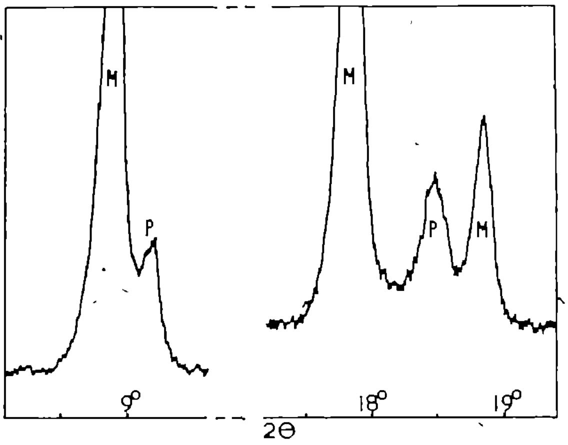

Fig. 1

The basal spacings of the co-existing muscovite and paragonite, calculated from the 002 peaks on a diffractometer trace, are 9·946 Å and 9·612 Å, respectively. Rosenfeld *et al.*[1] report the basal spacings of co-existing muscovite and paragonite from two biotite zone rocks to be: (1) 9·981 Å and 9·623 Å; (2) 9·975 Å and 9·623 Å. Paragonite is about 5 per cent of the total white mica. The diffractometer traces over the 001 and 002 peaks using copper *K* radiation are reproduced in Fig. 1.

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CRYSTALLOGRAPHY

Molecular Structure of Tutin

TUTIN ($C_{15}H_{18}O_6$) was first isolated by Easterfield and Aston[1] and identified as the convulsive poison present in the leaves and seeds of the New Zealand species of *Coriaria*, a shrub known by the Maori as 'toi toi'. The molecular structure of tutin is now inferred from an X-ray crystal structure analysis of α-bromo-iso-tutin.

Tutin is one of a series of chemically and pharmacologically similar compounds of which picrotoxinin ($C_{15}H_{16}O_6$)[2,3] and coriamyrtin ($C_{15}H_{18}O_5$)[4] have been most intensively studied. The structure (I) for picrotoxinin was proposed by Conroy[5,6] and this was confirmed by X-ray crystallographic studies which also determined the absolute configuration of the molecule[7,8]. On the basis of the picrotoxinin structure and chemical degradation studies, Karyone and Okuda proposed structures (II) and (III) for coriamyrtin and tutin[4] respectively. Further chemical evidence[9] led to the dilactone structure (IV) for tutin proposed by Johns and Markham.

The crystal structures of four bromo-derivatives have been examined in order to determine their suitability for complete structure analysis by the heavy atom method. The crystal data for these compounds are given in

Table 1

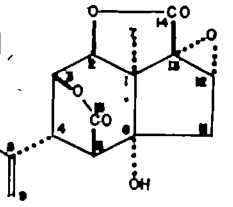
Compound	Formula	Crystal system	a (Å)	b (Å)	c (Å)	d_{obs} g/cm³	$d_{x\text{-}ray}$ g/cm³	Z	Space group	x_{Br}	y_{Br}	z_{Br}
α_1-Bromotutin	$C_{15}H_{17}O_6Br$	Orthorhombic	7·28	8·77	23·31	1·67	1·67	4	$P2_12_12_1$	—	0·25	0·125
α_2-Bromotutin	$C_{15}H_{17}O_6Br$	Orthorhombic	7·70	7·92	24·60	1·63	1·63	4	$P2_12_12_1$	—	0·25	0·083
α-Bromo-iso-tutin	$C_{15}H_{17}O_6Br$	Trigonal	8·461	—	36·61	1·65	1·63	6	$P3_12$ or $P3_22$	0·56	0·08	0·182
α-Bromotutinone	$C_{15}H_{15}O_6Br$	Orthorhombic	13·83	13·58	7·84	1·68	1·69	4	$P2_12_12_1$	0·17	0·14	0·12

Table 1. Lattice parameters were measured from Weissenberg photographs taken with copper K radiation and the crystal densities were measured by the method of flotation in a mixture of bromoform and carbon tetrachloride.

The two crystalline modifications α_1-bromotutin and α_2-bromotutin, here reported for the first time, are presumably analogous to α_1- and α_2-bromopicrotoxinin[10]. Although not required by space group symmetry, the bromine atom positions, as determined from Patterson projections, were found to be approximately at $y = 1/4$ in both the α_1- and α_2-bromotutin structures. This would complicate a structure analysis by introducing spurious symmetry into Fourier syntheses of electron density with the bromine atoms used as phase-determiners. A three-dimensional structure analysis of α-bromo-iso-tutin, rather than α-bromotutinone, was undertaken because of the uncommon trigonal crystal symmetry of the former structure.

Complete three-dimensional intensity data (1,988 non-symmetry-related reflexions) were recorded on Weissenberg multi-films and the intensities were estimated visually. The bromine atom position was deduced from sections of the three-dimensional Patterson synthesis, and the first three-dimensional Fourier synthesis with bromine phases clearly revealed the positions of all the carbon and oxygen atoms (Fig. 1). Although normally it is difficult at this stage of an analysis to distinguish between oxygen and carbon atoms from the peak densities in the approximate electron density maps, the configuration in the exo epoxy ring was evident from the formation of an intermolecular hydrogen bond of length 2·7 Å from the hydroxyl at C_2 to the epoxy oxygen. Structure factors calculated for all reflexions with an overall temperature factor of 2·5 Å² gave an R factor of 0·28. After two cycles of full matrix least-squares refinement with individual isotropic atomic temperature factors, R was reduced to 0·18. The refinement is being continued.

I



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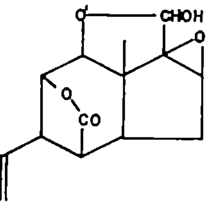

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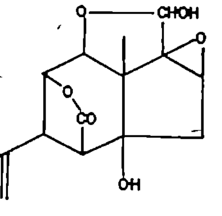

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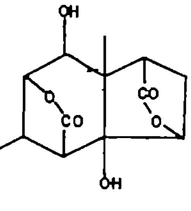

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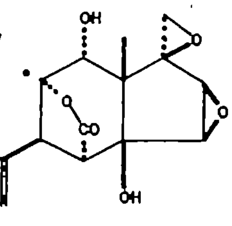
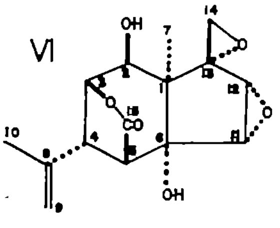


VI



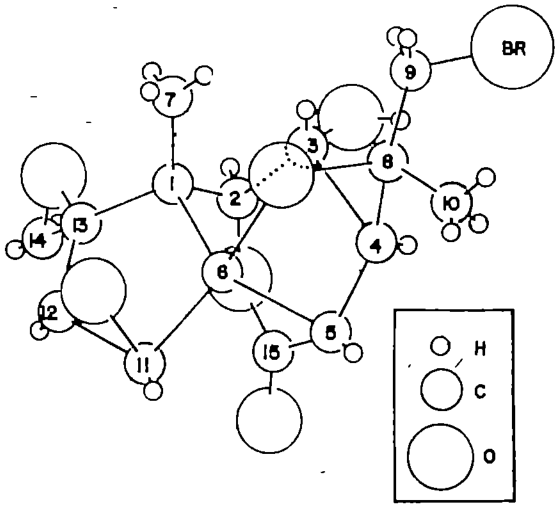


Fig. 1

If, as chemical evidence suggests, the tutin series differs from the iso-tutin series only in that the lactone ring closes at the C_3 hydroxyl, rather than the C_5 hydroxyl, then tutin has the structure (V) or its mirror image (VI). The absolute configuration of the molecule has not yet been determined, but by analogy with the absolute configuration of picrotoxinin[8], this may well be (VI). As in the case of picrotoxinin, the molecule features a framework of interlocking rings in a conformation which has complicated the determination of its structure from chemical and spectral evidence. Thus, in the iso-tutin series, the lactone grouping and C_2 and C_5 with their attached hydrogens shield the two epoxy rings, particularly the 11, 12 ring, from rearward nucleophilic attack.

This work was supported by grant B-3742 from the U.S. Public Health Service, National Institutes of Health.

Note added in proof. A crystal structure analysis of α-bromo-isotutinone by MacKay and Mathieson (C.S.I.R.O., Melbourne, Australia), which is in progress, confirms the molecular framework as reported here.

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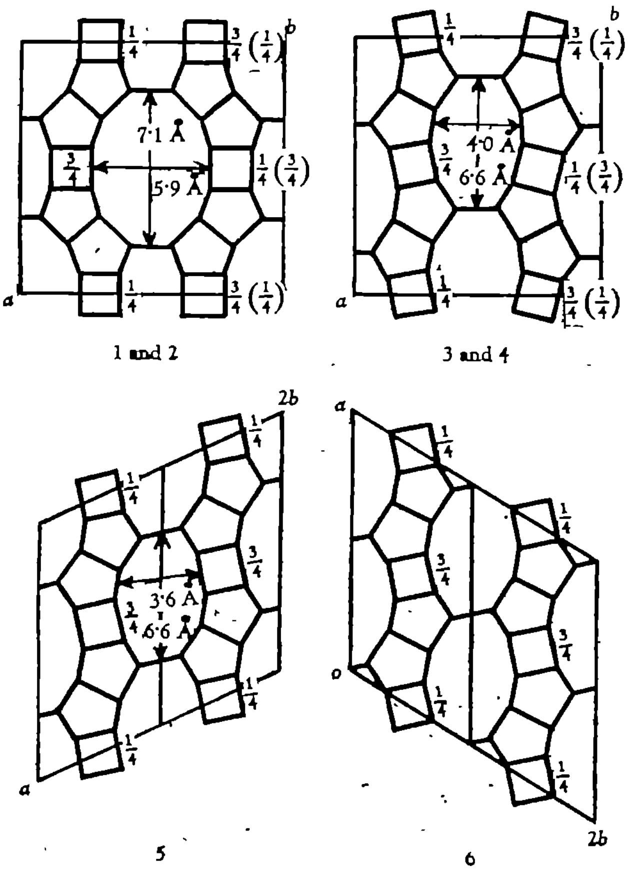
Possible Structures related to Mordenite

Meier[1] has recently given a structure for the fibrous aluminosilicate mordenite, containing strongly bonded chains of SiO_4 or AlO_4 tetrahedra. The chains, which have the unusual feature of containing a proportion of five-membered rings, are linked laterally to form a three-dimensional framework. All apical oxygen atoms are shared between two tetrahedra. In addition Meier and Gottardi[2] have shown that an alternative-side linking of the same chains can account for the structure of dachiardite. This raises the question whether there are more structures which can be built from these chains. A little consideration will show that there is, in fact, an endless number. However, some arrangements involve rather improbable linkings and result in large unit cells, while others can be obtained only by distorting the natural bonding distances within the chains. If both these types are omitted there are just six structures which have undistorted chains and unit cells not encompassing more than four chains.



1 and 2 3 and 4

5 6

Fig. 1. The six simple structures based on the mordenite-type chain. The numbers 1–6 correspond with those in the first column of Table 1. Heights of the four-membered rings are indicated, and for structures (2) and (4) the figures in parentheses must be read for the layer on the right. Approximate free diameters for the large channels are given

Fig. 1 shows the six structures projected down the axis of the chains with the unit cells thinly outlined. The choice of axes is kept as in mordenite, for both orthorhombic and monoclinic structures. *a* and *b* lie in the plane of the paper and *c*—the fibre axis—is perpendicular to this plane. Silicon (or aluminium) atoms are centred on corners while oxygen positions fall roughly halfway along each line. The mordenite chains (running normal to the plane of the paper) can be thought of as bonded together at intervals of $\frac{1}{2}c$ by the square four-membered rings, thus forming layers parallel to *ac*. These may then link together in one of two ways, in which a translation of $\frac{1}{2}c$ by alternate layers produces the second arrangement from the first. To show this the heights of the four-membered rings are indicated, choosing the origin as in mordenite.

In Table 1 the six arrangements are listed with their highest possible centric symmetries. The axes given are those found by calculation based on the mordenite cell. For dachiardite they compared with $a = 18{\cdot}73$, $b = 10{\cdot}30$ Å, $\gamma = 107°\ 54'$ for the natural mineral[3]; the *c*-axis, which is the chain repeat distance, equals 7·52 Å for each structure.

Table 1

No.	Structure Occurrence	Highest symmetry	*a* (Å)	*b* (Å)	γ
1	Mordenite	*Cmcm*	18·13	20·5	—
2	Unknown	*Immm*	18·13	20·5	—
3	Unknown	*Bmmb*	18·7	19·6	—
4	Unknown	*Bmmm*	18·7	19·6	—
5	Dachiardite	*B2/m*	18·7	10·3	106° 45′
6	Unknown	*B2/m*	18·7	11·7	123°

Only two of these structures occur in Nature, but a few crystals giving electron diffraction patterns corresponding approximately to the body-centred structure No. 2 have been synthesized hydrothermally from aluminosilicate gels containing strontium[4], similar to those gels which yielded a strontium-mordenite.

I thank Prof. R. M. Barrer and Dr. W. M. Meier for advice.

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CHEMISTRY

Breaking Foams produced by Ionic Surfactants

A NEW and remarkably effective way of breaking foams produced by ionic surfactants has been discovered. In this connexion, it is necessary to distinguish between 'foams', which term, I suggest, be restricted to those systems stabilized by soluble surfactants at the air-water interface, and 'froths' which are stabilized by insoluble products at the air-water interface. When foams collapse, therefore, there is no visible residue whereas when froths collapse there is an insoluble scum produced.

If a temporarily stable foam produced using an anionic surfactant such as laurate or dodecylbenzenesulphonate has placed on it some foam produced using a surfactant of opposite charge, for example, trimethyldodecylammonium chloride, there is a dramatic interaction at those points where the two different foams meet, with mutual destruction. Furthermore, as foams are thermodynamically unstable, the quantity of the first foam destroyed by the addition of the second foam, which I propose calling a contra-foam, is very much greater than the amount of contra-foam added. For example, a vessel of 12-l. capacity was filled with a foam produced from dodecylbenzene-sulphonate. A blob of contra-foam, enough to cover a penny, was put on the top of the foam bed. Within a few seconds a cylinder, approximately of the diameter of the added contra-foam, was channelled right through the foam, leaving the remainder of the foam intact. Presumably the interaction takes place at the plateau borders, where the opposite charged surfactants meet and neutralize each other, but the instability, thus produced, proceeds some distance into the foam.

This is clearly due to opposite charge effects, because if foams produced from similarly charged surfactants or from non-ionic surfactants are contacted in the same way, there is no interaction at all, both foam and contra-foam remaining unchanged.

In a similar way, a foam produced from a cationic surfactant is quickly destroyed by using a contra-foam produced from an anionic surfactant.

By suitable placement of blobs of contra-foam, large amounts of foam can be made to collapse almost instantaneously using very little contra-foam. There is a slight residual scum, presumably produced as a reaction product between the oppositely charged surfactants, but the water substrate now shows no tendency to foam, showing that the surfactant from the collapsed foam does not re-enter the water in an active form.

In order to destroy an anionic foam it has been found that contra-foams produced from diamine surfactants are better than those produced from singly charged cationics, presumably because of the double charge. If the foam is simply sprayed with a dilute solution of the same contra-foam surfactant, the effect is not nearly so marked as when using a contra-foam itself. Presumably, the nature of foams is such that the opposite charges can get closer together more quickly if two foams are placed in juxtaposition.

For dealing with large masses of foam, a primitive foam gun was made. This consisted of a sintered glass porous plate fused into a 1·5 in. glass tube, which, about 6 in. above the porous plate, was bent to make an angle of 60°. A dilute solution of contra-foam surfactant is placed in the gun, bubbles generated by introducing compressed air, and using this, the contra-foam can be strategically directed so as to break the foam completely. In this way, it was possible, in a few minutes, to destroy completely a foam, which filled an open box, about 8 ft. cube. This foam was produced by a sewage effluent, and was probably due to the presence of dodecylbenzenesulphonate.

It is suggested that a method such as this might offer a means of dealing with some case of the nuisance foams produced by the so-called hard detergents. It should be emphasized that, so far, this method has only proved effective for dealing with foams, and not with froths, and quite obviously it has no use for breaking foams produced by non-ionic surfactants.

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Ion-Exchange Theory of Coagulation

RECENTLY, Mirnik[1] has proposed a "mass-action law" theory of colloid stability, as an alternative to the Derjaguin – Landau – Verwey – Overbeek theory, lately extended by one of us[2]. The purpose of this communication is to examine the validity of Mirnik's arguments and of his experimental evidence. We shall summarize his basic assumptions using simplified notation as follows.

(1) A coagulating adsorbed counter-ion (cation) M', of valency z', associates with z' stabilizing (potential-determining) univalent anions A (I^- in the case of negative silver iodide) to form a molecular unit on the surface of a negative colloidal particle. If the chemical potentials (per mole) of the molecule, anion and cation are $\mu_{AM'}$, μ_A and $\mu_{M'}$ respectively, then the mass-action law reads:

$$\mu_{AM'} = z'\mu_A + \mu_{M'} \qquad (1)$$

If, in the solution phase, $\mu^{\circ}_{M'}$ is the standard chemical potential and c' the concentration of the cation in mol./l. and the activity coefficient is equated to unity, then $\mu_{M'} = \mu^{\circ}_{M'} + RT\ln c'$. Further, if γ is the maximum concentration of cations in the adsorbed state and $(1-x)\gamma$ the actual concentration where x is a fraction, then Mirnik assumes that $\mu_{AM'} = \mu^{\circ}_{AM'} + RT\ln(1-x)\gamma$, where $\mu^{\circ}_{AM'}$ is the corresponding standard chemical potential and again the activity coefficient is put equal to one. Substituting into (1), we obtain Mirnik's relation:

$$\ln c' = -z'a + \ln(1-x)\gamma - \ln D'_0 \qquad (2)$$

where

$$\ln D'_0 = (\mu^{\circ}_{M'} - \mu^{\circ}_{AM'})/RT,\ a = \mu_A/RT \qquad (3)$$

This is essentially a Freundlich adsorption isotherm for the counter-ion M'. (Mirnik expresses his concentrations in g. equiv./l., in which case c' and γ should be multiplied by z', but this does not change the final form (2).) Packter[3] has also applied the mass-action law to ion-pair formation on the particle surface, although between a counter-ion and only one stabilizing ion. However, he has incorporated this into the Derjaguin–Landau–Verwey–Overbeek theory.

(2) According to experimental work by Herak and Mirnik[4,5], two counter-ions (for example, Co^{2+} and Eu^{3+}) can exchange on the surface of the silver iodide particles and the total amount of adsorbed ions in g equivalents remains constant. If equations (1)–(3) refer to one ion species and a double dash is used to denote the other, then the relations corresponding to (2) and (3) for the second species read:

$$\ln c'' = -z''a + \ln x\gamma - \ln D''_0 \qquad (4)$$

$$\ln D''_0 = (\mu^{\circ}_{M''} - \mu^{\circ}_{AM''})/RT \qquad (5)$$

From their experiments, Mirnik and Herak[5] propose the relation:

$$\ln c'/c'' = (z'' - z')a + \ln[(1-x)/x] \qquad (6)$$

where a is practically independent of the concentration of stabilizing ion in the solution. In order that (6) be consistent with (2) and (4) it is necessary that $D''_0 = D'_0$ although the standard chemical potentials in the expressions for D''_0 and D'_0 refer to different species. Furthermore, the constancy of a implies that the chemical potential of ion A in the solution is independent of its concentration whereas, for negative silver iodide sols, the variation in a is $\Delta a = \Delta\mu_A/RT = -2{\cdot}3\,\Delta pI$, where pI can vary from 1 to 7. Thus Mirnik's deductions cannot be valid, and we must conclude that at least one of the relations (2), (4) and (6) is incorrect. Mirnik apparently avoids the anomaly of fixed μ_A by postulating that the chemical potential of a stabilizing ion in the adsorbed state differs from that of the same ion in the solution phase, but this contradicts thermodynamics.

If $x = 1/2$ and $z'' = z'$ then on a $\ln c' - \ln c''$ plot the relation (6) represents a straight line having a slope of 45° and passing through the origin. However, an examination of Herak and Mirnik's work (Fig. 8, ref. 5) shows that the ion-pairs $Y^{3+} - La^{3+}$ and $Y^{3+} - Nd^{3+}$ give two lines of somewhat different slopes, although the authors have drawn a common line for the two. Neither of these two lines goes through the origin. This means that D'_0 differs from D''_0 even though the two ions M' and M'' have the same valency ($\ln(D'_0/D''_0) \sim 1$ for the pair $Nd^{3+} - La^{3+}$) and we may expect a greater difference with ions of different valencies. Also, the experimental results summarized in Tables 1 and 2 of the same work[5] show a spread in the values of a which cannot be neglected.

(3) The experiments of Mirnik and Herak cast doubt on the relations (2) and (4) in another way. Mirnik postulates a hypothetical adsorbable uncharged species exchanging with ion M''. If c_0 is the concentration of this species in the liquid phase and $(1-x)\gamma$ its concentration in the adsorbed phase, then in place of (6) we have:

$$\ln c''/c_0 = -z''a + \ln[x/(1-x)] \qquad (7)$$

This relation can also be regarded as an adsorption isotherm for counter-ion M' provided c_0 is known. The experimental adsorption isotherm for Eu^{3+} by Mirnik and Herak[6] resembles the Langmuir rather than the Freundlich adsorption law. In explaining this, they assume that c_0 is independent of x, in contradiction to (2), and consequently introduce two different forms for the same quantity c'' as a function of x. In earlier work, Težak[7] had already introduced the uncharged species and assumed that the concentration c_0 was so much greater than c'' that it could be regarded as constant.

(4) Perhaps the most controversial conjecture of Mirnik is that a water molecule forms a complex with two stabilizing anions (one for each hydrogen) and stabilizes the colloidal particle. This complex is treated in the same way as the species AM'; it has a concentration $(1-x)\gamma$ in

the adsorbed state and can interchange with the molecule AM'', where M'' is the coagulating ion. If $\mu_{(A,\,H_2O)}$ is the chemical potential of this negative ion complex of valency 2, $\mu^0_{(A,\,H_2O)}$ the corresponding standard chemical potential and μ_{H_2O} the chemical potential of water, then according to Mirnik:

$$\mu_{(A,\,H_2O)} = 2\mu_A + \mu_{H_2O} = \mu^0_{(A,\,H_2O)} + RT\ln(1-x)\gamma \qquad (8)$$

Mirnik's basic hypothesis is that the coagulation condition is given by:

$$\mu_{(A,\,H_2O)} = \mu_{AM''}(= \mu^0_{AM''} + RT\ln x\gamma) \qquad (9)$$

Making use of (8) μ_A is replaced by $(\mu_{(A,\,H_2O)} - \mu_{H_2O})/2$ in (5) and the relation (4) yields

$$\ln c''_{\text{coag}} = -z''a + \ln x_{\text{coag}}\gamma/D''_0 \qquad (10)$$

for the coagulation concentration c''_{coag}. Mirnik also assumes that $\mu^0_{(A,\,H_2O)} = \mu^0_{AM''}$, thereby again equating standard chemical potentials of different species, and therefore derives from (8) and (9) the curious result that at critical coagulation $x = x_{\text{coag}} = 0{\cdot}5$. If a is independent of the concentration of stabilizing ion (which we have shown to be incorrect) and both x_{coag} and D''_0 are independent of the valency z'', then (10) is a linear relation between $\ln c''_{\text{coag}}$ and the valency z'' of the coagulating ion. Mirnik interprets this as the Schulze–Hardy rule.

It seems that there cannot be a mathematically simple Schulze–Hardy rule which applies to sols in general[3]. Indeed, there are examples which follow other formulations, for example, for arsenic sulphide sols, $\ln c''_{\text{coag}} =$ constant $- 6\ln z''$ seems to be a good approximation. Ion exchange usually applies to two ions of the same sign whereas in Mirnik's theory the counter-ions exchange with electrically neutral water molecules. In this connexion, if the criterion (9) were acceptable, it would be more logical to express it in gram equivalents, so that the same numbers of stabilizing ions are neutralized in the exchange process. Hence this criterion should read $z''\mu_{(A,\,H_2O)} = 2\mu_{AM''}$ (so that $2\mu_{M''} = z''\mu_{H_2O}$). The determination of x_{coag} from this relation becomes uncertain because of the term $RT\ln\gamma$ and in general x_{coag} would depend on z''. There seems no physical justification for the value $x_{\text{coag}} = 0{\cdot}5$, and indeed Mirnik and Herak cannot fit their theoretical adsorption isotherm to their experimental one[6]. Furthermore, their theory does not explain how the water molecules can be stabilizing agents for both negative and positive sols.

As experimental evidence for their Schulze–Hardy rule Herak and Mirnik claim that the plots of "valency-logarithm coagulation value" and "valency-logarithm desorption concentration" at $x = 0{\cdot}5$ are parallel (Fig. 4, ref. 4). However, this does not follow from the example given by the authors because their coagulation concentrations (c_{coag}) of Na^+, La^{3+} and Th^{4+} appear to be in error. The value for Na^+ is too low and that for La^{3+} too high[8]. Indeed, Herak and Mirnik's own value of c_{coag} for Nd^{3+} is much lower (Fig. 5, ref. 4) and one would expect this to be very similar to the true c_{coag} for La^{3+}. Finally, as plotted, c_{coag} for Th^{4+} is also too high, which is probably due to the high pH at which the ions are hydrolysed giving species of lower valency. Thorium exists in solution as an ion of charge $+4$ only at $p\text{H} < 4$ (ref. 9), where its c_{coag} is lower than the value used by Herak and Mirnik. If the correct values of c_{coag} of all these ions are used, the "valency-logarithm coagulation value" line has a smaller slope than the "valency-logarithm desorption concentration" line, the ratio of the slopes being about 0·6, contrary to Mirnik's theory.

This theory is presented as an alternative to the widely accepted theory that colloid stability results from an interplay of electric double layer repulsion and van der Waals attraction between colloidal particles. In our opinion there is no reason why Mirnik's theory should be accepted as correct.

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Ionization by Charge Transfer of Adsorbed Gases in Mass Spectrometers

IN a recent investigation of anomalies in the ionization of different simple gases by mono-energetic electrons Marmet and Morrison[1] pointed out that charge exchange occurs in the ionization chamber of a mass spectrometer operating at low pressure, and therefore should be occurring on the walls. Investigating the ionization of nitrogen, they observed that an addition of water in the gas stream brought indeed a reduction of about 1·5 V in the appearance potential of N_2^+ ions, observed at about 14·1 V, whereas the normal first ionization potential of nitrogen is 15·58 V. They ascribed this result to a reaction such as:

$$N_2^* + H_2O^+ \rightarrow N_2^+ + H_2O \qquad (1)$$

On the other hand, recent shock-wave experiments on energy transfer by Clouston *et al.*[2] and sound propagation studies by Parbrook and Tempest[3] show that the cross-section for deactivation of vibrationally excited nitrogen is 10–100 times more effective in water–nitrogen collisions than in nitrogen–nitrogen collisions, suggesting that interactions between nitrogen and water molecules are highly probable as soon as the chance for them to meet within narrow distances is high enough. The studies show also that relaxation times for vibration are of the order of 10^{-4}–10^{-7} sec.

Lindholm[4] showed that the cross-section for charge exchange in ion impact phenomena is high if the energy required for ionization is close to the recombination energy of a given ionic state of the ion which takes up an electron.

Therefore we suggest that the reaction (1) be more accurately written in the following way:

$$N_2^* + H_2O^+(^2A_1) \rightarrow N_2^+(X^2\Sigma_g^+) + H_2O \qquad (2)$$

since the ionization potentials of water are 12·60 V ($^2B_1$), 14·35 V ($^2A_1$) and 16·34 V ($^2B_2$), as determined by Frost and McDowell[5], and an examination of Fig. 10 of ref. 1 shows the uncertainty is such that the appearance potential of N_2^+ might be as high as 14·5 V (corrected).

This of course does not explain why the appearance potential of nitrogen is so much reduced. Marmet and Morrison suggested that it is due to adsorption of nitrogen. Investigations on photoelectric effects of adsorption phenomena have indeed shown that the lowering of the ionization potential of the adsorbed species is not unfrequent, but the effect is much less important, being of the order of a tenth of a volt[6], than the one referred to here. Moreover, the adsorption of normal nitrogen molecules on a metal surface at 400° K should not be very important, particularly at pressures as low as 10^{-5}–10^{-6} mm.

For these reasons, we would prefer to think that a vibrational or electronic excitation of the nitrogen molecule takes place in the gas phase, by inelastic collisions with electrons, prior to adsorption. The vibrational quantum of nitrogen being 0·29 V (= 2,359 cm⁻¹) one should admit the molecules are in their 3rd or 4th vibrational level, the thermal energy being only about 0·04 V. A possible process for this vibrational excitation is the formation of a negative transitory N_2^- ion, loosing its electron and afterwards getting vibrationally excited. This may occur in the particular case of nitrogen as has been shown by Schulz[7].

The other possibility is an electronic excitation by the electron beam; this happens for higher electron energies, and Schulz[7] has distinguished the formation of $A^3\Sigma_u^+$, $a\,\Pi_g$ and $C^3\Pi_u$ excited states at approximately 8, 10 and 12 V. There is no way for the moment of deciding what state might be concerned here, but it may be pointed out that the first excited electronic states of nitrogen correspond to an electron jump into either a $\pi_y^*\,2p$, a $\pi_z^*\,2p$ or a σ^*2p orbital; these are all antibonding and should bring an increase of nuclear distance, resulting in a more polarizable and more easily adsorbed molecule.

Finally, the curve reported by Marmet and Morrison shows a hump about 1·2 V above threshold and it may be significant that the first excited state $A^2\Pi_u$ of the N_2^+ ion is also situated some 1·15–1·21 V above the ground $X^2\Sigma_g^+$ state[8].

To conclude, the anomalies observed may be explained on the assumption of excited states of ions taking part in the charge exchange process on the surface, after vibrational or electronic excitation of the nitrogen molecule in the gas phase by electron collisions. Experiments of charge exchange between nitrogen and water by Lindholm's ion bombardment technique should also prove useful in explaining these phenomena.

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Luminescence from the H—O₂ Reaction

THE spectrum of the HO_2 radical is of considerable importance and several unsuccessful attempts have been made to record it. The HO_2 radical has one more electron than the HNO molecule, which has an electronic transition at 7600 Å (ref. 1), and consideration of the molecular orbitals of HO_2 suggests that this radical should have a very similar electronic transition to that of HNO (ref. 2). The HNO spectrum was first obtained in absorption during flash photolysis experiments[1], but has more recently been observed in emission in the chemiluminescent reaction of hydrogen atoms with NO (ref. 3). The similarity of the bond dissociation energies of H—NO (49 kcal/mole)[3] and H—O_2 (46 kcal/mole)[4] suggests that hydrogen atoms might give a chemiluminescent reaction with oxygen, despite previous failures to find any electronic emission from this reaction.

Hydrogen atoms were produced by the dissociation of commercial hydrogen in two Wood's tubes and pumped through two side-arms on opposite sides of a reaction vessel 3·5 cm in diameter and 35 cm long connected directly to a 150 l./min rotary pump. Commercial oxygen was introduced through a third side-arm situated between the hydrogen inlets. The vessel was screened from direct light from the discharges, and mounted end-on directly in front of the slit of a Hilger 'Wave-length' spectrograph with *f*/10 glass optics and a focal length of 30 cm. The spectrum shown in Fig. 1 was photographed on hypersensitized Kodak II.*N* (I.R.E.R.) plates in 30 min, using a 100μ slit-width, and a partial pressure of each reactant of about 0·5 mm mercury. No visible glow was apparent. The intensity of the spectrum was extremely variable, and the relative intensity of hydrogen and oxygen atomic lines, due to partial entry of the discharge region into the reaction vessel, was also very variable, as shown by the comparison between the spectra in Fig. 1*a* and *b*, which were taken under almost identical conditions. However, the intensity distribution within the spectrum was reproducible. The erratic behaviour suggests that a trace impurity may be important in the production of the spectrum, either because the impurity supplies the excited molecules, or because the impurity (water ?) acts as a catalyst in the production of the excited molecules.

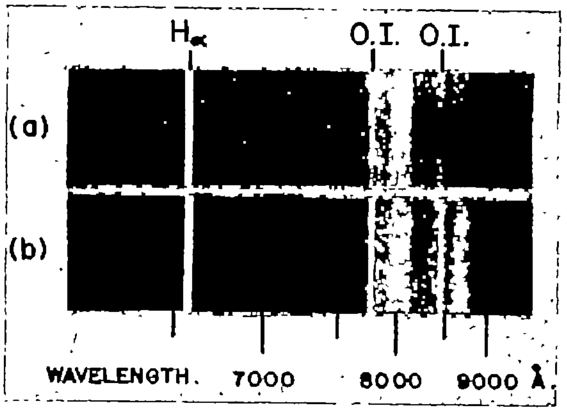

Fig. 1. The spectrum emitted in the reaction of hydrogen atoms with oxygen

The reaction between hydrogen atoms and ozone leads to the emission of near infra-red vibration-rotation bands of OH (ref. 5), and H_2O emits near infra-red vibration-rotation bands in the flame of oxygen burning in an atmosphere of hydrogen[6]. However, both these systems are of red-degraded bands, and their wave-lengths do not agree with those of the spectrum in Fig. 1, which shows prominent violet-degraded heads at 6853, 7149, 7562 and 8103 Å (± 10 Å). The coarse rotational structure of the HNO bands is violet degraded, due to an opening out of the HNO angle on electronic excitation, and a similar behaviour is to be expected for HO_2. Thus, although there is no proof of the carrier of the observed spectrum, it seems possible that this is the spectrum of the HO_2 radical.

A new reaction vessel is being built to accommodate a set of multiple reflexion mirrors, and it is hoped to obtain sufficient intensity for a higher resolution study of this spectrum.

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Measurement of Small Quantities of Calcium by Radiometric Precipitation

Radiometric precipitation is in principle an extremely sensitive method of analysis, but in practice the sensitivity is usually limited by the efficiency of separation of the precipitate and its parent solution[1,2]. An adaptation of the technique of chromatography affords a quick and simple method both of phase separation and of washing the precipitate with a small volume of liquid. I have used this method to measure concentrations of calcium as low as 10 p.p.m. in less than 1 mg of solution.

In this method equal quantities of nitric acid containing the calcium sample and of 1 per cent fluoride solution labelled with radiofluorine are measured in a length of fine bore polythene tubing attached to a microsyringe and then mixed and temporarily stored in a small polythene bubble. The solution is then spotted on a strip of chromatographic paper where calcium fluoride is precipitated when its *p*H is raised by blowing on ammonia. This method of raising the *p*H is much to be preferred to that of adding liquid as the latter tends to move the solution without mixing well with it. After precipitation some 1,000 p.p.m. fluoride solution is allowed to run along the paper until the excess activity has moved away from the precipitate, which remains stationary. A fluoride solution has to be used for the irrigation, rather than water, as about 10^{-3} ml. runs through the precipitate and this quantity of water would dissolve 10^{-7} g of calcium from calcium fluoride. Finally the paper is dried and the piece containing the precipitate is cut off so that the activity of the precipitate can be measured.

The quantity of calcium in an unknown sample must be determined from a calibration curve of standard calcium samples (Fig. 1) because, although the activity is directly proportional to the calcium concentration over a range of several hundredfold, the measured activities of precipitates from standard calcium solutions containing 1 per cent of fluoride are roughly half of those expected on the grounds of chemical equivalence and the known specific activity of the fluoride. Some of the activity is lost by exchange of fluoride during the irrigation and some of the calcium is precipitated as calcium hydroxide when the *p*H rises abruptly. The first reaction causes only a uniform reduction in the activities of a set of precipitates, as would the second if the rate of absorption of ammonia were the same for each sample. However, random variations in the quantity of fluoride precipitated with calcium are reduced to about 5 per cent if phosphoric acid, a powerful buffering agent, is present in the solution at a level of a few per cent. The precipitate with buffer is again a mixture, as the phosphates of calcium are only slightly soluble, but its composition will be determined mainly by the ratio of fluoride and phosphate concentrations. Further, at this level phosphoric acid has the advantage of swamping the effect of any phosphate in the unknown calcium samples.

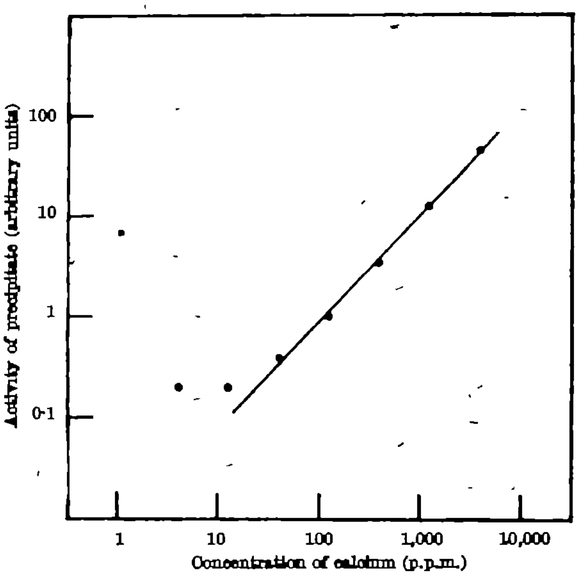

Fig. 1. Labelled fluoride precipitated with calcium

Any substance which is present in the unknown sample and is capable of forming an insoluble compound with either calcium or fluoride might, in principle, interfere with this method of analysis. However, small quantities of anions can be masked in the way described here for phosphate: by the addition of a larger quantity of the interfering anion to all the calcium solutions so that the precipitate is a mixture of constant composition. Interference by substances with fluorides of low solubility is troublesome; the effect of magnesium, for example, is complex and if it is present at a level of more than one-tenth of the calcium concentration the method is unreliable.

Before use the chromatographic paper must be washed in a dilute solution of either ethylenediamine tetraacetic acid or hydrochloric acid to remove a background contamination which is probably calcium. Even so, traces of fluoride stick to the paper limiting the sensitivity of the analysis. Solutions containing less than 10 p.p.m. of calcium, the level at which the activity of the precipitate is comparable with the background activity left in the paper, must be concentrated. This can be done without risk of contamination by freezing the solution in an unsealed polythene bubble and then drying under vacuum.

The principle of this method should be widely applicable; the main limitations being probably in handling small quantities of liquid and in finding suitable chromatographic paper.

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Phosphorescence in Plastics

A recent communication from Oster *et al.*[1] reported their finding of room-temperature phosphorescence in plastics containing dissolved aromatic hydrocarbons or dyes.

We wish to direct attention to work in the laboratories of the General Post Office Engineering Research Station in 1957 and onwards which resulted in the preparation of phosphorescent plastics having long decay times at room temperature. The work has been referred to elsewhere[2] but has not hitherto been described in the chemical literature and may be of interest to those engaged in the examination of luminescence processes.

In a search for cheapened forms of phosphors suitable for mail-coding applications, various organic systems were examined. It was found that enhanced intensities and prolonged decay periods were given at room temperatures when certain types of activating molecule were reacted simultaneously with the major components of aminoplastic resins. Since the resins formed from cyanuric acid and formaldehyde were more easily ground to a fine powder (suitable for use in printing inks, etc.), most of the results were obtained with this system. The amino-resin phosphors have been patented[3], and two such powders have been successfully used in field trials of automatic mail facing and sorting.

We find that the most effective activators contain one or more aromatic rings with substituent groups capable of reacting to form part of the resin molecule. Generally, —OH or —NH$_2$ groups are present but condensed ring hydrocarbons having active positions also give phosphors, though of lower activity. In all cases, the presence of —SO$_3$H groups augments the luminescence and shifts both the peak excitation and luminescence towards longer wave-lengths. Some of the phosphors exhibit luminescence which is visually detectable (in the dark) for up to 40 sec after ultra-violet irradiation. The greatly enhanced intensities and durations suggested that these systems might be qualitatively different from those previously reported. No detailed study has been made of the chemical or radiative processes involved, but some activators were structurally modified in an effort to establish if chemical linkage with the resin matrix were essential. The results indicate that this is so. Thus, cyanuric acid containing 1 per cent of *p*-hydroxy-diphenyl yields a formaldehyde-condensation product showing luminescence, in the range 4300–6300 Å, which is visually detectable for about 20 sec after irradiation at 2537 Å. If the *p*-hydroxydiphenyl is replaced by di-*p*-diphenyl ether or methyl-*p*-diphenyl ether there is no visually detectable delayed luminescence after irradiation with long- or short-wave ultra-violet light. Also, the phosphorescence imparted by *p*-phenylene diamine is slightly exceeded by that of the (nuclear substituted) 2,3,5,6-tetra-methyl derivative but no phosphorescence is observed if *N,N,N′,N′*-tetra methyl *p*-phenylene diamine is used. The blockage of the —OH and —NH$_2$ groups has in each case resulted in a drastic reduction of the phosphorescent properties, with no obvious change in the fluorescence.

The work is insufficient to justify firm conclusions, but it does seem probable that these materials differ qualitatively from the solutions which have been generally studied. Oster *et al.*[1] give some evidence for specific interactions between activating molecules and the polymeric matrix. It may be that the amino-resins provide an environment specially favourable to high phosphor efficiencies, even for dissolved species, but it also appears that the purely physical effects of a rigid matrix are here augmented by chemical bonding of the activating centres to the main network of the amino-resins.

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Acid Leaching of Uranium and Thorium Carbides

IN a recent communication by Sasaki *et al.*[1] attention is directed to the use of the carbides of uranium, plutonium and thorium as nuclear fuels and to the problems associated with processing and recovery of the irradiated fuel. It is revealed that in the acid leaching of thorium carbide (ThC$_2$) with nitric acid of concentrations below 4 N gaseous hydrocarbons are evolved, whereas at concentrations above 4 N organic material is produced, and evidence is presented that this organic matter does not have a C—H bond as indicated by the results of infra-red analysis. The formation of organic material during the acid leaching of uranium monocarbide has also been observed by Simpson *et al.*[2] and the presence of this could constitute a hazard in the uranium recovery process, although its formation may be avoided by prior hydrolysis of the carbide in water and subsequent dissolution of the resulting oxides in nitric acid[3].

We have examined the reaction of uranium monocarbide with nitric acid (6 N) and after removal of uranium from the resulting solution with tributyl phosphate in benzene[4] the aqueous raffinate was evaporated and the residue esterified with diazomethane. Chromatography of the mixed esters on alumina from benzene solution gave hexamethyl mellitate, which was identified by analysis and by comparison with an authentic specimen. Further elution of the column gave intractable resins. In one experiment involving the dissolution of uranium carbide in nitric acid (6 N) the solution was left for six weeks before processing and oxalic acid separated from the reaction mixture. An X-ray analysis of the uranium monocarbide utilized in the leaching process did not reveal the presence of free graphite and we suggest that the reaction proceeds by a breakdown of uranium carbide with formation of graphitic carbon which is then oxidized to oxalic and mellitic acids.

$$UC + 2O \rightarrow UO_2 + C$$

Mellitic acid was also isolated (as the hexamethyl ester) from the reaction of nitric acid (6 N) with uranium dicarbide (UC$_2$), uranium sesquicarbide (U$_2$C$_3$) and the carbides of thorium (ThC, ThC$_2$ and Th$_2$C$_3$).

In a typical experiment uranium monocarbide (519 g) and nitric acid (6 N; 4 litres) were allowed to react for twenty-six days and filtered from insoluble material. The aqueous filtrate was extracted until free of uranium with a 20 per cent solution of tributyl phosphate in benzene which had been equilibrated with nitric acid (6 N). Evaporation of the aqueous raffinate under reduced pressure gave a residue (12·7 g) which was suspended in ether and treated with an excess of ethereal diazomethane. Filtration from insoluble matter (24 g, containing 13·5 per cent of the initial carbon) and evaporation of the filtrate gave a mixture of esters (13 g) which was chromatographed on alumina from benzene solution. Elution with benzene furnished hexamethyl mellitate (5·1 g). Further elution of the column with ether, ether–methanol and methanol gave intractable mixtures. Approximately 30 per cent of the initial carbon was evolved as carbon dioxide during dissolution in nitric acid. Together with a 4·3 per cent yield of oxalic acid isolated in one case, the foregoing products account for 83 per cent of the carbon present initially. The optimum material balance (95 per cent) was secured in the case of uranium sesquicarbide, which afforded 38 per cent carbon dioxide, 10 per cent mellitic acid and 46 per cent other acids (unidentified: yield estimated as 'mellitic acid'). The corresponding yields for the other carbides were approximately: UC$_2$: 29, 8, 25; ThC: 47, 5, 23; ThC$_2$: 33, 3, 8 and Th$_2$C$_3$: 27, 1, 15 per cent.

We thank Mr. K. Hartley and Dr. G. Maclennan of the U.K. Atomic Energy Authority for directing our attention to the problem, Dr. H. B. Bell of the Royal College of Science and Technology for the X-ray analysis and Mr. T. Boyle for assistance. We also thank the U.K. Atomic Energy Authority for a grant and the supply of carbides.

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BIOCHEMISTRY

Starch-gel Electrophoresis of Pig Serum Proteins

MANY variations of the original technique of Smithies[1] for starch-gel electrophoresis of proteins have been developed, notably the vertical gel[2], the discontinuous buffer system[3], and two-dimensional applications[4,5]. The latter method has been used by Ashton[6] in examining pig serum proteins; it has one disadvantage in being time-consuming, requiring two electrophoretic runs, and at the most only two samples can be compared at once on normal apparatus.

In the investigation of pig serum reported here a discontinuous buffer system was used in a vertical apparatus, which enables ten samples to be characterized during one day's operation, with a separation into 22–26 components. A 12 per cent gel made up in buffer consisting of 0·010 M boric acid and 0·006 M *tris*, *p*H 8·6 (at 5°), is poured into the ends of the apparatus and allowed to set before trimming off to leave a rectangular hollow, into which the second gel is poured. This is made up in 0·25 M sucrose, 0·0015 M citric acid, 0·020 M *tris*, *p*H 9·1 (at 5°). The slots are formed in this gel using a slot-former as described by Smithies[2]. Electrophoresis is carried out for 6 h at 300 V, 15–20 m.amp in a room kept at +1°. The gel temperature at the end of a run is usually about 5°.

Blood samples have been taken at slaughter (age approximately 6 months) and allowed to clot overnight before pouring off the serum and centrifuging to remove any red cells. Serum is stored at −20°, with no apparent deterioration in 6 months as observed on the starch gel.

Typical patterns are shown in Fig. 1, with a convenient nomenclature of the bands given in Fig. 2. Polymorphism in the pre-albumins, α-globulins and β-globulins[6] has been found, and no two sera have yet shown identical patterns. The chief β-globulin patterns (variations in bands *B2*, *B3* and *B4*) are shown in the first three samples, (*a*), (*b*) and (*c*), type (*a*) being the more common. Sample (*d*) shows an example of a serum as yet found only in one particular litter of Large White pigs, and sample (*e*) the only serum so far obtained from an Essex breed; in both of these band *B6* is lacking or only present to a comparatively small extent. Sample (*f*) is from an 8-week-old animal, in which it can be seen that the γ-globulins *G3–5* are not yet fully developed[7]. Differences between the main breeds (Large White, Landrace, Wessex, Welsh), if any, are very small, yet the genetic polymorphism results in patterns of quite different appearances within one litter. It is probable that a statistical analysis of the various patterns will show certain forms as being more common in one breed than in another, and similar correlations should be possible with other characteristics of the animals[8].

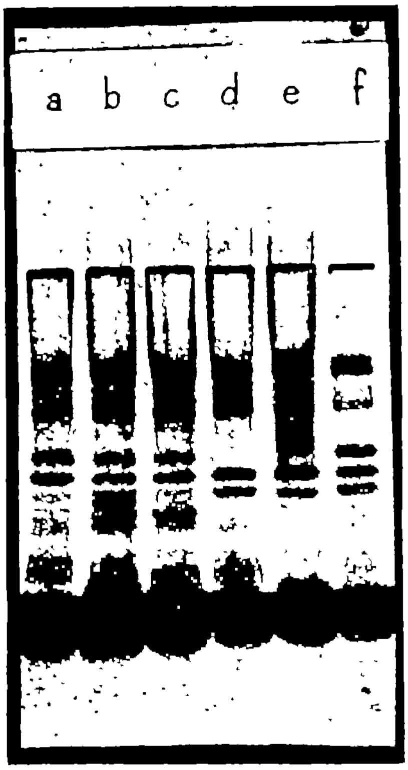

Fig. 1. Electrophoretic patterns of pig sera on starch-gel

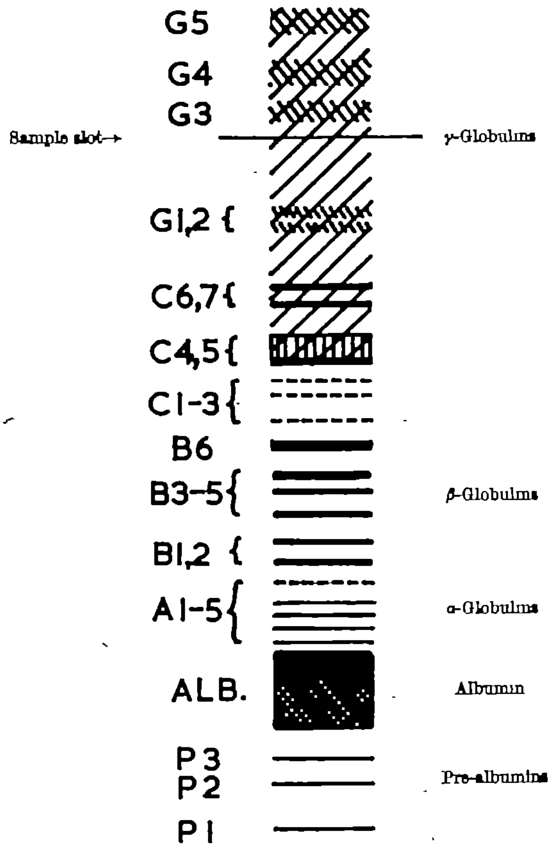

Fig. 2. Composite pattern of bands after electrophoresis, showing nomenclature used

Use of different buffer systems, for example, a triple discontinuity in the gel, results sometimes in better separations; with one system the albumin band has split into at least six components. But these methods are not always better for a complete comparison of all the protein bands, and so the system described here has been used for standard electrophoretic runs. Preliminary results with beef serum have yielded up to 36 bands using this standard method.

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Post-mortem Lability of Skeletal Muscle Proteins

As a result of the post-mortem conversion of glycogen to lactic acid, the pH of skeletal muscle falls from its *in vivo* value of about 7·3 to about 5·5, the actual level attained depending on several factors, such as the physiological state of the animal before death, the type of muscle and the temperature during glycolysis[1,2]. It has recently been shown in pig muscle that, where the rate of pH fall is fast and a relatively low pH is therefore attained while the temperature is still high, there may be considerable denaturation and precipitation of sarcoplasmic proteins[3]. It is also well known in enzyme preparatory procedures that lowering of the pH of extracts from animal tissues precipitates particulate material and nucleoprotein[4].

Giles[5], Tsuyuki[6] and others have used vertical starch-gel electrophoresis to separate sarcoplasmic proteins of various species. These were extracted from muscles which in some cases had already attained a low pH, however; it is thus likely that certain components had been denatured, were not extracted and did not appear on subjecting the extracts to starch-gel electrophoresis. The present investigations, in which a similar technique was used, have clearly indicated that certain sarcoplasmic proteins are unstable *post mortem*; precipitation of even small amounts of these can inhibit the normal extraction behaviour of muscle protein fractions[3,7].

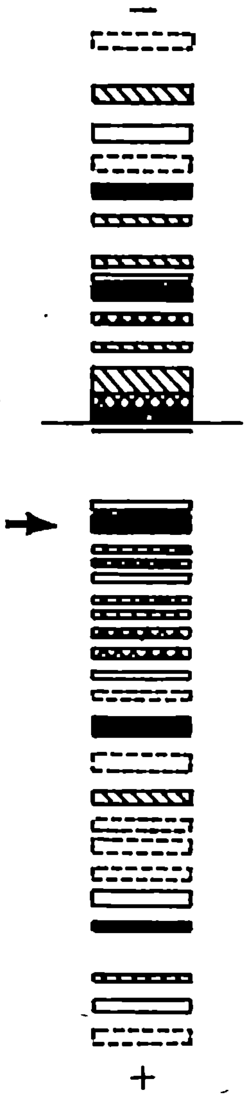

Fig. 1. Composite pattern of bands from beef muscle sarcoplasmic protein after starch-gel electrophoresis

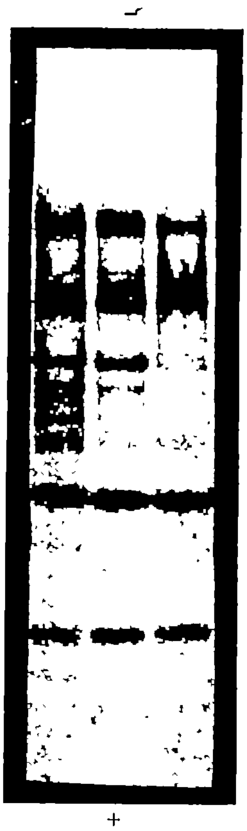

Fig. 2

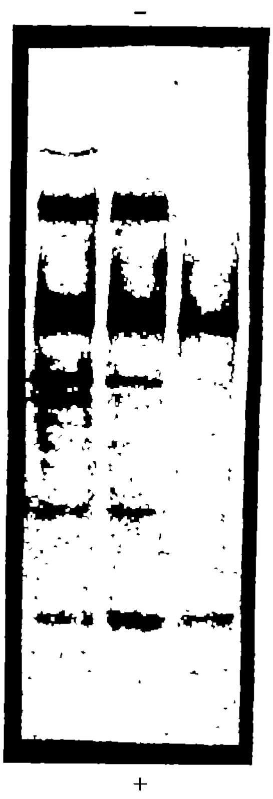

Fig. 3

Fig. 2. Electrophoretogram of beef muscle sarcoplasmic proteins. Left to right: extract of pre-rigor muscle; extract of muscle which was held at 0° for 20 h *post mortem*; extract of muscle which was held at 37° for 4 h *post mortem*

Fig. 3. Electrophoretogram of pig muscle sarcoplasmic protein. Left to right: (*a*) normal pig muscle; (*b*) 'white muscle disease' pig muscle; (*c*) severe 'white muscle disease' pig muscle

Beef l. dorsi muscle was obtained immediately after slaughter and had been chilled to 0° C. within 1 h of death. Samples were held at 0° C for 20 h, at 37° C for 4 h, or used at once. Homogenates were extracted with distilled water, after adjusting the pH to 7·0 with M *tris*, and the extracts were subjected to starch-gel electrophoresis. Some 35 bands can be obtained from beef muscle; these are diagrammatically represented in Fig. 1. In Fig. 2, it can be seen that several components are removed completely or very much diminished by the fast post-mortem glycolysis which occurs at 37° C in comparison either with the initial condition (before post-mortem glycolysis had proceeded appreciably) or with muscle in which post-mortem pH fall has been relatively slow (that is, at 0° C). Even in extracts prepared from muscles which have had a slow rate of pH fall, there are minor differences compared with those from the initial material of high pH, and it is evident that at the normal ultimate pH of muscle—5·5—certain sarcoplasmic proteins are already denatured and immobile.

Fig. 3 demonstrates that the major component of pig muscle sarcoplasmic proteins which migrates towards the anode at pH 8·5, and corresponds to a similar major component in electrophoretograms of beef muscle extract (arrowed in Fig. 1), and to band 9 in those of rabbit muscle (as tabulated by Hartshorne and Perry[8]), is markedly affected by the high-temperature/low pH combination. All these samples were prepared from post-rigor pig l. dorsi muscle; samples (*b*) and (*c*), however, were obtained from pigs having 'white muscle disease', in which the pH dropped rapidly *post mortem*, while the temperature was still high[8]. This caused considerable denaturation and insolubility of this sarcoplasmic component; some other proteins affected are also clearly demonstrated. Sample (b) shows some variation from the other two which may be attributable to breed differences.

Precipitation of the 'pH 5' proteins[9], by lowering the pH of sarcoplasmic extracts, produces a fraction which, on re-solution at pH 7·5, corresponds for the most part with the components most affected by the high-temperature treatment. It is concluded that *in situ* isoelectric precipitation of these proteins renders them more susceptible to heat denaturation. Most of them are still extractable on raising the pH to 7·0 providing that the temperature had not been high during the post-mortem pH fall.

Thus, it has been shown that several protein components of the sarcoplasmic complex are unstable and either denature, or are isoelectrically precipitated by the post-mortem pH fall in muscle, both if this is extensive (low ultimate pH) and/or fast. Many of the protein constituents are completely stable, however, and show no diminution in the starch-gel patterns, under these conditions.

This work was financed by the U.S. Department of Agriculture (grant No. *FG–UK*–101–59).

Note added in proof. The major component referred to (arrowed in Fig. 1) has been identified as creatine phosphoryltransferase in both pig and beef muscle. It is not, however, found in the 'pH 5' proteins.

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Spectrophotometric Assay of Ascorbic Acid Oxidase

The method generally used for assay of ascorbic acid oxidase depends on the fact that, within a certain range of enzyme concentration, the rate of oxygen consumption during ascorbic acid oxidation is proportional to the amount of enzyme present[1]. Daglish[2] and Hewitt and Dickes[3] proposed the utilization of the ultra-violet absorption peak of ascorbic acid at 265 mμ as a quantitative measurement of ascorbic acid.

A utilization of the ultra-violet absorptive properties of ascorbic acid is proposed for measuring ascorbic acid oxidase activity spectrophotometrically at 265 mμ, by following the diminution of the absorption peak of the acid. Fig. 1 shows the spectra of 0·1 and 0·2 μmole of ascorbic acid in 3 ml. in a 1-cm cuvette (curves A and B respectively) and after enzymatic oxidation (curve C). The enzyme preparation alone gave the spectrum shown in curve D. From these results it can be seen that the decrease in absorption at 265 mμ can be adopted for determining the rate of enzymatic oxidation of ascorbic acid.

A Beckman *DK2* recording spectrophotometer equipped with a time drive was used in this work; however, a *DU* spectrophotometer may be used. Small oranges, *Citrus sinensis* (1–3 cm diam.), were found to be high in ascorbic acid oxidase and were used as a source of the enzyme.

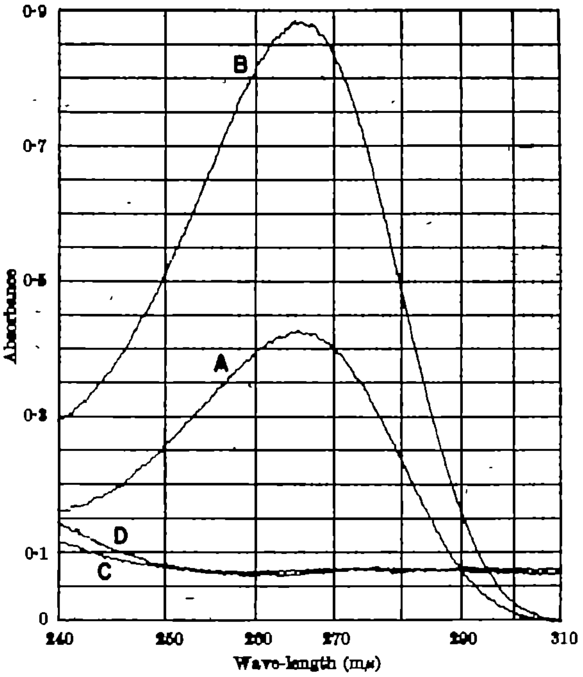

Fig. 1. Absorption spectra of ascorbic acid before and after enzymatic oxidation. The spectrum of 0·1 μmole and 0·2 μmole of ascorbic acid are shown (curves A and B). Curve C is the resulting spectrum of curve B after oxidation by 10 μl. of the enzyme preparation containing ascorbic acid oxidase. Curve D is the spectrum of 10 μl. of the enzyme preparation alone. The reference cell contained 3 ml. of 0·1 M phosphate buffer at pH 5·6 and the sample cell 3 ml. with 0·1 M phosphate buffer containing ascorbic acid and/or enzyme

Details of the enzyme preparation and purification appear elsewhere[4]. In general the tissue was macerated with 5 parts (w/v) 0·1 M phosphate buffer pH 6·5 in a Potter homogenizer. The homogenate was centrifuged at 3,000g for 15 min. The supernatant was retained for enzyme assay or purification. All procedures were carried out at 0–3° C.

The assay was carried out in silica cuvettes with a 1-cm light path at 25° C. Ascorbic acid in 0·5 μmole quantities in 3 ml. of 0·1 M phosphate buffer at pH 5·6 (ref. 4) was added to both the sample cell and the reference cell to compensate for autoxidation and large absorbance values of the ascorbic acid utilized.

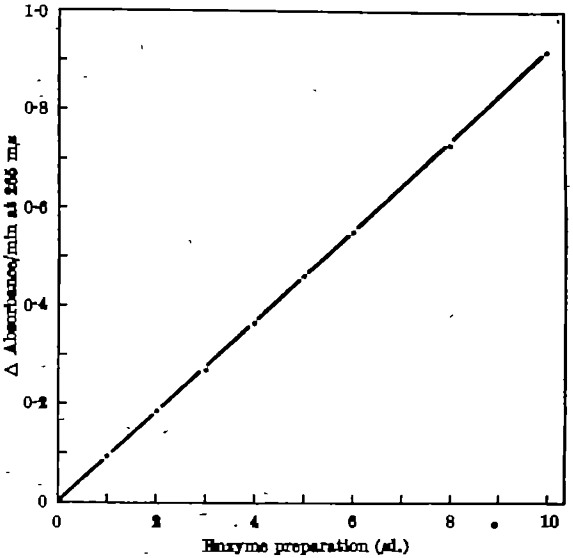

Fig. 2. Proportionality of enzyme activity (change in absorbance) to increased enzyme concentration. The procedure outlined in the text was followed with variation in concentration of the enzyme preparation as indicated. Ascorbic acid concentration was 0·5 μmole in both the reference and sample cell in a total volume of 3 ml. of 0·1 M phosphate buffer at pH 5·6

At zero time, various amounts of the enzyme preparation were added to the reference cuvette, thus allowing for a positive increase in the change in absorbance due to the oxidation of ascorbic acid. This change was recorded continuously at 265 mµ using a time-drive at 1 in./min. The rate was constant and could be extrapolated back to zero time.

The relationship between the amount of enzyme activity (change in absorbance per minute) and enzyme concentration can be seen in Fig. 2.

Absorbance results obtained spectrophotometrically were converted to establish enzyme units obtained manometrically (10 µl. oxygen/min)[1]. Temperature corrections were made on 10 µl. oxygen at 25° C and the corrected volume was converted to µmoles of molecular oxygen. One enzyme unit is equivalent to 0·405 µmole oxygen/min. Ascorbic acid requires only 1 atom of oxygen per molecule oxidized. Therefore, 0·81 µmole of ½ oxygen/min is utilized per enzyme unit. From Daglich's results[2] ascorbic acid has an $E^{1\%}_{1\,\mathrm{cm}}$ of 760 at 265 mµ. From this it was calculated that ascorbic acid has an absorbance of 4·4 per µmole in a 3-ml. volume. Therefore, 1 enzyme unit (0·81 µmole of ½ oxygen/min) would be equivalent to an absorbance change of 3·58/min. Manometric and spectrophotometric assay of enzyme units of the same enzyme preparation gave 11 and 12 enzyme units/ml. respectively.

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Presence of Phosphate-mediated Cross-linkages in Hard Tissue Collagens

It has often been suggested that covalent interchain cross-linkages contribute to the stability of collagen fibrils[1,2]. Gustavson[1] speculated that ester bonds exist between free carboxyl groups and the hydroxyl groups of the hydroxy amino-acids. The work of Gallop *et al.*[3] and Hormann *et al.*[4] has substantiated this proposal, implicating carbohydrate or sugar cross-linkage as well. We have found in our work with dentine collagen evidence for still another type of cross-linkage which might apply to the collagens of the calcified tissues, bone and dentine.

Bovine dentine collagen, decalcified with ethylenediamine tetraacetic acid (EDTA) at *p*H 7·5, was found to exhibit markedly different swelling and solubility characteristics in comparison with purified bovine corium collagen. For example, bovine corium collagen swells maximally to 700 per cent at *p*H 2·3 in salt free hydrochloric acid, whereas dentine collagen has no appreciable swelling in the entire acid-range. Bovine corium collagen can be rendered soluble to the extent of 8 per cent by isoionic heating at 60° C for 1 h. With bovine dentine collagen less than 2 per cent is solubilized under the same conditions after 20 h. These results obviously suggest a difference in the mode or extent of the intermolecular stabilization of these two types of collagen.

Comparative chemical analyses showed both collagens to have very similar amino-acid compositions and carbohydrate contents. However, the purified decalcified dentine collagen had a phosphorus content which, expressed in phosphate residues (HPO_4), averaged 0·4 per cent by weight. This phosphorus could not have been the result of residual calcium hydroxyapatite since the calcium/inorganic phosphorus ratio was 0·002 in the purified dentine collagen, compared with a ratio of 2·12 in the undecalcified dentine and 2·16 in pure calcium hydroxyapatite. Since the phosphorus could not be removed by prolonged EDTA extraction or dialysis, it is apparently present in a covalently bound form, presumably as the phosphate. The phosphate content noted here would correspond to 4 phosphate residues per 1,000 amino-acid residues, or to 12 potential cross-linkage sites per tropocollagen monomer unit. Phosphorus is essentially absent in the bovine corium collagens.

The phosphorus-containing moiety is present in a relatively stable form. On conversion of the purified, decalcified dentine collagen into gelatin by heating in water at 110° C overnight it was found that the non-dialysable gelatin still maintained two-thirds its original phosphorus content. Venom and spleen phosphodiesterases, used at their usual optimal *p*H, were ineffective in degrading the purified dentine collagen. The acid swelling and solubility properties of the treated collagen were unchanged after this treatment. However, these results certainly do not rule out the presence of phosphodiester linkages, since the highly organized collagen structure may mask the phosphate groups and diminish the enzyme activity. Likewise, the specificity of these enzymes may not be appropriate; all phosphodiesters are not equally good substrates for every phosphodiesterase[5]. In this regard it is pertinent to note that dentine collagen is also particularly resistant to the action of the usual protein denaturants (for example, 8 M urea) which have a strong denaturing action on bovine corium collagen. Work is in progress on the more accessible phosphate groups in the non-dialysable gelatins obtained by high-temperature hydrolysis. Investigations with oxidizing agents such as sodium periodate suggest that the phosphate may be associated with the carbohydrate moieties in dentine collagen.

These results lead us to suggest that the unusual properties of dentine collagen may be ascribed, in part, to the presence of cross-linkages mediated by phosphate groups. Perlmann[5] has described phosphate esters of three types in other proteins and peptides, —O—P—O—, —N—P—O—, and pyrophosphate, involving serine, threonine and arginine, and possibly carbohydrate. In addition, the hydroxy amino-acids peculiar to collagen, hydroxyproline and hydroxylysine should be considered as potential sites for phosphate ester formation.

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Heterogeneity of Urinary Acid Mucopolysaccharides

687 mg of crude urinary acid mucopolysaccharides (preparation *A*) were precipitated from 30 litres of normal human urine according to methods previously described[1].

587 mg of *A* were incubated with crystalline papain at 37° for 70 h in phosphate buffer *p*H 6·30, 0·2 M, containing ethylenediamine tetraacetic acid 0·001 M and cysteine 0·005 M. The digest was applied to a 30 × 1 cm column of 'Dowex 1 × 2' (200–400 mesh, acetate form) and elution was performed with 200 ml. of acetate buffer *p*H 5·90, 0·1 M, followed by 200 ml. of 4 M sodium chloride. The

Table 1. ANALYSES OF CRUDE URINARY MUCOPOLYSACCHARIDES (PREPARATION *A* AND *B*) AND OF THE 10 PEAKS ELUTED FROM 'ECTEOLA' COLUMN

Preparation	Weight (mg)	Hexosamine (per cent)	Hexuronic acid (per cent)	N (per cent)	S–SO₄ (per cent)	Glucosamine (per cent)*	Molar ratio, hexosamine = 1·00: Hexuronic acid	N	S–SO₄
A	687	14·0	10·0	8·75	1·76	Not performed	0·62	7·7	0·62
B	132	23·2	25·4	5·90	2·90	20	1·00	3·2	0·74
'ECTEOLA'									
Peak 1	3·0	19·6	17·0	5·30	0	17	0·80	3·5	0
Peak 2	4·85	21·7	25·6	7·80	0	13	1·10	4·6	0
Peak 3	7·00	20·7	27·0	10·40	0·94	27	1·30	6·7	0·27
Peak 4	5·72	20·8	26·5	6·40	1·90	14	1·20	4·2	0·55
Peak 5	10·40	21·4	27·2	10·20	1·87	23	1·15	6·1	0·48
Peak 6	16·10	22·3	26·4	9·20	1·43	24	1·05	5·2	0·36
Peak 7	20·40	25·9	26·0	7·80	1·90	14	0·93	3·9	0·43
Peak 8	24·20	25·0	27·0	6·40	4·40	10	1·00	3·3	1·00
Peak 9	25·60	24·6	26·4	8·00	3·50	27	0·96	4·0	0·80
Peak 10	13·20	21·7	21·0	7·70	3·80	28	0·82	4·6	1·00
Total weight material in peaks 1–10	130·70								
Average values for peaks 1–10		22·3	25·0	7·93	2·5	20	1·04	4·6	0·62

* The balance is per cent galactosamine.

4-ml. fractions collected were scanned for absorption at 280 mμ and for hexuronic acid content with the carbazole reaction. The protein material was eluted with the acetate buffer; the hexuronic acid-containing material, eluted with 4 M sodium chloride, was concentrated, dialysed, dried (132 mg recovered) and analysed (preparation *B*). The latter was then applied to a 40 × 4 cm column of 'ECTEOLA' modified cellulose (reagent grade; Brown Co., Berlin, New Hampshire), which was washed and eluted stepwise with solutions of increasing chloride concentration, according to the technique described by Anseth and Laurent[2].

Table 1 gives the weight and the analyses of preparations *A* and *B* and of the 10 peaks eluted from the 'ECTEOLA' column.

The 10 peaks were pooled as follows: peaks 1 and 2 = pool 1; peaks 3, 4, 5, 6, 7 = pool 2; peaks 8 and 9 = pool 3; peak 10 = pool 4.

The material from each pool was dissolved in water, electrodialysed and precipitated with 3 volumes of 95 per cent ethyl alcohol containing 0·3 per cent barium acetate. It was then delivered to a 12 × 2 cm column of cellulose, previously equilibrated with 80 per cent ethyl alcohol containing 0·3 per cent barium acetate. Elution was performed with a decreasing alcohol gradient[2] and the peaks were localized by the carbazole and anthrone reactions.

In each chromatographic run, anthrone-positive material was eluted between 80 and 40 per cent alcohol concentration and carbazole-positive material between 40 and 10 per cent alcohol concentration.

The anthrone-positive material from the four pools was combined, electrodialysed, dried and analysed (Table 2). An aliquot was hydrolysed and used for bi-dimensional ascending paper chromatography, using butanol 3, pyridine 2, water 1·5 as first solvent, water-saturated phenol as second solvent and aniline phthalate as the spray for development. The presence of galactose, glucose and arabinose was revealed.

Table 2. MOLAR RATIO OF THE FRACTIONS OBTAINED FROM POOLS 1–4 BY ALCOHOL FRACTIONATION

Fraction		Hexosamine*	Hexuronic acid	N	S—SO₄	Hexose	Glucosamine (per cent)
Anthrone-positive		1·00	0·30	4·00	not performed	1·85	95
Pool 1	*A*	1·00	0·90	3·70	0		13
	B	1·00	0·95	6·40	0		35
	average	1·00	0·92	5·05	0		24
Pool 2	*A*	1·00	0·94	4·30	0·40		35
	B	1·00	1·10	1·50	0·44		17
	C	1·00	1·10	1·85	0·34		19
	D	1·00	1·00	2·60	0·37		44
	average	1·00	1·03	2·56	0·39		28·7
Pool 3	*A*	1·00	1·00	2·30	0·65		9·5
	B	1·00	1·20	1·75	0·44		4·3
	C	1·00	1·00	1·65	0·83		12·5
	D	1·00	0·94	7·10	1·15		35
	average	1·00	1·03	4·00	0·77		15·3
Pool 4	*A*	1·00	0·60	8·40	0·88		32
	B	1·00	0·95	4·00	1·00		7·8
	C	1·00	1·20	2·90	0·94		11·5
	D	1·00	1·20	4·20	1·00		40·5
	average	1·00	0·98	4·90	0·95		23

* Hexosamine is arbitrarily made equal to 1·00.

The carbazole positive material of each pool was eluted in a pattern suggesting the presence of several partially resolved peaks and it was separated accordingly into arbitrary fractions (*A*, *B*, *C*, *D*). Analyses of the different fractions are presented in Table 2.

When the average molar ratio for each pool is compared with the average molar ratio of the 'ECTEOLA' peaks which constituted the pool, a fairly good agreement is observed, with the exception of the nitrogen value for pool 2, which is considerably lower than the average nitrogen value of peaks 3–7 from the 'ECTEOLA' column.

Although a separation of the glucosamine and galactosamine-containing mucopolysaccharides was not achieved with these techniques, nevertheless the results obtained are interesting in two respects.

First, the presence in normal human urine of a mucopolysaccharide containing glucosamine and neutral sugars is suggested. Whether this represents keratosulphate or the prosthetic groups of a glycoprotein cannot be stated at this moment. The small amount of material available (less than 1 mg) did not permit further purification for infra-red analysis or sulphate determination. The fact, however, that the material was retained on the 'ECTEOLA' column and was eluted with increasing salt concentration indicates the presence in it of strongly anionic groups.

Second, analyses of the hexuronic acid-containing fractions clearly indicates a different degree of sulphation. This fact suggests that a mechanism for the desulphation of acid mucopolysaccharides may exist, despite the failure, thus far, to identify the system responsible for it.

Recently, Kaplan and Meyer[3] have injected intravenously, in humans and dogs, highly purified acid mucopolysaccharides at a dose of approximately 1 mg/kg and have followed the ensuing urinary excretion of the polymer injected. Their finding that the small percentage of the material excreted was chemically identical to the injected polysaccharide is in contrast with our data and a possible explanation may be found in the substantial difference between the two experimental conditions. The hexuronic acid-containing fractions isolated in our study have a considerable amount of non-hexosamine nitrogen. This fact in conjunction with the observation of Kaplan and Meyer suggests that desulphation may occur at or near the natural site of occurrence of acid mucopolysaccharides, or at least prior to their entry into the circulation. Even if it is assumed that a highly purified mucopolysaccharide could be accepted as a substrate by the system responsible for desulphation, the question remains as to whether the injected polymers are able: (*a*) to reach the site of the specific desulphating system and (*b*) to compete for it with the already present natural substrates.

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Purification of Human Growth Hormone on 'Sephadex G-200'

WE have previously reported the preparation of human growth hormone (HGH) from acetone powders of pituitary glands[1]. The hormone preparations were obtained in high yield and proved satisfactory in clinical investigations but were by no means electrophoretically homogeneous. We wish to describe now a simple and reproducible method for further purification of this material which has resulted in a growth hormone preparation of markedly increased specific biological activity. This preparation, although still not homogeneous, was shown to be substantially purified by the criteria of disk electrophoresis and ultracentrifugation. No serum protein contamination could be detected when this purified preparation was assayed by immunological techniques. A second fraction obtained by this procedure showed some growth hormone activity and in addition possessed considerable luteinizing hormone (LH) potency. This fraction was analysed by immunological techniques and found to contain serum albumin and a smaller amount of serum globulins.

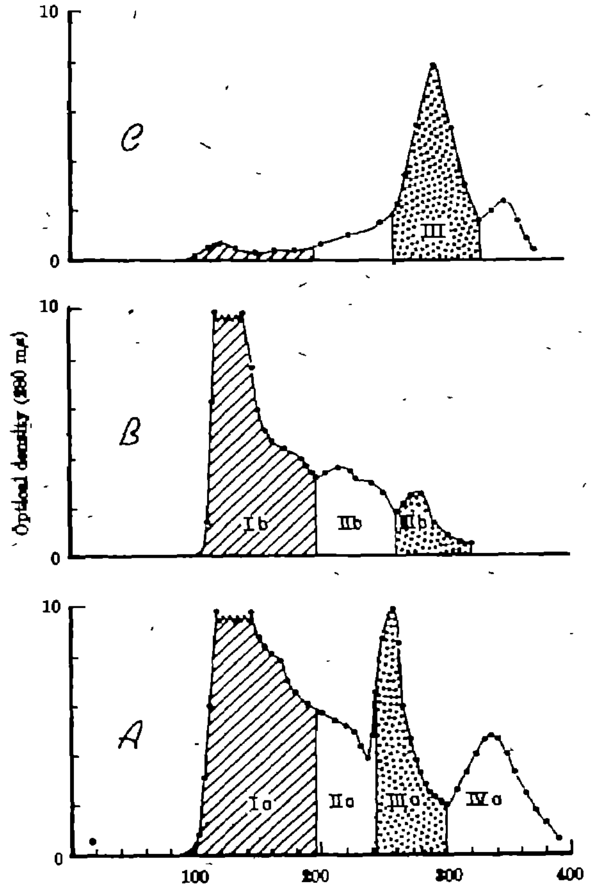

Fig. 1. *A*, Initial chromatography on 'Sephadex G-200'; *B*, chromatography of combined fractions Ia and IIa; *C*, chromatography of combined fractions IIIa and IIIb

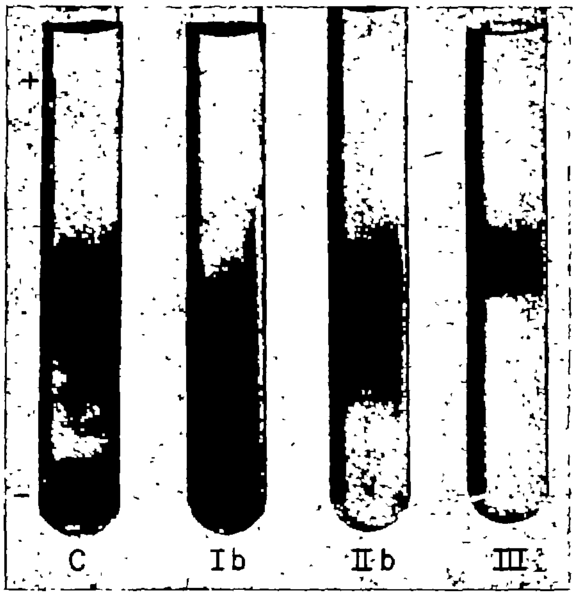

Fig. 2. Disk electrophoresis patterns of starting material (*C*) and fractions I*b*, II*b* and III

In a typical experiment, 1 g of a human growth hormone concentrate (fraction *C*)[1] was dissolved in 17 ml. of ammonium–formate buffer, *p*H 9·2 (0·1 M ammonia, 0·05 M formic acid), and placed on a column of 'Sephadex *G*-200' (1·8 × 120 cm) which had been equilibrated with the same buffer. This buffer was also used for eluting the applied material. The effluent diagram is shown in Fig. 1. Eluate was collected in 7-ml. cuts at 1-h time-intervals. Protein concentrations were estimated by measuring absorption at 280 mμ. The effluent portions were grouped into fractions I*a*, II*a*, III*a* and IV*a* after analysing numerous individual cuts by disk electrophoresis[2,3]. Fraction IV*a* was discarded since it contained mainly salts and nucleic acids. Each of the other combined fractions was lyophilized and then desalted by gel filtration on 'Sephadex *G*-25'. All the material eluted prior to fraction III*a* was combined and again passed over a column of 'Sephadex *G*-200', yielding fractions I*b*, II*b* and III*b*. The effluent diagram resulting from this procedure is also shown in Fig. 1. On the basis of electrophoretic analyses, fractions III*a* and III*b* were combined and again put through a column of 'Sephadex *G*-200' to remove any remaining quantities of fractions I*b* and II*b*. The purified growth hormone thus obtained was designated fraction III as shown in Fig. 1. Fig. 2 shows the patterns which were obtained by disk electrophoresis. Fraction III exhibits considerable improvement in purity when compared with starting material. In addition, fraction III was white in appearance and its solution was essentially colourless, while fractions I*b* and II*b* possessed the same brown, hæm-like colour as the starting material.

The yields and biological activities of various fractions are summarized in Table 1.

All hormone assays were performed by standard procedures previously described[1]. Protein concentration was measured by a modification of the phenol-biuret method as described by Sutherland *et al.*[4].

Ultracentrifugal analyses of fractions II*b* and III at one protein concentration (1·4 per cent) showed sedimentation

Table 1. YIELDS AND BIOLOGICAL ACTIVITIES OF FRACTIONS

Fraction	Yield* mg/protein/g starting material	HGH activity U.S.P. units/mg protein	LH potency X standard†	Prolactin‡ I.U./mg protein
Starting material		0·8–0·9	0·1	2–5
Ib	450–500	0·1	< 0·01	N.D.
IIb	100–150	0·2–0·3	1–1·5	<0·5
III	250–300	2–2·5	< 0·01	4

* Average of 30 experiments.
† Armour LH standard 227–80.
‡ Prolactin assays were provided by Dr. A. Segaloff.
N.D., Prolactin activity was not determined.

constants ($S_{20,w}$) of $4 \cdot 9 \pm 0 \cdot 1$ and $2 \cdot 3 \pm 0 \cdot 1$, respectively. Both fractions sedimented as single boundaries.

It is of interest that fraction II*b* containing luteinizing hormone activity and the human growth hormone fraction (III) were easily separable on 'Sephadex *G*-200', but were not separated by disk electrophoresis. When a 1 : 1 mixture of these two fractions was analysed by disk electrophoresis the main electrophoretic component of fraction II*b* was superimposed on the major component of fraction III. This we found to be true with both 7·5 per cent and 15 per cent acrylamide gels. Whether the prolactin activity of the highly purified fraction III is due to an intrinsic property of human growth hormone or to contamination with prolactin cannot yet be ascertained, since this preparation still contains two significant electrophoretic components.

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Effect of Carbon Tetrachloride Intoxication on the Activity of Enzymes of the Urea Cycle in the Liver of the Rat

THERE are many indications that amino-acid metabolism is altered following administration of carbon tetrachloride[1–3]. Smuckler *et al.*[1] have shown that there is an impairment of incorporation of amino-acid into protein as early as 1 h after administration of carbon tetrachloride, probably associated with changes in the structure of the endoplasmic reticulum. The level of activity of ornithine transcarbamylase in the blood also rises rapidly following this type of liver damage[4] while at longer time-intervals there is a decrease in the activity of glutamic dehydrogenase[5] and glutamic-pyruvic transaminase[6].

In investigating the changes following carbon tetrachloride intoxication the measurement of a series of enzymes linked in a metabolic cycle or chain is perhaps of greater interest than that of individual enzymes. The activity of urea cycle enzymes in rats treated with carbon tetrachloride are of significance not only in relation to changes in the metabolism of amino-acids but also because of the different intracellular localization of the enzymes in this cycle. The first two, carbamyl phosphate synthetase and ornithine transcarbamylase, are mitochondrial enzymes and the remainder, arginine synthetase, the rate-limiting step, argininosuccinase and arginase, are in the soluble fraction of the cell[6]. The pattern of change in the activity of these enzymes at different time-intervals after carbon tetrachloride administration was followed in an attempt to correlate the biochemical alteration with the known effects of carbon tetrachloride on cell structure[1,7,8].

The results of these experiments, summarized in Table 1, show that as early as 90 min after treatment of the rats with carbon tetrachloride there was an increase in the two mitochondrial-linked enzymes, carbamyl phosphate synthetase and ornithine transcarbamylase, both as units/g liver and as total activity. At 3 h this effect was even more striking. The other enzymes of the urea cycle, located in the soluble fraction of the cell, remain completely unchanged.

At longer time-intervals (24 h after administration of carbon tetrachloride) the activities of carbamyl phosphate synthetase and ornithine transcarbamylase fell, both in relation to the elevated values at 3 h and, in the case of the former enzyme, in relation to the control value. The specific activity (units/g liver) of the other three enzymes of the urea cycle decreased considerably; there is, however, a large increase in weight of liver at this time following carbon tetrachloride administration, and the total activity of these enzymes in the liver was significantly decreased only in the case of the arginine synthetase system. Flores *et al.*[3] found that the total activity of both arginine synthetase and arginase were decreased.

Recknagel and Litteria[9] have shown that carbon tetrachloride reaches its maximal concentration in the liver at about 2 h after the administration by stomach tube. The present results, showing a significant rise in the activity of carbamyl phosphate synthetase and ornithine transcarbamylase at this time-interval, are among the earliest changes reported in mitochondrial enzymes in carbon tetrachloride intoxication. Rossi and Zatti[10] have reported that the incorporation of phosphorus-32 into liver phospholipids *in vivo* rises at this stage and this may be closely related to some modification of mitochondrial structure. There is, however, no evidence from electron microscopy studies of extensive alterations in mitochondrial morphology as early as 90 min after administration of carbon tetrachloride, and it is generally thought that the first biochemical and morphological alterations are in the endoplasmic reticulum and that the mitochondrial damage takes place later[1,2,7,8].

We thank Dr. R. G. Westall for a supply of argininosuccinate and the Wellcome Foundation for a grant to one of us (F. R.). This work was supported in part by a grant

Table 1 EFFECT OF CARBON TETRACHLORIDE INTOXICATION ON THE ACTIVITY OF ENZYMES OF THE UREA CYCLE IN RAT LIVER

	No. of animals	Units/g liver				
		Carbamyl phosphate synthetase	Ornithine transcarbamylase	Arginine synthetase	Argininosuccinase	Arginase
Control animals	12	250 ± 16	14,190 ± 960	56 ± 4	208 ± 12	98,360 ± 6,340
Time after CCl_4 administration						
90 min	8	348 ± 33	18,880 ± 1,440	67 ± 7	231 ± 10	107,800 ± 13,400
Fisher's *P*		0·016	0 011	N.S.	N.S.	N.S.
180 min	8	383 ± 31	20,480 ± 900	60 ± 5	224 ± 7	92,500 ± 7,370
Fisher's *P*		0·002	< 0·001	N.S.	N.S.	N.S.

Enzyme activities were determined by the method of Brown and Cohen (ref. 11). A unit of enzyme activity is defined as that amount which catalyses the production of 1 μmole of citrulline or urea/h under the assay conditions. The values are expressed as means ± *S.E.M.* Fisher's *P* values for the comparison of the control and experimental groups are given, a difference being considered significant if this is no greater than 0·05. N.S. indicates that the results are not significantly different. The rats were starved overnight and carbon tetrachloride in liquid paraffin (1 : 1 v/v) was given by stomach tube (0·5 ml./100 g body-weight) with the rat under light ether anaesthesia. The control animals were similarly treated with 0·25 ml. liquid paraffin (B.P.)/100 g body-weight.

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Visualization of Different Diphospho- and Triphospho-pyridine Nucleotide Dehydrogenase Isozymes on an Agar-gel Plate

We have been looking for a quick and relatively cheap method of screening dehydrogenase isozymes, after electrophoresis. For this purpose, we chose agar gel as a support. We recognize that there is a greater diffusion on agar gel than on starch gel, and also that there is some difficulty in slicing the gel before detecting the isoenzymes. Nevertheless, we prefer agar gel because: (1) it allows a relatively quick electrophoretic partition; (2) it is transparent when dry; (3) it allows the use of the Grabar and Williams technique of immunoelectrophoresis[1].

For this medium, we developed a convenient tetrazolium method which: (1) limits the diffusion during the reaction period: (2) requires a minimal quantity of reaction components, some of them being very expensive; (3) can be used rapidly for different purposes on the same plate.

So far as we know, the method described here seems quicker, cheaper and more versatile than some other isozyme methods using tetrazolium salts[2,3].

The electrophoresis was carried out in a 1 per cent agar gel ('Agar Noble', Difco), in 0·05 M phosphate buffer at *p*H 7 for a good partition of slow components, or in *tris* buffer 0·05 M when phosphate ions are to be avoided. The gel was poured on photographic glass plates (13 × 18 cm for 4 samples, or 18 × 24 cm for 8 samples). Each sample was inserted in a 20 × 3 mm slit cut out of the agar gel with a special punch. The preparation was kept for 105 min in a field of 6 V/cm in the cold (+ 4°). The plate was then brought to laboratory temperature. It was covered with a wooden board which has been wetted before application so that it will stick to the agar gel. This little board outlined 4 or 8, 25 × 150 mm slits which lay over the fractionated samples. In each slit of the board was poured a solution containing the buffer, some substrate, coenzyme, oxidation inhibitor electron transporter (phenazine methosulphate) and tetrazolium salt, in 1 per cent agar gel.

For the detection of lactic dehydrogenase in plasma, 0·1 ml. of plasma was mixed with 0·1 ml. of 2 per cent agar gel. During the time the electrophoresis is being run in phosphate buffer at *p*H 7, the following solutions was prepared:

(*a*) Aliquots of a stock solution of the following compositions, prepared beforehand, and stored at 35° C in 5 ml. vials, were taken from the freezer: sodium lactate, 30 mg; diphosphopyridine nucleotide (Sigma), 2 mg; sodium azide, 10 mg; 3(4,5 dimethyl thiazolyl 1,2)2,5 diphenyl tetrazolium bromide (ATT: Mann), 2 mg (dissolved in 0·2 ml. acetone); *tris* buffer (0·05 M, *p*H 8·2), 2 ml.

The solution was transferred to a hæmolysis tube and warmed to 42° C. To this was added 2 ml. of 2 per cent agar gel in water, then 0·2–0·5 mg of sodium phenazine methosulphate (Sigma). After quick mixture, it was poured in one of the slits and immediately covered, to protect the complex from light.

After incubation for 1 h at 37° C in a damp atmosphere or 2 h at laboratory temperature, the isozyme pattern was very apparent. If desired it could be covered with a thin layer of 1 per cent agar gel in order to obtain a flat surface. Afterwards, the plate was fixed for 2 h in 2 per cent acetic acid. The preparation was then covered with filter paper and dried at 37° C.

The plate with the dried film could be kept indefinitely in the dark, and filed as a laboratory record.

We applied this system to visualize the isozymes of lactic acid dehydrogenase of normal and pathological plasma, as well as the isozymes of lactic, malic, isocitric, glutamic, β-hydroxybutyric and glucose-6-phosphoric acid dehydrogenases in rat liver homogenates. In addition, we applied our system after plasma immunoelectrophoresis. We are now examining the coloured precipitation lines obtained to determine whether they are enzymatically active.

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PHYSIOLOGY

Uterine Relaxant Properties of Bradykinin *in vitro*

At the present time, there is no effective means of blocking uterine contractions in order to prevent premature labour. A drug which would block uterine contractions may reduce the incidence of prematurity. Of all infant deaths occurring at the New York Hospital in 1961, 72·5 per cent were prematurely born.

Bradykinin was synthesized by Boissonnas, Guttmann and Jaquenoud[1] and was shown to be a nonapeptide. Elliot, Horton and Lewis[2] demonstrated the great sensitivity of the isolated rat uterus to bradykinin as indicated by increased contractility. Because of this constrictor response in the rat, Berde and Saameli[3] investigated the effect of bradykinin on the human uterus *in situ*. Intravenous injections of 2 μg/kg were without any effect on the human uterus. These authors concluded that bradykinin had no constrictor activity on the human uterus. Because of its known relaxant effect on smooth muscle, bradykinin in various concentrations was applied to human uterine muscle. Strips were placed in an organ bath of 5 c.c. capacity containing oxygenated mammalian Krebs's solution[4] which was kept at constant temperature and *p*H.

Bradykinin was applied 112 times to 32 non-pregnant uteri and 52 times to 13 pregnant uteri at the time of section. The effect on tonus, frequency and amplitude maximum was recorded after a pattern of spontaneous or induced contractions was established. A reduction in at

Table 1. Effect of Bradykinin on the Human Uterus *in vitro*

	Concentration of bradykinin (μg/ml.)						
	0·2	0·4	0·6	0·8	1·0	1·2	1·4
Non-pregnant: Incidence of response	11/20 55%	27/32 84·5%	17/18 94·5%	29/30 96·5%	12/12 100%		
Pregnant: Incidence of response	6/10 60%	12/12 100%	3/4 75%	13/13 100%	6/6 100%	4/5 80%	2/2 100%

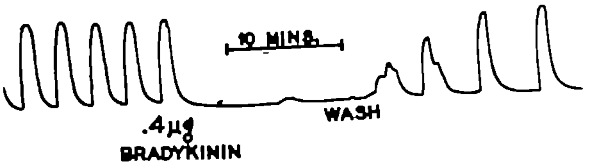

Fig. 1. In the non-pregnant uterus prompt relaxation following bradykinin with recovery is demonstrated

least one of these variables was considered a relaxant response. In the non-pregnant uterus a concentration of 0·2 μg/ml. produced a significant relaxant response in 57 per cent of the tests. At levels of 0·8 μg/ml. a maximum response in the non-pregnant uterus resulted in a complete inhibition of all contractions. In the pregnant uterus, as indicated in Table 1, slightly higher concentrations were required to inhibit contractility. Oxytocin-induced contractions required slightly higher levels of bradykinin than the spontaneous variety. This was particularly true in the pregnant muscle. Fig. 1 shows a low concentration of bradykinin-inhibiting contractions of the non-pregnant uterus in the presence of oxytocin and a return to the original pattern following a wash.

The high potency of this compound on human uterine muscle with its inhibiting action on contractions is of considerable interest. A study of bradykinin on *in situ* human uterus is indicated to determine its possible effect in preventing the onset of premature labour. The known hypotensive effect of bradykinin makes it unlikely that its use alone may be practical. A combination of a hypertensive agent, such as angiotensin, with bradykinin may provide effective therapeutic agent.

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Effect of Hydrogen Ion on the Rates of Migration of Sodium and Potassium Ions: Possible Relationship to Magnetism

I have read with interest the recent communication of Salminen[1] on the effect of hydrogen ions on the differential rates of migration of sodium and potassium ions. I believe this may relate to an experiment which I have performed demonstrating a differential effect of external magnetic fields on the concentration of these ions across a semipermeable membrane.

A 4-in. length of 'Cellophane' tube of the sort used in the artificial kidney apparatus was filled with the usual dialysate solution for blood lavage, the ends were sealed and it was suspended in a beaker of the same solution. The pole of a permanent magnet capable of lifting a 125-lb. weight of steel was placed against the 'Cellophane' tube so that the strongest magnetic field was inside the tube. After incubation for 9 h the concentration of ions in the two compartments was determined by standard methods available in the clinical laboratory. The experiment was repeated five times, and in each instance the results were similar to those given in Table 1.

Table 1

	Mg	Ca	K	Na	Cl	HCO₃
'Cellophane' tube	3·5	4·5	4·9	160	122	*
Beaker	3·5	4·5	4·4	151	123	*

All values in m.equiv./l.
* Present but not determined.

The sodium, potassium and chloride ions were always more concentrated in the 'Cellophane' tube than in the surrounding bath. This difference could be increased greatly by decreasing the volume of solution inside the 'Cellophane' tube. The increase of potassium was always proportionately greater than the sodium. The failure to demonstrate any effect on magnesium and calcium may have been due to formation of unionized complexes with bicarbonate.

I have surmised that the movement of electrons along the chains of respiratory enzymes in the mitochondria and the concurrent movement of hydrogen ions in the same direction generate electromagnetic fields, as do other electric currents, and that these magnetic forces may be a determining factor in the characteristic proportions of intracellular ions, and thereby related to the mechanism of the 'salt pump'.

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Dr. George's communication contains no mention of control experiments without a magnetic field. Thus it is open to question whether the results, interesting as such, are related to some factors other than the magnet. The fact that the experiments were performed with dialysate fluid made for blood lavage suggests that its P_{CO_2} would have been higher than that of the air. The loss of carbon dioxide from the beaker might have produced a higher gradient of H⁺ in the 'Cellophane' tube, resulting in an accumulation of other cations in this phase, as was found, for example, by Teorell in his classical work[1]. That the effect of H⁺ on the rates of simultaneous non-equilibrium migration of Na⁺ and K⁺ is different was experimentally found in my communication referred to by Dr. George.

A theoretical treatment of these problems is in progress by our group, using the concepts of irreversible thermodynamics.

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Effect of 3:5:3′-triiodothyroacetic Acid on the *In vitro* Oxygen Uptake of Leucocytes of Hypopituitary Patients

Neither L-3 : 5 : 3′-triiodothyronine (T_3) nor 3 : 5 : 3′-triiodothyroacetic acid (TRIAC) affect the uptake of oxygen by leucocytes from normal individuals studied *in vitro* with the direct method of Warburg with a modified Krebs's No. 2 buffer. When, however, these *in vitro* investigations are carried out with leucocytes from patients with primary hypothyroidism, an immediate stimulation of oxygen uptake can be demonstrated with TRIAC although no effect is produced by T_3 (ref. 1).

The opportunity was taken to carry out similar investigations on the leucocytes from 5 other patients who were also hypothyroid. In these patients, however, the hypothyroid state was the result of hypopituitarism and there was clinical and investigational evidence of the other expected endocrine deficiencies.

Blood was collected in heparinized siliconed tubes according to the method of Bisset and Alexander[4]. The leucocytes were isolated after sedimentation with dextran (6 per cent w/v in 0·9 per cent sodium chloride solution). From 80–100 ml. of blood from each patient sufficient leucocytes were obtained to set up 4 flasks, each of which contained approximately 40–50 million leucocytes in Krebs's No. 2 buffer. TRIAC in two concentrations, 25 and

50 µg/100 ml. medium, was added to duplicate flasks. The oxygen uptake of the leucocytes was examined by the direct method of Warburg using air as the gas phase. Readings were taken every 15 min. The results were expressed as the uptake of oxygen in µmoles/h/10^7 leucocytes.

Table 1. UPTAKE OF OXYGEN BY LEUCOCYTES (µMOLES/H/10^7 LEUCOCYTES)

	Control	µgm TRIAC/100 ml. medium 25	µgm TRIAC/100 ml. medium 50
1.	0·088	0·090	0·089
2.	0·100	0·103	0·100
3.	0·112	0·107	0·112
4.	0·084	0·077	0·079
5.	0·078	0 078	0·098

The results are shown in Table 1. Each value is the average of 2 readings. Patients 1–4 were receiving no therapy. Although they were hypothyroid, the uptake of oxygen by their leucocytes failed to show the stimulation produced by TRIAC which is usually found in patients with primary hypothyroidism. Patient 5, however, although he was also hypothyroid, was receiving maintenance treatment with cortisone. Unlike the other patients, there was a significant increase in the uptake of oxygen by his leucocytes as a result of incubation with TRIAC. It is possible that, in this patient, cortisone facilitated the access of the thyroid hormone to the site of action. The absence of the expected stimulation of oxygen uptake by TRIAC in patients with untreated secondary hypothyroidism may therefore, in part at least, be related to the secondary adrenal deficiency. Our findings at present do not permit further speculation, but once more emphasize the importance of the inter-dependence of the endocrine glands.

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Stimulation of Secretion of Insulin by Adenosine-triphosphate

IN previous papers[1–4] we showed that different metabolites, both *in vivo* and *in vitro*, produce insulin secretion which we measured by the method of the epididymal fat of the rat. In view of some of these results we decided to ascertain whether the intravenous injection of ATP was a stimulant to insulin secretion; although we are aware that the point of view actually held is that the metabolic processes affected by this nucleotide are intracellular.

Animals from our rat colony (140–160 g) were intravenously injected with ATP 1·7 × 10^{-3} M (total volume 0·25 ml., *p*H 7) and killed immediately after the injection (10–20 sec.). The evaluation of the plasma insulin effect and glucose uptake of the diaphragm and epididymal fat were carried out following the method already described[3]. As control of the effect of the ATP injection, we injected the same quantity of hydrolysed ATP (1·0 hydrochloric acid for 10 min at 100° C) and other experiments were also realized with hydrolysed ATP versus control.

As is shown in Table 1, the injection of ATP produces secretion of insulin, which shows itself by a greater plasma insulin effect and the increase of the glucose uptake of the diaphragm and of the epididymal fat. This action is similar to, although much speedier than, that obtained when we used glucose and *l*-leucine injections. On the other part, the hydrolysed ATP has no effect. The results obtained show the need to know the mechanism of action implied by possible extra-cellular activity of this nucleotide.

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Table 1. MEANS OF INSULIN ACTIVITY OF BLOOD PLASMA, GLUCOSE UPTAKE BY DIAPHRAGM AND ADIPOSE TISSUE AFTER ATP INJECTION

Injection	Insulin activity of blood plasma mg/100 of tissue*		Glucose uptake by diaphragm mg/100 of tissue*		Glucose uptake by adipose tissue mg/100 of tissue*	
ATP (1·7 × 10^{-3} M)	0·240 ± 0 016 (16)	$P < 0·01$	0·436 ± 0·016 (32)	$P < 0·01$	0 290 ± 0 017 (30)	$P < 0·01$
Control (hydrolysed ATP 1 7 × 10^{-3} M)	0·157 ± 0·018 (13)		0 382 ± 0 013 (32)		0·202 ± 0·018 (30)	
Hydrolysed ATP 1·7 × 10^{-3} M	0·186 ± 0·017 (18)	$P > 0·05$	0·448 ± 0·008 (66)	$P > 0·05$		
Control	0·189 ± 0 022 (10)		0·456 ± 0·015 (35)			

* All values are means ± *S.E.* The values in parentheses represent the number of experiments.

Transport of Potassium Ions in the Rat's Intestine

THE transport of sodium ions across the epithelium of the small intestine of the rat seems to be an active process (for a review of the evidence see Durbin, Curran and Solomon[1]). This active transport of sodium ions may be responsible for the potential difference existing across the gut wall, the mucosal side of which is negative with respect to the serosal side. In the jejunum the amplitude of this potential reported in the literature ranges from about 8 mV (refs. 2, 3) to 12 mV (ref. 4); the potentials in the ileum are smaller[3].

Evidence for the active transport of ions other than sodium across the epithelial membrane is slight and the possibility has frequently been discussed that many cations and anions move passively along their electrochemical gradients[1,2,5,6]. We have been interested in the transport of potassium ions by the epithelial cells of the rat's jejunum and ileum. From measurements of the concentration of this ion on both sides of the cellular barrier between mucosa and serosa, together with determinations of the transcellular potential, we have come to the conclusion that the total electrochemical potential of the potassium ions is the same on both sides of the epithelial membrane. This provides experimental evidence for the suggestion that the transport of potassium ions is a passive process and there is no need to postulate an active transport mechanism.

The rat's small intestine was divided into 'jejunal' and 'ileal' portions and the lumen was perfused *in vitro* by the method described by Gilman and Koelle[7]. The perfusion fluid was Krebs's bicarbonate solution gassed with 95 per cent oxygen and 5 per cent carbon dioxide, at *p*H 7·4 and at 37° C. It flowed at a rate of 5–7 ml./min; this provided adequate oxygenation and presented to the mucosal surface a fluid of constant composition. The relationship between the potassium concentrations of the fluid presented to the mucosal surface and that transported by the epithelium to the serosal side was obtained in experiments in which the gut segments were maintained in a chamber where the serosal surface was exposed to 95 per cent oxygen, 5 per cent carbon dioxide and water vapour. Transported fluid, escaping from the serosal surface, cut blood vessels and lymphatics, was collected so that a direct analysis of the composition of the fluid actually transported could be made. Table 1 shows that for both the jejunum and ileum the potassium concentration in the fluid transported to the serosal side was less than that in the perfusing mucosal fluid (as already observed by Smyth and Taylor[8]), both when the latter concentration was 6 mM and when it was 12 mM.

Table 1

	Chemical experiments			Electrical experiments
	Mucosal potassium concentration (mM)	Mucosal potassium concentration / Serosal potassium concentration*	Potassium equilibrium potential† (mV)	Measured potential mucosa–serosa† (mV)
Jejunum	6	1·56 ± 0·03 (25)	11·8 ± 0·5	11·6 ± 0·8 (9)
	12	1·54 ± 0·03 (7)	11·6 ± 0·3	11·2 ± 0·5 (6)
Ileum	6	1·46 ± 0·05 (23)	9·7 ± 0·9	9·3 ± 0·7 (6)
	12	1·52 ± 0·02 (7)	10·9 ± 0·8	10·2 ± 0·6 (6)

Figures are means and are *S.E.*'s; Nos. of experiments are given in parentheses.

* Determined in sample collected over period of 1 h.

† Mucosal side negative.

Potential measurements were made with calomel electrodes with saturated potassium chloride bridges (Beckman fibre junction reference electrodes) feeding into a low grid current cathode-follower. In the experiments in which potentials were measured the gut segments were immersed in 25 ml. of fluid of the same composition as that used to perfuse the lumen of the gut. The serosal electrode dipped into this pool and thus made contact with the whole of the serosal surface of the segment being studied; the mucosal electrode dipped into the fluid perfusing the lumen. The electrical potential measurements were thus made in separate experiments and under somewhat different experimental conditions from those where the chemical composition was determined. This measured potential was assumed to be a reliable measure of the potential which existed across the gut wall in the chemical experiments for the following reason. There is ample evidence that the gut transports well in the presence or absence of bathing fluid on the serosal side[7-9]; thus in the electrical experiments although the composition of the bulk of the fluid on the serosal side was Krebs's solution (or a Krebs's solution modified by transported fluid) the fluid immediately outside the serosal pole of the epithelial cell (that is, the fluid important with respect to the potential difference across the epithelium) would have had the composition of the transported fluid, as in the chemical experiment. The advantage of using the two procedures was that the potential and the chemical determinations were each made under conditions which permitted the greatest accuracy of measurement.

In six experiments on the jejunum the potential initially recorded (within 10 min of mounting the gut in the perfusing apparatus) was 11·8 ± 0·9 mV; during the next 20 min the potential rose to a maximum of 12·7 ± 1·0 mV and thereafter declined to a value of 9·8 ± 1·2 mV at the end of 1 h. In the ileum the corresponding potentials were 9·2 ± 1·2, 10·3 ± 0·7 and 8·2 ± 0·9 mV. This falling off of the potential did not seem to be due to any change in the composition of the serosal fluid; when this fluid was replaced by fresh Krebs's solution there was little or no change in the potential. In each experiment six potential measurements were made at intervals of about 10 min during the first hour and the value of their arithmetical average determined. The mean of these average values in all the experiments is given in Table 1, for experiments in which the potassium concentration in the Krebs's solution perfusing the lumen was normal (6 mM) and for experiments in which it was twice this value.

Correlation between chemical and potential measurements. If potassium is free to diffuse across the gut wall then at equilibrium for this ion the concentration difference observed must be associated with a potassium equilibrium potential (E) the magnitude of which is given by the relationship, $E = \frac{RT}{F} \log [K]_m/[K]_s$, where R is the gas constant, T the absolute temperature, F Faraday's constant and $[K]_m$ and $[K]_s$ are the mucosal and serosal concentrations of potassium respectively. The average ratio of $[K]_m/[K]_s$ in 25 experiments on the jejunum (using 6 mM potassium Krebs's solution) was 1·56 ± 0·03; the mean value of the potassium equilibrium potential obtained by averaging the individual potentials calculated from each experimentally determined ratio was 11·8 ± 0·5 mV. The corresponding values for 23 experiments on the ileum were 1·46 ± 0·05 for $[K]_m/[K]_s$ and 9·7 ± 0·9 mV for the mean potassium equilibrium potential. As can be seen from Table 1 the predicted potentials are very nearly the same as the potential differences actually measured (11·6 ± 0·7 mV for the jejunum and 9·3 ± 0·8 mV for the ileum) when averaged over an hour to correspond with the chemical measurements which were made on samples collected during an hour. This close agreement between the predicted and measured potential difference was also obtained in the experiments using 12 mM potassium Krebs's solution.

The net difference in electrochemical potential for the potassium ion on the two sides of the gut wall, $\frac{RT}{F} \log [K]_m/[K]_s - E$, is thus practically zero. Whereas this finding does not exclude the possibility that potassium is actively transported, the simplest explanation of our results is that potassium ions move passively across the wall of the jejunum and ileum and that their distribution is determined by a pre-existing potential across the gut which is at present believed to result, according to the work cited here, from the active transport of sodium ions.

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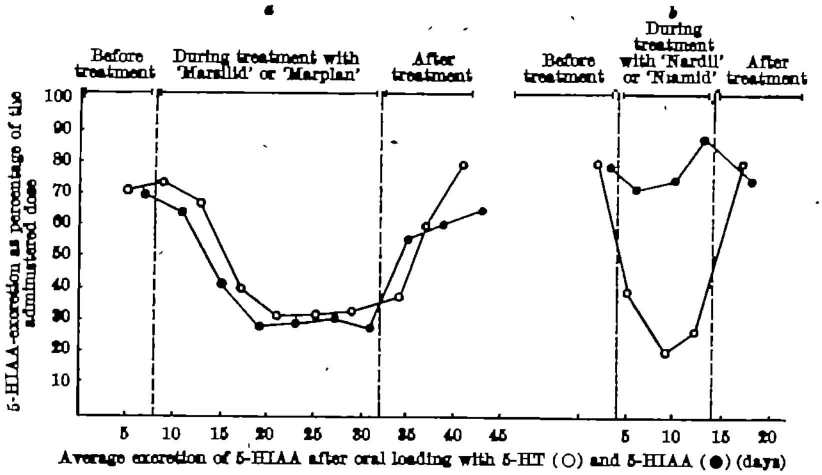
PHARMACOLOGY

Variable Action of Monoamine Oxidase-inhibiting Hydrazines on Serotonin Metabolism

Recently, van Praag and Leijnse[1] reported an interesting finding concerning monoamine oxidase-inhibiting hydrazines 'Marsilid' (iproniacid) and 'Marplan' (isocarboxazid) on serotonin metabolism. They found that



Measured in six subjects, before, during and after treatment with 'Marsilid' or 'Marplan' (from van Praag and Leijnse)

Measured in four subjects, before, during and after treatment with 'Nardil' or 'Niamid'

Fig. 1

during treatment with these drugs the increase in the excretion of 5-hydroxyindolylacetic acid (5-HIAA) in urine after oral feeding of 5-HIAA decreased in the same way as after oral administration of serotonin (5-hydroxytryptamine = 5-HT) (Fig. 1*a*). They concluded from this result that:

(1) Monoamine oxidase-inhibiting hydrazines have a similar and practically synchronous effect on 5-HIAA excretion after loading with serotonin and 5-HIAA.

(2) This effect is brought about through the same mechanism of action.

(3) The action of the so-called monoamine oxidase-inhibiting hydrazines on the serotonin loading test cannot be a result of the inhibition of monoamine oxidase.

In re-examining the work of van Praag and Leijnse, I have obtained the same results for 'Marplan'. I have not investigated 'Marsilid' due to the danger of toxic side effects. However, I feel that the conclusions drawn by van Praag and Leijnse are erroneous. I have found that treatment with two other monoamine oxidase-inhibiting hydrazines, 'Niamid' (isonicotinyl-benzylcarbamylethylhydrazine) and 'Nardil' (phenylethylhydrazine), decreases 5-HIAA excretion only after loading with serotonin while, however, the 5-HIAA excretion after loading with 5-HIAA itself is not influenced (Fig. 1*b*).

The conclusions are:

(1) Only 'Marsilid' and 'Marplan' among the four monoamine oxidase-inhibiting hydrazines influence 5-HIAA excretion after loading with serotonin or 5-HIAA in the same way. 'Niamid' and 'Nardil' reduce the 5-HIAA excretion only after loading with serotonin.

(2) I believe that it is, therefore, improbable that the effect of 'Marsilid' and 'Marplan' on 5-HIAA excretion after loading with serotonin and 5-HIAA is exerted through the same mechanism.

(3) The action of monoamine oxidase-inhibiting hydrazines on the serotonin loading test is certainly an expression of monoamine oxidase-inhibition, for those monoamine oxidase-inhibitors not belonging to the hydrazine series, such as benzyl-methyl-propinylamine, prevent the formation of 5-HIAA from serotonin in the same way. An action of these monoamine oxidase-inhibitors on 5-HIAA excretion after loading with 5-HIAA was not observed[2].

I should like to interpret the action of 'Marsilid' and 'Marplan' on 5-HIAA excretion after loading with 5-HIAA as a side effect of these two substances. The results of Stock and Westermann[3] also point in this direction. They found that inhibitors of monoamine oxidase exert a pharmacological action that is often independent of their capacity to block the enzyme. These authors are of the opinion that the side effects of monoamine oxidase-inhibitors can be of just as much importance in their ability to bring about other pharmacological, biochemical and, possibly, also therapeutic actions as their ability to inhibit monoamine oxidase.

Further investigation will be needed to clarify if the decrease in the 5-HIAA excretion after loading with 5-HIAA is an effect of 'Marsilid' and 'Marplan' without therapeutic meaning, or if there is a relationship to clinically important symptoms such as toxic side effects. With the introduction of new monoamine oxidase-inhibitors in the future, the behaviour of 5-HIAA excretion after loading with 5-HIAA must be examined.

The decrease in 5-HIAA excretion after oral loading with 5-HIAA brought about by 'Marsilid' and 'Marplan' is of general interest, since it is possible that 5-HIAA is not the end product of serotonin catabolism, but is further transformed in the body under certain conditions. Preliminary results indicate a change in the indole ring. Paper chromatographic investigation of urine after loading with 5-HIAA shows a strong colour reaction with Ehrlich's and diazo reagent corresponding in position on the chromatogram to 5-HIAA. With treatment with 'Marplan' after loading with 5-HIAA, it is no longer possible to detect diazo or Ehrlich-reactive material in the chromatogram[2,4].

Methods. 5-HIAA excretion in urine was determined by the method of Udenfriend *et al.*[5,6]. Urine was quantitatively collected for 8 h after loading with serotonin or 5-HIAA. Those factors interfering in the determination were eliminated[6,7]. Serotonin was administered as serotonin creatinine sulphate. Both serotonin and 5-HIAA were dissolved in a few millilitres of hot water. The doses were 0·15 mg/kg body-weight 1,5-hydroxytryptophan and equimolar amounts of serotonin and 5-HIAA.

Two tablets of 'Niamid' or 'Nardil' were administered mornings and evenings.

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Diminution of Imipramine Mortality in Hypothyroid Mice

A PREVIOUS report described enhancement of imipramine toxicity in hyperthyroid mice[1]. This work had been prompted by the observation of a clinical case in which the simultaneous exhibition of imipramine and thyroid hormone had preceded a bout of paroxysmal auricular tachycardia[2]. Although we had no similar clinical experience to commend our present work, we nevertheless considered it of interest to test the effect of hypothyroidism on imipramine mortality.

100 adolescent male Swiss–Webster mice were obtained. 50 were given a standard laboratory diet and 50 were

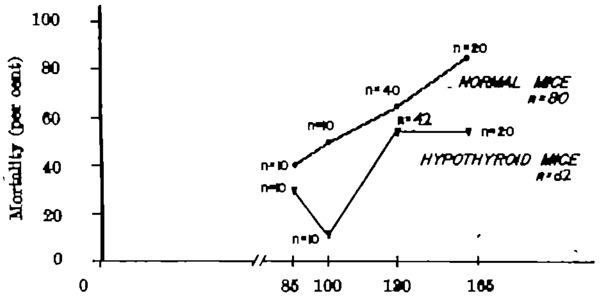

Fig. 1. Intraperitoneal imipramine acute (2 h) mortality in adult, male, Swiss–Webster white mice

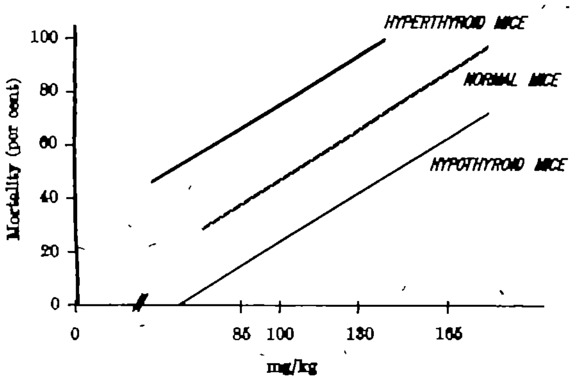

Fig. 2. Idealized imipramine dose–mortality curves in adult, male, Swiss–Webster white mice according to thyroid status

treated in the same way except that 0·2 per cent propyl thiouracil (PTU) was added to their drinking water. All mice were weighed at intervals of 48 h. Throughout this preparatory period the PTU-fed mice showed retarded gain in weight. On the sixteenth day these mice were found to use slightly less oxygen per unit time than the normal mice. At this time, using spontaneous behaviour as a criterion, five judges were able to identify cages containing PTU-fed mice versus 'normal cages' 15 times out of 25 tries (not significant).

The same day the mice were injected intraperitoneally with a solution of lyophilized imipramine in saline (10 mg/ml.). At the dose of 100 mg/kg, 5 of 10 normal mice died; 1 of 10 PTU-fed mice died. At the dose of 130 mg/kg, 12 of 20 normal mice died; 12 of 20 PTU-fed mice died. At the dose of 165 mg/kg, 17 of 20 normal mice died; 11 of 20 PTU-fed mice died. On the basis of these preliminary data we suspected that hypothyroidism reduced imipramine mortality. We attributed our failure clearly to demonstrate the phenomenon to the probability that the PTU-fed mice had been only mildly hypothyroid.

Another group of mice were chosen for further study. 30 were given a standard diet and 32 were given 0·2 per cent PTU water for 30 days. At the end of this time the PTU-fed mice were incontrovertibly hypothyroid. They had gained less weight than normals, their coats were dull and yellowish, and they appeared sluggish. At the dose of 85 mg/kg, 4 of 10 normal mice died; 3 of 10 PTU-fed mice died. At the dose of 130 mg/kg (the non-discriminating dose in the first experiment), 13 of 20 normal mice died; 12 of PTU-fed mice died. All deaths occurred within 2 h of injection. The mice that died, in both classes, showed pre-terminal hyperactivity, convulsions and occasional opisthotonous, as had hyperthyroid mice[1].

Results from the two experiments are combined in Fig. 1. The χ^2 value for an over-all analysis of difference is 4·30 ($P = < 0·05$).

Fig. 2 presents in idealized form the results of work recorded here plus the results from ref. 1. In mice, intraperitoneal imipramine presents a sloping dose–mortality curve. The curves for hyper-, hypo- and eu-thyroid mice are parallel over the dose-range tested. Hyperthyroid mice are significantly more sensitive, and hypothyroid mice significantly less sensitive, than euthyroid mice, to the mortal effects of imipramine.

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HÆMATOLOGY

Synthesis of Hæm in Normal and Leukæmic Leucocytes

RECENT investigations suggest that the formation of hæm from iron and protoporphyrin is enzymatic in avian erythrocytes[1–4], rat liver[5,6] and canine muscle[7]. The hæmoproteins, such as catalase, peroxidase and the cytochromes, exist in leucocytes[8]; however, the origin of hæm for these proteins is not apparent. The present study concerns the formation of hæm in a leucocyte system.

Leucocytes were isolated from human blood which was collected in siliconized glassware containing heparin. The blood was mixed with an equal volume of 4·5 per cent dextran, and allowed to sediment until the plasma rich leucocyte layer and the erythrocyte mass were clearly separated. The leucocyte layer was removed and centrifuged at 320*g* for 5 min. The sedimented leucocytes were resuspended in 1·5 vol. of de-ionized, distilled water and homogenized in a motor-driven glass homogenizer for 5 min at 4°. 5-ml. aliquots of homogenate, obtained from $2·1 \times 10^8$ to $1·5 \times 10^9$ leucocytes, were added to reaction mixtures which contained 0·13 μc. ferrous citrate (iron-59), 0·5 μmole protoporphyrin, 10 μmoles cysteine and 1,000 μmoles *tris* buffer *p*H 7·8, in a final 10-ml. vol. with 0·15 M sodium chloride. This mixture was incubated for 4 h at 37° and the reaction stopped with 0·1 M potassium cyanide. Carrier hæm was added, the total hæm was isolated, and the radioactivity of the recrystallized hæm was determined. Hæm synthesis was measured as the per cent incorporation of added radioiron into hæm (per cent uptake iron-59). Synthesis was considered to be significant if the total counts per minute of the isolated hæm were greater than twice background and the per cent uptake of iron-59 was greater than 2 per cent. In each experiment, 5 ml. of saline and 5 ml. of a hæmolysate, prepared from the patient's sedimented erythrocytes, were substituted as controls for the leucocyte homogenate. Incorporation of radioiron under these conditions was always less than 1 per cent. 5 ml. of an active avian hæmolysate was also included as a control in each experiment and the uptake of iron-59 was always greater than 5 per cent.

The results are shown in Table 1. When leucocytes isolated from normal individuals were used little, if any, hæm synthesis occurred and the uptake of iron-59 was less than 1·0 per cent. Similarly, when leucocytes from two patients with chronic myelocytic leukæmia, two patients with chronic lymphocytic leukæmia, and one patient with monocytic leukæmia were used, the uptake of iron-59 was less than 2·1 per cent. However, if the source of leuco-

Table 1. Hæm Synthesis in Leucocytes

Exp. No.	Patient	Diagnosis	Total No. of blasts	Percentage uptake ^{59}Fe
1	G. K.	Normal	0	0·4
2	T. G.	Normal	0	1·0
3	D. C.	Normal	0	0·5
4	T. S.	Normal	0	0·2
		Chronic leukæmia		
5	B. Bu.	Myelocytic	$0·04 \times 10^9$	0·2
6	B. Ba.	Myelocytic	0	0·1
7	J. T.	Lymphocytic	0	0·1
8	A. H.	Lymphocytic	0	2·1
9	H. H.	Monocytic	0	1·4
		Acute leukæmia		
10	N. L.	Myeloblastic	$1·1 \times 10^9$	29·9
11	A. G.	Myeloblastic	$3·3 \times 10^9$	11·5
12	C. C.	Myeloblastic	$3·1 \times 10^9$	4·9
13	L. W.	Lymphoblastic	$1·3 \times 10^9$	26·9
14	B. C.	Lymphoblastic	$3·7 \times 10^9$	6·9

cytes was from patients with either acute myeloblastic or acute lymphoblastic leukæmia, considerable synthesis of hæm occurred. When leucocytes from 3 patients with acute myeloblastic leukæmia were used the uptakes of iron-59 were 29·9, 11·5 and 4·9 per cent. In two experiments with leucocytes from patients with acute lymphoblastic leukæmia, the uptakes of iron-59 were 26·9 and 6·9 per cent.

Since the enzymatic synthesis of hæm is similar in avian erythrocytes[1–4], rat liver[5,6], and canine muscle[7], a preliminary characterization of this reaction was performed with leucocytes from patient L. W. Preheating the leucocyte homogenate to 80° for 10 min resulted in complete loss of activity. Optimal activity occurred at approximately pH 7·8. When the concentration of the homogenate was increased, there was a proportional increase in per cent uptake of iron-59. The rate of the reaction was linear for the first 30 min and approached a maximum after incubation for 4 h. As regards these characteristics, the synthesis of hæm in leucocytes appears to be similar to that described for other tissues[1–7].

In summary, hæm synthesis can be demonstrated in leucocytes obtained from patients with acute leukæmia, but not in leucocytes obtained from normal individuals or patients with chronic leukæmia. This difference probably results from the high concentration of lymphoblasts or myeloblasts in the blood of patients with acute leukæmia.

These findings are interesting in view of Vannotti's observation that leucocytes isolated from patients with acute leukæmia could convert δ-aminolævulic acid to porphobilinogen and then to porphyrin[9]. However, when leucocytes were isolated from normal individuals, patients with infections, or pregnant women, δ-aminolævulic acid was converted to porphobilinogen, but porphyrin formation was not observed.

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Hypohaptoglobinæmia in an American Negro Family

With the demonstration that random population pairs and dizygotic twins show greater intra-pair variability than monozygotic twins in serum haptoglobin (Hp) level, evidence for the genetic regulation of this protein has been presented[1]. This work also demonstrated that the normal values for haptoglobin phenotypes Hp 2–2 and Hp 2–1 are lower than those for Hp 1–1. If these differences are genetically determined, a similar mechanism involving the *Hp* 2 allele may be operative in a benign, genetically determined deficiency of serum haptoglobin[2–4]. A family of Dominican descent in which nine members drawn from two generations had haptoglobin-levels ranging from 6 to 52 mg/100 ml. and Hp 2–1 and Hp 2–2 phenotypes has also been reported[5]. On the basis of the available pedigrees, inheritance of this trait via a recessive haptoglobin allele (Hp^{0}) or a simple non-allelic dominant has been excluded. Although so far all Caucasians with hypohaptoglobinæmia have been, when typable, phenotypically Hp 2–1 or Hp 2–2, a strong association between the Hp 2^{m}–1 phenotype and haptoglobin depression exists in the American Negro[6]. We have studied an American Negro family, containing a pair of monozygotic twins in which hypohaptoglobinæmia unrelated to hæmolysis existed throughout an entire generation of offspring.

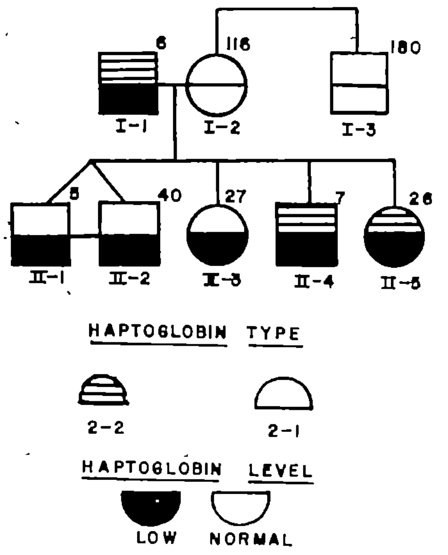

Fig. 1

The methods used in this investigation to exclude hæmolysis and hæmoglobin abnormalities and to confirm legitimacy have been described elsewhere[6]. A complete blood count was normal in all family members except II–1, who was in the nephrotic phase of glomerulonephritis when studied. Serum haptoglobin was measured on serial specimens by the peroxidase–guaiacol method of Connell and Smithies and haptoglobin phenotyping performed after vertical starch gel electrophoresis in borate buffer at pH 8·6 (refs. 7, 8). The family pedigree and haptoglobin phenotypes are presented in Fig. 1. Haptoglobin values are given as a suprascript for each family member. Age ranged from 17 to 52 years. The values considered subnormal are distinctly abnormal in our laboratory for Hp 2–1 and Hp 2–2, and are below the mean normal values obtained by Bayani-Sioson *et al.* using the peroxidase–guaiacol determination. Haptoglobin depression was found in 6 family members all having Hp 2–1 or Hp 2–2 phenotypes. Values ranged from 6 to 45 mg/100 ml. in these individuals. Despite his nephrotic syndrome, the characteristic rise in α-2-globulin was not observed in II–1 and haptoglobin depression was found in his otherwise normal identical twin. Although marked proteinuria may be associated with considerable haptoglobinuria, only trace quantities of haptoglobin could be identified in the urine of II–1 (ref. 9). It is noteworthy that renal biopsy specimens obtained percutaneously from II–1 and II–2 failed to reveal abnormal iron deposition in the renal parenchyma as might result with chronic hæmoglobinæmia.

These results indicate that genetically determined haptoglobin depression is not invariably associated with the Hp2^{m}–1 phenotype in the American Negro. Although the mechanisms involved are unclear, they would appear to be discrete from the *Hp*2^{m} allele, and as yet manifest only in the presence of the *Hp*^{1} allele.

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HISTOLOGY

Enzymatic Maturation of Skeletal Muscle

IN adult human and mammalian skeletal muscle there are two types of fibre. One is rich in oxidative enzymes and poor in phosphorylase and usually of smaller diameter, while the other group is rich in phosphorylase and poor in the oxidative enzymes[1]. In two cases of benign congenital hypotonia[2] and in a case of central-core disease[3] it was noted that all the fibres had a uniform enzyme activity.

The lack of differentiation of the muscle fibres in some diseases may represent a failure of maturation of the muscle. The investigations recorded here were undertaken to determine whether the muscle is already differentiated at birth.

Human muscle was obtained at autopsy from the gastrocnemius and quadriceps of full-term infants who had died within 24 h of birth. Muscle was taken from the limbs and trunks of freshly killed animals (guinea pig, rabbit, rat and hamster) less than 24 h old.

All specimens were mounted in a transverse plane on a slice of cork, rapidly frozen in liquid air and approximately 5μ sections were cut on a cold microtome at −25° C. Sections were mounted on coverslips and the following enzymes investigated: phosphorylase, DPN diaphorase, DPN-linked lactate dehydrogenase, and adenosine triphosphatase.

In full-term human infants the muscle was already differentiated at birth. The muscle fibres were much smaller than in adult muscle and were rounded in transverse section. They were arranged in bundles. The fibres rich in phosphorylase were of slightly greater diameter than those rich in the oxidative enzymes. The adenosine triphosphatase activity also showed variation, and in individual fibres it correlated with the phosphorylase activity. In autopsy muscle the adenosine triphosphatase and oxidative enzymes gave consistently positive results, but the phosphorylase was sometimes negative.

In the guinea pig, rabbit and hamster there was also differentiation of the muscle fibres at birth. This was most striking in the guinea pig where the fibres were polygonal in shape and arranged in bundles and resembled adult muscle. In the rabbit and hamster the differentiation was less marked, and the fibres tended to be rounded and were not so clearly grouped into bundles.

In the rat the fibres were not differentiated at birth. The investigation was then extended to include rats aged 1–14 days. Differentiation usually occurred between 7 and 10 days of age. This varied from one group of muscles to another, and in some muscles was noticeable with the adenosine triphosphatase reaction before the others. In some instances, particularly in the abdominal muscles, there was evidence of focal differentiation at 2 or 3 days of age. The rate of differentiation also seemed to be influenced by nutrition and general growth since rats from smaller litters tended to be larger and more mature and their muscle showed earlier maturation. By 14 days the muscle resembled that of the guinea pig at birth and appeared to have reached full maturity.

The difference in maturation in these animals may be partly accounted for by their length of gestation and their degree of general maturity at birth. The guinea pig with a 68-day gestation period has a more mature fur at birth and is much more mobile than the others. The rabbit (30–32 days' gestation) has a better-developed fur and is more active than the rat (gestation 21 days). The hamster, although having a shorter gestation (16–19 days) than the rat, also appears to be more active at birth, and its fur develops more rapidly. No direct comparison can be drawn in humans.

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PATHOLOGY

Electrophoretic Mobility of Human Tumour Cells

IT has been suggested that one of the characteristics of embryonal[1], proliferating[2], or neoplastic[3] mammalian cells is the presence of a high surface negative charge, as measured by cell electrophoresis. Data on fresh human tumour cells have not been available, however, except for studies of leukæmic cells[4,5]. The present investigation was designed to accumulate cell electrophoretic mobilities from a variety of fresh human tumour tissues.

Human tumour tissue was available from surgically excised specimens; in all instances the neoplastic tissue was dissected immediately following removal from the patient. Care was taken to select tumour tissue which was completely viable and without evidence of cyst formation, degeneration, hæmorrhage, inflammation or ulceration; it was selected to avoid adjacent normal tissues. The tumour tissue fragments thus obtained were placed in cold, sterile, isotonic saline solution within 5–10 min after removal from the patient; in some instances specimens of corresponding normal tissue from the surgical specimen were similarly treated. The tissue fragments were then washed with cold isotonic saline, followed by fine mechanical separation with eye scissors. These fragments, suspended in saline, were then passed through either a small Latapie mill or graded sieves. Sedimented tissue debris and cell clumps were discarded. The cell suspension was then suspended in sucrose–phosphate buffer.

The electrophoretic apparatus and general technique was similar to that described by Eylar *et al.*[6]. The rect-

Table 1. Tumour Cell Mobilities

	Specimen	Mobility	E.S.U./cm^{-2}
1	Adenocarcinoma of rectum	1·14	2,280
2	Adenocarcinoma of breast	1·19	2,380
3	Adenocarcinoma of kidney	1·26	2,520
4	Squamous carcinoma of lung	1·21	2,420
5	Squamous carcinoma of lung	1·32	2,640
6	Adenocarcinoma of colon	1·30	2,600
7	Adenocarcinoma of colon	1·26	2,520
	Normal epithelium of colon	1·34	2,680
8	Adenocarcinoma of colon	1·29	2,580
	Normal epithelium of colon	1·47	2,920
9	Leiomyoma of stomach	1·61	3,220
10	Osteosarcoma of maxilla	1·57	3,140
11	Leiomyoma of uterus	1·55	3,100
	Normal myometrium	1·55	3,100
12	Leiomyoma of uterus	1·46	2,920
13	Blast cell leukæmia: large cells	1·62	3,240
	Blast cell leukæmia: small cells	1·52	3,040

Mean negative mobilities, corrected to viscosity of water at 25° C., are expressed as μ/sec/V/cm. S.D. < 0.05.

angular cell, placed in a thermostat at 25 ± 0·01° C, was in the lateral position. Readings were taken at either the first or second stationary levels. The current was 4·0 m.amp, and the electrodes were silver, silver chloride. The buffer contained 0·201 M sucrose, 0·012 M disodium hydrogen phosphate, 0·003 M sodium dihydrogen phosphate and 0·0441 M sodium chloride; the pH was 7·0 and the ionic strength 0·083. An average of 20 or more mobilities was determined on each specimen. The apparatus was checked by daily runs of human red cells; in this apparatus, human red cells have an average negative mobility of 1·64 μ/sec/V/cm and Ehrlich ascites cells 1·21 μ/sec/V/cm.

The results (Table 1) show that epithelial malignant tumour cells (specimens 1–8) do not reveal a particularly high (negative) electrophoretic mobility; indeed, specimens 7 and 8 suggest that the tumour cell mobilities may be lower than corresponding normal tissue cells. On the other hand, connective tissue tumours (specimens 9–13) exhibit a fairly high mobility. The blast cell leukæmia buffy coat contained two cell populations according to size and electrophoretic mobilities.

The significance of these results is, at present, obscure. Present investigations in this laboratory are being carried out to elucidate the nature of tumour cell surface charges by enzyme treatment (neuraminidase, hyaluronidase), anion blocking (organic bases, polyvalent cations) and cation blocking.

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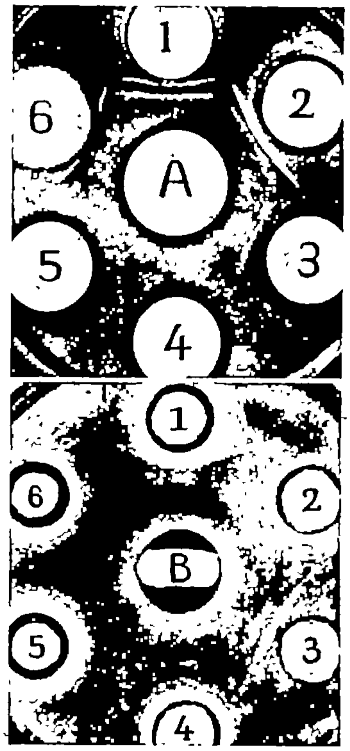
Production of a Highly Tissue-specific Antiserum against a Particular Sub-fraction separated from Kidney Microsomes

Immunological studies on tissue- or organ-specificity have been seriously handicapped by difficulty in obtaining an antiserum highly specific to a particular tissue or organ. Though the microsomes of several organs such as liver and kidney are known as the sites of so-called 'organ-specific' antigens[1], the antigenic composition of the microsomes is extremely complex[2]. They usually contain a number of 'non-specific' components, besides 'organ-specific' ones[3]. Recently, one of us[4] has reported that the deoxycholate extracts of kidney microsomes can be separated into several immunologically distinct sub-fractions, one of which contains 'kidney-specific' components. In the present communication the antibody-patterns of antisera produced against the separated sub-fractions will be described.



Figs. 1 and 2 Central wells: *A*, antiserum against *SF* : serum against *SF* 45. Peripheral wells: 1, *D*-fraction of kidney microsomes; 2, *SF* 20 of kidney microsomes; 3, *SF* 45 of kidney microsomes; 4, *SF* 80 of kidney microsomes; 5, *S*-fraction of kidney homogenates; 6, *D*-fraction of liver microsomes

The microsomal fraction isolated from chicken kidneys was treated with 0·5 per cent deoxycholate and was centrifuged at 105,000*g* for 90 min. The supernatant thus obtained (*D*-fraction) was separated into three sub-fractions by means of the fractional precipitation with ammonium sulphate after much the same procedures as previously given[4] though somewhat modified. These were: (1) the proteins precipitated between 10 and 20 per cent saturation of ammonium sulphate (sub-fraction 20); (2) the precipitate between 25 and 45 per cent saturation (sub-fraction 45); (3) the precipitate between 60 and 80 per cent saturation. Preliminary results on the chemical composition of each sub-fraction indicate that sub-fraction 20 and sub-fraction 45 contain most lipids of the microsomes, probably representing the materials of the membrane of endoplasmic reticulum, while lipid-poor sub-fraction 80 may be the content of the microsomal vesicles[4].

Each sub-fraction, after being dissolved with water and dialysed against it, was injected into rabbits with adjuvant technique. Ring precipitin tests with different dilutions of both test-antigens and the antisera revealed that each antiserum contains relatively high concentrations of the

Table 1

	Maximum antigen-dilutions to give a visible precipitate*			Maximum antiserum-dilutions to give a visible precipitate†		
Antisera / Antigens	Anti-*SF* 20	Anti-*SF* 45	Anti-*SF* 80	Anti-*SF* 20	Anti-*SF* 45	Anti-*SF* 80
D-fraction of kidney	80	160	80	16	32	32
D-fraction of liver	40	20	160	8	4	64
Sub-fraction 20	320	80	—	64	8	—
Sub-fraction 45	80	640	40	8	64	32
Sub-fraction 80	20	80	640	1	8	128

* Each undiluted test-antigen contains *c*. 1·0–1·5 mg protein/ml.
† Each test-antigen contains *c*. 30–50 μg/protein/ml.

antibodies against the homologous antigen (Table 1). The results were well confirmed by double diffusion test in agar. Antigenic component to cause a strong line in reaction with the antiserum against sub-fraction 20 is commonly distributed to *D*-fractions of both kidney and liver, but is scarcely detectable in sub-fractions 45 and 80 as well as in the unsedimentable fraction (cell sap, sub-fraction) of the kidney homogenates (Fig. 1). Therefore, this type of antisera can be said as highly 'fraction-specific' but not 'kidney-specific'. The antiserum against sub-fraction 45 is highly 'kidney-specific'. In reaction with sub-fraction 45 and *D*-fraction of kidney, it produces a strong precipitate, identical line to which is scarcely perceptible against other test-antigens prepared from the homologous and the heterologous organs such as liver and lung (Fig. 2). Besides, this antiserum is contaminated with several minor antibodies to produce a few weak lines, some of which occur also against sub-fraction 20 or sub-fraction 80. The antiserum against sub-fraction 80 has quite different antibodies from those contained in the foregoing two types of antisera. Strong lines produced by anti-sub-fraction 80 against the homologous antigen are identical to those developed against *D*- and sub-fractions of both homologous and heterologous organs. The antibodies contained in anti-sub-fraction 80 may be predominantly directed against such components that are neither 'microsome-specific' nor 'kidney-specific'.

The antiserum against sub-fraction 45 was conjugated with rhodamine sulphate[5] and used as a histochemical reagent to stain the freshly frozen sections of kidney, liver and lung of embryonic chickens (16–17 days) and newly hatched ones. Only the epithelial cells of the kidney tubuli bind the conjugated antiserum.

Since these antisera, the antibody patterns of which were briefly described here, make it possible to detect particular molecular groups contained in kidney microsomes, they may be valuable tools to follow sub-cellular or molecular changes occurring in the course of embryonic differentiation as well as of carcinogenesis of kidney.

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Age Changes of the Thymus in Two High-leukaemia Strains of Mice, *SL* and *AKR*, and in their F_1 Hybrids

Metcalf[1] has shown that the thymus from both sexes of the high-leukaemia strain of mice, strain *AKR*, greatly exceeds in weight that from the low-leukaemia strain *C3H* mice. Furthermore, it was noted that the weights of the *AKR* thymus show a pronounced sex difference, the thymus of females being much heavier than that of males. Arnesen[2] has also observed that the weights of the thymus from strain *AKR* exceed those from the low-leukaemia strain *WLO*. He also pointed out that the adrenal cortex in strain *AKR* contains a smaller amount of lipid-staining material as compared with the tissue of the latter strain.

In the present work, two high-leukaemia strains *AKR* and *SL* and their F_1 hybrid mice bred in our laboratory were used. The origin of these two inbred strains of mice has already been described[3].

A comparative study of the pathology of leukaemia in the two high-leukaemia strains, *AKR* and *SL* (ref. 3), showed that the majority of cases of lymphoid tumours in *AKR* were of thymic origin, whereas in strain *SL* spontaneous leukaemic tumours of thymic origin were not observed. The tumours more frequently originated in the mesenteric lymph node.

All experimental animals were separated according to sex and maintained in an air-conditioned room kept at approximately 23° C with free access to 'Oriental Compressed Diet *CMF*' and water, and were not subjected to any type of treatment. The animals were killed with ether at designated intervals after birth and the thymus, spleen, and mesenteric lymph node were dissected free of extraneous tissues and weighed on a torsion balance. The kidney and liver were also weighed. After weighing, all tissues were fixed in 10 per cent formalin, embedded in paraffin, and stained with hæmatoxylin and eosin for histological study. All animals included in the tabulation were confirmed to be free from leukæmia or other significant diseases. Each age group of either sex consisted of more than five mice.

The maximum weight of the thymus was reached in strain *AKR* at sixty days after birth in the females and at thirty days in the males. In strain *SL*, the age for attaining maximum thymus weight was somewhat earlier than in strain *AKR* and was at forty days in the females and at twenty days in the males (Fig. 1). It was of particular interest to note that sexual maturity in females, as determined by the vaginal smear method, is reached earlier in strain *SL* (thirty-four days of age) than in strain *AKR* (thirty-eight days of age) and that the age of onset of involution of the thymus was well correlated with the time of sexual maturity. In agreement with Metcalf's observations, a pronounced sex difference in the thymus weight was clearly demonstrated in the two strains of mice: the thymus glands of females weighed much more than those of males. A significant sex difference (examined using Student's *t* series at the 0·05 level) was noted from forty days after birth in strain *AKR* and from thirty days in strain *SL*. Although the results obtained with F_1 hybrid mice are incomplete, the shape of the thymus-growth curves was similar to that of the parental strains. From Fig. 1, it is also evident that the *AKR* thymus is greatly hyperplastic when compared with that of strain *SL* of the same sex.

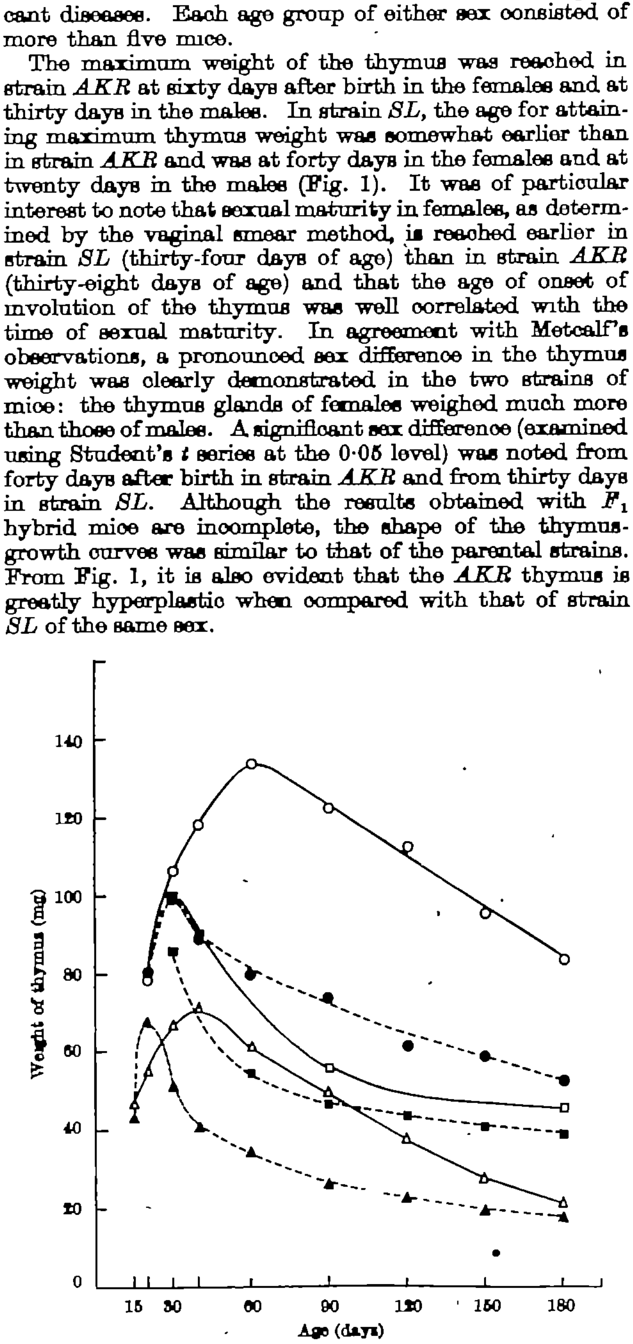

Fig. 1. Changes in weight of the thymus with age. ● - - - ●, *AKR*, male; ▲ - - - ▲, *SL*, male, ■ - - - ■, (*AKR* × *SL*)F_1, male. Continuous lines and open symbols represent corresponding females

Table 1. AVERAGE PROPORTION OF THE MEDULLA IN THE THYMUS

Strain	Male Age (months) 1	 6	Female Age (months) 1	 6
AKR	16·5% (5)*	21·2 (8)	14·2 (6)	27·7 (8)
SL	28·5 (7)	38·2 (6)	23·6 (6)	44·1 (6)
(*AKR* × *SL*) F_1	23·0 (6)	28·5 (5)	22·3 (7)	32·3 (5)

* Total number examined.

Sections of the thymus were projected on white paper and the outlines of the cortex and the medulla were traced. The percentage of the area occupied by the medulla in the thymus was calculated. The results obtained at one and six months of age are presented in Table 1. The relative size of the medulla seems to increase with advancing age. It was also interesting to find a difference in histology of the thymus between the two strains examined: the relative size of the medulla being larger in strain *SL* than in *AKR*, and the values obtained in the F_1 hybrid mice were intermediate between those of the two parental strains.

A detailed study of the histology of the lymphoid tissues from the two strains of mice is now in progress, to investigate preleukæmic lesions and the earliest stage of leukæmic proliferation in the thymus and/or other lymphoid tissues.

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Encephalitogenic Factor in Experimental 'Allergic' Encephalomyelitis

SEVERAL attempts to isolate purified encephalitogenic factor(s) capable of producing experimental 'allergic' encephalomyelitis (with Freund's adjuvants) have been reported[1-5]. Recently, Kies, Gordon, Laatsch and Alvord[6] were successful in obtaining a derivative from guinea pig brain active in doses of 1–2 μg; and Robertson *et al.*[7] obtained a preparation which showed "high . . . activity even at five gamma doses".

A material capable of producing disease in twelve out of twelve guinea pigs (about 500 g weight) when 1 μg was injected with Freund's adjuvants has been isolated from fresh human post-mortem brain. It was obtained by a method differing from that of Kies *et al.*[3] only in so far as special precautions were taken throughout to maintain an acid *p*H (refs. 2–5). On passing through a 'Sephadex *G*100' column this material gave five components, most of the activity being contained in the second (largest) fraction. Electrophoretic examination showed that the original material comprised a major and a minor moiety migrating towards the cathode at *p*H 5. The most active eluate from the Sephadex column migrated as a single component in the same direction, provided that the *p*H was again maintained at 5·0. If the *p*H were altered to 9·0 then this purified material showed one major and five minor bands.

An analytical ultracentrifuge analysis, kindly carried out by Dr. R. A. Kekwick of the Lister Institute, showed only one component in both the crude and the column-purified encephalitogenic material. It seems probable that relatively small molecules (20,000–50,000 mol. wt.) are involved.

The non-specific antigens shared by brain with various other tissues[8,9] can still be demonstrated in both the crude and the purified encephalitogenic factor by the Ouchterlony plate method. Obviously then, despite the high activity of the final material, non-active contaminant yet remains.

An attempt has been made to demonstrate circulating antibody to the encephalitogenic factor both in experimental 'allergic' encephalomyelitis of guinea pigs and in human multiple sclerosis cases by the tanned red blood cell technique. So far, no statistically significant difference from controls has been demonstrated in either case. Nor have serial estimations of the serum titre of encephalitogenic factor-antibody in animals which developed experimental 'allergic' encephalomyelitis after the inoculation of freeze-dried brain plus Freund's adjuvants demonstrated a rise in antibody level.

Since the development of experimental 'allergic' encephalomyelitis has in general been linked with the evolution of a delayed tuberculin type sensitivity to the brain used as antigen, skin sensitivity to encephalitogenic factor has been tested in animals suffering from the disease. This sensitivity has been compared with that developed to the saline supernate from fresh brain emulsion, known to be non-encephalitogenic. While guinea pigs suffering from experimental 'allergic' encephalomyelitis (induced by freeze-dried brain plus Freund's adjuvants) show definite sensitivity to encephalitogenic factor ($P < 0·001$), they do so also to the inactive supernate referred to here ($P < 0·001$). Moreover, sensitivity of such animals to supernate is greater than it is to encephalitogenic factor ($P < 0·05$–$0·02$). These results suggest that many of the skin hypersensitivity observations hitherto published may relate to material which is non-encephalitogenic.

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RADIOBIOLOGY

Effect of Fluoroacetate on Uptake of Yttrium-91 in Rabbits

STUDIES of yttrium-binding by bone powder and bone extracts have indicated that the presence of citrate might explain the uptake of yttrium-91 in bone[1]. The experiments recorded here were undertaken to determine whether the citrate content of tissues could be correlated with uptake of yttrium, and for this purpose the uptake of yttrium in the soft tissue of the rabbit was investigated after giving sodium fluoroacetate to the animal prior to the yttrium.

Several workers[2-4] have examined citrate accumulation in the soft tissues of rats at various times after injection with non-lethal doses of fluoroacetate. They were able to observe a 10- to 20-fold increase in concentration of citrate in tissues such as heart, spleen and kidney, but no significant increase in bone[4,5]. Similar investigations of citrate accumulation in the soft tissues of rabbits have not been reported. In my experiments Dutch rabbits received intraperitoneal injections of sodium fluoroacetate; the dose ranged from 0·1 mg/kg to 1·0 mg/kg body-weight.

The rabbits either died or were killed by anaesthetizing and cutting the jugular vein, and the citrate content of the spleen, liver, kidneys and heart was analysed by the method of Taylor[6]. The results are shown in Table 1. Whereas in the rat all the soft tissues except liver show elevated citrate-levels, in the rabbit the heart was the only organ in which a large increase in citrate content could be demonstrated.

Table 1. CITRATE-LEVELS IN SOFT TISSUES OF YOUNG RABBITS AFTER INTRAPERITONEAL INJECTIONS OF FLUOROACETATE

Dose (mg/kg)	Liver	Spleen	Kidney	Heart	Remarks
		(μg/g wet tissue)			
Control	20	50	30	70	—
0·1	20	50	40	120	Killed after 4½ h
0·1	30	50	40	110	Killed after 2 h
0·5	20	50	140	850	Died after 3 h
0·5	30	70	70	680	Died after 1½ h
1·0	30	50	100	300	Died after 1 h

In the experiments where yttrium was injected after giving fluoroacetate, a series of 5 fluoroacetate injections at half-hourly intervals (total dose 0·5 mg/kg body-weight) were given prior to intravenous injection of carrier-free $^{91}YCl_3$ (dose 45–80 μc./kg body-weight). The animals were killed 10 min and 1 h after receiving $^{91}YCl_3$, and the tissues were analysed for citrate and yttrium-91 content. Control rabbits received saline injections in place of fluoroacetate. Although at least a 6-fold increase in citrate concentration could be demonstrated in the heart, there was no similar increase in yttrium-91 content. The content of yttrium-91 in the liver, kidney, heart, spleen, blood, femur and tibia were examined and at both time intervals there was good agreement between the values for the control rabbits and those receiving fluoroacetate injections. It was therefore concluded that in soft tissues there was no correlation between yttrium uptake and citrate content. In bone, however, under normal conditions, this argument may not hold, since the citrate may be present in a different form—as a polypeptide complex[7], or as a calcium salt[8,9].

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Change of Phytol into Chlorophyll *a* in *Chlorella vulgaris*

Chlorella vulgaris was grown in sterile wort agar under constant illumination. When the alga had reached maximum growth it was collected and pooled using a sterile solution[1].

After filtration and several washings the plants were resuspended in the same solution used for washing to which 2-^{14}C-glycine (specific activity 5·69 mc./mM (Radiochemical Centre, Amersham, England)) were also added. About 9·2 g wet algae, and 260 ml. of the solution containing 12 μc. of labelled glycine were placed quickly in each of 7 Erlenmeyer flasks. The flasks were whisked from time to time. Incubation was stopped after the following periods: 1·5, 3·0, 4·5, 6·0, 8·0, 12·0, 21·0 h. The cells were collected by centrifugation, washed alternately with saline and with saline plus 1 per cent of inactive glycine and stored in deep freeze (−50° C) until the chlorophyll could be extracted, following the procedure described by Strain and Manning[2]. The separated chlorophyll *a* was again chromatographed on cellulose (solvent benzine + 0·5 per cent propanol). The prepared chlorophyll had an absorbance ratio at 660 mμ and 428 mμ of 1·27 ± 0·02. Amounts of chlorophyll, spectrophotometrically quantitized, were added with carbon carrier and their activity measured using the gas counting technique[3]. Then the pheophorbide was prepared as outlined in ref. 4, and assayed again for its specific radioactivity in the same way as that used for the chlorophyll. The molar extinction coefficients used were: 8·80 × 10^4, and 5·27 × 10^4 for pheophorbide[5,6].

Table 1

Exp.	Material	Incubation time (h) 1·5	3·0	4·5	6·0	8·0	12·0	21·0
(1)	Chlorophyll	264	1,100	520	690	5,280	824	1,150
	Pheophorbide	90	245	262	393	505	510	512
	Phytol*	174	855	258	297	4,775	314	638
(2)	Chlorophyll	223	247	136	220	241	231	308
	Pheophorbide	33	52	70	71	100	103	98
	Phytol*	190	195	66	158	141	128	210

Specific activity in disintegrations/(min × μM) at different incubation times (relative error < 3–4 per cent).

* Calculated from those of chlorophyll and pheophorbide.

The results of two experiments are presented in Table 1. It can be seen that the carbon-14 incorporated in the second experiment is smaller and that the relative heights of the two peaks are also different; but the specific activities are the same. We believe the age of algal culture explains this: the algae used for the first experiment were collected after two weeks and those of the second experiment after one month. It must be remembered that the metabolic activity of chloroplasts changes with the age The specific activity of chlorophyll *a* reaches two maxima at about 2·5 h and 8 h, and at the end of the incubation it is increasing again. The specific activity of pheophorbide increases closely according to an exponential law, and reaches a saturation value at about 8 h of incubation. Therefore, the activity peaks of chlorophyll are due to the phytol.

These results may be explained by the following hypothesis:

(1) The specific activity of glycine decreases during the course of the experiment: the specific activity of pheophorbide reaches a constant value at about 8 h incubation and it was shown that the *Chlorella* could be cultivated for 5–6 days, at least, in the solution used. Therefore, if the specific activity of pheophorbide does not increase after 8 h, it must be argued that the glycine cannot be labelled at this time.

(2) The glycine is significantly converted in other compounds: we found the phytol to be radioactive. But we have also direct proof of this fact. In the second experiment an eighth sample was made, from which, after 20 min incubation, the dinitrophenylhydrazones of pyruvic and ketoglutaric acids were isolated, separated from themselves, and assayed for their specific activities. The activity found in these two compounds was about 5 per cent of that of the 2-^{14}C-glycine used in the experiment.

(3) There is an exchange reaction between the free phytol and that linked to chlorophyll: we found that the specific activity of phytol reaches a maximum value and then decays, contrary to pheophorbide. It can be demonstrated that the specific activities of precursor and product have the same values when the specific activity of the product had reached its maximum[4]. Obviously, if a product has more than one precursor, the specific activity of the product must have more than one maximum, because this relation must be verified for every precursor. The results

show that there are, at least, three sources of carbon-14 coming from 2-^{14}C-glycine for the phytol.

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BIOLOGY

A Germplasm-transmitted Alteration of Histocompatibility in the Progeny of Homograft Tolerant Mice

The *in vivo* co-existence of cells of two genotypes achieved in the state of actively acquired tolerance inspired this investigation to determine if any detectable changes occurred in progeny of tolerant mice secondary to the exposure of maturing germ cells to a chimæric environment.

A litter of 5 C_3H mice was injected intravenously during the first 24 h of extra-uterine life with 2–4 × 10^6 nucleated cells from the spleen of an A strain adult male donor to produce tolerance after the model of Billingham, Brent and Medawar[1]. When they reached 30 days of age, the 4 animals, weaned from the injected litter, were grafted with full-thickness abdominal skin measuring 1·5 × 2 cm from A male donors. These grafts were placed on the dorsum of host mice, rotated 180°, and observed for gross signs of acceptance or rejection. At 60 days post-injection, the litter of 2 males and 2 females showed 1 male and the 2 females bearing healthy appearing A strain allogeneic grafts. The tolerant male was then placed in a breeding cage with $(A \times C_3H)F_1$ hybrid female mice and afterwards produced 7 litters of the back-cross progeny hereafter known as $ZBC_{S_1}(C_3H_{\text{tolerant}}\ A♂ \times (A \times C_3H)F_1 ♀)$. Non-injected C_3H males bred with $(A \times C_3H)F_1$ hybrid females produced the back-cross control group of ZBC mice and breeding between $(A \times C_3H)F_1$ hybrid males and females produced the $(A \times C_3H)F_2$ hybrid. Additional control groups were formed by using inbred C_3H, A, and $(A \times C_3H)F_1$ hybrid mice. An $(A \times C_3H)F_1$ hybrid breeding female in our colony spontaneously developed a mammary adenocarcinoma which was used with an 18th transplant generation A strain mammary adenocarcinoma to test the acceptance by the various groups of mice. The latter tumour had been transplanted among A strain animals only, during its course of development, to avoid any of the changes in histocompatibility known to occur in tumours grown in donor and host of different genotypes previously described by Barrett and Deringer[2]. A cellular suspension of each neoplasm was prepared by mechanical homogenization of 2 g of tumour in 10 c.c. of saline and was then diluted to a final concentration of 20–25 × 10^7 nucleated cells per c.c. All test animals were injected with 0·1 c.c. of this suspension subcutaneously, injecting the $(A \times C_3H)F_1$ hybrid tumour in the dorsal neck region and the A tumour in the right inguinal area. The animals were checked weekly for the presence of tumour until time of death. The results are summarized in Table 1.

The back-cross population bred from the tolerant male and $(A \times C_3H)F_1$ hybrid female showed an $(A \times C_3H)F_1$ hybrid tumour incidence at time of death of 91·3 per cent although 5 weeks after tumour inoculation there were 45 nodules palpable in the 46 ZBC_{S_1} mice and at 7 weeks there were 43 palpable growths. There were 13 palpable A strain tumour nodules at 5 weeks of growth, 17 at 7 weeks and 14 large tumour masses at time of death. The animals succumbed to their malignancy between 6–14 weeks following injection. In the other groups of animals, no instance of growth of tumour followed by regression was noted. The incidence of the A and $(A \times C_3H)F_1$ hybrid neoplasms in the A, C_3H, $(A \times C_3H)F_1$, ZBC, and $(A \times C_3H)F_2$ groups followed the expectations of susceptibility determined by multiple dominant histocompatibility factors which are formulated in the theory of the genetic basis of transplantation[3,4]. The acceptance of both A and $(A \times C_3H)F_1$ hybrid tumour in the ZBC_{S_1} mice, however, compared with the ZBC mice can be seen to be significantly different.

The explanation for an almost uniform acceptance of the hybrid tumour by the ZBC_{S_1} and only a 30 per cent rate of take of the A tumour is not clear. If the inherited change in the back-cross progeny of the tolerant mouse involved the acquisition of an A complex of antigens it would be expected that the A tumour be accepted with the same incidence as the $(A \times C_3H)F_1$ hybrid tumour. The change in the progeny, however, may involve the failure of response to A antigen in the hybrid dose while the tumour from the inbred animal presented enough of an antigenic stimulus to prevent its acceptance in some of the animals.

Owen[5] and Stone *et al.*[6] have demonstrated that the progeny of chimæric bulls possessed blood cells of one genotype only and thus further evidenced the cattle chimerism as a phenomenon of transplantation of hæmatopoietic tissue during pre-natal existence. It is not known, however, whether these animals would have responded with the production of agglutinins after challenge with cells of the non-transmitted blood type of their sire[7]. Further study is in progress to determine the nature of the change described in this report. The length of its heritability would give information whether the germ cells of the tolerant animal are functioning with a replicating chemical messenger of non-genetic material which renders a degree of tolerance to its cells until diluted out through successive cell divisions. The recent demonstration in *Drosophila* by Fox[8] of a one generation modification of a specific type protein in progeny lacking the appropriate chromosomal segment, but present in their female parent, may have a mechanism parallel to the alteration of histocompatibility seen in these experiments.

Elucidation of the nature of the tolerant state as well as the study of other types of cellular changes secondary to

Table 1. Allogeneic Tumour Susceptibility in Back-cross Progeny of C_3H Tolerant A ♂

No. of animals	Strain host	Strain Tumour donor	Takes	%	Strain Tumour donor	Takes	%
14	A	$(A \times C_3H)F_1$	0	0	A	14	100
8	C_3H	$(A \times C_3H)F_1$	0	0	A	0	0
9	$(A \times C_3H)F_1$	$(A \times C_3H)F_1$	9	100	A	9	100
31	$(A \times C_3H)F_2$	$(A \times C_3H)F_1$	2	6·5	A	5	16·1
39	ZBC*	$(A \times C_3H)F_1$	3	7·7	A	0	0
46	ZBC_{S_1}†	$(A \times C_3H)F_1$	42	91·3	A	14	30·4

* $ZBC = C_3H$ ♂ × $(A \times C_3H)F_1$ hybrid ♀

† $ZBC_{S_1} = C_3H_{\text{Tolerant}}\ A$♂ × $(A \times C_3H)F_1$ hybrid ♀.

All animals (45–90 days old) were inoculated subcutaneously with 2·0–2·5 × 10^7 tumour cells in saline suspension. The $(A \times C_3H)F_1$ hybrid tumour was a spontaneous neoplasm and the A tumour was in its 18th transplant generation.

exposure with allogeneic cells may be possible with this type of system. In addition to determining the length and specificity of the change, it will be important to determine whether the alteration noted involves the transfer of genetic material, the precursor system of antigenic specificity or, more likely, whether these animals have an alteration in the cellular system involved in the recognition of *A* strain antigens.

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Rumen Function in the Camel

IN May 1962, samples of strained rumen contents obtained from 25 camels in the Alice Springs area of Central Australia were made available for analysis. The animals had all been born in the area (the last importation of camels into Australia was made in 1886)[1]. The area had been suffering from drought conditions for the previous seven years, but the camels were in reasonable physical condition and apparently reproducing satisfactorily. British breeds of cattle are normally grazed in the area; but their numbers had been greatly reduced during the drought and many of the survivors were in poor condition.

The *p*H of the rumen contents were determined in the field before straining through cheese cloth, and the fluid was preserved with mercuric chloride for transport to the laboratory.

Other chemical methods were as follows: total nitrogen—Kjeldahl technique using selenium as the catalyst; total volatile fatty acid—steam distillation through a Markham still after acidification with sulphuric acid[2]; proportions of volatile fatty acids—gas chromatographic technique of James and Martin[3].

Although a full botanical analysis of the contents of the rumina was not made, leaves of *Eucalyptus gamophylla* predominated and were associated with small quantities of *Acacia aneura* and *Acacia rempeura*. A diet of this composition would be low in protein as eucalyptus leaves contain approximately 1 per cent nitrogen and acacia leaves 1·5 per cent nitrogen[4].

Ruminants re-cycle nitrogen into the rumen from the plasma via the saliva and by diffusion through the rumen wall[5,6]. Schmidt-Nielsen *et al.*[7] have shown that on a low nitrogen intake camels are very efficient at conserving body nitrogen by limiting urea excretion in the urine, and if re-cycling also occurs in the camel these mechanisms would result in higher rumen nitrogen and more active fermentation than would otherwise be possible.

The results which were obtained are summarized in Table 1.

Table 1. CHEMICAL CONSTITUENTS OF THE RUMEN FLUID

	Concentration	*S.D.*	Range	No. of samples
Volatile fatty acids (mM/l.)	133·8	25·0	98·1–185·0	25
Total nitrogen (mg/l.)	726	254	360–1,230	23
*p*H	6·4	0·3	4·8–6·8	25

Proportion of volatile fatty acids (per cent)

No. of samples	Acetic	*S.D.*	Propionic	*S.D.*	Butyric and higher	*S.D.*
10	77·1	4·6	16·4	2·3	6·5	3·8

Table 1 shows that the rumen contents were low in nitrogen and high in volatile fatty acids. The latter does not necessarily mean that the rate of fermentation of the diet was rapid. The rumen contents were relatively dry and apparently similar to those described by Schmidt-Nielsen *et al.*[8]. Camels do not have rumen papillæ[9]. Both these factors could slow down the rate of absorption of volatile fatty acid from the rumen.

The proportions of volatile fatty acids are similar to those produced in the rumina of sheep or cattle by any high roughage low nitrogen diet[10], and the microbial population of the rumen had a similar morphological appearance to that present in sheep on low nitrogen diets, but no protozoa were seen.

Camels graze a wider range of top feed than cattle because of their greater height, and the evidence presented here suggests that the camel can maintain quite satisfactory rumen function on this diet. Cattle can be forced to eat eucalyptus leaves under serious drought conditions, but even if the top feed eaten by camels were made available to them it would not constitute a suitable maintenance ration.

Differences in the rate of passage of food through the alimentary tracts of camels and cattle could be of interest particularly in view of the results presented here and the fact that the camel does not have an omasum[11].

The samples were obtained and processed in the field by Mr. A. J. Mackenzie, leader of the University of New England Exploration Society's Expedition to Central Australia. This work was supported by research grants from the Australian Wool Research Committee and the University of New England.

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Immunogenic Activity of the Larval Stages of *Haemonchus placei*

IT has been shown in calves which have experienced a substantial initial infection of *H. placei* that they develop a high level of immunity to further larval challenges and that the removal of a mature adult burden with phenothiazine therapy appears to delay the development of the immunity[1,2]. More recent work where calves had mature *H. placei* surgically implanted into the abomasum indicated that the implant conferred no immunity to the recipient calves to a challenge with 10,000 infective larvæ[3].

The development of the anthelmintic 'Neguvon', which is highly effective against all parasitic stages of *H. placei*[4], has made it possible to stop an infection at selected stages. In the present investigation, 'Neguvon' was used to terminate artificial infestations in calves on the twelfth day when the moult from the 4th to 5th stage occurs. Thirty calves were reared parasite free and infected with 3rd-stage infective *H. placei* larvæ to compare the immunity produced to a challenge of 10,000 infective 3rd-stage larvæ, by two infections of 3rd and 4th stages and

Table 1. DESIGN OF EXPERIMENT

Group	No. of calves	Treatment on day 0	12th	33rd	45th	66th	108th
1	7	10,000 3rd-stage larvæ orally	'Neguvon' treatment	10,000 3rd-stage larvæ orally	'Neguvon' treatment	10,000 3rd-stage larvæ orally	Killed
2	7					10,000 3rd-stage larvæ orally	Killed
3	3	10,000 3rd-stage larvæ orally		10,000 3rd-stage larvæ orally		10,000 3rd-stage larvæ orally	Killed

two infections of 3rd, 4th and 5th stages. Details of the treatments accorded to the three main groups of calves in the experiment are shown in Table 1. A further six groups of calves were used to establish the infectivity of the larval batches used and the efficacy of the treatment with 'Neguvon'.

Table 2. MEAN POST-MORTEM WORM COUNTS

Group	4th-stage larvæ	5th-stage adults	Total worm burden
1	157	2,198	2,355
2	57	2,374	2,431
3	33	10	43

The mean post-mortem worm counts for the three groups are given in Table 2. Control groups showed that at the time of treatment with 'Neguvon', on the twelfth day post-infection, the larvæ had developed to the late 4th stage or very early 5th stage. The results indicated that little effect on the course of a challenge infection, other than slight delayed development, resulted from the host's previous experience of a double infection consisting mainly of the 3rd and 4th stages of the parasite, while a continuance of the original infection to include the patent 5th stage produces a high level of immunity. These and previous results[3] appear to emphasize the importance of the early 5th stage of *H. placei* in the production of immunity in the bovine; but further work is necessary to consider the effects of time, length of infection, larval moults and other possible immune stimuli.

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Energy Utilization of Poor Quality Roughage by Water-deprived Steers

RECENT, but as yet unpublished, work by Livingston, Payne and Friend[1] and Payne and Livingston[2] has shown that cattle are able to reduce their nitrogen output on a low nitrogen intake treatment when water consumption is either voluntarily or compulsorily restricted. At the suggestion of these authors it was decided to extend this work to make a preliminary examination of the energy as well as the nitrogen balance of water-deprived animals.

Table 1

$$\frac{\text{Energy retention (cal/24 h)}}{\text{Digested energy (cal/24 h)}} \times 100$$

	Ayrshire 1	Ayrshire 2	Boran 1	Boran 2
12 per cent crude protein and *ad lib.* water	39·5	43·1	39 1	32·5
4 per cent crude protein and *ad lib.* water	−18·7	−19·3	−4 9	−19·7
4 per cent crude protein and water deprivation	1·7	1·0	−1·8	2 1

Two sets of identical steer twins, one *Bos taurus* (Ayrshire) and the other *Bos indicus* (Boran), were used in this experiment. The rations, which were fed to all four animals, were: (1) 12 per cent crude protein ration of hay and concentrates with *ad lib.* water; (2) 4 per cent crude protein ration of hay to appetite with *ad lib.* water; (3) 4 per cent crude protein ration of hay to appetite with 96 h water deprivation.

A change-over period of three weeks was allowed between the different rations. Oxygen consumption, carbon dioxide output and methane production were measured for each animal on each ration at 20° C and 50 per cent relative humidity in a closed-circuit respiration chamber (Rogerson[3]). Urinary and fæcal nitrogen and carbon were determined.

The ratio of metabolizable to digested energy was slightly higher for the Ayrshires than for the Borans, due principally to a greater loss of urinary energy by the latter, but the ratio did not seem to be affected by water deprivation in either breed.

The change from 12 to 4 per cent crude protein ration with *ad lib.* water resulted in a decline of some 60 per cent in digestible energy intake and caused about a 20 per cent fall in the animals' heat production. The heat production of both Ayrshire steers and one of the zebus exceeded the metabolizable energy by 20–25 per cent. The heat production of the other zebu exceeded its metabolizable energy by 6 per cent. When fed the same 4 per cent crude protein diet but allowed water for only 30 min every 96 h, the heat production of the three steers previously in negative energy balance was reduced, while the other steer remained relatively unchanged. All four animals were then in approximate energy equilibrium.

Water balance data suggested that, although on 96-h water deprivation, the animals were not under severe stress. The average daily water intake over the period of three weeks on 96-h deprivation was 5·5 l.

Under the conditions of this experiment the results suggest that cattle on a low protein roughage diet might be better able to maintain themselves in positive energy balance if subjected to some degree of water restriction.

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Propylene Phenoxetol as a Fish Anæsthetic

PROPYLENE phenoxetol has been found to be a suitable anæsthetic for fish at this laboratory. This substance has been used as a preserving agent by Owen and Steedman[1,2] and as a narcotic for relaxing animals prior to fixing for histological examination[3]. As an anæsthetic it has, like *M.S.222*, the advantage over urethane of not being a known carcinogenic substance. I have used it to quieten live fish when they are being weighed, and also as a narcotic to relax fish so that they may be fixed with the fin rays extended.

Two experiments with plaice, *Pleuronectes platessa* L., have been carried out which show the strength of the solutions to use under different conditions.

Small plaice of 16·5–20 cm were used at 6·5° C in 3 l. of solution each of 0·0025, 0·01, 0·025 and 0·05 per cent propylene phenoxetol. In the two weakest solutions the fish never became completely anaesthetized even after 6 h, though they were considerably quietened. In 0·01 per cent propylene phenoxetol the plaice were limp and did not curl when turned over after 30 min, and even after 6 h they recovered in 20–30 min when returned to clean sea-water. In the 0·025 per cent solution the plaice were again completely anaesthetized after 30 min, but they were not able to recover after 6 h in the solution. They took 30 min to recover their normal activity after 1 h exposure to the anaesthetic. In the strongest solution, 0·05 per cent, the fish were quite limp, and opercular movements had stopped after 30 min, but within 6 min of being transferred to clean water they were able to recover, and even after 1 h in the propylene phenoxetol recovery was possible. After 3 h in this solution the fish were dead.

The second experiment was carried out with larger fish of 27–33 cm in three strengths of propylene phenoxetol solution at 15° C. In the weakest solution, 0·005 per cent, the fish took 2 h to become completely anaesthetized and were unable to recover after 3 h in it. In 0·01 per cent propylene phenoxetol the plaice were limp after 20 min and could recover after 2 h in the solution, but not after 3 h. In the strongest solution, 0·025 per cent, the fish appeared completely anaesthetized after 5 min and were able to recover after 30 min in the propylene phenoxetol but not after 1 h.

From the experiments it is concluded that, for anaesthetizing fish for a period of more than 3 h, propylene phenoxetol must be used with great care since the strength of the solution and the temperature are critical. On the other hand, the experiments also show that it is a useful anaesthetic for periods of up to 1 h when a solution of 0·01–0·025 per cent can be used at normal laboratory and aquarium temperatures with fish of a wide range in size.

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Tubularia crocea L. Agassiz in British Waters

RECENTLY some colonies of a tubularian hydroid were submitted to the British Museum (Natural History) by Dr. S. Markowski. The colonies were found attached to a rope at the intake of Cliff Quay Power Station, near Ipswich, on the River Orwell in July 1959. In general appearance the colonies resembled *T. larynx* in having a stem height of about 5 cm.

However, careful examination of the ripe female gonophores revealed that instead of the usual four, blunt, rounded tentacular rudiments, the gonophores possessed 6–8 laterally compressed ridges. The species was therefore something unusual for the British fauna and it was identified with *Tubularia crocea* L. Agassiz[1], a species of cosmopolitan distribution in warmer waters.

The latter species has previously been reported from British waters—by E. T. Browne[2], on the stern of a sailing ship in Plymouth Sound. This ship had arrived direct from Iquique, Peru, in September 1895, and it is evident that some colonies survived in Plymouth Sound for some years because two colonies were found in 1907. They were examined by Browne and are briefly recorded[3]. Browne's manuscript notes state that two specimens were found on the bottom of the laboratory boat on October 21, 1907; one of these polyps possessed the eight apical ridges on the female gonophores. Later (October 25), a special search of the buoys in the Sound yielded a large colony with gonophores containing actinulae. Browne noted that in the hydranth. the hypostome, stomach, collar and the spadix in the gonophores were a deep crimson. From a comparison of *T. crocea* and *T. larynx* side-by-side he noted that whereas the collar and neck of the hydranth were deeply pigmented in the species *crocea*, it did not, on the other hand, show any conspicuous coloration in that part in *T. larynx*. Other differences in trophosome indicated by Browne may be summarized as follows:

T. larynx	*T. crocea*
Expanded distal tentacles laterally compressed with sharp inner edge with inconspicuous narrow band of nematocysts.	Expanded distal tentacles with inner edge more rounded with broader nematocyst band.
Proximal tentacles with few scattered nematocysts.	Proximal tentacles thickly covered with nematocysts, more or less arranged in clusters.

A search of the British Museum collections did not reveal any genuine material of *T. crocea* from North American waters but a chance examination of the type material of *Tubularia polycarpa* Allman[4], from Coquimbo, Peru, yielded abundant fertile polyps. The gonophores of these, too, possessed the characteristic apical ridges in the female gonophores and in the male gonophores the tentacle rudiments were reduced to a slightly raised ring around the aperture: these characteristics indicate that this species must now be regarded as synonymous with *T. crocea*.

One other species, *Tubularia mesembryanthemum* Allman, remains to be considered. This was described and figured by Allman from the Gulf of Spezia at the same time that he described *T. polycarpa*. In his diagnosis of *T. mesembryanthemum*, Allman mentions that the female gonophore possesses "eight compressed, crest-like apical processes, which are replaced in the male by four small rounded tubercules". In the Ipswich material (but not Browne's Plymouth colonies) male gonophores have been seen. Agassiz declared that in his *T. crocea* there were no tubercules but whether *T. mesembryanthemum* is a distinct species on account of the four small rounded tubercules remains to be elucidated. The species which Bedot[5] recorded from Roscoff as having "les tubercules et les côtes longitudinales caractéristiques" thus retains the name *T. mesembryanthemum* unless the latter species proves to be identical with *T. crocea*.

It is significant that the occurrence of *Tubularia crocea* in a new British locality should be in the vicinity of a power station which uses sea water for cooling purposes.

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Beginning of the Accumulation of Vanadium during the Early Development of the Ascidian *Phallusia mamillata* Cuvier

WHEN the uptake of vanadium by the tunicate *Phallusia mamillata* was investigated, it was observed[1] that small (young) animals (< 20 g fresh weight) accumulated the metal far more intensively than adult ones. This gave rise to the question at which stage of their development the animals become capable of absorbing vanadium. Furthermore, an attempt was made to find out whether this phase coincides with the differentiation of the blood cells, because in *Phallusia mamillata* the vanadium is present almost exclusively within specific blood cells, that is, vanadocytes.

Unfertilized and fertilized eggs (fertilization rate 80–90 per cent) were incubated in sea-water with an addition of nearly carrier-free vanadate-[^{48}V] at 18° C in the dark. The unfertilized eggs contained no more than 5 per cent of the total activity (10^6 c.p.m./ml. sea-water) even after an incubation period of 40 h. The activity of vanadium-48 is of the same order in all stages of development until the larva are about to hatch, that is, during 18 h (Fig. 1). Not until the appearance of swimming larva (up to 90 per cent of the fertilized eggs) does the accumulation of vanadium rise. During the following 18 h in single samples up to 80 per cent of the total activity offered was absorbed. At that time the absorption of the tail of the larva, which was now attaching itself to a substrate, had already begun. Further uptake of vanadium-48 was now reduced, obviously in consequence of the decreased concentration of vanadate in the medium.

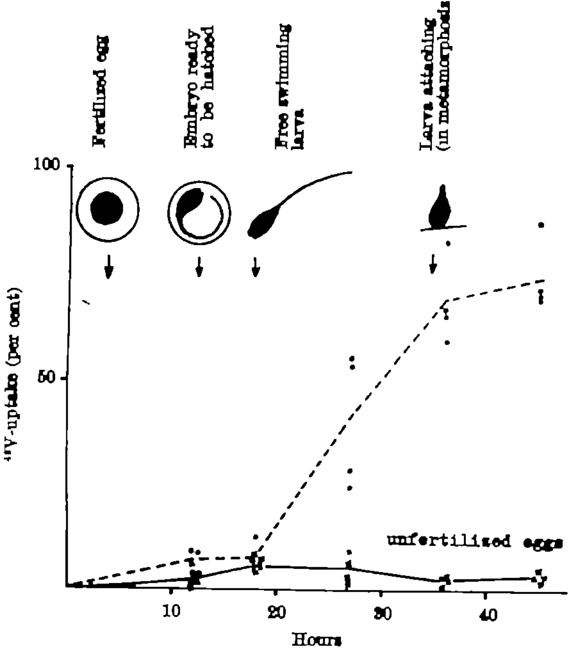

Fig. 1. The uptake of vanadate-[^{48}V] by *Phallusia mamillata* during development. ^{48}V-addition: 50 × 10^6 c.p.m./50 ml. sea-water containing an average of 8 × 10^4 fertilized resp. unfertilized eggs. Development at 18 ± 0·5° C in the dark during careful rocking (12 rocking movements/min). Four parallel samples; 5 ml. of each sample were withdrawn at the times indicated and the centrifuged material was washed three times with sea-water and measured by a scintillation counter

If it is assumed that the larva, the epidermis of which is already covered with a cuticle, absorbs the vanadium in the same way as the adult animals do[1], namely, through the branchial sac, it can be concluded that the gill slits develop during the larval stage[2], by which the branchial cavity may communicate via the atrial apertures with the outer medium. According to Kowalevsky[3] hæmatoblasts have separated at that time from the mesoderm and from the 'Darmdrüsenblattzellen' of the tail and migrated to the sides of the intestine, which now has opened into the cloaca.

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Leeches as Endoparasites of Frogs

Leeches are adapted by their structure and physiology to an ectoparasitic, blood-sucking mode of life. Some have secondarily become predators, but none has obvious adaptations to endoparasitism. Those that enter the upper respiratory tracts of birds and mammals to suck blood from the mucous membrane usually remain only long enough to get their meal. It is therefore interesting to find that at least one species has been found living internally in frogs, usually in the lymph space beneath the skin but sometimes in the coelom. The leech is a species of *Philaemon*, possibly *P. grandidieri* Blanchard. The frogs are of six species and were taken in primary montane forest from the central mountains of the Australian Trusteeship Territory of New Guinea. They were at an altitude of 4,600–10,500 ft., between 143° and 146° E. on a latitude of approximately 6° S.

Rana grisea van Kampen (1 specimen infested) and *Hyla angularis* Loveridge (5 specimens) are ground-dwellers which rarely travel far from water. *Hyla becki* Loveridge (3 specimens) and *Hyla* n.sp. Tyler[1] (6 specimens) are normally found among low vegetation which they occasionally climb. *Nyctimystes kubori* Zweifel (1 specimen) and *N. narinosa* Zweifel (2 specimens) are typical tree-climbing frogs. Except for *Rana grisea* little is known of their breeding habits but it is unlikely that any exhibit arboreal spawning or direct development. They probably congregate at water for breeding and the leech may well attack them there. None of the frogs shows a scar marking the point of entry, so the leeches must have entered when they were very small and have lived and grown for a considerable period within the host. The jaws of the leech

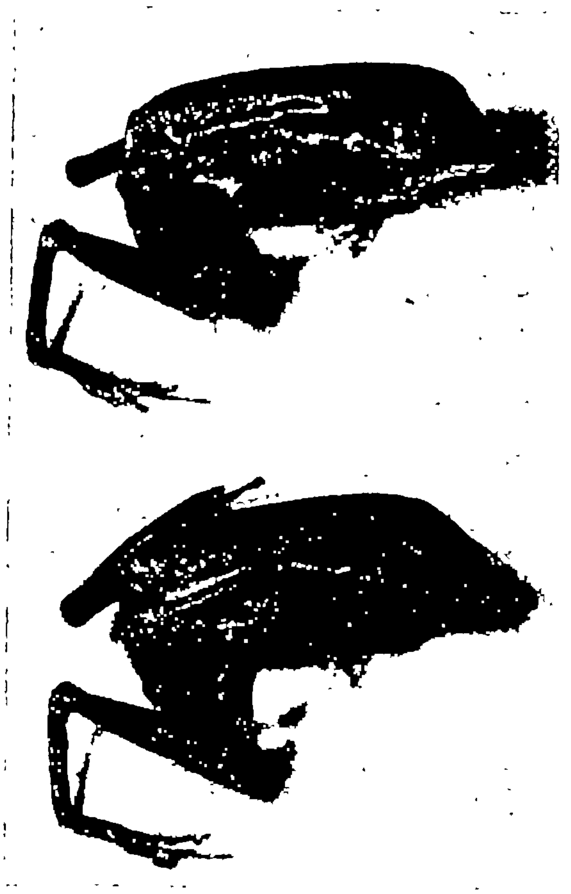

Fig. 1. Above, preserved specimen of *Hyla becki* showing the bulge in the dorsal surface caused by the leech in the subcutaneous lymph space. The split at the posterior end is a post-mortem effect. Below the same specimen with skin incised to show the leech in position. (*Photo* B. N. Donetil)

are well adapted to piercing skin; but it is surprising to find that almost one-third of the specimens had penetrated the muscles of the body-wall and settled in the coelom.

The leech shown in Fig. 1 is capable of taking almost the whole of the blood of the frog in one meal, and a prolonged endoparasitic relationship is possible only if the leech has modified its feeding habits to the extent of taking little and often. Those in the coelom would also have to adapt themselves to life in a very low oxygen concentration.

There is one previous record of such a phenomenon. Richardson[2] described the occurrence of *Batracobdella picta* (Verrill) in the dorsal subcutaneous lymph space of *Rana catesbiana* in Canada. In this case the entry seems to have been accidental and abnormal, for the leech is well known in the free-living state, and only two cases of the association were discovered from many hundreds of frogs examined. *Philaemon* is known as an ectoparasite in Madagascar, Australia, the Samoan Islands and Juan Fernandez[3], but we have discovered no records from New Guinea. Leeches are hermaphrodites and this might be regarded as an advantage in an endoparasite, but cross-fertilization is normal and the eggs are deposited in a cocoon. It therefore seems likely that either the leeches leave their hosts to breed or there is a breeding population of free-living leeches which has not yet been discovered. In either event this curious phenomenon throws interesting light on the origins of the endoparasitic habit.

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Emerson Effect in Isolated Chloroplasts

The oxygen production resulting from flash illumination of chloroplast fragments was determined, using the method previously described[1]. Spinach leaves were ground in 0·35 M sodium chloride, 0·037 M magnesium chloride, 0·01 M ascorbate and 0·1 M *tris* buffer adjusted to *p*H 7·6. After centrifugation, the chloroplasts were washed once in the grinding medium but without ascorbate and broken in the same medium diluted ten times. The reaction mixture contained, in a final volume of 2 ml., chloroplast fragments containing 0·15 mg chlorophyll and the following (in μmoles): potassium ferricyanide, 3; sodium chloride, 70; magnesium chloride, 10; adenosine triphosphate, 1·5; inorganic phospate, 30; *tris* buffer, 50, adjusted to *p*H 7·4. Throughout the measurements the reaction mixture was maintained at 5° ± 1° C and was flushed with nitrogen gas containing less than 1 p.p.m. oxygen. Flashes, from Philips photoflux bulbs, were of sufficient intensity to saturate the reaction ($2·8 \times 10^{-4}$ lumens, 35-msec duration). The change of oxygen yield per single flash was used to determine the rate of decline of activity of the chloroplasts with time.

It has been shown previously[1], with white light, that the yield of oxygen from a single flash is greatly increased when an algal suspension is illuminated with very low intensity continuous light. These experiments have been repeated using isolated chloroplasts with monochromatic flash and background illumination. The flash was filtered through a GAB filtra flux interference filter with transmission either at 644 ± 13 mμ or 697 ± 7 mμ. The continuous light was passed through a *D*.290 Hilger and Watt's small grating monochromator, with the slit width adjusted to give a band-width not greater than 5 mμ. The intensity was approximately the same at all wave-lengths. The chloroplasts were illuminated with continuous monochromatic light of a selected wave band until a steady rate of photosynthesis was obtained; then the additional oxygen produced by a single flash was measured. The yield of oxygen from the flash as a percentage of the yield obtained in darkness without any continuous illumination is plotted against the wave-length of the continuous light and is shown in Fig. 1. With a flash at 697 mμ, the greatest yield of oxygen was obtained when the continuous light was at 651 or 675 mμ, with a maximum effect at 675 mμ. With a flash at 644 mμ, the greatest yield was obtained when the continuous light was at 675 or 700 mμ. The wave-length for the maximum enhancement of the yield from the flash was unchanged when the intensity of the continuous light was varied several fold

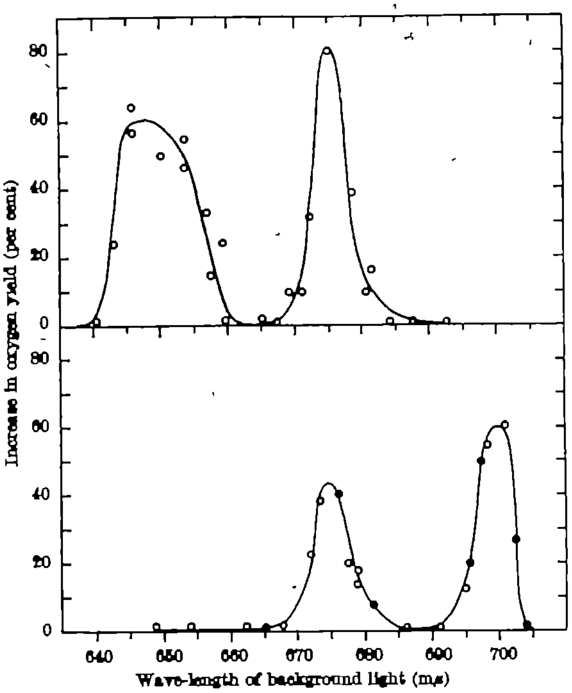

Fig. 1. Percentage increase in yield for a monochromatic flash at 697 (upper) or 644 (lower) as a function of the wave-length of the continuous monochromatic illumination. The percentage is expressed as the increase in yield compared with a flash given in darkness

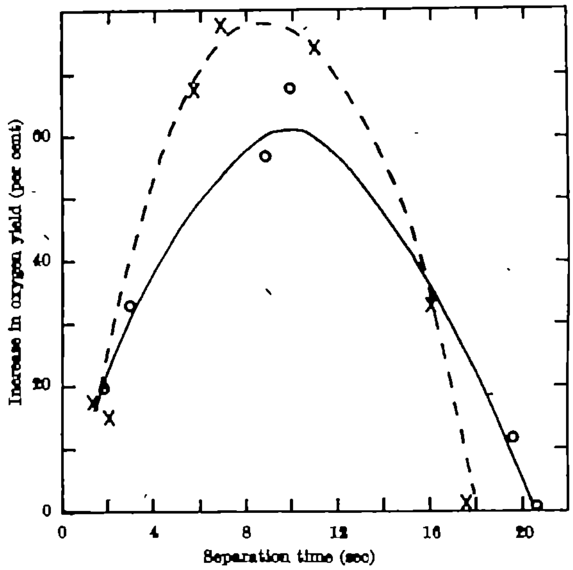

Fig. 2. Yield per flash as a function of the time-intervals between the cessation of illumination and the flash. ——, Yield from a flash of 697 mμ following illumination at 656 mμ; – – –, yield from a 644-mμ flash following illumination at 700 mμ

The marked dependence of oxygen yield on wave-length indicates the existence of different chlorophyll forms which must have sharp absorption bands.

In other experiments the chloroplasts were illuminated by a single flash given at a measured time-interval after the continuous background light was turned off. Fig. 2 shows the yield of oxygen from the flash as function of the time between the cessation of the background illumination and the flash. Even 8 sec after the continuous light was turned off, the yield of oxygen from the flash was increased compared with that from the same flash given after a prolonged dark period. The results were appreciably the same whether the flash was at 697 mμ and the background light at 651 mμ, or the flash at 644 mμ and the background at 700 mμ.

These results show that isolated chloroplasts reducing ferricyanide exhibit the same type of enhancement effect as has previously been observed with photosynthetic plants[2], and with *Chlorella* reducing quinone[3]. Furthermore, they demonstrate in spinach leaves, chlorophyll forms which absorb at essentially the same wave-lengths as those observed by French and Fork[4], in leaves of different plants and in green algae, using the differential spectrophotometer. If two pigment systems are postulated (one absorbing at 675 and 700 mμ and the other at 651 and 675 mμ in spinach) catalysing two separate photochemical reactions the products of which interact in photosynthesis, a lifetime for the products of several seconds is indicated. Our observations were at 5° ± 1° C, whereas those of French were at room temperature. French found no enhancement, in *Chlorella*, of the yield at 670 mμ as a result of simultaneous illumination with 650 mμ. When studying the reactions of isolated chloroplasts the effect of a light-induced respiration, if present at all, may be smaller than with whole cells. It is not clear as yet whether this apparent difference in wave-lengths is due to the difference in temperature or to the complications introduced in a respiring system. Witt[5] has observed absorption changes consequent on illumination and suggested that the longest lifetime of any intermediate in photosynthesis is of the order of 10^{-2} sec. Our work suggests the existence of a long-lived intermediate which may not be detectable by the method of difference spectroscopy used by Witt, but which is required in the reactions leading to the production of oxygen.

We are grateful to the Royal Society for a Government grant-in-aid of this investigation. One of us (P. M. B.) holds a postgraduate studentship of the University of London. We are also grateful for a grant from the Central Research Fund of the University of London.

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ENTOMOLOGY

Insecticide absorbed by the Central Nervous System of Susceptible and Resistant Cockroaches exposed to Dieldrin

PENETRATION and metabolism studies carried out in this Department on susceptible and resistant houseflies, using the sulphur analogue of dieldrin or a non-insecticidal bromine derivative of aldrin[1]; aldrin, dieldrin, isodrin or endrin[2]; failed to reveal any differences between the strains which would explain the mechanism of resistance. There is some evidence that dieldrin acts on the central nervous system of susceptible insects[3] and it seemed possible that dieldrin failed to reach the sensitive cells of this system in the resistant insect due to some internal local barrier, for example, a dieldrin impermeable neural lamella.

In order to investigate this, dieldrin resistant and susceptible *Blattella germanica* were treated topically with dieldrin (10 μg in 2 μl. acetone) on the dorsal abdomen and kept at 25°. The susceptible insects became prostrate within about 6 h of treatment; but the resistant ones were unaffected. The intact cockroaches were rinsed externally in acetone/petroleum ether (20 : 80 by vol.) to remove unabsorbed insecticide and the nerve cords dissected out. The nerve cords were homogenized with acetone–petroleum ether and the dieldrin in the extract

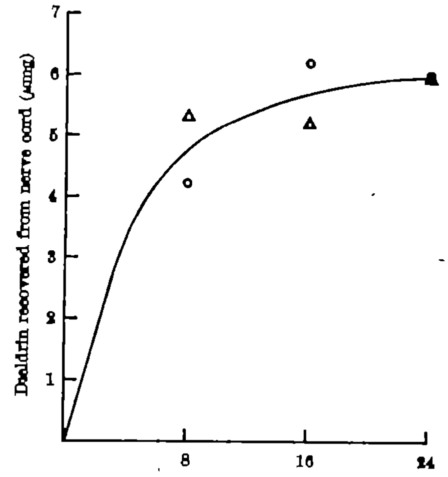

Fig. 1. Dieldrin uptake by susceptible and resistant *Blattella germanica*. ○, Dieldrin found in susceptible nerve cord, △ dieldrin found in resistant nerve cord

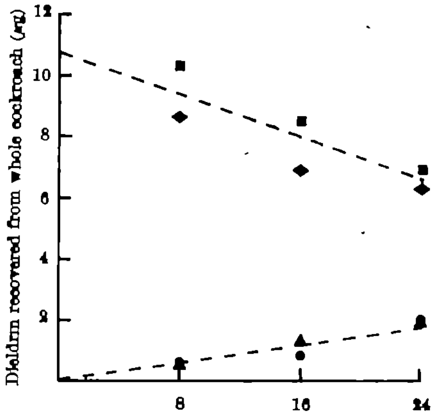

Fig. 2. Dieldrin uptake by susceptible and resistant *Blattella germanica*. ■, Dieldrin found on outside of resistant insect; ◆, dieldrin found on outside of susceptible insect; ●, dieldrin found in tissues of susceptible insect; ▲, dieldrin found in tissues of resistant insect

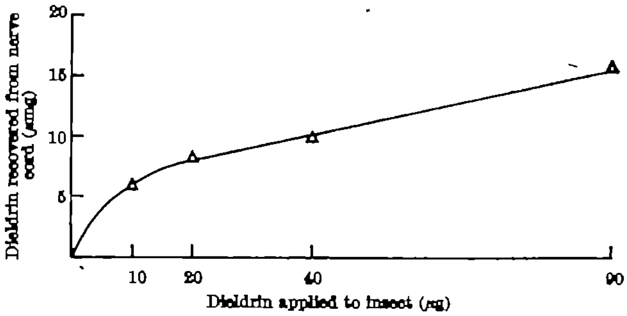

Fig. 3. Dieldrin uptake by resistant *Blattella germanica*. △, Dieldrin in nerve cord of resistant insect

assayed by gas-liquid chromatography. The results are shown graphically in Fig. 1. Increasing doses of dieldrin were topically applied to the resistant strain and the animals left for 24 h before the insecticide in the nerve cord was assayed (Fig. 3). None of these insects was showing any signs of poisoning. Penetration into the whole insect was studied in both strains, Fig. 2.

From Figs. 1 and 2 it will be seen that there were no differences between the amounts of unchanged dieldrin recoverable from the tissues either of the whole animal or the nerve cord in the resistant or susceptible cockroach.

Fig. 3 shows that in the resistant animal the concentration of dieldrin in the nerve cord increased with increased topical application of insecticide.

These results are not consistent with the existence of a dieldrin-impermeable membrane around the nerve cord of the resistant animal.

These and earlier investigations therefore suggest that resistance is not due to metabolism or to lack of penetration of the insecticide, but rather to the ability of the resistant insect to withstand higher concentrations of unchanged dieldrin within the central nervous system itself without showing any signs of poisoning.

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Differential Growth Response of the Male and Female Rice Moth to Antibiotic Supplements

During the course of the nutritional studies[1] on the rice moth, *Corcyra cephalonica* Staint., to determine the comparative value of several commercially available antibiotic feed supplements, it was observed that in some cases male moths of *Corcyra cephalonica* Staint. exhibited a higher growth response than the females receiving dietary antibiotics in their larval stages. A survey of the literature failed to disclose specific observations on this phenomenon among insects, although Palafox and Rosenberg[2] observed that male chicks respond in a more satisfactory manner to antibiotic feed supplements than the female.

The culture of *Corcyra* was maintained as described earlier[3]. For experimental purposes 10 in. × 2 in. glass vials were filled with 120 g of crushed 'juar' (*Sorghum vulgare*). Weighed quantities of the antibiotics penicillin, oleandomycin and terramycin were mixed in the diet at the rate of 100 p.p.m. by thoroughly distributing the premix in the vials, containing the food. Premix was prepared in glucose in convenient amounts for efficient mixing. The control groups received an equal amount of glucose only in the diet. Fifty freshly laid eggs (24-h layings) were introduced in the experimental vials containing different antibiotic-feed supplements. Six replications were used for each treatment. Moths emerging from the different treatments, after completing their development in the different antibiotic-feed supplements, were sorted out for males and females. Ten males and an equal number of females were weighed individually from each replication to assess the growth response in the sexes. Results are summarized in Table 1. The growth indexes shown in Table 1 were obtained by dividing the average body-weights of either males or females in each experimental group by the mean body-weight of the respective control group.

Table 1. Comparative Growth Indexes of Male and Female as Influenced by Antibiotic Feed Supplements

Supplement	Growth Indexes (%) Male	Growth Indexes (%) Female
Penicillin	112·2	106·4
Oleandomycin	99·5	100·7
Terramycin	108·3	103·2
Control	100·00	100·00

From these results it is seen that the males fed on penicillin-feed supplements grew 12·2 per cent heavier than their controls, and those fed terramycin grew 8·3 per cent heavier, whereas the females grew only 6·4 per cent and 3·2 per cent heavier than their respective controls. On analysis, F value between sex growth indexes was highly significant ($P < 0.01$). Oleandomycin proved ineffective in enhancing the growth of either male or female.

I thank Mr. B. N. Jha, vice-chancellor, for encouragement, and Dr. B. K. Srivastava, entomologist to the Government of Rajasthan, Jobner, for guidance. I also thank M/S. Dumex Private, Ltd., Bombay, for gifts of the antibiotics.

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A Modified Window-trap for Assessment of Fumigant Insecticides in Experimental Trap-huts

We are assessing the toxicities of new insecticidal compounds as part of a World Health Organization programme to evaluate possible substitutes for the chlorinated hydrocarbons to which anopheline mosquitoes in many countries have become resistant. The experimental trap-hut of the Muirhead-Thomson type[1] is used for this assessment as it is a research tool of proved value. There are, nevertheless, a number of weaknesses in its design that lead to errors in assessing insecticide-toxicity:

(1) Nearly all resting is on the roof so that the toxicity of the roof-substrate has an over-riding effect on overall mortality.

(2) In the presence of a fumigant insecticide an unassessed proportion of mosquitoes die simply due to confinement in the atmosphere of the insecticide in the window-trap, whereas under natural conditions they would have escaped from the hut and survived.

(3) An unassessed proportion of hut-entering mosquitoes leave through the eaves.

Investigations of these three points are in progress in our outstations at Magugu in Tanganyika, and at Taveta in Kenya. Errors due to the first point have been largely overcome by assessing mortalities in huts with different types of roof-surfaces[2]. Errors due to the second point have been greatly reduced by a simple technique which is the subject of this communication. The third point is still unresolved, but is receiving attention.

Table 1. Mortality of Blood-Fed Mosquitoes in Window-Traps (W.T.) with Funnels of Cotton Mosquito Netting and of Sheet Polythene

Age of dispenser (days)	Exposure period (h)	Species of mosquito	Percentage mortality with number studied in parentheses: W.T. with netting funnel	Percentage mortality with number studied in parentheses: W.T. with polythene funnel
72	4	*Aedes aegypti*	0 (55)	2 (62)
2	2	,,	59 (51)	0 (59)
2	2	,,	14 (61)	0 (57)
64	4	*A. gambiae*	85 (68)	0 (71)
16	2	,,	16 (38)	21 (34)
16	2	,,	11 (52)	0 (58)
24	4	,,	97 (38)	0 (37)
28	4	,,	76 (39)	0 (36)
32	4		0 (42)	2 (40)

[illegible]

The normal type of window-trap, fitted to an experimental hut, is supported on a frame that fits into a window 1 ft. square. There is an exit funnel of cotton mosquito netting, with a mouth 1 in. in diameter, and a trap of similar netting which has sides and a back 1 ft. square. Thus in a hut with a fumigant insecticide such as 'Dichlorvos' there is a diffusion of vapour from the hut into the window-trap through an aperture virtually 1 ft. square since the vapour diffuses readily through the mosquito netting. By changing the funnels of netting for ones of polythene sheet (250 gauge) the area for diffusion of 'Dichlorvos' vapour was reduced from approximately 1 ft.^{2} to 1 in.^{2}, while the exit aperture for mosquitoes remained the same size. The results in Table 1 show that this modification greatly reduced the mortality of blood-fed mosquitoes confined in the window-traps. The percentage mortalities are for 24 h after exposure and are corrected against control mortalities by Abbott's formula[3]. Complementary chemical determinations, using a method to be published later, showed that the concentration of 'Dichlorvos' in the window-traps with plastic funnels was of the order of 0·005 μg/l., compared with 0·020 μg/l. in traps with netting funnels.

The modified window-trap has now been incorporated into our routine trials with experimental huts to assess the toxicity of 'Dichlorvos' to naturally entering mosquitoes.

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MICROBIOLOGY

Deoxyribonucleic Acid Type attributable to a Bacterial Endosymbiote in the Protozoon *Crithidia (Strigomonas) oncopelti*

In the course of an investigation of the occurrence of two paths of lysine biosynthesis[1,2], tracer and enzyme experiments provided evidence that the trypanosomatid flagellate, *Crithidia* (*Strigomonas*, *Herpetomonas*) *oncopelti*, contains a bacterial endosymbiote[3,4]. This evidence was supported by electron microscopy: the inferred endosymbiote shows structures consistent with a cytoplasmic membrane, a cell wall, and nuclear zones, and endosymbiotes which are dividing, as judged by the presence of a constriction, exhibit membrane septa[4,5]. The protozoon can be 'cured' of its endosymbiote with penicillin[4,5]. It was suggested that the endosymbiote furnishes lysine and probably other metabolites to the protozoon, which in turn provides a protective environment for the endosymbiote[3,4]. This suggestion took into account: (*a*) the exceptionally simple nutritional requirements of the protozoon, which were recorded by Newton[6]; (*b*) the presence of 'bipolar bodies' within the protozoon, which were described by Newton and Horne[7] and afterwards recognized as the endosymbiote[3,4]. Attempts to demonstrate deoxyribonucleic acid (DNA) in the bipolar bodies had given inconclusive results[7].

In parallel experiments, DNA samples from *C. oncopelti* and other protozoa were examined for their base composition by their buoyant density in a caesium chloride gradient[8,9]. The buoyant density is known to be linearly related to the mole per cent guanine plus cytosine (G + C) composition of DNA, and may correlate with the taxonomic position of the organism involved[10]. For *C. oncopelti*, three DNA bands were observed in the caesium chloride gradient (Fig. 1): a quantitatively major one, corresponding to a density of 1·709 g/c.c. and presumably derived from the protozoan nucleus, and two minor ones with densities of 1·699 g/c.c. and 1·691 g/c.c. The base compositions corresponding to these densities are 50, 40 and 32 per cent G + C, respectively. Accordingly, it was of interest to examine the possibility that one of the minor bands represents DNA from the endosymbiote.

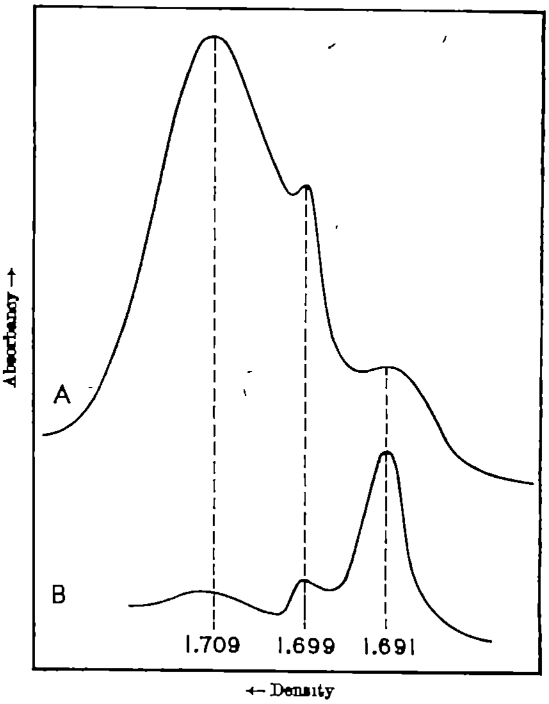

Fig. 1. Banding patterns of DNA from cells of endosymbiote-containing *C. oncopelti* (*A*) and from isolated cells of the endosymbiote (*B*)

Preparations of the endosymbiote were obtained as follows. Cells of *C. oncopelti* were collected during active growth on peptone–liver extract–glucose medium and were suspended in 0·2 M (potassium) phosphate buffer at *p*H 7·0, made 0·25 M in sucrose. The suspension was treated with glass beads in a Mickle disintegrator until approximately 80 per cent of the protozoan cells were disrupted, and was then fractionated by centrifugation; the material sedimenting (for 10 min) between 900 and 1,200*g* was collected. Microscopic examination indicated that this fraction consisted largely of the endosymbiote, along with some fine debris. This fraction was suspended in 0·2 M phosphate at *p*H 7·0, containing 0·25 M sucrose and 0·01 M magnesium sulphate, and was treated with deoxyribonuclease to destroy, so far as possible, any contaminating protozoan DNA. The treated suspension was made 0·04 M in trisodium citrate, and was centrifuged. The sedimented material was washed by centrifugation with a solution containing 0·15 M sodium chloride, 0·25 M sucrose, and 0·015 M trisodium citrate, and was then suspended in a solution containing 0·15 M sodium chloride and 0·015 M trisodium citrate. The resulting suspension was lysed by addition of 20 per cent sodium lauryl sulphate (0·5 part by volume per part of suspension), and was heated at 55° C for 10 min. The lysed suspension was subjected to the procedure for the extraction of DNA, essentially as previously described[11].

The banding patterns of the DNA obtained from the endosymbiote-containing protozoa (Fig. 1*A*) and from the isolated endosymbiotes (Fig. 1*B*) indicate that the minor band of density 1·691 g/c.c. of tracing *A* and the major band of tracing *B* represent DNA from the endosymbiote. The calculated base composition (32 per cent G + C) for the DNA from the endosymbiote resembles that for the DNA from various bacteria, pleuropneumonia-like organisms, and kappa[8,10,11]. The origin of the bands of density 1·699 g/c.c., representing minor components in tracings *A* and *B*, remains unknown. The narrow width of these bands, which may reflect a high degree of homogeneity or high molecular weight or both, suggests that these bands may correspond to DNA from specialized homogeneous structures or a virus. If so, the cæsium chloride density gradient technique would be useful in investigating not only bacterial endosymbiotes, but also other inclusions exhibiting genetic continuity within a cell.

This work was aided by grants from the National Science Foundation and the U.S. Public Health Service, and by a contract between the Office of Naval Research, Department of the Navy, and Rutgers, The State University. The strain of *C. oncopelti* used was kindly furnished by Dr. F. G. Wallace.

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VIROLOGY

Lettuce Necrotic Yellows Virus

A DESTRUCTIVE virus disease of lettuce, causing extensive crop losses sometimes as high as 100 per cent. was recognized in Victoria in 1954 as being distinct from the lettuce mosaic disease. Until 1959, however, all attempts to transmit the virus to lettuce with aphids which commonly infest lettuce, with thrips, leaf-hoppers and sap inoculation methods were unsuccessful. In that year a virus was transmitted mechanically to *Nicotiana glutinosa* L. from many diseased lettuce plants and from apparently normal common sowthistle (*Sonchus oleraceus* L.) plants. These isolates were, at first, incorrectly identified as mild variants of the tomato spotted wilt virus[1], because of the remarkable similarity between the diseases caused by the two viruses on some common hosts, including lettuce. The tomato spotted wilt virus has been isolated occasionally from lettuce; but it is of minor significance in Victoria, Australia, possibly because common strains found here on tomato and other hosts do not appear to infect lettuce in Nature.

The causal virus, which appears to be distinct from any previously described, is to be named lettuce necrotic yellows virus. The virus is unique in its host and vector relationships. It is not seed-borne in lettuce, or any other host so far examined, but it is widely distributed in Nature on *S. oleraceus*, which is its only known source. It is transmitted specifically, and most efficiently after moulting, by the aphid *Hyperomyzus lactucae* L., which breeds only on *Sonchus* spp. although currants (*Ribes* spp.) are reported[2] to be an overwintering host, and may be the original source of the virus. This possibility is being investigated. The aphid transports the virus to lettuce on which it feeds transiently, but not preferentially. It dies without multiplying when caged on lettuce for several days.

In addition to the hosts already mentioned, the virus has been experimentally transmitted to and recovered from *Datura stramonium* L., *Gomphrena globosa* L., petunia, tomato and spinach. Attempts to infect *Nicotiana tabacum* L. var. *xanthi* and Turkish have not been successful. Lettuce is extremely difficult to infect by sap inoculation, and mechanical transmission of the virus from lettuce to lettuce has not been achieved.

Sowthistle, lettuce and *Lactuca serriola* L. are the only hosts from which the virus has been recovered in Nature.

Thermal inactivation of the virus in expressed sap from *N. glutinosa* occurred between 52°–54° C after 10-min exposure. Its longevity *in vitro* ranged from 2 to 8 h for individual isolates. These differences may be due to different concentrations of the virus rather than to strain differences.

There is evidence that the lettuce necrotic yellows disease can be effectively controlled by eradicating sowthistles in the close vicinity of lettuce crops.

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GENETICS

A New Chromosome Number and Karyotype in *L. radiata*

CYTOLOGICAL investigations in several species of *Lycoris* have been carried out by a number of workers[1-4]. The somatic chromosomes here are fairly large in size and few in number, reported to be between 12 and 33. Of these 12, 13, 14, 15, 17, 22, 29 + 1 *B*, 30 and 33 have already been counted. In karyotype analysis the chromosomes could be classified broadly into *V* and rod-shaped types No species have been found with all *V* chromosomes in their complement but those with 22 and 33 chromosomes have been observed to have rod chromosomes with subterminal constrictions only (*L. haywardii*, *L. radiata*, *L. sanguinea* and *L. sprengeri*). Among those with both *V* and rod chromosomes in their complement some have rods of the nearly terminal constrictions only (*L. aurea*, *L. chinensis*, *L.* '*sperryi*' and *L. traubii*), others have rods with subterminal constrictions (*L. albiflora* and *L. elsiae*), still others have rods with subterminal and nearly terminal constrictions (*L. caldwellii*, *L. squamigra*, *L. incarnata* and *L. houdyshelii*). In *L. chinensis* one *V* chromosome was observed to be much shorter in size (designated small *v*) than the rest of *V* chromosomes[4].

In *L. radiata* somatic chromosome numbers have been reported with 22 and 33 chromosomes and the usual karyotype consists of only rod chromosomes with subterminal constrictions (Fig. 1*A*). Recently, while making cytological observations on a number of bulbs labelled as *L. radiata*, one was found to have a chromosome number and karyotype quite different from the others. Here 31 rod chromosomes (all with subterminal constrictions), 1 *V* chromosome and 1 small chromosome with nearly median constriction were observed (Fig. 1*B*). Hence, $2n$ number here could be counted as 32 + 1 *B* or supernumerary chromosome. This small chromosome was much shorter in size in comparison with the rest of the chromosomes. Previously only one species of *Lycoris* has been found with a *B* or supernumerary chromosome, that is, in *L. incarnata* with 29 + 1 *B* (ref. 5). In all the other bulbs 33 rod chromosomes were observed with subterminal constrictions (Fig. 1*A*). All these bulbs with 33 rod chromosomes and that

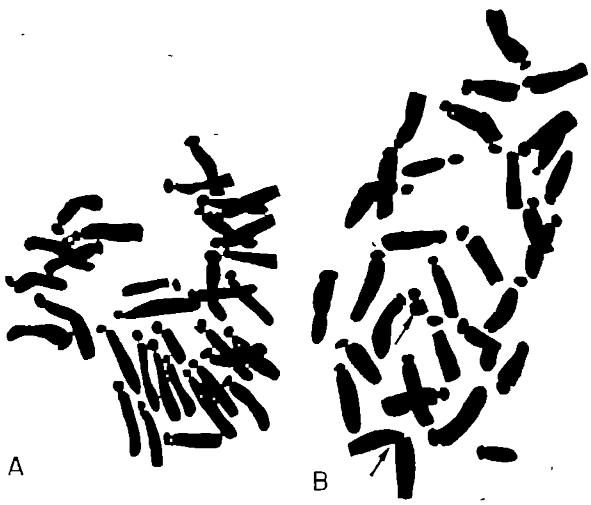

Fig. 1. *A*, *Lycoris radiata*, somatic metaphase with 33 rod-shaped chromosomes (× *c*. 1,100); *B*, *L. radiata*, somatic metaphase with 31 rod-shaped, 1 *V*-shaped and 1 *B* or supernumerary chromosome. *V* and *B* or supernumerary chromosome shown by arrows (× *c*. 1,100)

with 31 *R* + 1 *V* + 1 *B* chromosome had leaves like those in *L. radiata* but none of these has flowered yet.

With regard to the finding of a *V* chromosome in *L. radiata* the mechanism of the formation of a *V* chromosome from two rods with subterminal constrictions through reciprocal translocation or fusion can be taken into account. The formation of a *V* chromosome by these methods has been suggested by Cave and Bradley[9] in *Miersia chilensis*. Although the exact nature of the origin of a *B* or supernumerary chromosome is not known, Darlington's[10] idea regarding the origin of this type of chromosome from an *A* seems relevant.

This new chromosome number and altered karyotype observed in *L. radiata* once more shows the rôle of structural change in speciation and in the formation of chromosome races in *Lycoris* (Table 1).

Table 1. Chromosome Number and Karyotype in *Lycoris*

Lycoris taxa	Types of chromosomes *V*	*R*		Chromosome No.	Possible potentialities in rod chromosomes
aurea	10	2		12	22 *R*
	9	4		13	22 *R*
	6	8		14	22 *R*
	7	8		15	22 *R*
'sperryi'	7	8		15	22 *R*
traubii	10	2		12	22 *R*
	9	4		13	22 *R*
chinensis	7	9		16	22 *R*
straminea	6	10		16	22 *R*
albiflora	5	12		17	22 *R*
elsiae	5	12		17	22 *R*
haywardii		22		22	22 *R*
radiata		22		22	22 *R*
sanguinea		22		22	22 *R*
sprengeri		22		22	22 *R*
caldwellii	6	21		27	33 *R*
squamigera	6	21		27	33 *R*
incarnata	4	25	1 *B*	29 + 1 *B*	33 *R* + 1 *B*
houdyshelii	3	27		30	33 *R*
radiata	1	31	1 *B*	32 + 1 *B*	33 *R* + 1 *B*
		33		33	33 *R*

I thank Dr. W. S. Flory, jun., for his guidance during the course of this investigation. I also thank Dr. A. K. Sharma for advice.

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MISCELLANEOUS

Storage of Standardized Radioactive Solutions

There is considerable interest in the storage of radionuclide solutions in polythene bottles. Keith, in a recent communication[1], indicated the losses of solvent through the walls of polythene ampoules. The experience of our laboratory is comparable in this respect, but it might be noted that the rate of loss was extremely high from concentrated hydrochloric acid solutions where apparently gaseous hydrochloric acid was being transferred through the walls. We believe that the following information on other types of losses will be of interest.

The stability of nuclide solutions in polythene bottles and possible losses through adsorption on the walls have been questioned many times. A series of tests was performed to determine if appreciable losses occurred in the normal use of these bottles for solution storage and handling. The results obtained should be considered only as a guide and appropriate tests carried out as needed.

Six nuclides (cæsium-137, cerium-144, zirconium-95, iodine-131, ruthenium-106 and barium-140) were obtained from Oak Ridge, aliquots taken, diluted and stored in sealed polythene ampoules (6 ml. capacity) for a period of from 10–63 days.

Nuclide	Carrier	Acid	Percentage retained after rinse Water	Acid
^{137}Cs				
	–	–	0	
	×	–	0	
	–	×	0	
^{144}Ce				
	–	–	5	0·6
	×	–	0	
	×	–	0	
	–	×	0	
	–	×	0	
^{95}Zr	–	–	48	36
	×	–	0*	
	–	×	1·2	0·8
^{131}I				
	–	–	26	25
	×	–	17	17
	–	×	26	26
^{131}I with Na_2SO_3 at *p*H 8			0	
^{106}Ru				
	–	–	35	15
	×	–	26	26
	–	×	19	23
^{140}Ba				
	–	–	0	
	×	–	0	
	–	×	0	

* With either zirconium or niobium or with both as carriers.

In each case, duplicate samples were prepared in water, with carrier (1 mg/ml.) and with 0·1 N hydrochloric acid solution, except in the case of iodine-131 where sodium sulphite (*p*H at 8) was used, and zirconium-95 where oxalic acid was used.

The samples were γ-counted in a 3·25 in. × 5 in. sodium iodide well-crystal, with a 1 in. × 1·75 in. well surrounded by a stainless steel, mercury-filled shield. All samples were counted for 5 min and a counting error of at least 1 per cent was maintained. The average counter background was 225 c.p.m. After the initial count, the drawn stems of the ampoules were cut off, the bottles rinsed four times with warm water, and re-counted. In the case of residual activity, the bottles were re-washed four times in a 1 N hydrochloric acid solution and re-counted.

The results are shown in Table 1. In every case except one, the nuclide may be kept in solution by addition of appropriate acid or carrier. Ruthenium adsorbs appreciably on polyethylene and even at best 25 per cent will remain on the walls.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 25

Royal Society of Medicine, Library (Scientific Research) Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Mr. H. Palmer: “The Pharmaceutical Industry—What it is and What it Does”.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. John D. Woods and Mr. Brian B. Matthews: “Archaeology Under the Sea”.

Monday, March 25—Wednesday, March 27

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2)—Convention on “H.F. Communication”.

Tuesday, March 26

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. D. C. Pepper: “Polymerization of Styrene by Strong Acids”.

Society of Instrument Technology, Measurement Technology Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. L. Finkelstein: “Principles of Measurement”.

Tuesday, March 26—Thursday, March 28

Institute of Metals (at Church House, Great Smith Street, London, S.W.1)—Spring Meeting.

Royal Institution of Naval Architects (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1)—Spring Meeting.

Tuesday, March 26—Friday, March 29

Effluent and Water Treatment Exhibition and Convention (at Seymour Hall, Seymour Place, London, W.1).

Wednesday, March 27

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. J. Smagorinsky: “The General Circulation” (Symons Memorial Lecture).

Institute of Fuel (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. I. Krzymuski: “Economics of Air Cooling in a Refinery”.*

Royal Institute of Chemistry, London Section (in the Lecture Theatre, Science Museum, South Kensington, London, S.W.7), at 5.30 p.m.—Discussion Meeting on “Education and Training in Chemistry”.

British Institution of Radio Engineers (Joint meeting with the Institution of Electrical Engineers, at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. R. L. Gregory: “Instruments in Experimental Psychology”.

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. D. H. Ward: “Thinking Statistically in the Food Industry”.

Wednesday, March 27—Saturday, March 30

Society of Environmental Engineers (at the Mechanical Engineering Department, Imperial College of Science and Technology, Exhibition Road, London, S.W.7)—Symposium and Exhibition—“Environmental Testing: The Key to Reliability in Industry”.

Thursday, March 28

Institution of Mechanical Engineers, Applied Mechanics and Lubrication and Wear Groups (at 1 Birdcage Walk, Westminster, London, S.W.1)—All day Symposium on “Fatigue in Rolling Contact”.

Plastics Institute (at the Wellcome Building, Euston Road, London, N.W.1), at 4 p.m.—Symposium on “Reinforcements other than Glass Fibres”.

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. A. Charlesby and Mr. P. G. Garratt: “Radiation Protection in Irradiated Dimethylsiloxane Polymers”; Mr. A. Charlesby and Mr. J. Morris: “Enhanced Ionic Polymerization of Styrene Induced by Radiation”.

Institution of Mechanical Engineers, Industrial Administration and Engineering Production Group (at the College of Science and Technology, Manchester), at 6.45 p.m.—Mr. L. A. Williams and Mr. R. J. Finlayson: “Engineering Market Research Theory and Practice”.

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at the College of Further Education, Harlow), at 7.30 p.m.—Mr. R. C. Worster: The Effect of Volute Shape on Centrifugal Pump Performance”.

Thursday, March 28—Friday, March 29

Biochemical Society (in the Department of Biochemistry, University College, Gower Street, London, W.C.1), at 4.30 p.m. on Thursday—Annual General Business Meeting; 10 a.m. on Friday—Original Communications.

Friday, March 29

Royal Society of Medicine (at 1 Wimpole Street, London, W.1), at 10 a.m.—All-day Symposium on “Chemotherapy of Cancer”.

British Mycological Society, Plant Pathology Committee (in the Large Physics Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 10.45 a.m.—Symposium on “Experimental Techniques and Methods in Plant Pathology”.

Society of Environmental Engineers (at Imperial College, London, S.W.7), at 2.30 p.m.—Annual General Meeting.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. L. S. Penrose, F.R.S.: “Limitations of Eugenics”* (The Woodhull Lecture).

Monday, April 1

Royal Institution (at 21 Albemarle Street, London, W.1), at 5.30 p.m. Mr. Norris D. McWhirter: “Facts and How to Find Them”.

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on “Passenger Handling Within Large Buildings”.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—“Tribes of the Xingu”—Films with commentary by Mr. Adrian Cowell.

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. Coates: “Modern Aspects of Phosphorus Compounds”.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer (preferably with a knowledge of isotope techniques and enzyme studies) in the Department of Biochemistry, for duties involving teaching at postgraduate level and research—The Secretary, Institute of Psychiatry (University of London), The Maudsley Hospital, Denmark Hill, London, S.E.5 (March 27).

Lecturer (with a good honours degree or equivalent qualification in mathematics or statistics and postgraduate research or industrial experience) in the Department of Mathematics—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff (March 30).

Lecturers or Assistant Lecturers (two or three posts) in the Department of Zoology—The Registrar, The University, Bristol (March 30).

Reader in Polymer Chemistry or Technology—The Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff (March 30).

Technician in the Department of Genetics, for work concerned with the culture of micro-organisms—The Registrar, The University, Liverpool 3, quoting Ref. No. CV/277/N (March 30).

Assistant Lecturer (with special interests in petrology and mineralogy) in Geology—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 5).

Biochemist in the Endocrine Unit—The Registrar, The University, Liverpool, quoting Ref. No. CV/276/N (April 6).

Lecturer in the Department of Electric Power Engineering—The Registrar, The University, Liverpool, quoting Ref. No. CV/278/N (April 6).

Junior Lecturer (preferably with a registrable medical qualification and an interest in histology) in Physiology—The Registrar, Trinity College, Dublin 2, Republic of Ireland (April 8).

Assistant Lecturer in Botany or Zoology—The Secretary to the Trustees, Magee University College, Londonderry, Northern Ireland (April 10).

Second Chair of Applied Mathematics in the Newcastle Division of the University of Durham—The Secretary to Senate, University Office, 1A Kensington Terrace, Newcastle-upon-Tyne 2 (April 10).

Demonstrator in Pharmacology—Dr. T. D. Kellaway, Secretary of the Appointments Committee, Department of Pathology, The University Tennis Court Road, Cambridge (April 11).

Lecturer in Botany at the Royal College, Nairobi, to teach angiosperm taxonomy and plant ecology to London B.Sc. (General Honours) level and help with other courses in the Department—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 12).

Lecturer (with an appropriate degree in applied science in industrial chemistry or chemical engineering) in Industrial Chemistry at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, April 12).

University Nuclear Physics Research Fellows (Ph.D. standard, with special aptitude for research in high or low energy nuclear physics)—The Registrar, The University, Liverpool, quoting Ref. No. CV/279/N (April 13).

Junior Lecturer in Botany—The Registrar, Trinity College, Dublin 2, Republic of Ireland (April 15).

Senior Lecturer or Lecturer in Mechanical Engineering at the University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, April 15).

Senior Lecturer or Lecturer in Pathology in the Faculty of Medicine at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

Senior Lecturer or Lecturer in Veterinary Preventive Medicine and Veterinary Public Health in the Faculty of Veterinary Science of the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

Chair of Theoretical Physics tenable in the Durham Division of the University—The Registrar and Secretary, Durham Colleges, 38 North Bailey, Durham (April 17).

Reader in Geophysics in the Durham Division of the University—The Registrar and Secretary, Durham Colleges, 38 North Bailey, Durham (April 17).

Second Chair of Geography tenable in the Durham Division of the University—The Registrar and Secretary, Durham Colleges, 38 North Bailey, Durham (April 17).

Experimental Officer (with a degree in physics or in textiles and preferably some postgraduate experience assisting in knitting research) in the Division of Textile Physics, Commonwealth Scientific and Industrial Research Organization, Ryde, Sydney, Australia, to assist in a study of dimensional stability and mechanical properties of cloth knitted in a variety of ways—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 465/177 (April 20).

Research Officer (with at least a university honours degree in science, or equivalent qualifications, together with some years of appropriate postgraduate research experience, and preferably experience in reaction kinetics surface or solid-state chemistry, and/or vacuum techniques) IN THE DIVISION OF COAL RESEARCH, Commonwealth Scientific and Industrial Research Organization, North Ryde, N.S.W., Australia, to undertake fundamental research on the mechanism of catalysis of the reactions of carbon with oxidizing gases—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/428 (April 20).

LECTURERS IN MECHANICAL ENGINEERING—The Secretary of University Court, The University, Glasgow (April 22).

SENIOR LECTURER (graduate in veterinary science with postgraduate experience in bacteriology) IN VETERINARY BACTERIOLOGY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, April 26).

POSTDOCTORAL RESEARCH FELLOW (organic chemist) IN CHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea (May 1).

SCIENTIFIC DIRECTOR (with a higher degree, preferably a doctorate, and considerable experience in marine zoology or a related experimental field) OF THE GREAT BARRIER REEF COMMITTEE'S RESEARCH STATION at Heron Island (University of Queensland)—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, May 3).

MICROBIOLOGIST/BIOCHEMIST (male, with a good honours degree and relevant postgraduate experience, preferably in biochemistry), to collaborate with plant pathologists in the study of host–parasite relations in fungal and bacterial diseases of fruit trees, particularly the biochemical factors associated with resistance—The Secretary, East Malling Research Station, Maidstone, Kent (May 6).

LECTURER OR ASSISTANT LECTURER IN THE DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Leeds 2 (May 25).

ASSISTANTS (honours graduates) IN THE DEPARTMENTS OF MATHEMATICS, PHYSICS AND CHEMISTRY—The Headmaster, Atlantic College, St. Donat's Castle, Llantwit Major, Glamorgan.

BACTERIOLOGIST (with a first- or second-class honours degree, and previous research experience), to carry out fundamental research in food microbiology; and a BIOCHEMIST (with a first- or second-class honours degree), for research on lipids in relation to food—The Secretary, Low Temperature Research Station, Agricultural Research Council, Downing Street, Cambridge.

CHAIR OF INORGANIC CHEMISTRY; and CHAIR OF THEORETICAL PHYSICS—The Registrar and Secretary, The University, Bristol.

CHIEF TECHNICIAN (with wide experience in either chemistry or biochemistry laboratories, the control of staff, maintenance and organization of laboratories and the purchase of equipment and materials) IN THE NEW DEPARTMENT OF BIOCHEMISTRY—The Secretary, Biochemistry Department, Imperial College of Science and Technology, London, S.W.7.

DEPARTMENTAL DEMONSTRATOR (preferably with some experience in either chemical analytical methods, emission spectroscopy, or X-ray crystallography) IN MINERALOGY IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY—The Reader in Mineralogy, Department of Geology and Mineralogy, University Museum, Oxford.

MASTER TO TEACH CHEMISTRY at all levels—The Headmaster, School House, Rugby.

PRINCIPAL LECTURER; and a SENIOR LECTURER IN THE PHYSICS DEPARTMENT—Clerk to the Governing Body, Northern Polytechnic, Holloway London, N.7.

RESEARCH ASSISTANT (B.Sc., preferably with experience of radioactive isotope tracer work), to engage in studies of calcium metabolism in the Medical Unit—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

RESEARCH ASSISTANT IN THE DEPARTMENT OF ANATOMY, for work on the electron-microscopy of cells in tissue culture—The Secretary, University College, Gower Street, London, W.C.1.

RESEARCH ASSISTANTS (honours graduates or students in their final honours year) IN CHEMISTRY, to work under the direction of Dr. W. Gerrard mainly in the field of organometallic and inorganic polymer chemistry—Dr. W. Gerrard, Head of the Department of Chemistry, Northern Polytechnic, Holloway, London, N.7.

SENIOR TECHNICIAN (with wide experience in the organization of chemistry or biochemistry laboratories) IN THE NEW DEPARTMENT OF BIOCHEMISTRY—The Secretary, Biochemistry Department, Imperial College of Science and Technology, London, S.W.7.

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EMIGRATION OF SCIENTISTS FROM GREAT BRITAIN

FOR the past ten years the Advisory Council on Scientific Policy has from time to time expressed concern over the emigration of highly qualified research scientists, particularly to the United States, and the consequent effect on Britain's scientific manpower. An inquiry in 1954–55 showed that, during the academic years 1947–52, about 2 per cent of Britain's output of postgraduate chemists, among whom the loss was then thought to be most significant, had taken employment in the United States, but there was then no evidence that an undue proportion of the best graduates had left the country. In 1956 the Advisory Council sought further information, and the subject was fully discussed in the Council's report for 1956–57 and more briefly in its report for 1957–58. Since then, a Joint Board of the Civil Service Commission and the Atomic Energy Commission has been established to interview in North America candidates for Government posts in Britain, and this Joint Board is also interviewing candidates for research fellowships in universities and colleges in Britain offered by the Department of Industrial and Scientific Research.

Although conflicting views were frequently expressed as to the extent and seriousness of the loss of scientists to Britain, in view of the continuing shortage of scientific manpower the loss could not be ignored, and a year ago a report from the Institute of Physics and the Physical Society, listing 71 prominent physicists who had emigrated to the United States, expressed grave concern as to the effect on schools of physics in Britain (see *Nature*, 193, 911; 1962). In 1962 the Council of the Royal Society appointed an *ad hoc* Committee, with Sir Gordon Sutherland as chairman, to collect information on the subject. This report, covering the ten-year period up to May 1962, has now been published*. It is limited to scientists who were normally resident in, and who obtained a Ph.D. degree in, Britain. It is commendably brief, but it does not deal with immigration into Britain, and although a 96 per cent response was obtained from the 563 professors and other heads of departments who were consulted, its data are probably representative rather than comprehensive, nor does the report touch on the reasons determining either emigration or return to Britain.

The Committee concluded that the annual rate of permanent emigration of recent Ph.D.s is now at least 140 per annum, or about 12 per cent of the total output in anatomy, bacteriology and microbiology, biochemistry and biophysics, botany, chemistry, engineering, genetics, geology and geophysics, mathematics, metallurgy, pharmacology, physics, physiology and zoology. Of these, about 60 go to the United States, more than 20 to Canada, and 35 to other Commonwealth countries. This represents a three-fold increase in the decade, and, if those going abroad temporarily are included, the annual rate of migration of recent Ph.D.s to the United States is now at least 260, or more than 22 per cent of the output in the subjects investigated. The corresponding figures for all other countries are 157 or 13·5 per cent.

The annual rate of permanent emigration of university staff has also increased three-fold in the same period and is now about 60, or 1 per cent of the total staff in Britain in the subjects covered. Of these, some 25 go to the United States and about 25 to Commonwealth countries. The total permanent emigration of Ph.D.s and university scientists to the United States is thus at least 83 per annum in the subjects covered, and a recent report from the Natural Science Foundation, *Scientific Manpower from Abroad* (NSF-62-24), estimates the immigration of scientists and engineers from the United Kingdom in 1961 as 575, compared with 291 from Germany and 842 in 1957. Of these, about 400 are engineers and the figures include B.Sc.s and others. The decline in immigrants from the United Kingdom, however, is much less than that in immigrants from Germany, and it is probable that a significant percentage of the 1,200 scientists or engineers who enter the United States from Canada each year came originally from Britain.

Perhaps the most significant feature of the survey is the marked increase in the proportion of recent Ph.D.s on university staffs permanently emigrating to the United States; about 4 per cent of those awarded Ph.D.s emigrated in 1952, but the proportion rose to 8 per cent in 1957 and has remained steady at about 7 per cent in the past four years. Moreover, in the past five years Britain has lost a number of outstanding scientists to the United States, including nine Fellows of the Royal Society, and their departure has created gaps leading to difficulties in certain important fields of research in Britain. While the Committee did not obtain figures on the permanent immigration of scientists and engineers to the United Kingdom, it considers some balance might be struck between emigration to and immigration from the Commonwealth. Permanent immigration from the United States is negligible, and there is no suggestion anywhere in the report that the gross loss in quality is in any way discounted. What is certain is that the absolute emigration figures must be larger than those given in the report, although, since 36 out of the 475 departments to which questionnaires were sent failed to reply, certain ratios and annual comparisons may be slightly in error.

Over the decade the number of Ph.D.s going permanently to the United States has varied from 24 in 1952 to 61 in 1958 and the incomplete total for 1962 was 48. There is no doubt as to the marked upward trend of this emigration, and among chemists it has increased, on the average, by a factor of between 1·5 and 2, but even more among physicists. The emigration to other Commonwealth countries, which at least doubled in the same decade, included a large number of geologists, zoologists, botanists and biochemists, whereas chemists and physicists predominate in the flow to the United States.

Information on scientists with a Ph.D. degree who went abroad, mostly temporarily with a fellowship, etc., is less complete. Of 1,053 who did so (753 to the United States) in the five years 1957–61, 545 are known to have returned (410 from the United States), but the present location of 365 is uncertain. This temporary emigration to the United States is largely in chemistry and physics. Of the permanent loss of 389 university scientists, which increased from 19 in 1952 to 55 in 1961 and in 1962 was ~~64~~—a total known to be incomplete—the report observes that the loss to the United States was greatest in physics, chemistry, mathematics and biochemistry, in that order, and to Canada in chemistry and physics. Of the research institutions, the National Physical Laboratory, the National Engineering Laboratory, the Radio Research Station, the Services Electronics Research Laboratory, the Royal Naval Scientific Service and the Medical Research Council lost some 74 members of their scientific staff; this sample should discount the argument that conditions in the universities alone are inadequate to offset the attractions of the United States. Even in the Government service the 'drain' could be quite serious, and it would be rash to dismiss the argument of the Institution of Professional Civil Servants that this is to be attributed to short-sighted and illogical Government policy on remuneration for the Scientific Civil Service as without some justification.

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* *Emigration of Scientists from the United Kingdom.* Report of a Committee Appointed by the Council of the Royal Society. Pp. 32. (London: The Royal Society, 1962.)

In its general remarks in this report, the Royal Society Committee, after examining figures from the University Grants Committee for 1960–61, expresses the opinion that Britain is probably losing nearer 19 per cent than 13 per cent of new Ph.D.s each year and that these include an unduly high proportion of the most promising scientists. Reports from the interviewing board of the Civil Service Commission and the Atomic Energy Authority expressed the view that some 70 per cent of those interviewed were worthy of appointments, compared with 50 per cent of those interviewed by the Commission in Britain. It is also noted that some 20 Fellows of the Royal Society now hold what are probably permanent appointments in the United States, of whom nine went there between 1958 and 1962, and that of the 224 university scientists who emigrated to the United States in the period 1952–62, 45 held senior professorships or are in charge of important scientific laboratories. The Committee notes in passing that this loss of scientists represents to some extent the loss of a considerable investment in education which, in a House of Lords debate on February 27, Lord Hailsham put at £20,000 or more per scientist, but it regarded as much more serious the economic consequences of the loss to Britain of the leadership and the creative contributions to science and technology which they would have made in the course of their working lives.

That, of course, is the crux of the situation and the real reason for the concern which was expressed alike last year in the report of the Institute of Physics and the Physical Society and in the debate in the House of Lords on February 27. The report, in fact, establishes the welcome fact that science is still incontrovertibly international. What is new is that the country which attracts the majority of migrating scientists is now the United States and not Germany, and consequently, so far as Britain is concerned, there is no longer in language alone one important factor favouring ultimate return to the country of origin. That the United States should have displaced Germany in this way is, nevertheless, not to be regretted in itself, and, if in due course the United States were succeeded by France or the U.S.S.R. or some other country as the lodestone, that should be to the advantage rather than the disadvantage of science as a whole. There is, in fact, very little that in short term a Government can do to affect the attractiveness of another country for its own scientists. In the long term, it can only aim at improving the excellence of the facilities available for its own scientists and to hope that in due course its own institutions may become something of a Mecca of science in their turn.

Ideally, of course, the institutions which provide the Meccas of scientists should vary from discipline to discipline in different countries, not simply because that situation might well provide a rough balance between emigration and immigration, but even more through the greater opportunities of cross-fertilization of ideas that could result. The growth of international institutions in science if wisely fostered could well contribute considerably to that end. Even as it is, the extent of the absolute loss is by no means easy to compute, and it is possible to write off much too hastily as a complete loss the £20,000 or so invested in the education of a scientist who permanently emigrates. Nor can one stress the vital importance of Britain's contribution to the development of the emerging territories and press for a larger contribution in education and research in those countries and at the same time claim that those who go overseas to serve those needs are no longer of any value to Britain. In any event, it is scarcely sound to argue that because a scientist has been educated at public expense he should remain *ipso facto* in the country responsible for it, particularly if there is any substance in the contention that the country is failing to provide appropriate opportunities or conditions of service commensurate with his ability.

Besides preserving some sense of proportion, it is obviously important that the Government should be clear as to the reasons which determine such emigration and be prepared to take whatever measures may, in the long run, help to restore a balance in numbers between emigration and immigration if, in fact, the loss is such as to threaten the staffing and efficiency of Britain's own institutions. It is one thing to say that it is immaterial where research is conducted since the results are available for all: it is another thing if difficulty is found in recruiting staff of the quality which the research institutions need or who are capable of attracting and inspiring students who, in turn, themselves will become leaders of research. It may, indeed, be a compliment to Britain that so many of her scientists should be sought by the United States, and Britain can be rightly proud of her record of Nobel prizewinners. That, however, should not induce complacency as to the maintenance of the conditions which, in the past, have fostered such excellence, or weaken the determination to do all that is possible to ensure that Britain produces an increasing number of such men and women whether they remain in Britain or not.

It should be noted that the Council of the Royal Society regards the report as purely fact-finding and does not propose to make any further comment on it at present. The Committee on Scientific Manpower is still considering a sample report from the 1961 census which indicated that there were some 40–50 per cent more scientists and technologists in Britain than had hitherto been anticipated. However, there is still no reason to believe that Britain is turning out enough scientists for her own needs; the Royal Society report clearly suggests that Britain is not providing sufficiently for the best. As to whether that is due to the absence of sufficient facilities is a matter of some dispute. Lord Todd, in his important speech in the House of Lords debate, was inclined to agree that this might be one factor, though he laid nothing like the stress on this factor that Lord Chorley did afterwards. Lord Chorley argued that the conditions of work, including equipment, was one of the decisive reasons determining emigration. Lord Chorley, incidentally, was inclined to argue that the other main reason was financial.

Prof. W. Mansfield Cooper has long since expressed strong disagreement with the view that either higher salaries or better facilities are dominant factors, but has been much more seriously concerned at the possible loss of outstanding young scientists because they find insufficient opportunities and prospects in Britain. This is a view freely reflected in individual comments on the Royal Society's report. It is widely believed that there is a lack of opportunities in Britain, and particularly that there are insufficient posts for creative work. It is also suggested that there is little enthusiasm for new ventures, a charge which is brought against industry as much as or more than against Government establishments.

Lord Todd wisely remarked in the debate that Britain could not hope to check emigration or restore a better balance between emigration and immigration simply by increasing her own postdoctoral fellowships. So long as the American economy kept expanding, the flood of American postdoctoral fellowships was likely to continue at its present level and Britain would suffer loss by emigration. He was disheartened, not because young scientists went abroad to widen their experience, but rather because so many sought it in the United States rather than in Europe, and he thought it would be worth a substantial effort to encourage more to go to Europe. He also directed attention to the element of chance which, as in his own career, might determine whether or not a scientist remained abroad, and pointed out that part of Britain's response in this situation must, in the first instance, be to seek to expand her economy and raise her national income. It was not simply a matter of providing more scope in the universities; a quickening of the pace and a change in the scope of research in Britain's industry might be even more important.

There is a further factor that deserves attention which was noted by Dr. I. W. Wark in an address last October on "Scientific Research as a Career" (see *Nature*, 197, 737; 1963). Dr. Wark, in discussing Australia's own problem of emigrating scientists, referred to the danger that the emigrating scientist might so make his changes even abroad as to acquire neither the variety of experience nor responsibility that would really fit him to exercise high responsibility. Dr. Wark expressed certain definite reservations with regard to overseas experience and study at the start of a research career which deserve serious attention, though it is difficult to see exactly what action could be taken on them officially. They are directed primarily at the Research Fellow himself, or at the departmental heads under which the research fellowship may be held.

One other point which was raised in the statement issued last year by the Institute of Physics and the Physical Society should also be considered. This is the frustration which is often experienced and the excessive time consumed in endeavouring to obtain grants for research projects. The Earl of Halsbury referred specifically to this in the House of Lords, instancing a recent observation by Prof. A. B. Pippard on the deteriorating morale in laboratories which resulted from delays, perhaps as much as six months, in obtaining grants for new apparatus needed to follow up a discovery. The Earl of Halsbury strongly supported Prof. Pippard's plea for some sort of discretionary fund placed at the disposal of the universities, but subject to subsequent audit and inspection, which would enable them to draw funds rapidly to permit swift response to new ideas. The statement of the Institute of Physics and the Physical Society advocated something similar, but was gravely concerned as to the ability of British universities at present to obtain adequate financial resources for new equipment and sufficient posts of responsibility. However, it recognized that British universities could offer a degree of freedom and a sympathetic atmosphere to new ideas comparable with those of the best Government and industrial laboratories in Britain, or even with American laboratories.

That, by implication, means a further look at the whole question of the administration of research, and the debate did, in fact, elicit a plea for a speedy report from the Trend Committee. Nor was this the only point at which the debate put the question of the emigration of scientists into its proper perspective. The Earl of Halsbury said frankly that it was most important not to do the right thing for the wrong reason. The real reason for making opportunities for young men was that it was the right thing to do. Britain should be a land of opportunity for the young, and the spirit of adventure should not be quenched, for such would only induce emigration; this view was strongly endorsed by Lord Hailsham in his reply on the debate. Britain should do whatever was necessary to improve science all the way through; she should improve her universities in size and pattern throughout, and thus make the whole quality of life more attractive.

Sir Gordon Sutherland and his colleagues have not written the last word on the emigration of scientists; rather they are likely to have stimulated an even closer debate on the reasons for emigration and the measures that should be taken, not to repress emigration but to ensure that Britain produces and retains a sufficient number of scientists of the highest ability to meet her own needs and those of the Commonwealth countries, including especially the emerging nations. It shows that science is still a truly international activity and that migration is to be welcomed not feared. What is to be feared is the short-sightedness, the parsimony and rigidity which fail to provide the conditions in which a creative activity can flourish and instead lead to frustration and waste of high ability. The importance of the inquiries now being conducted by the Trend Committee, the Slater Committee on research into natural resources and of that since initiated into the supply and use of technicians is again attested. Equally relevant are the findings of the Zuckerman Committee on the management and control of expenditure on research and development, and, it is hoped, those which the Robbins Committee is expected soon to make available.

In the face of all this material calling for close study and urgent imperative action, it would be reassuring to find the Minister for Science being equipped with larger and more commensurate staff for the purpose than loaded with fresh and essentially irrelevant responsibilities. When a responsible committee of physicists can express the considered opinion that, for the first time in Britain's scientific history, conditions in British universities are failing to attract physicists of the front rank the time for complacency—or flippancy—is past. Britain can no longer continue to rely on the elasticity of the arrangements in the past which have often proved so outstandingly successful, as, for example, in the creation of the Unit of Molecular Biology of the Medical Research Council with its recent Nobel prizewinners. If it is wise to await the result of the inquiries already in hand before modifying the present system, the facts revealed by this inquiry into the emigration of scientists attest the urgency of the situation and seem to point to one or two temporary expedients. What is essential is that the Minister for Science should make sure that, when the results of the present inquiries are at last available, decision and action should not be further delayed because it is found that further facts are required. Creative thinking should clear the way for swift action, including that of educating the public as to what is involved, in which the United States has in the past year or two made such striking and even revolutionary advances.

Moreover, the United States gives generous financial support to scientists and scientific institutions which not only stimulates, but sometimes also initiates, scientific advances and discovery not only in Great Britain but also in many other countries, both advanced and emerging. This is evidenced in practically every issue of *Nature*.

In other words, while recognizing that the recent Royal Society report should stimulate Britain to put her own scientific house in order (with an essential change of heart at the Treasury), it must be acknowledged that the United States is not only taking, in the form of scientific personnel, but also is generously giving, through her Foundations, Government departments, etc., and thus encouraging and supporting the advancement of science far beyond her own frontiers. This surely reveals the right attitude towards science as an international discipline which ignores national boundaries and differences in race, colour or creed.

MACHINES FOR HIGH-ENERGY NUCLEAR PHYSICS

Particle Accelerators

By Prof. M. Stanley Livingston and Dr. John P. Blewett. (International Series in Pure and Applied Physics.) Pp. xvi+666. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1962.) 135s. 6d.

THIS book is a welcome addition to the literature of particle accelerators. There are many excellent review articles on the subject varying from semi-popular expositions to highly technical treatises. Until recently, however, comprehensive books on particle accelerators have been almost non-existent. Yet the audience for such a book is wide, including as it does the accelerator physicist, the engineer, the nuclear physicist and to some extent the non-specialist.

In this respect the authors of *Particle Accelerators* have succeeded in producing a reasonable balance of all those aspects. Above all, they manage to convey something of the excitement and wonder which still adheres to accelerator building. To quote from their introduction: "The thrill of discovery is the reward of exploration", and again, "The pleasure and excitement of the working crew when a new accelerator is first tuned in to set a new voltage record is something to be remembered for a life-time". Comments like those and anecdotes, now part of the historical development of accelerators, give the book a warmth rarely found in treatises on technology.

Production of high-energy beams of charged particles in the laboratory began in the early 1930's with the Cockcroft and Walton d.-c. generator, the Lawrence cyclotron and the Van de Graaff electrostatic generator. Their impact on nuclear physics was tremendous and by 1940 these machines were commonplace in university laboratories. After the Second World War, the discovery of the principle of phase stability, a better understanding of dynamical principles, coupled with war-time advances in microwaves and electrical engineering, initiated a new family of large accelerators. These were the frequency modulated cyclotrons, the electron and proton synchrotrons and the betatron. There is no need to elaborate the significant contributions these accelerators have made in elementary particle physics during the past fifteen years. The largest of those accelerators are the *Bevatron* at Berkeley, and the 10-GeV machine at Dubna, near Moscow. Later this year similar machines will come into operation at the Rutherford Laboratory in Britain and at the Argonne Laboratory in the United States. This type of accelerator is now referred to as a constant gradient machine.

The success of these machines stimulated designers to seek new methods for increasing both the energy and intensity of existing accelerators. This search was rewarded by the discovery in 1953 of the principle of alternating focusing by Courant, Livingston and Snyder, and at an earlier date by Christophilos. Within the past few years three proton synchrotrons of this design have come into operation—a European machine at CERN, Geneva, of 25 GeV, an American machine at Brookhaven of 28 GeV and a Russian machine in Moscow of 7 GeV. The Russians are also building a 70-GeV machine which should come into operation in 1964. In addition, an electron machine of 5 GeV, directed by Livingston, has come into operation within the past few months. A similar machine should come into operation in Hamburg later this year.

This brief account of accelerator installations is only indicative of present-day activity in this field and does not include the very large number of smaller machines. Even so, the search for new techniques still goes on. This has led to a new family, still in the design stage, now referred to as fixed-field alternating gradient accelerators. Design studies in extending the range of the present alternating gradient machines also suggest that these could be extended to 1,000 GeV with a diameter of several miles. The pressure to build such a machine is likely to grow rapidly in the near future.

This 'story' of accelerator development is very well told in the present book. Both authors have participated in the advances of this field and have an intimate knowledge of many of these machines. Livingston, for example, was associated with Lawrence in the early 1930's in cyclotron development. After the War he helped to design the *Cosmotron* and the *AGS* at Brookhaven, and recently has directed the electron synchrotron project at Cambridge, United States. Blewett has also been associated with the construction of both the *Cosmotron* and the *AGS*. In particular, he has been responsible for the proton linear accelerator injector and for directing recent design investigations in a large international accelerator project.

In their book, separate chapters are devoted to each type of accelerator mentioned here. There are also special chapters on ion sources, particle dynamics, the principle of phase stability, magnet design and shielding. A strong sense of historical development accompanies the description of each machine, which makes enjoyable and stimulating reading. The amount of detailed information recorded by the authors is quite remarkable. In a subject which has always demanded a happy alliance between physicist and engineer the authors have succeeded in giving due weight to each. To the specialist in one or other of these fields the style may at times be too racy, and certain aspects may be treated rather superficially for the book to be a satisfactory formal text-book, but what it lacks in this respect it gains in information and enthusiasm. It is a book that everyone associated with accelerators, as designers, as builders or as users, will profit from and enjoy reading.

W. Walkinshaw

COAGULATION OF THE BLOOD

Human Blood Coagulation and Its Disorders

By Dr. Rosemary Biggs and Dr. R. G. Macfarlane. Third edition. Pp. xxiv+474. (Oxford: Blackwell Scientific Publications, 1962.) 52*s*. 6*d*. net.

THE five years since the last edition of this book have brought a greater clarity to the field of blood coagulation: it has not been necessary to postulate the existence of any new clotting factors, but much light has been shed on the inter-relationships of those factors the existence of which in normal blood is now firmly established. Important recent work has been concerned with the activation of the coagulation mechanism by contact of blood with glass and other foreign surfaces, the changes which platelets undergo during clotting, the relationship of thrombosis to coagulation and the fibrinolytic system. All these developments are lucidly and critically discussed in the new and extensively revised edition of this excellent book.

The form of the book remains unaltered: the first part is devoted to the theoretical aspects of human blood coagulation and the second to the diagnosis and treatment of coagulation defects. There is an excellent new introductory chapter to the second part which will enhance the value of the book to the practising physician and clinical pathologist, and the technical appendix has been amplified. The most important practical advance in the treatment of blood coagulation disorders in recent years has been the authors' own work on the preparation and clinical use of concentrates of antihæmophilic globulin and Christmas factor, and the use of these is fully described.

It is a pleasure to record that the standard of production is vastly improved. In spite of the inclusion of much new material, the book has been kept to the same size, partly by the use of slightly smaller print and by the omission of the summaries at the end of each chapter, which served no very useful purpose in the previous editions. The figures are now excellent and the bibliography is greatly improved by being collected in a single alphabetical series; only the index leaves room for considerable improvement in subsequent editions. This book remains unchallenged in its field; it is not only indispensable to all workers on blood coagulation, but also so well balanced and readable as to be highly recommended to non-specialists.

R. M. Hardisty

TISSUE TRANSPLANTATION

Ciba Foundation Symposium on Transplantation

Edited by G. E. W. Wolstenholme and Margaret P. Cameron. Pp. xii+426. (London: J. and A. Churchill, Ltd., 1962.) 56*s*.

THIS volume is dedicated to the memory of the late Peter A. Gorer, whose brilliant work on the serology and serological genetics of homograft reactions is briefly surveyed in the Chairman's opening remarks. In closing these remarks Prof. P. B. Medawar states "... let us not forget that we are still quite ignorant of the proximate cause of the death of any homograft" and "... we know of no harmless and lastingly effective way to subdue the homograft reaction in adult animals".

The sixteen papers presented at this symposium contain little that offers immediate hope of negating these statements although obvious progress is being made in the understanding of some of the immunological aspects of tissue transplantation. The opening papers deal with the considerable recent progress made in the difficult problem of identification and characterization of tissue antigens. Further practical progress is reported in the attempts to ameliorate runt disease in mice. Notable contributions to the theoretical advance are the papers presented on the role of the thymus, the quantitative nature of immune tolerance and an investigation into the clonal selection theory.

In view of the increasingly successful efforts being made in the field of human organ transplantation, it is disappointing to find that, with one exception, the communications are confined to studies on small rodents. Nevertheless, the symposium is of obvious value to any worker in the field of tissue transplantation, as a convenient summary of recent progress in the non-clinical aspects of this subject.

W. J. Dempster

NATURAL AND SYNTHETIC COLOURING MATTERS

Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields

Edited by T. S. Gore, B. S. Joshi, S. V. Sunthankar and B. D. Tilak. Pp. xxvii+659. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 171*s*. 6*d*.

THE book contains a collection of papers and reviews most of which deal with recent progress in the chemistry of natural and synthetic colouring matters, but a few are concerned with the wider field of plant chemistry and general organic chemistry. The authors in many cases are of world-wide reputation, both in the field of theoretical and experimental organic chemistry. Some of the papers follow the pattern of those found in the journals of the various chemical societies, with the exception that all the authors start with a reasonable introduction to their subject-matter, a thing which is rarely found in some present-day journals, thus making it easier for the reader to follow and understand the work under discussion. Some of the papers even go so far as to include an experimental section along with analytical results. In the reviews, a particular field is surveyed over a number of years and in the majority of cases right up to 1960. In many instances suggestions are made for further investigations which still require to be carried out in the reviewed field.

About one-third of the book is devoted to the chemistry of oxygen heterocyclic and related natural occurring compounds, while a quarter deals with various interesting synthetic dyes and intermediates, the preparation and properties of which, in a number of cases, are treated from a physical chemical point of view. I am pleased to note that the editors have not allowed the reviews concerned with the chemistry of dyes to become pages of recipes. Other parts of the book are concerned with some topics found in the following fields; the azulenes, diterpenes, alkaloids and carcinogenic hydrocarbons. The book succeeds in presenting an up-to-date and complete account of the included topics in the light of modern theory, the subject-matter being presented in a clear and concise fashion. In a book of this type it is quite easy for the editors to omit a topic which some readers might believe should have been included, but I think that they have made a very good selection.

Though most of the book is concerned with up-to-date work, the occasional review describes some older significant and outstanding work in a particular field, especially among some of the naturally occurring pigments, an example being the work of Hooker on the chemistry of lapachol. A few pages are even devoted to Hooker himself, an extraordinary man and a true master of experimental investigation.

It follows, therefore, that the potential market for this book will be limited, but for specialists in the fields mentioned, and for libraries it will provide a valuable summary of information and a worthy addition to the literature of organic chemistry. It will also be of interest to some students specializing in organic chemistry, as well as to chemists and technologists concerned particularly with synthetic dyes. The overall production is excellent, and the diagrams and formulæ are clear and easy to follow. This book was compiled to commemorate the contribution to organic chemistry of Prof. K. Venkataraman on the occasion of his sixtieth birthday.

R. LIVINGSTONE

HISTORY OF THE INTELLIGENCE TEST

Studies in Individual Differences

The Search for Intelligence. Edited by James J. Jenkins and Donald G. Patterson. (The Century Psychology Series). Pp. xiv+774. (London: Methuen and Co. Ltd., 1961.) 63*s*.

THIS volume brings together for the first time sixty-six previously published papers, covering the historical development of intelligence testing. The authors range from Sir Francis Galton (1869) to Dr. Guilford (1959). In terms of its professed aims—that of helping students—the collection must be judged a success; nowhere else can so many formative contributions be read with such ease.

Nevertheless, it is a sobering moment when a research field commemorates its ancestors in this way, and one which invites more general comment. First impressions are undoubtedly favourable. Almost all these articles reflect a high level of technical competence; and some of them, Spearman's for example, are works of virtuosity. Second impressions are less comforting. We are reminded of how long ago our present methods of testing were established, and how little we have added during the past forty years to our knowledge of their practical value. By the end of the First World War, intelligence tests were already established as valid instruments of general classification. Fryer (1922) shows, for example, that there is relatively little overlap in intelligence quotient between "dentists" and "labourers", between "clergymen" and "sheet metal workers". Since 1922, the mental testing movement has expanded out of all recognition, and there has been a luxuriant growth of theories about the structure of the intellect, but our tests and our knowledge of their predictive efficiency are in most essentials the same as they were when Fryer's article was written. By normal technological standards then, this is an area of conservatism, even of stagnation.

Another disconcerting impression is one of dullness. Thinking is presumably the human being's most interesting accomplishment, so it must be of some significance that, in our efforts to measure it, we have generated a literature of such tedium. Throughout its considerable length, this volume contains barely a handful of references to individual people tested—although Terman's work is an important exception in this respect—and even fewer instances of their thinking in real life situations. Indeed, in some of the more recent articles, scarcely a shred of human subject-matter remains. In view of the statistical sophistication that mental testers nowadays take for granted, this suggests not merely a neglect of natural observation but an actual flight from it. Paradoxically, it seems, the study of human thinking has become a haven for the kind of scientist who finds it natural to have as little personal contact with the people he tests as possible. The dangers of such a situation are obvious. Individuals, particularly those who are exceptions to a given rule, are invaluable to psychologists as a source of criticism. Their loss of interest in individual people may thus account not only for the dullness of the intelligence tester's work but also its conservatism.

Surprisingly early on, intelligence testers seemed to lose the inventiveness which characterized early pioneers like Binet. Covertly, they seemed to assume that all the essentials of intelligence could be measured with the tests to hand. Yet the briefest glance at the intelligence quotients of any group of successful brain-workers—research physicists for example—would have shown them that this was false. Many brilliantly successful men and women have relatively unimpressive intelligence quotients; many who succeed brilliantly at intelligence tests are relatively unimpressive in every other respect. It follows, at the higher levels at least, that the measurement of intelligence is an area crying out for innovation.

LIAM HUDSON

CIRCUMPOLAR PLANTS

The Circumpolar Plants

1. Vascular Cryptograms, Conifers, Monocotyledons. By Eric Hultén (Kungl. Svenska Vetenskapsakademiens Handlingar, Bd. 8, No. 5). Pp. 275. (Stockholm: Almqvist and Wiksell, 1962). 65 kronor.

PROF. HULTÉN is well known to everyone interested in phytogeography for the excellent distribution maps he has published of the plants of north-west Europe and, more recently, for his work on amphi-atlantic plants. In all these maps a great deal of information is conveyed with remarkable clarity by means of a combination of shading, individual symbols and lines.

The present volume is in a sense a continuation of the one dealing with the amphi-atlantic plants and follows the same general plan; in fact, as Prof. Hultén himself observes, no clear distinction can be drawn between the amphi-atlantic and circumpolar types of distribution.

The species included range from those, such as *Colpodium vahlianum*, which are nearly confined to the arctic, to others, for example *Adiantum capillus-veneris*, which reach their northern limit in England. They all have wide, though often strongly disjunct, distributions in the northern hemisphere.

In general, a broad view is taken of what constitutes a species and not infrequently the areas of groups of closely similar species are shown on one map. This practice has the advantage of bringing out certain features of circumpolar distribution, some of the most striking of which are discussed in the introduction. The discontinuities, it is suggested, are partly due to climatic preferences and partly to historical factors, such as the former existence of a large ice-dammed fresh-water lake in the Obi Basin. The not infrequent occurrence of a fairly uniform series of populations in the north paralleled by a ring of distinct subspecies or closely related species on mountains much further south is very plausibly explained in terms of climatic changes during the Quaternary period.

It is evident that over large areas information about the flora is inadequate so that both distributional data and taxonomy are unsatisfactory. This is a limitation which has to be accepted for the present, and it is to be expected that these maps will provide a stimulus for further work.

There are inevitably errors and omissions in a work of this size, even in regions the flora of which is as well known as that of the British Isles. Perhaps the most striking of these is in the map of *Potamogeton natans*, where only four localities, all in the extreme north, are shown though the plant is, in fact, recorded from every part of the country. Two other less obvious but perhaps more serious errors are the inclusion of records for *Eriophorum alpinum* and *Carex bicolor* from Scotland, neither of which is now accepted as correct.

These are, however, minor blemishes in a valuable and well-produced work, which would be more convenient to use if the pages had been cut by machine.

T. G. TUTIN

IONOSPHERIC CONSEQUENCES OF THE EARTH'S ORBITAL ECCENTRICITY

By Sir EDWARD APPLETON, G.B.E., K.C.B., F.R.S.
Principal and Vice-Chancellor, University of Edinburgh

THE Earth moves in an elliptical orbit and is therefore not always equidistant from the Sun. The Earth–Sun distance is, in fact, 3·4 per cent greater in early July than it is in early January, at which latter period the Earth makes its closest annual approach to the Sun. The result of this orbital eccentricity is that solar electromagnetic radiations, as received on the Earth, are 6·5 per cent weaker in early July than in early January. The effect of this annual variation of solar radiation intensity is, of course, small, and cannot be detected in measurements of, say, atmospheric temperature because of the influence of large-scale air movements. A recent attempt to detect the effect on the ionosphere has, however, been successful.

The stratum of the ionosphere selected for examination was the E Layer, because of the known regularity of its behaviour. This is an ionospheric layer in which the rates of electron production and electron loss are sensibly equal, so that the electron density of the layer is in a quasi-stationary condition[1]. This is especially the case at noon, and that is why noon measurements of peak electron density alone were used in the search for an annual effect.

The raw material used in the investigation has been the corpus of regular ionospheric measurements made, over recent years, by the world's ionospheric stations. More specifically it consists of measurements of the hourly critical penetration frequency of the E Layer, fE, as disclosed by the method of vertical radio sounding; since, from this quantity, it is possible to calculate the peak electron density of the E Layer, $N_m(E)$. In order to discover whether the annual variation of the intensity of the incoming solar ionizing radiation was recognizable in the measurements of $N_m(E)$ it was necessary to ensure that such measurements referred to a constant value of the Sun's zenith distance, χ, throughout all the months of the year. This meant that noon measurements of peak electron density made at a fixed site were unsuitable, because of the change of the value of χ at that site with the season. Instead, it was necessary to use measurements from a large number of stations all over the world and plot curves showing the dependence of $N_m(E)$ on χ, for noon conditions during each month. From such curves there could be read off the values of $N_m(E)$ for any selected value of χ for any particular month.

Another feature of the treatment of the data consisted in removing the influence of the variation of sunspot activity from month to month during the course of the year. In a subsidiary investigation it has been found that $N_m(E)$ increases substantially proportionally to increase of R, the Zurich sunspot number. This result prompted the suggestion of using the average value of $N_m(E)$ for the eleven years of a sunspot cycle as the representative value for a given hour of the day in a given month. Two advantages accrue from such an operation. In the first place the effect of the variation of R from one month to the next has been sensibly eliminated; while, secondly, there has been a statistical advantage in using more data in the inquiry. The sunspot-cycle period selected for this averaging process consisted of the years 1949–59 inclusive, and so included the period of the International Geophysical Year, 1957–58. In addition, it was found that, for the year 1954 exceptionally, the value of R was extremely small and roughly constant over the months of the year. It was therefore possible to make an additional search for the annual effect using the results of this one year only.

By using the averaged results for the period 1949–59, or the results for the year 1954 alone, it was possible, then, to find the value of $N_m(E)$ for each month of the year, under both constant-χ and constant-R conditions. In this way one might hope to detect an annual variation of $N_m(E)$ resulting from the annual variation of the ionizing flux of radiation, now thought to be solar X-rays, which is responsible for the E Layer formation.

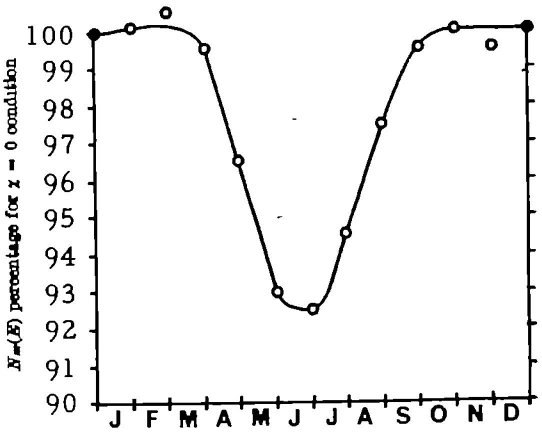

Fig. 1 Relation between the peak electron density of the E Layer, $N_m(E)$, and the month of the year, for vertical-Sun conditions

In Fig. 1 are plotted the various values of $N_m(E)$ for the sunspot cycle 1949–59 under conditions of constant χ and constant R. The values are expressed as percentages of the average value for December and January, since the Earth approaches closest to the Sun on January 3. Evidence of the expected annual effect is at once recognizable, the value of $N_m(E)$, under conditions of both constant χ and R, being less during June–July than during December–January. As is stated in the legend to Fig. 1, the values of $N_m(E)$ for each month relate to conditions of vertical Sun, that is, $\chi = 0$. This choice was made to eliminate any possible effect of a purely seasonal character. The average value of the Zurich sunspot number, throughout the months of the year, for the period 1949–59 was roughly 90, a figure about half that representative of sunspot maximum conditions.

Since the curve depicted in Fig. 1 portrays an annual variation of the type we should expect, due to the Earth's orbital eccentricity, we are encouraged to compare the magnitude of the effect found in the E Layer with that to be expected on theoretical grounds. As has already been stated, we know that the intensity of the solar ionizing rays must be 6·5 per cent less during June–July than during December–January. Undoubtedly, therefore, the rate of electron production in the E Layer will follow an annual variation of the same magnitude. But the value of the resulting ionospheric electron density depends on the rate of electron loss as well as on the rate of electron production. If the rate of electron loss is describable by an attachment law, for example, we should expect $N_m(E)$ to vary directly with the intensity of the solar radiation flux. If, on the other hand, electrons disappear according to a recombination law, we should expect $N_m(E)$ to vary as the square root of the intensity of the solar flux. In the former case we should expect an annual effect, in $N_m(E)$, of 6·5 per cent; in the latter case of 3·25 per cent. Now it happens that the experimental results shown in Fig. 1 indicate an annual variation of 7·5 per cent, which might be taken as indicating the operation of an attachment law. Other investigations, however, suggest that the law describing the rate of electron loss in the E Layer is one intermediate between that of attachment and recombination, in which case the experimentally observed effect is larger than that wholly attributable to the Earth's orbital eccentricity.

The general result of the investigation reported here must therefore be that the effect of the annual variation of the Earth–Sun distance has been shown to be recognizable in ionospheric measurements; but the existence of a smaller additional annual variation, due to some unknown cause, cannot be entirely excluded.

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[1] Appleton, E. V., and Lyon, A. J., *Physics of the Ionosphere*, 20 (Physical Society, London, 1955).

TOWARDS A SOUTHERN COMMONWEALTH OBSERVATORY

By Dr. S. C. B. GASCOIGNE
Mount Stromlo Observatory, Canberra, Australia

THE arguments for setting up a major optical telescope in Australia, possibly under joint United Kingdom–Australian control, have received much recent publicity and are well known[1]. Briefly, they are that from Australian latitudes one can see the third of the sky inaccessible to northern observatories, this third containing such strategic objects as the Magellanic Clouds and the galactic centre; and that if progress is to be made with the tremendous problems created by the radio astronomers it is essential that the Commonwealth Scientific and Industrial Research Organization radio installation at Parkes, New South Wales[2], and the large Mills cross being built at Hoskinstown[3], be matched by a reasonably equivalent optical facility—the 210-ft. dish has already far outstripped the capabilities of the 74-in. telescope at Mount Stromlo. These arguments are strong, much more so than may generally be realized; a telescope of the size proposed, 120 or 150 in., could make a decisive impact on astronomy, one out of all proportion to its size and cost. My purpose is to show why this is so.

Two problems at the heart of present-day astronomy, and two to which a considerable part of the time of this telescope would be devoted, are those of evolution and magnetic fields. Evolution has been a preoccupation with astronomers for the past hundred years, but real progress has been possible only since the recognition, explicitly by Bethe in 1938, of the thermonuclear conversion of hydrogen to helium as the primary source of stellar energy. Since then the subject has developed rapidly; now we can estimate ages for galaxies, clusters and for many individual stars; we have a reasonable idea of how stars are formed, evolve and decay; we can suggest processes by which heavier elements are built up from hydrogen, and see how stellar and interstellar matter may be steadily enriched by the creation of these heavier elements. Equally, the subject abounds in unsolved problems: What are the dynamics of star formation? How do stars evolve in their later stages? What is the place of variable stars in the evolutionary scheme? How do we explain the abnormal chemical abundances which continually seem to be cropping up? And so on.

Magnetic fields in galaxies were unknown fifteen years ago. Their existence was first suggested by consideration of cosmic-ray energies, then by observations of interstellar polarization, but the recognition of their true importance had to wait for Shklovsky's work in 1953 on synchrotron radiation—the emission of electromagnetic radiation by high-energy particles spiralling in magnetic fields. The synchrotron process not only explains, in a unified way, the broad features of interstellar polarization, galactic coronal emission and cosmic rays, but also provides the major clue to our understanding of radio galaxies like Cygnus A, and of spiral structure, a spiral arm being perhaps nothing more than a gigantic tube of magnetic force. It is now clear that magnetic fields are intimately concerned with the structure and dynamics of galaxies—the magnetic and kinetic energies of our galaxy, for example, are roughly equivalent—and we begin to see how, given the mass, angular momentum and magnetic energy of a galaxy, we may be able to trace its evolutionary history and so see how galaxies have become differentiated as regards both structure and stellar content. To achieve this understanding is an ultimate goal of astronomy; and there seems no place where it can better be attained than in the Magellanic Clouds. It is here that we hope to gain the overall view, the correlation of evolutionary processes with morphological and dynamic features, as well as the detailed insight into the outstanding evolutionary problems themselves. What some of these problems are, and where progress is held up for want of more telescope power, I will now describe.

The Magellanic Clouds are near—ten times nearer than the Andromeda nebula—and rich in neutral hydrogen, emission nebulæ, super-giants, peculiar stars, variables of all kinds, young clusters and old clusters, all these objects—an important point—at much the same distance from the Sun. It is because of this that the Clouds provide such an unparalleled opportunity for investigating evolutionary processes. From the young clusters we can find the present rate of star formation, and learn something of the factors such as density, turbulence and dust content of the pre-stellar medium on which the actual process of star formation is believed to depend. One need here is for radial velocities of individual cluster stars. From the old clusters we can, in principle, determine both an age and an approximate chemical composition, and so build up a picture of how element formation has proceeded in the Clouds. We may be able to go further, and answer such questions as: Are the Clouds younger than the galaxy? How long have they been associated with the galaxy? Progress here depends rather critically on our ability to measure magnitudes and colours for stars of 20th magnitude or fainter; at present we cannot reach to much below 19, and it is difficult to see how this figure can be improved.

Better abundance determinations can be made from analyses of stellar spectra, taken at high enough dispersion (~ 15 A/mm); but with existing spectrographs (which are as efficient as any) such spectra can be obtained only for very luminous stars in the Clouds, and these, because of their super-giant characteristics and resulting instability, are unsuitable for abundance determination. Variable stars have always played a central part in astronomy, as indicators of distance, and, more recently, of the age and evolutionary status of the populations in which they occur. The Magellanic Clouds contain thousands of variables; again, some of the most critical, the short-period cluster variables, are just above the limit of detection of a 74-in. telescope, and can be investigated only slowly and with difficulty. Finally, an optical, as distinct from a 21-cm kinematic, picture of the Clouds can be built up from spectroscopically determined radial velocities of Cloud stars, and this is essential to any understanding of the large-scale structure. In the galaxy we have radial velocities for about 12,000 stars; in the Clouds, for 200, each the product of several hours' work on a 74-in reflector. Here the need is not so much to go fainter as for a more rapid accumulation of data.

Important though the Magellanic Clouds may be, the basis on which all stellar astronomy rests is detailed studies of the much nearer objects in the galaxy, and most of the work of the northern observatories is directed towards this end. The southern third of the galaxy has, of course, its share of objects of special interest, either as prototypes or prime examples of a particular class, or because they exhibit peculiar or significant properties (in astronomy the peculiar is almost always significant). These objects include some unique gaseous nebulæ, especially the Gum and Eta Carinæ nebulæ; three of the brightest cepheids in the sky, all with unusual properties; hundreds of open clusters; the majority of the globular clusters; and innumerable peculiar stars, single or multiple. One of these, an extraordinary object, appears to consist almost entirely of hydrogen and rare earths, with a small admixture of inert gases and calcium. I mention it first because it is unique, and presents astrophysicists and nuclear physicists with one of their most formidable problems in this field; secondly, because of the limitations of existing telescopes we cannot observe it at high enough dispersion to enable us to determine the atmospheric parameters which are essential to any theoretical discussion.

There are, of course, problems peculiar to the southern part of the galaxy as such. We want to delineate the major structural features, especially the spiral arms; to map the galactic field; to explore the kinematics of the galaxy to greater distances and in greater detail; and to determine the distance and something of the nature of the galactic centre. Here, as in the preceding section, existing equipment is more adequate, and much progress has been made. But the kinematic problem—in this context the measurement of the radial velocities of remote galactic objects—will always require the greatest telescope power available, and the results are essential to the proper interpretation of the 21-cm observations being made at Parkes. Progress on the galactic centre, which in Australian latitudes passes through the zenith, is also much to be desired, especially since the radio astronomers have shown this to be a region of most unexpected complexity. Even with a major telescope, however, there will be difficulty in penetrating the dust clouds which lie between us and the galactic centre.

Passing now beyond the local system, the most challenging problem, and indeed one of the most exciting in modern physics, is that presented by the radio galaxies. A typical source, Cygnus *A*, radiates 10^6 times as much radio power as a normal galaxy, though at visible wavelengths the powers are comparable; it is about 300,000 light-years across—twice the distance from the Galaxy to the Magellanic Clouds—and contains, in the magnetic field and associated particles, the energy equivalent of 10^8 supernova explosions, an appreciable part of the available nuclear energy of the whole galaxy. Ten years ago a suggestion that such an object actually existed would have been dismissed as preposterous. Now we regard it as a magnetic field stretching far into intergalactic space, together with a source which can inject into it colossal numbers of relativistic particles, the particles, of course, supplying the radiation by the synchrotron process. The problems of the origin and maintenance of the field, and the energy supply of the source, are completely unsolved. Most radio galaxies have a complex structure, which seems to bear little resemblance to that of the optical galaxies with which they are identified. As is well known, this identification is a matter of great difficulty, even for the formidable northern combination of the Cambridge and Owens Valley interferometers and the 200-in. telescope. To match the appreciably more powerful Parkes and Hoskinstown installations, at Mount Stromlo we have only the 74-in. reflector. Already it is clear that quite powerful radio sources can barely be detected with the 74-in., let alone examined in any sort of structural or spectroscopic detail. Nevertheless, it is largely from such identifications and such investigations that progress must come. As Burbidge remarked of the synchrotron process: "The key arguments leading to our theoretical understanding came from predictions and observations of the *optical* radiation from the Crab nebula and the jet in M 87". (The italics are his.) Australia has here the opportunity of achieving a historical advance in a field it has made peculiarly its own. It would be a national tragedy if this opportunity were allowed to pass to one of the countries already known to be planning their own large southern telescopes.

Two questions remain. First, will the development of image intensifiers make large telescopes unnecessary? The short answer is that an image tube promises to be effectively a high-speed photographic plate, fast enough to cut exposures by a factor of 10 or 100. As such, its major immediate application will be in spectroscopy, where it promises to effect a revolution. The detection of faint stars and galaxies is another matter, governed by the joint effects of the brightness of the night sky and of photon statistics, and in this field it will be much longer before the image tube can compete with the photomultiplier, a virtually noiseless device of equally high quantum efficiency. The cost of developing such a tube will be comparable with that of a great telescope.

The second question is: Will satellites make terrestrial observatories obsolete? In one sense this has already been answered by the planning and active construction of a number of major telescopes, most of them either in the United States or the U.S.S.R. The first satellites are to observe that part of the ultra-violet spectra of bright stars and interstellar matter which is inaccessible from the surface of the Earth; this task can be done in no other way. When it comes to the measurement of faint stars and galaxies near the limit of big optical telescopes the position is different. The satellites are now competing directly with conventional telescopes. They will have the advantages of dark skies and small images (small because they will be undisturbed by atmospheric variations), but to capitalize on these must overcome enormous technical problems, just one of which will be the necessity for maintaining pointing accuracies in the region of 0·01 sec arc over extended periods. Difficulties mean money, and while the point has been made before, one cannot refrain from mentioning that the Venus probe (there were two) is reported to have cost £10,000,000. This sum would pay for an observatory of the scale advocated here at least twice over; the scientific return from the observatory will surely be many thousands of times greater.

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ARE CRYPTOVOLCANIC STRUCTURES DUE TO METEORITE IMPACT?

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THIS article is a brief summary of the main geological argument contained in a detailed paper on cryptovolcanic structures which is to appear in the *American Journal of Science*. In view of the present lively interest in possible evidences of meteorite impact it is important to make available the areal setting of major cryptovolcanic structures which casts serious doubt on the present tendency to ascribe their origin to meteorite impact.

The term 'cryptovolcanic' was applied in 1905 by Branco and Fraas[1] to the small, circular Steinheim Basin in south-west Germany. In 1933, I (ref. 2) described essentially

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similar, but more deeply eroded, roughly circular structures in the United States, and reviewed the evidence concerning three others which are only incompletely exposed. Since then, other comparable areas of intense local deformation have been discovered. The second edition of the *Tectonic Map of the United States* marks the location of 14 cryptovolcanic structures[3], but more have come to light since it was published.

These structures consist of: (1) a central uplift within which the strata are highly contorted and broken up, surrounded by (2) a more or less continuous ring-shaped depression which tends to be bounded and cut by faults. In the Steinheim Basin, this depression is concealed by fresh-water lake deposits. Branco and Fraas ascribed the origin of the Steinheim Basin to volcanic activity. Some of the intensely shattered limestones within the central hill are full of peculiar cone-shaped bodies representing conical fracture surfaces densely covered with bifurcating ridges and grooves which radiate from the top of the cone. Because of this highly characteristic pattern, the authors called these bodies "Strahlenkegel", interpreting them as products of violent shock. I found similar shatter cones in the centre of the largest of the American cryptovolcanic structures, the Wells Creek Basin[4]. Since 1933, the idea that cryptovolcanic structures might be the result of meteorite impact has steadily gained favour among American geologists[5]. Suspecting that shatter cones might be used as evidence of shock from above, Dietz visited the majority of the American cryptovolcanic structures and found that shatter cones are present in most of them. By a bold jump in logic, he suggested that shatter cones may be diagnostic of meteorite impact[6].

His reasoning received unexpected and powerful support from the discovery of coesite and stishovite in the Ries Basin[7]. Coesite and stishovite are heavy phases of silica. They occur in minute amounts in the glassy phase of 'bombs' of partially fused gneissoid and granitoid basement rocks which abound in deposits of suevite, a tuff-like rock made up of comminuted silicate materials and glass shreds. The Ries Basin, the largest known European circular structure of problematic origin[8], differs from the typical cryptovolcanic structures in having no central uplift and no shatter cones. Instead of the central uplift, there is a deep, roughly circular depression from which all overlying sedimentary rocks (Permian to upper Jurassic) and several hundred feet of the crystalline basement have been removed and scattered over the surrounding basin shelf and on to the undisturbed surface of the surrounding plateau, in a mixture of large fragments up to hill-size in a matrix of finely ground rock powder. Where the floor of the surface on which this 'Bunte Breccie' rests is exposed it is covered with scratches and grooves which point toward the centre of the structure. Shattered rock masses and 'Bunte Breccie' lie scattered up to a distance of 20 km (12 miles) from the outer rim of the basin (or 29 km (18 miles) from the edge of the central depression), the average size of the fragments growing finer with distance. Reich's detailed seismic refraction studies[9] have shown that the floor of the central circular depression, with a diameter of 8 km, is essentially flat.

The structure of the broad ring-shaped 'shelf' between the edge of the surrounding undisturbed plateau and the central depression is largely hidden beneath a thick blanket of fresh-water limestones deposited in a lake formed when the outlet of the valley of the Wörnitz River was filled with ejecta from the Basin. (Remnants of this fill still cling to the valley side below the town of Harburg.) Reich's seismic studies suggest a disordered mosaic of blocks of concealed pre-Tertiary rocks on either side of a horseshoe-shaped ridge of displaced gneissoid basement rock, perhaps a 'damped-wave' effect like that suggested by the structure of the Wells Creek Basin[10]. In the Ries, however, the central part was blown out, not merely boosted up. The most striking aspect of the Ries Basin is thus the superficial character of the disturbance. Drilling in two cryptovolcanic structures in North America indicates that there, too, deformation was limited to shallow depths.

The roughly circular outline with the greatest deformation in the centre, the violent disruption of the rocks in the centre, the shallowness of the disturbance, common association of shatter cones with cryptovolcanic structures, other structural features suggesting origin through shock, such as narrow fissures filled with rock powder, and above all, the presence of high-temperature–high-pressure phases of silica, all find such a simple and elegant explanation in meteorite impact that geochemists and geophysicists, and a rapidly growing number of geologists, tend to assume the problem solved.

No doubt most, if not all, geochemical and geophysical (tectonic) features of individual cryptovolcanic structures could have been produced by meteorite impact. But were they? Discussion based on these features neglects a fundamental point: If meteorite impact created the structures, their incidence in time and space must be consistent with the hypothesis, that is, they must be distributed at random. This means that they must not bear any demonstrable relation to structural axes in the Earth's crust, nor to features the formation of which was accompanied by deep-seated volcanic activity.

In the forthcoming paper, I apply this test to the largest cryptovolcanic structures of Europe, America and Africa: the Ries Basin, the Wells Creek structure, and the Vredefort Dome (accepting the application of the term 'cryptovolcanic' to this structure by supporters of the hypothesis of meteorite impact).

The choice of the largest example in each continent is independent of any structural considerations and makes use of the outstanding examples quoted by the advocates of origin by meteorite impact—in the Ries Basin especially because of the presence of coesite and stishovite in the partially melted gneissoid fragments in the suevite tuff associated with it, in the Wells Creek Basin and the Vredefort Dome because of the conspicuous development of shatter cones. In all three cases, inspection of the regional setting reveals conclusively that each lies on a major regional structural axis aligned with one or several structures involving localized mafic and ultramafic igneous rocks.

Fig. 1 shows three essentially circular structures lined up in south-western Germany at intervals of approximately 50 km: the Ries Basin (21–24 km in diameter) with coesite in glass from partially molten basement, the Steinheim Basin (3·5 km in diameter), the type locality of shatter cones, and the Urach region (about 40 km in diameter) where more than 250 tuffisite pipes perforate the Jurassic plateau like a sieve. In the Urach area, most of the pipes are filled with finely ground-up rock material (chiefly limestone) with varying proportions of lapilli of melilite basalt; basalt occupies a few of the pipes[11].

The line connecting these three unusual areas essentially parallels: (1) the axis of a geomagnetic high which reflects a gentle buried pre-Triassic topographic ridge; (2) the axis of the young flexure along which the Mesozoic rocks of the Jura Plateau of southern Germany bend down beneath the Miocene and younger rocks of the Swiss foreland of the Alps. On the map (Fig. 1) this is shown by the shoreline of the marine upper Miocene sea which can be recognized to-day as a distinct low marine cliff, with pholadid borings and layers of barnacle fragments in the adjoining marine beds. Moreover, two facts link the Ries with the other two structures aligned with it: (1) all three structures came into being in uppermost Miocene time, a time of widespread volcanic activity in western Europe; (2) drill holes in both the Ries Basin[12] and the Urach area[13] reveal a geothermal gradient almost three times as great as that which is normal for western Europe, and as high as that of Thermopolis, Wyoming[14] (which is some 145 km (90 miles) from Yellowstone Park, famous for its young volcanism and active geysers).

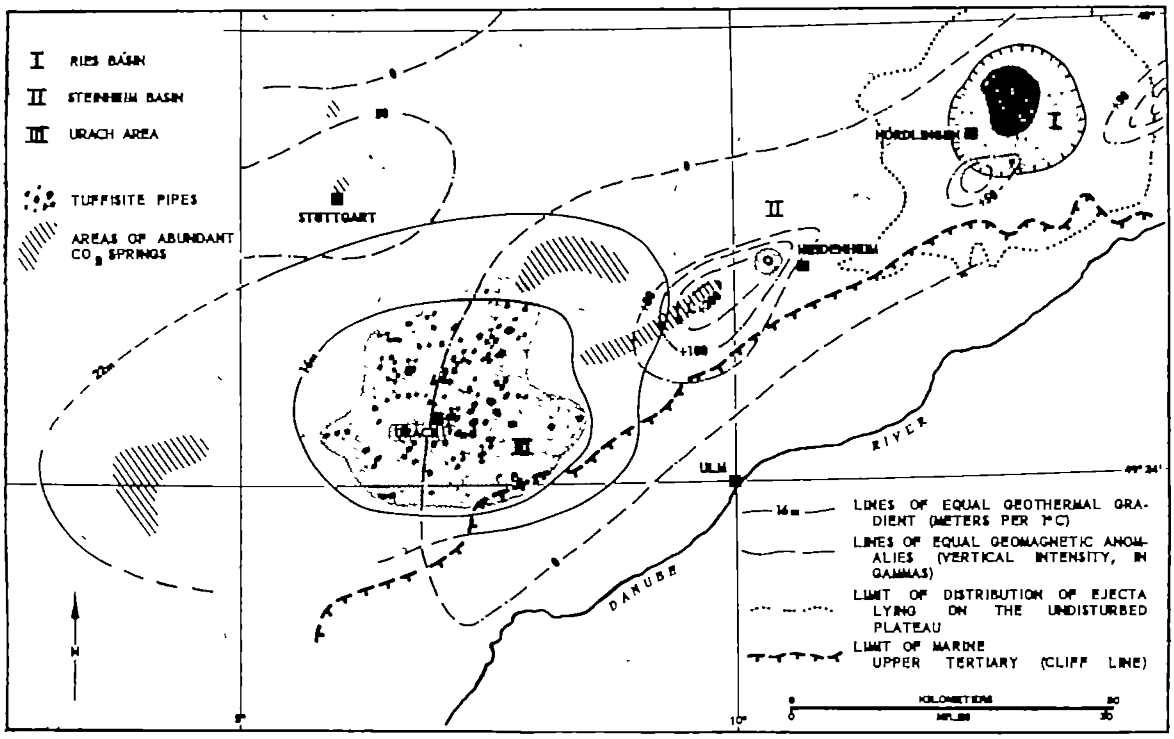

I RIES BASIN
II STEINHEIM BASIN
III URACH AREA
TUFFISITE PIPES
AREAS OF ABUNDANT CO$_2$ SPRINGS
STUTTGART
NÖRDLINGEN
I
II
HEIDENHEIM
+100
URACH
III
ULM
RIVER
DANUBE
48° 24'
N
9°
10°
16 m LINES OF EQUAL GEOTHERMAL GRADIENT (METERS PER 1°C)
LINES OF EQUAL GEOMAGNETIC ANOMALIES (VERTICAL INTENSITY, IN GAMMAS)
LIMIT OF DISTRIBUTION OF EJECTA LYING ON THE UNDISTURBED PLATEAU
LIMIT OF MARINE UPPER TERTIARY (CLIFF LINE)
0 KILOMETERS 50
0 MILES 30

Fig. 1. Map of the structural axis on which lie the Ries Basin, Steinheim Basin and the Urach area of breccia pipes. (Data from Georg Wagner (ref. 28); Gerold Wagner (personal communication); Neumann (ref. 29); Reich and Horrix (ref. 9); Carlé (ref. 13))

Yet two of these structures, the Ries Basin and the Steinheim Basin, are supposed to be the result of the impact of a giant meteorite and a minor satellite respectively, while the Urach region is the product of deep-seated volcanic activity.

The hypothesis of meteorite impact demands that by pure chance two meteorites, a giant accompanied by a satellite, hit the Earth in line with a wholly independent major terrestrial structural axis precisely at a time when volcanism was active throughout western Europe, and that the larger of the two, also by chance, produced by impact approximately the same amount of heat as that of the volcanic Urach region so that, after some 6–8 million years, the thermal gradient is as great to-day as that around Thermopolis, Wyoming.

The Wells Creek Basin stands out among American cryptovolcanic areas by its size (diameter about 13·7 km = 8·5 miles); by the intensely broken up condition of the rocks in the uplifted central area which form a veritable megabreccia; and by the poorly developed, but distinct, anticlinal ring between the outer limits of the structure and the central uplift, suggestive of an elastic damped wave effect[15].

The Wells Creek Basin is aligned with two structures, the Hicks Dome and the Avon area, along a prominent anticlinal flexure zone comparable with that which connects the Ries and Steinheim Basins with the Urach area. In Fig. 2 (ref. 16) this curved axis is left blank. To the north, the Pennsylvanian formations dip very gently north-north-eastward into the Illinois Carboniferous basin. To the south, the Cretaceous–Tertiary formations dip equally gently into the head of the Mississippi embayment. In the centre of this curved flexural axis, opposite the head of the embayment, lies the Hicks Dome, an oval, faulted, domed area of about 14·5 km (9 miles) average diameter, characterized by breccia pipes and dikes rich in mafic to ultramafic volcanic material (one dike carries radioactive yttrium–thorium monazite)[17]. From Hicks Dome, dozens of thin, more or less vertical breccia dikes with varying amounts of basic volcanic material cut the Mississippian rocks in a south-south-easterly direction, on top of and parallel to the axis. The size of the small mafic dikes south-south-east from Hicks Dome is greatly exaggerated in Fig. 2 (ref. 16) in order to make them stand out. They seem to end at the southern border of the detailed geological map of the western Kentucky fluorite district, from which the representation is taken[16]. They grow smaller south-south-westward, but may well extend farther than shown on the map.

About 145 km (90 miles) to the south-south-east of the Hicks Dome, three diminutive craterlets filled with Cretaceous sediments trend north-north-westward a short distance beyond the Wells Creek Basin, that is, essentially in the same direction as the basic dikes farther north, and, more important, in the direction of the anticlinal flexure zone (Fig. 2). Dr. Wilson[18], who described them, called them impact craters, caused by small meteorite fragments running ahead of the master meteorite. Here, as in the Ries–Steinheim–Urach line, it is assumed that a giant and baby meteorites hit the ground in line with the axis of an independent major flexure zone.

About 168 km (105 miles) west-north-west of the Hicks Dome lies the Avon area[19]. It is broadly oval, with the long axis measuring about 17·5 km (11 miles) and, surprisingly, it duplicates the Urach area. So far, 78 breccia pipes have been found. All are quite small, some contain only ground-up limestone fragments in a limestone powder, like some in the Urach region; but most of the pipes contain fragments of granitoid rocks carried up from the basement together with lapilli of melilite basalt which are practically identical to those of the Urach region[20]. Through the courtesy of its chief geologist, Dr. Frank G. Snyder, I have been able to examine the cores from two holes drilled by the Saint Joseph Lead Company in the Avon area. The increase in basaltic lapilli and in granite fragments downward is strikingly shown as the drill holes approach the crystalline basement and, in one case, penetrate it deeply.

Here then, of three structures lying on a major flexure zone (of purely terrestrial origin), one is supposed to be the product of meteorite impact, while the other two are undoubtedly volcanic in origin.

I cannot accept a hypothesis which holds that in two continents multiple meteorites (in one case a giant and a

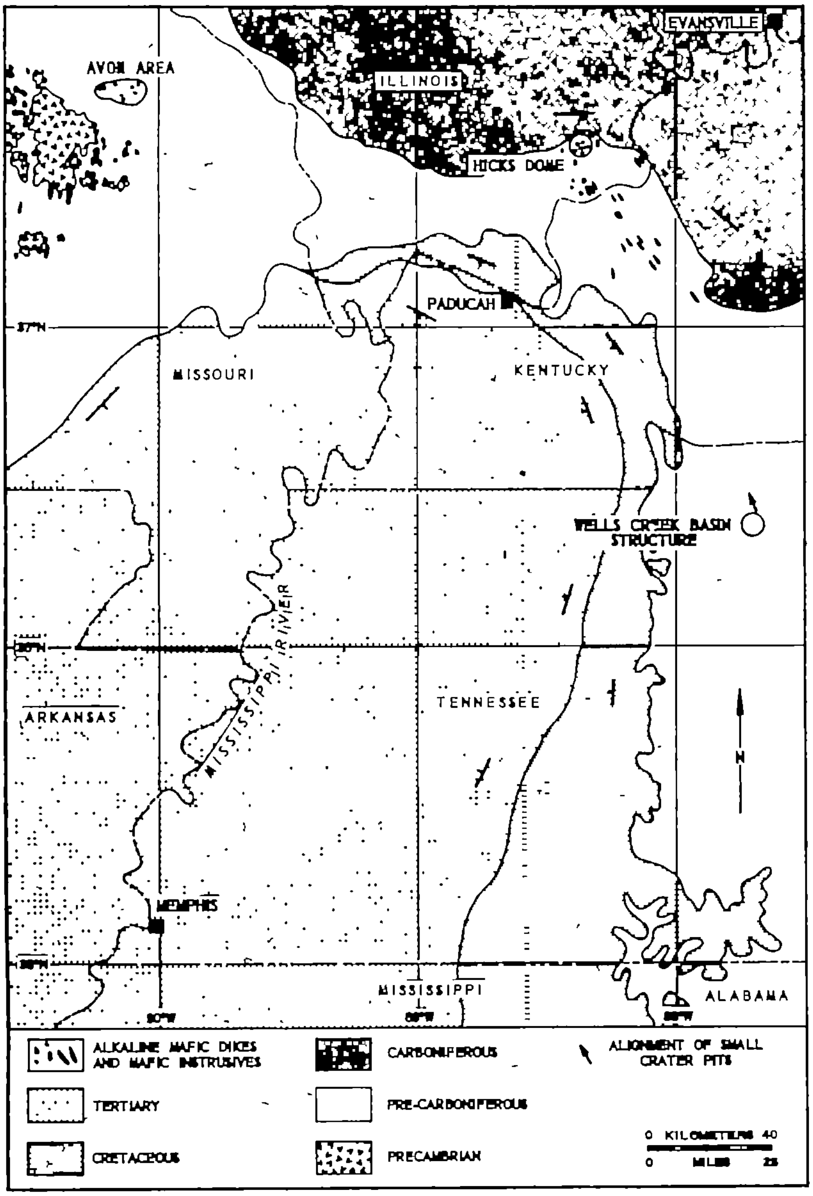


Fig. 2. Map of the structural axis on which lie the Wells Creek Basin, the Hicks Dome and the Avon area of breccia pipes. *Note*. Only the location of the structures and of mafic alkaline dikes (exposed or cut in drill holes), breccia pipes and the trend of three diminutive crater-like pits are shown. All structural details, especially the complex fault system of the Illinois–Kentucky mining district, are omitted. (Data from Heyl and Brock (ref. 16); Kidwell (ref. 19); and the *Tectonic Map of the U.S.A.*, second edit. (ref. 3))

satellite, in the other a giant and dwarf fragments) struck a clearly defined terrestrial flexure zone so that their impact scars are aligned parallel to its axis and with structures of proved volcanic origin.

Like the largest cryptovolcanic structures of Europe and America, the Vredefort dome lines on a major structural axis, but one of a wholly different nature. Fig. 3 (ref. 21) shows that it is one of four roughly circular areas (points 4–7) in the Orange Free State which lie on a straight line over 430 km (270 miles) long, trending toward the centre of the vast Bushveld area (point 3), in a north-north-easterly direction (about N. 27° E.). In Southern Rhodesia, a second structural axis (points 1 and 2) trends a little more northerly (N. 17° E.), also heading toward the centre of the Bushveld intrusive centre. This is the Great Dyke, one of the great ultramafic terranes of the Earth's surface. The total distance from the northern end of the Great Dyke to the Trompsburg structure (points 1–7) is more than 1,450 km (900 miles).

The Great Dyke[22] is a relatively straight-layered body of mafic and ultramafic rocks, over 530 km (330 miles) long and between 5 and 11 km (3–7 miles) wide. It was intruded into a terrane of crystalline rocks, chiefly granite, with occasional zones of metamorphic schists. Intrusion proceeded from four centres, each producing a sequence of similar structure and component rock types, but differing from each other in the number of layers, their thickness and distribution. In each complex, the component layers maintain a remarkably constant average thickness. All dip toward the centre of the Great Dyke from their borders, while longitudinally they plunge toward the centre of eruption in each complex.

Where seen in a few good exposures and confirmed by inclined drill holes that passed from the rocks of the Dyke at the surface into the granite at depth, the borders of the Great Dyke are fault contacts dipping inward at angles varying from 25° to 70°. On either side, the Great Dyke is paralleled by a long quartz gabbro dike which disappears where the central portion of the Dyke is most depressed. These companion dikes seem to be the last phase in the regional differentiation process.

In length, width and even in the northerly trend, the Great Dyke resembles the complex young grabens of the East African graben belt. These young grabens, of course, are much wider at the surface. But with border faults converging downward toward the base of the crust at angles averaging 45°, perhaps to a depth of 10 km or more in earlier Precambrian time, the border faults of the Great Dyke would converge to something like 4 km from a possible surface width of 24 km. Near the base of the crust, where the faults would tend to become merely boundaries between plastically moving rock masses, the ultramafic rocks of the sub-crust would tend to rise isostatically in response to the lowering of the surface of the graben. Repeated intrusions of ultramafic materials into the base of the sinking graben block seem feasible in view of the plastic rather than the viscous conditions of rock at moderate depth. The ultimate synclinal structure may be the result of final compaction and settling of the cooling rock body, perhaps combined with a last sagging of the graben.

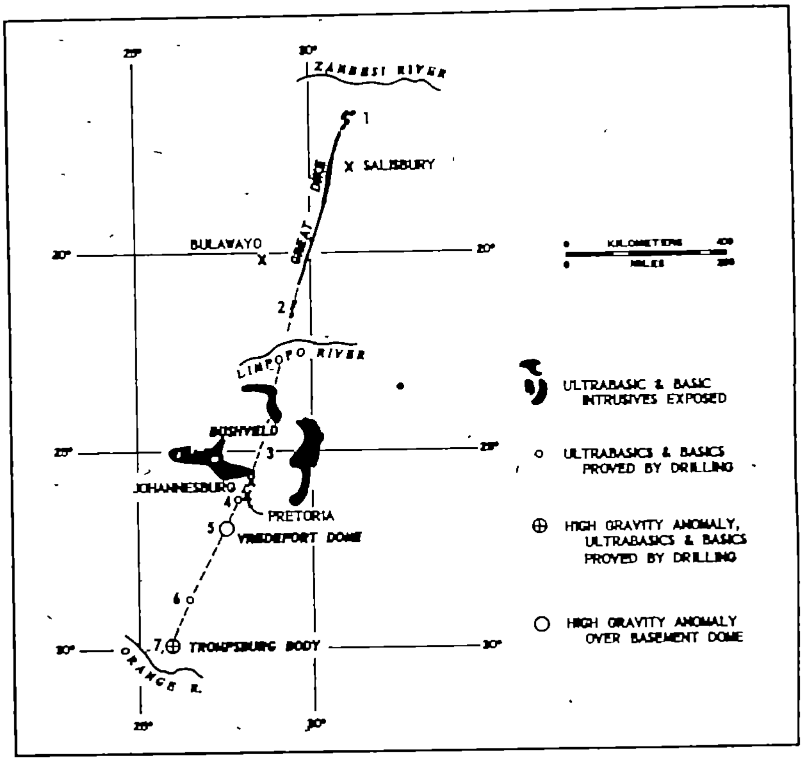
The coarse grain of the ultramafic rocks requires crystallization beneath a roof of substantial thickness. Remnants are preserved in bodies of schists measuring up to three miles across, where the Dyke cuts belts of metamorphosed sediments.

Recently a comparable complex was discovered[23] in the Zambesi valley, about 128 km (80 miles) west of the Great Dyke. It is some 40 km (25 miles) long and consists of rocks similar to those in the upper layers of the Great Dyke.

The Bushveld complex[24] of intrusive sheets and connecting dikes which lies at the centre of this vast alignment of mafic and ultramafic rocks (point 3), is about 400 km (250 miles) wide east to west, and not much less in a north–south direction. The combined thicknesses of these intrusions amount to 6–8 km (4–5 miles), wedged between layers of the thick Transvaal system (15,000–28,000 ft.); the lower horizons carry the same concentrations of platinum and chromium that are found in the Great Dyke.

The Trompsburg structure in the Orange Free State at the far end of the southern line of intrusives (point 7) is roughly circular, and, from the size of the gravity anomaly, its diameter is estimated to be 48 km (30 miles)[25]. It was first discovered by the high gravity and magnetic anomalies above it. Drilling showed that it contains the same mafic and ultramafic rocks which characterize the whole belt; the same was proved for the minor intrusions (points 4 and 6 on Fig. 3).

The Vredefort dome lies neatly in line with the whole belt of deep-seated intrusions. The high positive gravity anomaly above it indicates that heavy rocks underlie the unbroken roof of granite and infolded metasediments. The joint patterns on the exposed granite surfaces are those seen generally on broadly domed basement areas[26]. But the granite and overlying mantle of sediments are traversed by an intricate network of pseudotachylite veins—anastomosing dikelets of pulverized basement rocks, comparable with similar veins in the Ries, where they cut basement and sediment cover. Shatter cones—which are missing in the Ries—are found in abundance in most rock formations of the Vredefort dome[27].


25°
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ZAMBESI RIVER
1
X SALISBURY
GREAT DYKE
BULAWAYO
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20°
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LIMPOPO RIVER
BUSHVELD
3
25°
25°
JOHANNESBURG
4
PRETORIA
5
VREDEFORT DOME
6
7
TROMPSBURG BODY
30°
30°
ORANGE R.
25°
30°
KILOMETRES
MILES
ULTRABASIC & BASIC INTRUSIVES EXPOSED
ULTRABASICS & BASICS PROVED BY DRILLING
HIGH GRAVITY ANOMALY, ULTRABASICS & BASICS PROVED BY DRILLING
HIGH GRAVITY ANOMALY OVER BASEMENT DOME

Fig. 3. The Bushveld chain of intrusives (redrawn from Cousins, ref. 21)

To assume that a giant meteorite happened to strike a point precisely in line with structures produced by such large-scale deep-seated magmatic activity within the same general (earlier Precambrian) time interval, producing a structure of similar dimensions 35 km (22 miles) away, seems to me to carry the original hypothesis *ad absurdum*. In the case of the four cryptovolcanic structures discussed here, the hypothesis of meteorite impact seems, therefore, unacceptable. It follows that neither the presence of shatter cones nor of heavy phases of silica are criteria for meteorite impact. The full paper will discuss possible alternatives.

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SOME MICROSTRUCTURES OF COMPLEX MORPHOLOGY OBSERVED IN PREPARATIONS OF CARBONACEOUS CHONDRITES MADE UNDER STERILE CONDITIONS

By Dr. GREGG MAMIKUNIAN and Dr. MICHAEL H. BRIGGS*

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VARIOUS classes of microstructures in the 5–30µ size-range have recently been examined in samples of carbonaceous meteorites by Claus and Nagy[1–3], who have named the objects "organized elements". On the basis of detailed morphological studies and staining reactions with biological dyes, Claus and Nagy have suggested that the "organized elements" are microfossils of an extra-terrestrial life-form, indigenous to the meteorite parent-body.

Other workers who have also investigated samples of these meteorites and have identified structures that they consider to be indigenous fossil remains are Staplin[4], Reimer[5], Palik[6,7], Cholnoky[8], Skuja[9], Ross[10], Engels[11] and Timofeev[12].

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On the other hand, several scientists, after examining the structures in question, have claimed that identification as microfossils is premature. Thus Fox[13] has suggested that the objects are spheroids of non-biological organic matter, together with droplets of sulphur and contaminating terrestrial organisms.

Fitch *et al.*[14–16] have argued that the "organized elements" are various mineral grains and hydrocarbon droplets together with some contaminants. Deflandre[17] has similarly claimed that the objects are terrestrial contaminants or artefacts. Briggs[18], who examined sterile preparations of the meteorites, has suggested that some are mineral grains, while others are associations of sulphur with organic matter, probably of abiogenic origin. In a recently prepared report[19], we have identified what we believe to be terrestrial contaminants in sterile meteorite preparations. Mueller[20] has presented evidence that one class of "organized element" which displays a complex morphology is a rare limonite pseudomorph of troilite.

With this marked disagreement it is clear that the true nature of the "organized elements" will be solved only after prolonged examination by many different scientists competent in various fields. Thus, contributions from bacteriologists, palynologists, micropaleontologists, pathologists, crystallographers, histologists and organic chemists are necessary, and it is improbable that any single person is competent to identify microscopic objects in all these fields. As the meteorites are difficult to obtain and are only available for examination at present by a small group, we have prepared a catalogue of microphotographs[21] for wide circulation.

Table 1. DETAILS OF METEORITES

Name	Country of origin	Date of fall	Wiik's (1956) classification (ref. 22)
1. Orgueil	France	1864	I
2. Cold Bokkeveld	South Africa	1838	II
3. Murray	United States	1950	II
4. Haripura	India	1921	II
5. Mighei	Ukraine, U.S.S.R.	1889	II
6. Mokoia	New Zealand	1908	III
7. Felix	United States	1900	III
8. Dimmit	United States	before 1947	non-carbonaceous

Table 2. STERILIZATION PROCEDURE: PERCENTAGE ETHYLENE OXIDE AND RELATIVE HUMIDITY IN STERILIZING GAS MIXTURE

Position and time (h)	Percentage ethylene oxide	Temp. of ethylene oxide (°F)	Dew point (°F)	Percentage relative humidity
0	27·7	70·5	+49·5	46
1	27·5	72	+49	44
3½	27·6	75·5	+47·5	37
4½	27·8	72·5	+47	40
5¼	27·3	74	+50·5	43
5½	26·6	76	+51	41

In this article we present a selection of photographs of some microstructures of complex morphology which we have observed in sterile preparations of some of the meteorites.

A list of meteorites used in this investigation is given in Table 1.

All meteorite samples were examined to ensure that they were free of fusion crusts, museum markings and obvious surface contamination.

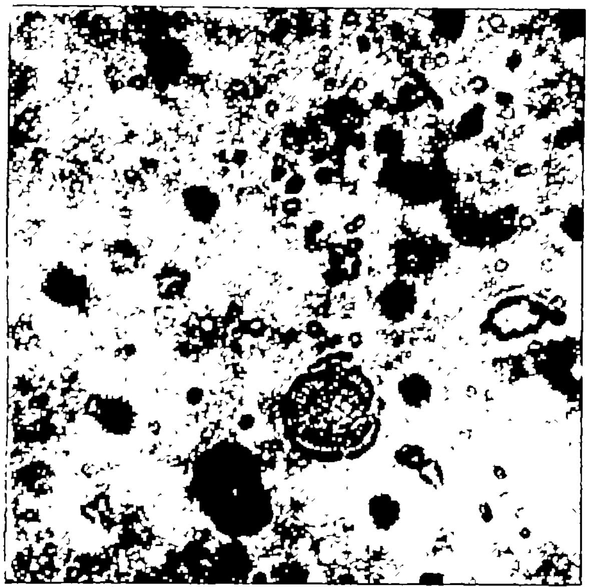

Fig. 1. Microstructure observed in Mokoia meteorite preparation. (× 630)

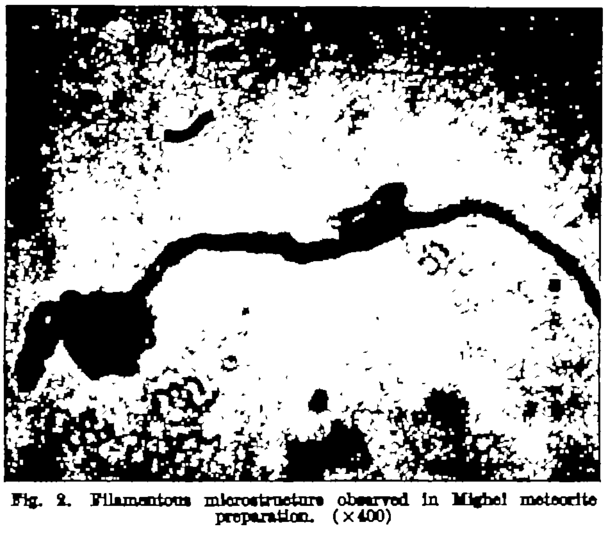

Fig. 2. Filamentous microstructure observed in Mighei meteorite preparation. (×400)

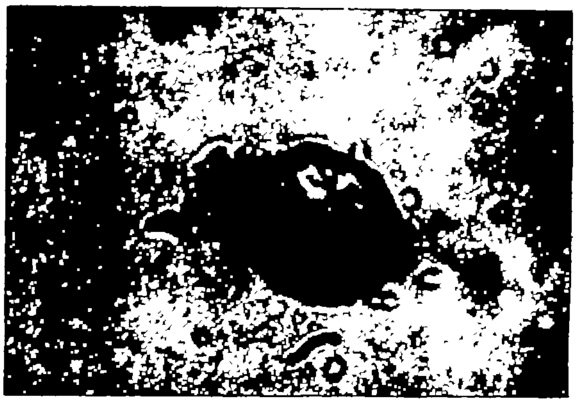

Fig. 3. Microstructure observed in Haripura meteorite preparation. (×630)

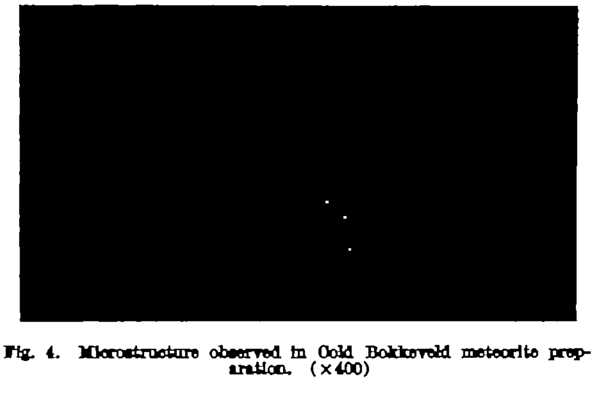

Fig. 4. Microstructure observed in Cold Bokkeveld meteorite preparation. (×400)

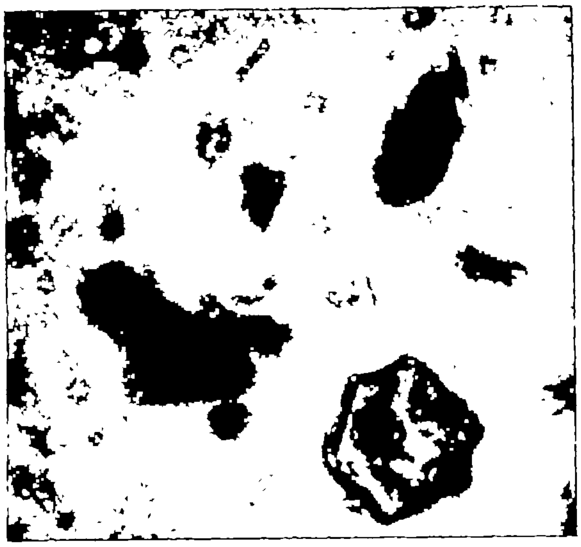

Fig. 5. Microstructure observed in Cold Bokkeveld meteorite preparation. (×630)

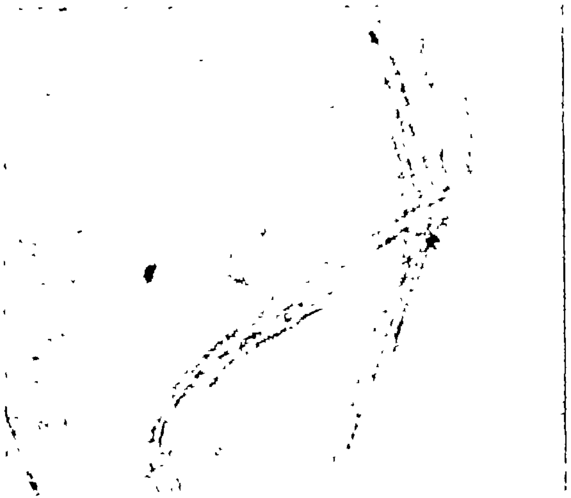

Fig. 6. Filamentous microstructures observed in Dimmit meteorite preparation. (×400)

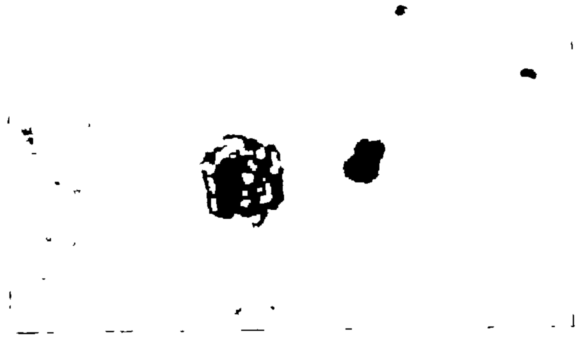

Fig. 7. Microstructure observed in Orgueil meteorite preparation (×1,000)

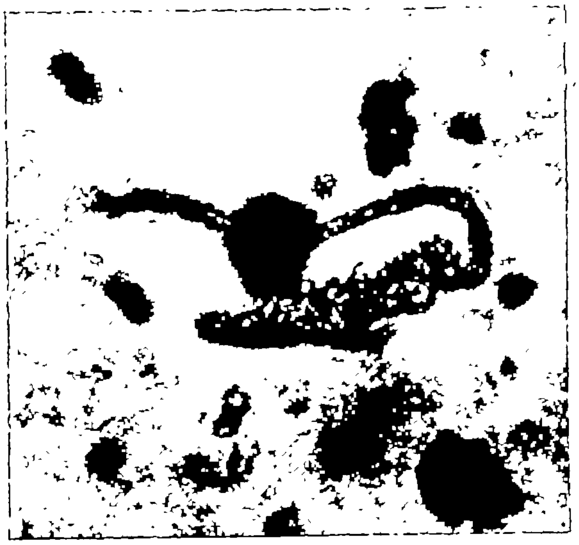

Fig. 8. Microstructure observed in Orgueil meteorite preparation. (×630)

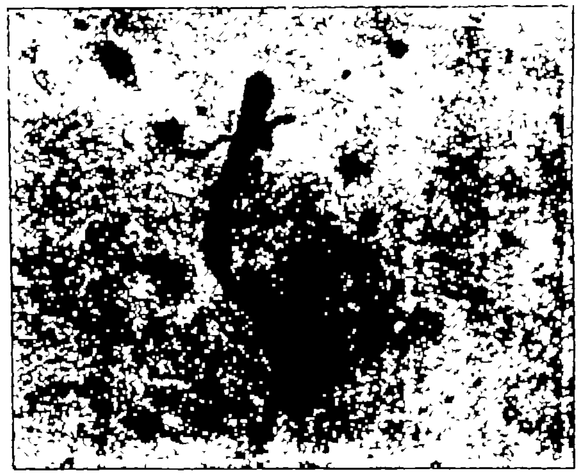

Fig. 9. Microstructure observed in Murray meteorite preparation. (×160)

Small pieces of each meteorite were separately placed in Petri dishes in a sterile glove-box. All other equipment used in preparation was also placed in the box. The interior of the box was then sterilized by ethylene oxide for 5·75 h. Table 2 shows the sterilizing conditions at intervals throughout this period. Ethylene oxide was removed by flushing with sterile nitrogen for 30 min. Microscope slides were prepared in the atmosphere of nitrogen.

All slides were prepared in the same manner. A small grain of meteorite was carefully powdered between glass slides, then mounted in 'Permount' beneath a coverslip. Six slides were prepared of each meteorite. Other slides were stained with saffranin or crystal violet before mounting. Control slides were made of the stains and 'Permount' without meteorite powder.

The remaining grains of meteorite were added to separate steril containers of 'Trypticase' growth medium, which were then sealed and incubated for 7 days at 37° C. After this time no growth was detected in any container.

Photomicrographs of some interesting structures observed in these preparations are shown in Figs. 1–10.

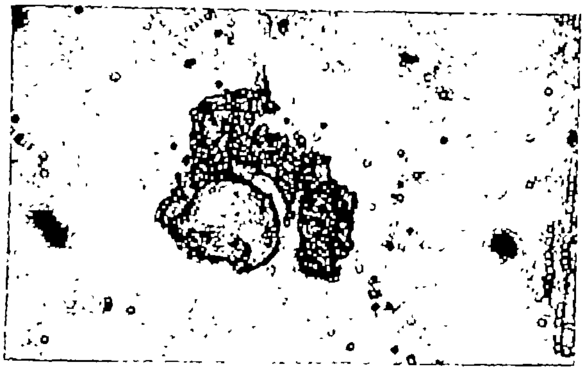

Fig. 10. Microstructure observed in Mighei meteorite preparation. (×400)

An examination of these illustrations demonstrates that all meteorite preparations used in this work contain a variety of rare microstructures in the c. 20-μ size-range which exhibit a complex morphology, and yet are not readily identifiable. It is our opinion that most of these objects are unusual mineral grains or terrestrial materials which have contaminated the meteorite during museum storage. However, conclusive identifications are not possible.

We thank the following for gifts of meteorite samples: Dr. W. A. Watters, New Zealand Geological Survey; the Director, Wanganui Museum; Dr. E. P. Henderson, Smithsonian Institution; Dr. E. Anders, University of Chicago; Dr. R. Marshall, Jet Propulsion Laboratory; the Director, Indian Geological Survey; the Director, Chicago Natural History Museum.

We also thank Mr. F. Morelli, Jet Propulsion Laboratory, for help in the preparation of slides.

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SPECIAL THEORY OF RELATIVITY

By PROF. HERBERT DINGLE

THE replies which have been received to my communication to *Nature*[1], in which I pointed out a contradiction in the special theory of relativity, are too numerous for individual publication, and the Editor has asked me to deal with them (other than that of Prof. Max Born, which appears on p. 1287 of this issue) collectively. Apart from trivial misunderstandings, the criticisms of my argument fall into a few classes which I will consider separately, but I should first say that only one correspondent heeds the last paragraph of my letter, despite its italics. I there asked, not for the reason why Einstein's statement differed from mine, of which I was aware, but why the former should be considered true and the latter false. The correspondent in question—the only one, in my view, who has really faced the problem—admits that no such reason can be found, and concludes that the conception of an objective rate–ratio of two clocks is impermissible. I cannot agree, but since space prohibits an analysis of this suggestion I justify my dismissal of it here by the fact that I am examining only Einstein's theory, and since he asked: "What is the rate of this clock, when viewed from the stationary system ?" and gave an answer in "seconds per second", it is clear that he accepted a rate–ratio as a meaningful conception.

The remaining correspondents make the following points (it will be remembered that my communication contained two statements, one by Einstein claiming that moving clocks run slow, and one by me claiming that moving clocks run fast): (1) In Einstein's statement the interval considered is that between the separation of the clocks and an event on the moving one, but mine refers to the interval between the separation of the clocks and an event on the stationary one. I have therefore myself introduced the contradiction which I ascribe to Einstein's theory. (2) The internal consistency of the Lorentz transformation can be demonstrated mathematically; it is therefore unquestionable. It gives a unique value for the time of every event in every inertial co-ordinate system, and so cannot possibly contain a contradiction. This reply is usually accompanied by a Minkowski diagram. (3) Both statements show how one clock appears to an observer on the other; they are not contradictory because they relate to different co-ordinate systems.

None of these replies meets the point of my criticism. I can best explain this, I think, by slightly amplifying the situation as described by Einstein, so as to make explicit some steps there tacitly assumed and make the situation more vividly imaginable.

$$\dot{Y} \rightarrow \underset{A}{.} \qquad\qquad \dot{X} \rightarrow \underset{B}{.}$$

A and X are twins who separate at birth at a speed v such that $\sqrt{(1 - v^2/c^2)} = 1/5$. Each carries a clock which reads 0 at the moment of separation and thereafter reads the age of its bearer. Ahead of A, in the direction of X's motion, and keeping at a constant distance from A, is another child B, born at the same moment as A in A's and B's common time system and carrying a similar clock synchronized with A's. Likewise, in the rear of X, and keeping at a constant distance from X, is a child Y, born at the same moment as X in X's and Y's common time system and carrying a similar clock synchronized with X's.

When X is 6 years old he passes B and they exchange photographs which have just been taken. B, and therefore A, is then 30 years old according to the Lorentz transformation. Further, when A is 6 years old Y passes him and they also exchange recent photographs. The Lorentz transformation then shows that Y, and therefore X, is then 30 years old. All assemble later and agree, on the evidence of the photographs, that A is 30 when X is 6 and X is 30 when A is 6. I call this a contradiction.

The readings can be set out in more detail: Tables 1 and 2 readily follow from the Lorentz transformation.

Table 1 corresponds to Einstein's statement, with A fixed at the origin of the K system and X at the origin of the k system, and we see that X ages more slowly than A, as he concludes. But we also have Table 2.

Table 1

		X is born	X meets B
'Stationary' (K) system	t =	0	30
'Moving' (k) system	τ =	0	6

Table 2

		A is born	Y meets A
'Stationary' (K) system	t =	0	6
'Moving' (k) system	τ =	0	30

Table 2 corresponds to my statement, with A and X still fixed at the origins of the same systems as before, and we see that A ages more slowly than X.

I can now deal with the above objections.

(1) Of course I have referred to a different pair of events from Einstein; vain repetition would be foolish. If a thing has contradictory aspects, you will not discover the fact merely by inspection of one, however often repeated. Einstein's statement and mine are neither paraphrases of one another nor two halves of a single argument. They are independent statements, running in parallel, not in series, and if one is valid, so is the other; but they lead to incompatible conclusions. There is no reason why the persons or clocks should be compared by Table 1 rather than Table 2. True, I do not get X's age directly but have to infer it from Y's; but Einstein does not get A's age directly but infers it from B's. If Y's photograph is not evidence of the age of X, thus discrediting my conclusion, then B's is not evidence of the age of A, thus discrediting Einstein's. It seems to me self-evident that any objection to the implications of Table 2 is *ipso facto* an objection to the corresponding implications of Table 1.

(2) The internal consistency of the Lorentz transformation is not in question. The figures in both tables, as the Minkowski diagram shows clearly, are unambiguous, and not, as numbers, conflicting; but no clock or person can behave in such a way as to fulfil their requirements. One correspondent urges that Einstein's theory is that the time of a distant event can be assigned only by definition and not by natural necessity, and a definition cannot be disproved. But that is not Einstein's theory; that merely clears the ground to make room for a theory (it was, in fact, at the time a piece of supreme intellectual insight). If that were all, then any fantastic figures could appear in the tables, and could not be disputed. But Einstein proposed a particular definition, and his theory was that actual clocks would conform to it. It required them to behave so that the figures in each horizontal line of Tables 1 and 2 were the readings of clocks synchronized according to his prescription, and those in each vertical line were related by the Lorentz transformation. That determined them uniquely. This was, and is, too difficult to test experimentally when it differs perceptibly from the Galilean requirements, but what Tables 1 and 2 show is that the clocks cannot conform to the theory, for in order to satisfy some of its requirements (Table 1) they must necessarily violate others (Table 2). Hence the theory fails—not because of mathematical inconsistency but because of inapplicability to the facts of nature.

(3) This point is met in my reply to Prof. Born (p. 1287); there has been no change of co-ordinate system. But, in any event, co-ordinate systems are merely means to an end; the contradiction here is in the end. Suppose A and B are black and X and Y white. Then what is proved is that blacks are men when whites are boys, and blacks are boys when whites are men. You do not change their skins when you bring them mentally to rest.

An analogy will pinpoint the problem. To find the effect of latitude on clock-rate I use two clocks differing only in latitude. Calculation then shows me that A reads 11 o'clock when B is observed to read 10, and that B reads 11 when A is observed to read 10. Neither clock goes backwards. I conclude that the calculation is wrong. Substitute 'uniform motion' for 'latitude' and 'special relativity theory' for 'calculation', and the analogy becomes an identity. The substitution is not invalidated by any differences between latitude and motion: write x for latitude and y for calculation, and the argument is unaffected. My question is: What other conclusion is possible? It is not a mathematical question: a mathematical answer is simply a sign that the point has been missed. Only a sentence is needed, and no correspondent gives that sentence.

Even a brief reflexion on the consequences of a disproof of the special relativity theory shows how fundamental they are. In the first place, 'space-time', as something having objective significance, is abolished, and 'universal time', independent of space, is restored—not, however, as a necessity of thought as in the old days, but as the only concept of time that makes possible a consistent description of nature. There is no limit to the velocities attainable by material bodies, and—unless there is, in fact, an ether of the Lorentz type which modifies bodies moving through it—there is no contraction of rods, slowing-down of clocks, increase of mass, and so on, accompanying motion.

Electromagnetic theory, on the other hand, needs revision, and three possibilities seem open. First, the *ad hoc* assumptions just mentioned, which were introduced by Lorentz to protect his equations from the assaults of observation, may be again called into service. This, however, would raise the new problem, as difficult as that which it displaces, of finding a reason for such arbitrary changes. Secondly, the converse process to Einstein's might be tried. His theory was devised to reconcile the equations of mechanics with those of the Maxwell–Lorentz electromagnetic theory by changing the independent variables in the former so that the equations obeyed the Lorentz transformation to which the latter were invariant. That having failed, the independent variables of the electromagnetic equations might be regarded as functions of the space and time co-ordinates of Newtonian mechanics, and the functions sought which would render those equations invariant to the Galilean transformation: this would be, in effect, to locate the 'space–time' fiction in electromagnetism instead of in mechanics and ordinary experience. It is a purely mathematical problem to discover whether such functions exist. If not, then the only remaining possibility would seem to be a fundamentally different electromagnetic theory, perhaps on the lines adumbrated by Ritz[2] or those which Faraday outlined and Maxwell mistakenly identified with his own[3], or something directly introducing quantum conceptions. This might seem inadmissible in view of the impressive evidence for the Maxwell–Lorentz theory, but it would be no more remarkable than the supersession of Newton's gravitation theory by Einstein's —which, incidentally, need not share the breakdown of the special relativity theory.

But, whatever may be the right solution, electromagnetic theory is so intimately involved in the whole of sub-atomic physics that the entire subject must be submitted to revision if the special relativity theory is disproved, and the importance of this is so great that a clear settlement of the question, before kinematical velocities not negligible compared with that of light become possible, is imperative. This demands a genuine effort to examine the matter with a completely unprejudiced mind. The general impression which these replies give is that the obstacles to the acceptance of my argument are mainly psychological: my communication has been read, not to see if what I say is right, but to see where it is wrong. If my critics could only manage to conceive the possibility that it might be right, I think they would at once see that it is; it is so very simple.

[1] *Nature*, 195, 985 (1962).

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[3] See *Philosophy of Science*, 27, 233 (1960).

OBITUARIES

Dr. L. B. Turner

DR. LAURENCE BEDDOME TURNER, who died in a nursing home on January 28, was born at Charlton, Kent, on April 6, 1886. He was among the earliest of the advanced engineers in Great Britain engaged in research on wireless techniques as applied to practical signalling by continuous electromagnetic waves using the thermionic valve.

L. B. Turner was educated at Bedford Grammar School and King's College, Cambridge, obtaining honours in the Mechanical Sciences Tripos in 1907. Following a year's research in the Engineering Laboratory, he was awarded the John Winbolt Prize for an essay on his work. After acquiring practical experience at the works of Messrs. Siemens Brothers, Woolwich, and at Siemens and Halske in Berlin, he entered the Post Office as an assistant staff engineer in 1910. Here he was engaged in experimental work, and in the design of ship and shore stations. During the First World War he was at the Army's Signal Experimental Establishment at Woolwich, where he designed field apparatus, including portable transmitters for short wave-lengths.

At the end of the War he left the Post Office to return to Cambridge as Fellow of King's College and director of studies in engineering. Later he became a technical adviser on the Postmaster-General's Committee concerned with the establishment of a world-wide long-wave wireless chain, of which the Hillmorton Station near Rugby, and that at Abu Zabal near Cairo formed the first link. While the Rugby Station, still in use, has proved to be an outstanding success as a reliable sending station of world-wide range, the principle of the chain as a whole was overtaken by the development of the short-wave beam system by Franklin and Marconi.

L. B. Turner became a corporate member of the Institution of Electrical Engineers in 1920, and was one of the founder members of the Wireless Section of the Institution. He was chairman of the Section for the session 1922–23, and in his inaugural address he gave an excellent review of the development of the thermionic valve during the early years of its application to wireless signalling. As a supplement to the address he gave an interesting account of some original work on spontaneous fluctuations in valve amplifiers. He patented in 1920 a two-valve circuit arrangement designed to amplify weak radio signals at the same time reducing the strength of prevailing atmospheric disturbances.

The remarkable developments in wireless communication during the First World War demanded new works of reference, and L. B. Turner was one of the first in the field with his *Outline of Wireless*, published in 1921. This was followed ten years later (1931) with his more comprehensive volume entitled: *Wireless: a Treatise on the Theory and Practice of High-Frequency Electric Signalling*. Both books were noteworthy for their clarity of description of the fundamental principles of the subject, with an adequate presentation of practical details.

The versatility of Turner's interests was, however, well demonstrated by a paper he read before the Institution of Electrical Engineers in 1937 on the principles of design of electrical thermostats, and containing a description of a mains-operated isothermal chamber constant to one-thousandth of a degree Centigrade.

During the Second World War he was with the Admiralty Signals Establishment at Portsmouth, where his work was excellent if less well known than during the First World War. He returned afterwards to Cambridge, where he was able to devote himself to further research. He was made a University reader in 1948.

R. L. SMITH-ROSE

Mr. A. Warmisham

ARTHUR WARMISHAM, who died on November 10, read mathematics in the University of Manchester under Prof. H. Lamb and Mr. (later Sir) Charles Darwin, and graduated M.Sc. In 1912 he entered the firm of Taylor, Taylor and Hobson (now Rank Taylor Hobson) as optical designer. In those days the number of university men (apart from chemists) entering industry was small—of mathematicians practically none. There was no technical training in optical design apart from a small course for sight-testing opticians at the Northampton Polytechnic (now the Northampton College of Advanced Technology), so that English optical designers (such as H. Dennis Taylor) had to teach themselves. So it was with Warmisham; practically the only books were Dennis Taylor's *System of Applied Optics*, based on Coddington, and von Rohr's *Formation of Images in Optical Instruments*.

With the First World War came the development of aircraft and the use of photography for reconnaissance. It was found that at the aperture (*f*/4·5) and field (35°) most suitable, English photographic lenses were inferior to one German lens (which, of course, was no longer available). It happened that Warmisham had been examining the 4-element type of anastigmat and had developed a lens remarkably free from coma over just this field and at this aperture, and this property was valuable in improving contrast in the image, facilitating the recognition of detail. The lens (and others) was tested by the Royal Flying Corps side-by-side with the German and found to be superior. It was accordingly extensively used in air photography, and appropriately named the *Aviar*.

After the War his task was to adapt the *Aviar* to professional use, and it became very popular. An apochromatic form was designed for process work.

Improved standards of definition were demanded by the cinematograph industry both for taking and projecting lenses. Warmisham designed a remarkable series of projection lenses of greater aperture to give the brighter picture required for the larger cinemas which were then being built. Another demand came with the introduction of talking films. Hitherto, variation in the scale of the image (for 'close-ups', etc.) was made by moving the camera to and fro, with visual focusing. This was objectionable when sound was recorded. All extraneous noises had, of course, to be eliminated. Warmisham visited the United States and there invented a lens of variable focal length. By variation of the separation of the components not only was the focal length changed (and with it the scale of the picture), but the image was kept automatically in focus throughout the range of movements, without shift of the camera. This was not the first 'variable focus' lens but the first to achieve an acceptable standard of definition over a wide range of focal lengths, at high aperture. 'Zoom' lenses, as they are called, are now widely used in cinematography, television and still photography and projection.

Warmisham published little, but he was a prolific inventor, as the files of the Patent Office show—in fact, he invented more than he could develop to marketable form. Many of to-day's well-known optical designers received their early training under his guidance, and the wide use and adaptation of his methods under present-day conditions in Great Britain and overseas is a tribute to his contribution to the art.

'A. W.', as he became known, was no respecter of persons and had a caustic yet humorous way of expressing somewhat candid opinions, but these were given with a Puckish grin that disarmed resentment. H. W. LEE

NEW FELLOWS OF THE ROYAL SOCIETY

At a meeting of the Royal Society on March 21, the following were elected to fellowship of the Society:

Dr. J. B. Adams, director of the Culham Laboratory of the U.K. Atomic Energy Authority, Abingdon, distinguished for his contributions to the design and construction of the 25-GeV proton-synchrotron at the European Centre for Nuclear Research, Geneva.

Sir Eric Ashby, Master of Clare College, Cambridge, distinguished for his outstanding services to the advancement of science and for his contributions to botany.

Prof. H. N. Barber, professor of botany in the University of Tasmania, Hobart, distinguished for his work in experimental cytology, population genetics and the genetics of flowering.

Prof. E. H. S. Burhop, professor of physics in University College, London, and at present at the Accelerator Division, CERN, Geneva, distinguished for his contributions to experimental and theoretical atomic and nuclear physics, particularly to the Auger effect and atomic collision processes.

Prof. H. G. Callan, professor of natural history in the University of St. Andrews, distinguished for his researches in the field of cytogenetics and for his analysis of the synthetic functions of chromosomes.

Dr. J. W. S. Cassels, Caley lecturer in mathematics in the University of Cambridge, distinguished for his contributions to the theory of numbers and in particular to Diophantine approximation and the arithmetic of curves of genus one.

Prof. T. N. George, professor of geology in the University of Glasgow, distinguished for his researches on the stratigraphy and palæontology of the Lower Carboniferous and the application of palæontology to the theory of evolution.

Prof. J. L. Gowans, Henry Dale research professor of the Royal Society, Sir William Dunn School of Pathology, Oxford, distinguished for his elucidation of the life-history and physiological function of the lymphocyte.

Dr. P. B. Hirsch, lecturer in physics in the University of Cambridge, distinguished for his researches with the electron microscope on imperfections in the crystalline structure of metals and on the relation between structure defects and mechanical properties.

Dr. J. H. Humphrey, head of the Division of Immunology in the National Institute for Medical Research, London, and deputy director of the Institute, distinguished for his immunological researches, in particular on the synthesis of antibody and the mechanisms of hypersensitivity.

Prof. P. L. Krohn, professor of endocrinology in the University of Birmingham, Department of Anatomy, distinguished for his researches on the transplantation of endocrine glands, particularly in its bearing on the ageing process.

Dr. D. Küchemann, deputy chief scientific officer, Royal Aircraft Establishment, Farnborough, distinguished for his theoretical and experimental work in aerodynamics, particularly in studies of wing-forms for supersonic flight.

Dr. M. S. Longuet-Higgins, senior principal scientific officer, National Institute of Oceanography, Wormley, Surrey, distinguished for his contributions to physical oceanography and especially for his investigations into ocean waves.

Dr. J. F. Loutit, director, Radiobiological Research Unit, Medical Research Council, Harwell, distinguished for his work in radiobiology, and particularly for his interpretation of recovery from radiation injury.

Dr. J. W. G. Lund, senior principal scientific officer, Freshwater Biological Association, Ambleside, Westmorland, distinguished for his work on freshwater biology, particularly on the taxonomy and ecology of algae.

Dr. Sheina MacAlister Marshall, principal scientific officer, Scottish Marine Biological Association, Marine Station, Millport, Isle of Cumbrae, distinguished for her researches on the productivity of the sea and on the bionomics of marine planktonic organisms.

Prof. P. T. Matthews, professor of theoretical physics in the Imperial College of Science and Technology, London, distinguished for his contributions to quantum field theory and the theory of elementary particles.

Dr. B. Y. Mills, senior principal research officer, Division of Radiophysics, Commonwealth Scientific and Industrial Research Organization, Sydney, Australia, distinguished for his contributions to radio astronomy and especially for the development of a novel form of high-resolution radio telescope.

Dr. C. G. Phillips, Fellow of Trinity College and reader in neurophysiology in the University of Oxford, distinguished for his work on the physiology of the central nervous system, in particular on the control of muscular movement.

Dr. J. A. F. Roberts, director, Clinical Genetics Research Unit, Medical Research Council, Hospital for Sick Children, London, W.C.1, distinguished for his researches into human heredity, particularly in relation to mental ability.

Dr. L. Rotherham, Board member for Research and Development, Central Electricity Generating Board, London, distinguished for his work on the manufacture of new metals and for his contributions to research associated with the development of the generation of nuclear power.

Dr. T. S. Stevens, reader in organic chemistry in the University of Sheffield, distinguished for his original researches in organic chemistry, particularly for his work on the mechanism of molecular rearrangements.

Dr. T. M. Sugden, reader in physical chemistry in the University of Cambridge, distinguished for his work on the detection and identification of transient species, radicals, ions and electrons, in flames and on the reaction mechanisms involved.

Dr. D. Tabor, lecturer in physics in the University of Cambridge and deputy director of the Research Laboratory for the Physics and Chemistry of Solids, Cavendish Laboratory. Distinguished for his original work on the physical and mechanical properties of solids.

Prof. A. J. C. Wilson, professor of physics in the University College of South Wales and Monmouthshire, Cardiff, distinguished for his contributions to X-ray analysis in the elucidation of the atomic structure of crystals.

NEWS and VIEWS

Royal Society Research Professorship: Prof. G. H. Beale, M.B.E., F.R.S.

THE first of the Royal Society research professorships, for which the Government made provision in 1962, has been awarded to Dr. G. H. Beale, of the Department of Genetics, University of Edinburgh. G. H. Beale was born in London in 1913, and graduated in botany at the Imperial College of Science and Technology in 1935. From then until 1940 he worked on flower pigments at the John Innes Horticultural Garden, being one of the group of workers who, under the inspiration of J. B. S. Haldane, made the first successful attempt to describe gene effects in biochemical terms. During the Second World War he served in the Army, spending two years in northern U.S.S.R. and another two in Finland. During 1946–47 he worked on bacterial genetics in Demerec's laboratory in Cold Spring Harbor, Long Island, and then moved to Indiana with a Rockefeller fellowship to study the genetics of *Paramecium* with Sonneborn. He returned to Great Britain in 1948, and joined the Institute of Animal Genetics at Edinburgh, where he has remained ever since, first as a lecturer and later as reader in the University Department of Genetics. He was elected a Fellow of the Royal Society in 1959.

Since his return to Britain, Prof. Beale has devoted his attention to *Paramecium* and in particular to the complex and revealing interplay between cytoplasmic factors and nuclear genes in controlling the appearance of antigenic substances. By the time his monograph on *The Genetics of Paramecium aurelia* appeared in 1954, he had already shown that the antigens are controlled by several gene loci, at each of which a number of alleles exist, and that it is some condition or 'state' of the cytoplasm that decides which of the gene-loci shall be active and bring about the appearance of its characteristic antigen. This is a system of the greatest theoretical importance, since it is exactly of the kind that has often been postulated to explain the processes of embryonic development, but has the advantage of being much more accessible to analysis, both genetical and biochemical, than any known embryonic system. In recent years considerable progress has been made in characterizing the chemical nature of the antigens; they are proteins, of rather high molecular weight, but quite accessible to detailed study. The nature of the cytoplasmic 'states' is more obscure; but Beale and his associates have recently discovered a quite unexpected type of cytoplasmic entity, namely particulate elements, which they name 'metagons', capable of persistence but not of multiplication in the absence of a corresponding gene, and involved in the maintenance of the symbiotic 'killer' particles which may inhabit the cytoplasm of *Paramecium*.

Pure Mathematics at Monash: Prof. G. B. Preston

PROF. G. B. PRESTON, associate professor of mathematics at the Royal Military College of Science, Shrivenham, has been appointed to the foundation chair of pure mathematics at Monash University. He was formerly a mathematical scholar of Magdalen College, Oxford, and has held an appointment at the Royal Military College of Science since 1949. During 1956–58 he was a visiting assistant professor at Tulane University, New Orleans, where he collaborated with Prof. A. H. Clifford on a monograph on the algebraic theory of semigroups. He was promoted to associate professor at Shrivenham in 1961 under the scheme whereby scientists in the Civil Service are rewarded for research work of special merit.

Scientists in Government Services

IN a written answer in the House of Commons on March 12 to a question regarding action on the recommendations of the Zuckerman report regarding transfer of research staff and interchange between posts in the Scientific Officer Class and in other Civil Service classes, the Financial Secretary to the Treasury, Mr. A. Barber, stated that these recommendations had been fully considered in the Interdepartmental Panel. There were already more than 500 posts in the scientific-officer class established at headquarters of the departments for scientific and general administration, and the use of scientists on this type of work was being extended. While the Committee's recommendations were unacceptable, experience provided little evidence that any significant number of scientists employed in the Civil Service who had a marked capacity for administrative work wished to be employed in this way.

The Parliamentary and Scientific Committee

THE annual report of the Parliamentary and Scientific Committee for 1962 records that the Sub-Committee, confined to Parliamentary members, set up to consider the future of research associations, reported in July, and the report has been submitted to the Minister for Science (Pp. 22. London: Parliamentary and Scientific Committee, 1963). It is hoped that the issues raised may be discussed by representatives of the Committee with the Minister in due course. Discussions and talks during the year covered the Gibb–Zuckerman report; the future of water supplies, water conservation, etc., in Great Britain; technical considerations relating to the Channel tunnel and Channel bridge; problems of diseases arising out of the use of new materials and processes in industry and agriculture; nuclear energy in Great Britain; Soviet science; and recent developments in connexion with mechanical engineering research in Great Britain.

The Microchemical Journal

THE Academic Press will continue to publish *The Microchemical Journal*, under the auspices of the American Microchemical Society (formerly Metropolitan Microchemical Society). The first six volumes were published by Interscience Publishers. The new editor-in-chief is Dr. Al Steyermark, of Hoffmann-La Roche, Inc., Nutley, New Jersey. He is assisted by four editors: H. A. Flaschka, Howard J. Francis, jun., Keiichiro Hozumi and Wolfgang J. Kirsten, and an advisory board. Starting with Vol. 7, 1963, the *Journal* will cover a broader field. Research articles of all phases of chemistry involving small-scale manipulation are eligible for consideration. Manuscripts dealing with both organic and inorganic work relating to the preparation, purification, separation, detection, determination, trace analyses, clinical chemistry, and all types of instrumentation—microscopy, chromatography (thin-layer, paper, gas), spectroscopy (infra-red, ultra-violet, mass, X-ray), nuclear magnetic resonance, and neutron activation, may be submitted to the editor-in-chief. During 1963, four issues will be published. The price for Volume 7 is 16.00 dollars. Further information can be obtained from the Academic Press, 111 Fifth Avenue, New York 3, or Academic Press, Berkeley Square House, London, W.1.

The British Museum (Natural History)

THE British Museum (Natural History) has recently issued the tenth edition of its short *Guide to the Exhibition Galleries* (Pp. vi + 38. London: British Museum (Natural

History), 1963). In general format and contents this is similar to the previous edition of 1962, and the difference is mainly due to four additional pages of text and two extra illustrations. This is chiefly an account of the recently opened botanical gallery and illustrations of their dioramas and wall cases. It is unfortunate that one illustration scarcely does credit to the attractive mineral barytes.

The Durban Museum and Art Gallery

The report for 1961–62 of the Durban Museum and Art Gallery records the completion of the new Hall of Marine Life and the start of five new displays in the entrance hall (Pp. 24. Durban: Durban Museum and Art Gallery, 1963). An illustrated guide to the three major modernized galleries has been issued and several collecting expeditions have been made by the staff. The list of papers published is an impressive one considering the size of the staff.

Department of Scientific and Industrial Research Grants, 1963

D.S.I.R. Research Grants, 1963, summarizes information regarding the Grants for Special Researches awarded by the Department of Scientific and Industrial Research (Pp. vi+9. London: H.M.S.O., 1963. 1*s*. 6*d*. net). These grants are given to supplement resources derived from the University Grants Committee or elsewhere and are not intended to meet the entire cost of research projects or to replace existing resources. The awards are for investigators who are already of acknowledged standing in their subjects. Grants may be given for the support of researches in astronomy, biology, chemistry, geology, mathematics, physics and the sciences between and adjoining these, and also in applied science and technology, and engineering; but applications for assistance in subjects in which the Agricultural Research Council, the Development Commission, the Medical Research Council or the Nature Conservancy are directly interested should be made to the appropriate body. Applications for grants in certain branches of the humanities and social sciences may also be considered where appropriate. Notes on Nato research grants and on senior visiting fellowships of the Organization for Economic Co-operation and Development are included.

Gordon Research Conferences, 1963

The Gordon Research Conferences, the purpose of which is to stimulate research in universities, research foundations and industrial laboratories by fostering the exchange of ideas and information, are to be held this year in four research schools in New Hampshire during the period June 10–August 30. Forty-eight informal conferences, each lasting five days, will be held, their subjects covering a wide range of topics in the physical and biological sciences. Residence and registration charges for each conference amount to 100 dollars. Applications should be addressed to, and further information can be obtained from, W. George Parks, director, Gordon Research Conference, University of Rhode Island, Kingston, Rhode Island.

"Notions of Niche and Competition Among Animals, with Special Reference to Freshwater Fish"

Dr. A. H. Weatherley, of the Department of Zoology, School of General Studies, Australian National University, Canberra, has written to the Editor stating that in his article under the above title, appearing on p. 14 of the January 5 issue of *Nature*, he erroneously attributed a definition of competition among animals by Dr. Alec Milne, Agricultural Research Council Unit of Insect Physiology, to Prof. J. L. Harper, University College of North Wales. Dr. Weatherley wishes to apologize for this error to both Prof. Harper and Dr. Milne.

University News:

Bristol

The following appointments to lectureships have been announced: B. M. Bird (electrical engineering); Dr. A. Keller (physics); Dr. R. H. Ottewill (physical chemistry within the Department of Physical and Inorganic Chemistry).

London

The following titles have been conferred: professor of the archæology of the Roman Provinces, on Mr. S. S. Frere, in respect of his post at the Institute of Archæology; professor of hæmatology on Dr. P. L. Mollison, in respect of his post at St. Mary's Hospital Medical School; reader in pharmacology, on Dr. J. R. Hodges, in respect of his post at the Royal Free Hospital School of Medicine; reader in biophysics, on Dr. R. Miledi, in respect of his post at University College; reader in physiology, on Dr. Patricia P. Scott, in respect of her post at the Royal Free Hospital School of Medicine; reader in human nutrition, on Dr. G. R. Wadsworth, in respect of his post at Queen Elizabeth College.

Monash

Prof. M. G. Marwick has been appointed to the foundation chair of anthropology and sociology in the University. Prof. Marwick is at present professor of social anthropology and head of the Department of Social Anthropology and African Administration in the University of the Witwatersrand. During 1962 he was Simon Senior Research Fellow in the University of Manchester.

Announcements

From January 1963, the Council of Scientific and Industrial Research, New Delhi, is issuing research communications in a series of four periodicals: *Indian Journal of Pure and Applied Physics*; *Indian Journal of Chemistry*; *Indian Journal of Technology*; and the *Indian Journal of Experimental Biology*. The *Journal of Scientific and Industrial Research* will continue with all the features included up to December 1962 in its Section *A*.

The Low Temperature Research Station, Cambridge, will hold open days during May 22–23, at which recent and current work will be displayed. Further information can be obtained from the Director, Low Temperature Research Station, Downing Street, Cambridge.

A Symposium on "Moulds for Reinforced Plastics", arranged by the Plastics Institute Reinforced Plastics Group, will be held in London on May 28. Further information can be obtained from J. N. Ratcliffe, Plastics Institute, 6 Mandeville Place, London, W.1.

The annual conference of the Ergonomics Research Society will be held at the College of Aeronautics, Cranfield, during April 1–4. Subjects under discussion will include: process control; workplace layout; machine tools; engineering production; the comprehension of engineering drawings; the effects of heat; the teaching of ergonomics; the future of ergonomics; criteria in ergonomics; the elderly worker in industry. Further information can be obtained from Mr. David Whitfield, Ergonomics Laboratory, College of Aeronautics, Cranfield, Bletchley, Bucks.

A symposium on "The Colorimetry of Near Whites", organized by the Colour Group (Great Britain), will be held at the Imperial College of Science and Technology on April 3. The programme will include: the measurement of whiteness; the scan-test reference instrument for reflectometry; the colorimetry of fluorescent whites; light sources for matching near whites. There will also be an exhibition of colorimeters, spectrophotometers, light sources, colour atlases, colour filters, colour vision tests and allied equipment. The group's first Newton Lecture entitled "The Rays are not Coloured" will be delivered by Prof. W. D. Wright. Further information can be obtained from F. J. B. Wall, Physics Research Laboratory, Ilford Ltd., Woodman Road, Brentwood, Essex.

THE BRITISH BROADCASTING CORPORATION

ANNUAL REPORT

THE annual report for 1961–62 of the British Broadcasting Corporation* welcomes the recent White Paper, *Broadcasting: Memorandum on the Report of the Committee on Broadcasting*†, in so far as it affects the B.B.C., while noting that the Government accepts responsibility to see that the Corporation secures sufficient income to carry out its additional tasks. With the report are published the accounts for the year ended March 31, 1962, showing an income from licence revenue of £36·7 million, expenditure of £13·55 million on operating the sound service, and £20·2 million on the television service, with a grant-in-aid totalling £7·38 million for the External Services.

Immediate steps were taken towards bringing into operation at the earliest possible date the Government's proposals for more hours of sound broadcasting, a second television programme (see following article), a start to colour television, more Welsh and Scottish television and more educational programmes for adults on the present television service. The growth of external broadcasting is particularly stressed, and over the past ten years the combined output of the twelve major countries broadcasting to external audiences has more than doubled, although in the same period the output of the Corporation has declined. The field is now led by the U.S.S.R., the Soviet satellite countries, the Voice of America and Communist China—the last mentioned displacing Britain from the fourth position in 1962. Much of the increased output is directed towards the Asian countries, those of Latin America and the emergent countries of Africa. For this reason, the Corporation warmly welcomes the Government's approval early in 1962 of a major programme of relay-transmitter building which will increase the range and effectiveness of some of the Corporation's transmissions overseas. Some services elsewhere, however, were cut, and the closing down of operations in the United States is particularly regretted. The Governors record their conviction that it is in the national interest that these external services should be maintained over a broad front. Reference is again made to selective jamming of the Corporation's Russian service and to deliberate full-scale jamming of the Hungarian, Rumanian, Bulgarian, Kuoyu and Cantonese services.

Commenting on sound programmes, the report notes the success which attended the concentration into brief space of a series of broadcasts on the Third Programme: for example, a series of scientific broadcasts on "Quanta and Reality" were repeated in a single evening, and five talks and a discussion entitled "Strategy of Survival" were concentrated into two evenings in one week. Much of the year was occupied with preparations for the first trans-Atlantic programme exchanges in association with the Post Office, other European broadcasting organizations and the sponsors of the American communications satellites. The Corporation was also engaged in extensive research into the potential of local broadcasting in Britain. The direct contribution to further education was mainly through broadcasts on Network Three, designed to meet the needs and interests of various minority groups; a new Saturday morning television series, "Science on Saturday", introduced in the summer of 1961, was a first step towards meeting the needs of those wishing to use television for the planned study of science by adults.

Reception in many parts of the United-Kingdom continued to be unsatisfactory because of interference from foreign stations, and although certain technical measures taken improved matters to some extent, with the present overcrowding of the medium wave-band there is nothing further that the Corporation can do to reduce the effect of the interference. The Corporation continued to develop its very-high-frequency services, which are virtually free from interference, and it is estimated that about 4·6 million very-high-frequency receivers are now in use. Much was done during the year to achieve greater reliability and precision in the performance of broadcasting apparatus, and transistor and printed circuit techniques promise great increased reliability and possibly considerable savings in maintenance. Preparations were made for the new series of experimental 625-line transmissions on the very-high-frequency Bands IV and V, including both black-and-white and colour television to begin in the autumn of 1962, and attention has also been turned to the propagation of ultra-high-frequency signals over the sea. With a change in the pattern of engineering recruitment, arising from an increasing demand for engineers in proportion to technical assistants, the Corporation has been less able to meet its full need of engineers by internal promotion. Besides a higher recruitment of qualified men, the Corporation has stepped up its scheme of sandwich courses, but the training facilities have been much more heavily loaded in consequence. Staff at March 31, 1962, numbered 17,125 full-time and 887 part-time, compared with 16,375 and 1,140 in 1960–61.

SECOND TELEVISION PROGRAMME

The opportunities in planning the British Broadcasting Corporation's second television programme are outlined in extracts from an address given at the University of Leeds by Mr. K. Adam, director of B.B.C. Television, and now published in the *B.B.C. Record* (No. 15; November 1962). The second programme is expected to start in April 1964 on 625 lines in very-high-frequency from Crystal Palace and to have reached the North of England by the summer of 1965. It is proposed to begin with at least 25 h of fresh material per week, concentrating on the peak hours each evening with extra hours at the week-end, and in any one evening to cater in depth for a special section of the total audience by, for example, a long play, a feature film, a full-length broadcast of sport, edited versions at length of political and scientific conferences or open-ended discussions. It is uncertain what transmission standards of colours will be used, and Mr. Adam pointed out that colour will always be very expensive and may never average more than one or two hours a night. It is hoped to recruit new writers and to encourage new styles of writing, and Mr. Adam also referred to the possible organization of programmes in collaboration with universities and learned societies.

Science must have its full place in this second programme, and a working group has been set up which will be visiting all regional headquarters to ensure that regional studios are used to their maximum capacity in 1964. In *B.B.C. Record* (No. 16; January 1963) further details are given of production plans which contemplate at least three more London production studios by April 1964, with a fourth to permit expansion to 30 h. Colour transmissions will start before the end of 1964 and the first major production studio at the Television Centre equipped for colour should come into operation in the autumn of 1965. Two-thirds of the population should be receiving the new programme in 1966. The Liaison Committee set up, with Mr. J. S. Fulton as chairman, to guide the Corporation in providing adult education programmes was to hold its first meeting on January 30.

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* The British Broadcasting Corporation. Annual Report and Accounts for the year 1961–62. (Cmnd. 1839.) Pp. 166+16 photographs. (London. H.M.S.O., 1962.) 10s. net.

† *Broadcasting: Memorandum on the Report of the Committee on Broadcasting*, 1960. Pp. 12. (Cmnd. 1770.) (London. H.M.S.O., 1962.) 1s. 3d. net. [See also *Nature*, 195, 927, 1962.)

In the first of a series of lunch-time lectures at Broadcasting House, published as *B.B.C. Lunch-time Lectures—I*, Mr. L. Miall discussed the future of B.B.C. Television after explaining why a new service must be started in what are, for the Corporation, quite new and more difficult broadcasting bands—the very-short- or the ultra-high-frequency bands known as *IV* and *V*. These bands will involve, in effect, new sets for viewers but will give much more space to accommodate new programmes. Mr. Miall emphasized that the process of changing over and building new transmitters, which will gradually carry the Corporation to all parts of the country, is a huge engineering task, involving a capital cost likely to exceed £40 million and building and operating more than 250 new transmitters as well as many low-powered booster transmitters. Co-siting with the Independent Television Authority is essential if the amenities of the countryside are not to be damaged excessively. In indicating some of the plans for new programmes, Mr. Miall concluded by stressing the necessity of avoiding any debasement of the medium.

Lecture 2 dealt with B.B.C. news and current affairs, and, after claiming that the Corporation endeavours to achieve a balance between what the public wants and what the maturer members of the public needed, Mr. D. Edwards forecast great developments in relation to science, which more and more provides news. The Corporation hopes to help the increasing number of scientists, and, besides building up the number of its science producers, he thought that the Corporation would provide in its second television programme a regular programme, doing for science what "Monitor" has done for the Arts. A third lecture, by M. Pulling, on "International Television" pointed out that viewers in Britain saw less of *Eurovision* than viewers in many other European countries. However, while the complete elimination of the 405-line standard in Britain and the 819-line system in France would take many years, an important improvement would result both in the quality of pictures from other countries seen in Britain and in those from Britain seen in other countries: benefit would begin with the start of the second television programme. Finally, the facilities offered by a successful satellite communications system seemed sufficiently attractive to justify the immense efforts required.

THE LEVERHULME TRUST

THE fourth report of the Leverhulme Trust covers the years 1959–61, in which grants and awards totalling £885,000 were committed as compared with £593,000 during the previous triennium[*]. While the expansion is due to a widening of the field of interest of the Trustees, the general policy remains one of initiating new ventures, and neither capital grants for buildings or equipment nor general appeals are considered.

The primary concern is still fellowships and scholarships, and, of the total, 42 per cent has gone to universities and other forms of higher learning and 21 per cent to medical research. Of the £371,150 for universities, £104,400 was for overseas visiting fellowships and £50,000 for fellowships in general research at the University of Liverpool. £6,600 went to the Department of Animal Husbandry and Veterinary Surgery at the University of Bristol for research into digestibility and into anaesthesia in domestic animals; £5,100 for biochemical research on the cell nucleus at the University of Edinburgh; £13,500 to the University of Hull for research fellowships on Commonwealth studies; £12,950 to the London School of Economics for scholarships and research awards; £15,000 to the Institute of Education, University of London, for an advanced training course concerned with the use of mass media, and £3,300 to the Institute's Research Unit for Student Problems. The University College of South Wales and Monmouthshire received £10,000 for business administration fellowships, the University College of Swansea £9,000 for research scholarships in social science, the University of Manchester £4,400 for an extension of research fellowships and studentships at Jodrell Bank Experimental Station, and £11,600 was provided for overseas visiting fellowships for the Manchester and Glasgow Colleges of Science and Technology. Besides £31,500 for a professorship in geology at the University of the Punjab, £9,000 went to the Kenya College of Social Studies for the salaries of two tutors, and £5,000 for overseas visiting fellowships for the universities of Holland.

Awards totalling £46,550 to learned societies and research institutions include £2,000 to the Acton Society for its inquiry into the role of the arts graduates in industry; £6,000 to the British Association for research on the Lowenfeld–Dienes method of teaching mathematics; and £19,900 to Political and Economic Planning for its study of trade unions in a changing society and £4,100 for its study of international government. The Institute of Community Studies received £4,000 towards various research projects which include various studies in depth of the experience of students at Cambridge, Manchester and Southampton of factors determining the choice of specialization, the compatibility of effective vocational training with a broader intellectual education, comparative quality of different methods of teaching, the effect of halls of residence, living in college, and unions and societies on vitality of the university and relations between staff and students, and the Institute of Strategic Studies £3,250 for the salary of a research assistant.

Of the awards totalling £184,900 for medical research, £90,000 was to the African Research Foundation in East Africa for cancer research, £28,000 to the East Grinstead Research Trust for a research fellowship and laboratory assistants, and £3,900 to the Department of Education of the Deaf, University of Manchester, for research on hearing aids for children. Grants for research fellowships included £5,150 to the Institute of Dental Surgery, £6,000 to St. Mary's Hospital Medical School, and £6,900 for a surgical research fellowship at Guy's Hospital. The Royal College of Surgeons received £12,000 for continuation of a research fellowship in the Faculty of Anaesthetists, and £6,000 for continuation of one in the Department of Physiology; the Institute of Laryngology and Otology £4,050 to extend research on the physiology of hearing and balance into the central nuclei and tracks in the brain, and University College Hospital Medical School £5,000 for the interchange of students between London and Ibadan. Various other grants were to the London School of Hygiene and Tropical Medicine, including £9,000 for secondment to Makerere College, Uganda, £3,000 to the Ross Institute, and £2,500 to the Human Nutrition Research Unit.

Besides awards of £3,000 for archaeology, miscellaneous grants included £12,000 to the Commonwealth Society for the Deaf for training African students as teachers of the deaf, £15,000 to the Royal Commonwealth Society for Voluntary Services Overseas, £7,500 to the Educational Interchange Council for Research Scholarships, and £32,500 to the National Bureau for Co-operation in Child Care.

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[*] The Leverhulme Trust. Fourth Report of the Leverhulme Trustees, 959–1961. Pp. 61. (London: The Leverhulme Trust, 1962.)

IMPERIAL COLLEGE SIERRA LEONE EXPEDITION 1963

THIS summer, Imperial College of Science and Technology is sending an expedition of undergraduate biologists to Sierra Leone. The main purpose of the expedition is to carry out a comprehensive study of the flora and fauna of the Northern Province. It is hoped to direct particular attention to the Scarcies River basin and, if possible, to the Loma Mountains. This will be the third expedition from the College to Africa in two years, others being to Nigeria and Ethiopia. The expedition is being made up of five botanists and three zoologists. Two members have had previous expedition experience and all the botanists have worked on the vegetation of the montane region of southern Yugoslavia. An extensive programme of collection and observation is being worked out. It is hoped to bring back several thousand specimens: these will be deposited either at the Kew Herbarium, the Commonwealth Mycological Institute, or the British Museum (Natural History).

In addition to the general survey, the expedition is undertaking work on behalf of the Food and Agriculture Organization. In particular, a survey will be made of indigenous food plants, and it is hoped to collect wild coffee plants of possible value for future taxonomic or breeding work. The zoologists will be primarily concerned in making observations and collections of the fauna of the area, and in particular on the Heteroptera and Coleoptera. A collection of Mollusca will be made to continue work by the Nigerian Expedition on the Mollusca of West Africa. Collections of mammal and bird parasites will also be made, and blood smears taken for pharmaceutical purposes.

The expedition will spend approximately eight weeks in Sierra Leone, and a full report will be published. If the aims of the expedition are accomplished then further expeditions may well continue the work in future years.

P. ROGERS

TROPICAL PARASITOLOGY

THIS symposium, the first regional symposium of its kind, was organized jointly by the Unesco South-east Asia Science Co-operation Office and the Department of Parasitology, University of Singapore. It was held at the Medical School, University of Singapore, during November 5–9, 1962. It was convened under the chairmanship of Prof. R. S. Desowitz. The purpose of the symposium was to provide an opportunity for an exchange of experiences and results among research workers in the field of tropical parasitology.

The conference was attended by delegates from Australia, Ceylon, Hong Kong, India, Indonesia, Japan, Malaya, the Philippines, Singapore, Taiwan, Thailand and Vietnam. These delegates, as well as representatives from the World Health Organization and the Food and Agriculture Organization, had been invited by Unesco to attend the conference. Dr. N. Ansari, chief of Parasitic Diseases Branch, represented the World Health Organization.

The symposium opened with an address of welcome by the vice-chancellor of the University of Singapore, Dr. B. R. Sreenivasan. This was followed by an introductory address by Mr. L. Matteson, director of the South-east Asia Science Co-operation Office, Djakarta.

The first day was taken up by a series of survey papers relating to the background and progress of work in the field of parasitology in the various countries included in the region. An interesting feature of these reports was their refreshing variety of approach. Each report, in its different way, described the background of endeavour which had resulted in the establishment of the various institutions where parasitology is studied throughout the South-east Asian region and gave details of the investigations which were being carried on. These reports constituted a valuable contribution and comprise not only a useful compilation pertaining to the history of parasitology in this region but also an index of personnel, institutions, and bibliographies.

Although the scope of these reports was by no means uniform, it was nevertheless a prominent feature that they emphasized the need for further co-operation and interchange of ideas among parasitologists in the South-east Asian region.

It was evident that there has been a marked improvement in the overall facilities available for research on parasitic diseases in the tropics. Places which were once little more than field stations have now become centres of basic parasitological research. The important implication of this is that the most up-to-date laboratory apparatus is now within relatively easy access to the front line of research in the parasitological field, so that direct investigations on the important disease-producing parasites can be conducted. Previously, it had so often occurred that the basic observations on morphology, physiology, immunology, etc., were restricted to parasites which could be maintained in the laboratory.

On the other hand, it was also evident that the old problems of disease and untimely death are still the main concern of parasitologists over most of the South-east Asian region. But the vigorous research programmes relating to the diagnosis and control of parasitic diseases now operating in many of the South-east Asian countries, coupled with the frequent visits being made by specialists in various fields, and the close liaison which has evidently been established with universities and research centres in other parts of the world, gives promise of a successful campaign against the protozoa, worms and arthropods which still cause such widespread disease in tropical regions.

This overall emphasis on medical parasitology is in contrast with the situation in Australia, where the parasites of domestic animals appear to comprise the most important application of parasitology, and where it is evident that funds for research in the field of veterinary parasitology far exceed those available for medical parasitology.

Three full days were devoted to research papers; these again showed a remarkable diversity of interest. Thus, there were papers of a clinical nature, some dealing with lesions and symptoms, others with method of control, epidemiology, and treatment. But there were also papers devoted to the basic principles of parasitology, such as the immunology of parasitic infections, the physiology and metabolism of parasites, as well as systematics, ecology, evolution, and phylogeny in relation to parasitic organisms.

The whole symposium achieved a balance of emphasis between, on one hand, the fundamentals of structure and function in relation to parasitism, and, on the other, the distress and hardship which parasitism involves.

It is not intended to summarize the content of these individual papers, because it has been arranged that a full report of the proceedings of the symposium will be published. Suffice it to say that the admixture of hypothesis, observation and application contributed to no small extent to the feeling which prevailed that we were all, in spite of widely differing specializations, primarily parasitologists.

The delegates were accommodated in the King Edward VII Hall of the University of Singapore near the Medical School. This contributed in a special way to the success of the symposium. Scientific meetings are becoming more and more intensive, consequently programmes are sometimes so full that there is virtually no time for discussion. This means that the younger delegates have little or no opportunity for discussion of their problems with their more experienced colleagues, who are quite understandably occupied with exchanging greetings with their old friends. This symposium showed the value of delegates living in the same residence and sharing the same table at meals. Ample opportunities were thereby available for discussion, and as a result, one really felt that this had been a conference in the true sense, because all delegates had had an opportunity to become acquainted. In this way many new friendships were made, and much valuable information was imparted between the meetings.

The last day was devoted to a discussion on future policy in relation to co-operation and interchange between parasitologists in the South-east Asian region. Several recommendations were made which, it is hoped, will help to formulate a policy which will ensure further meetings of this kind and possibly the formulation of a permanent panel for the region.

Delegates unanimously resolved to record their sincere appreciation of the services rendered to international science in the field of parasitology to Unesco coupled with the name of its regional representative, Mr. L. Matteson, and to the host country, Singapore, coupled with the name of Prof. R. S. Desowitz, for the excellent arrangements which had been made. All agreed that the conference had been an unqualified success.

J. F. A. SPRENT

COSMIC NUMBERS

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IT has been suggested many times (perhaps first by Weyl[1]) that there might be a close connexion between the separate domains of atomic or nuclear physics and cosmology. This view is admittedly intuitional and the only evidence in support of it so far comes from the numerical coincidences of large dimensionless 'cosmic numbers'. The most typical of these numbers are[2]:

$$\frac{R}{a_0} \simeq \frac{cT}{e^2/m_e c^2} \simeq 4 \times 10^{40} \tag{1}$$

$$F_1 = \frac{e^2}{G m_p m_e} = 0{\cdot}3 \times 10^{40} \tag{2}$$

In (1) the characteristic radius of the universe $R \simeq cT$ is expressed in terms of the classical electron radius $a_0 = e^2/m_e c^2$, where T^{-1} is Hubble's constant and $T \simeq 1{\cdot}3 \times 10^{10}$ light years is a characteristic age of the universe. The result is similar if the pion Compton wave-length (indicating the range of the nuclear forces) is used instead of the classical electron radius. In (2) the 'force ratio' F_1 is the ratio of the Coulomb and gravitational forces between an electron and proton. In (1) and (2) e is the charge, m_p and m_e are the masses, G is the gravitational constant, and c is the velocity of light. If g is the pion–nucleon coupling constant[3] ($g^2/\hbar c \simeq 15$) the ratio of the strong interaction between two nucleons, separated by a distance of approximately a_0, and their gravitational force is:

$$F_1 \simeq \frac{g^2}{G m_p^2} \simeq 0{\cdot}3 \times 10^{40} \tag{2'}$$

and this is an alternative expression for the force ratio F_1. If numbers such as $\alpha = e^2/\hbar c = 1/137$, $g^2/\hbar c \simeq 15$, and $m_p/m_e = 1{,}836$, are regarded as occupying a level of order unity, then we can write:

$$\frac{R}{a_0} \sim F_1 \tag{3}$$

It is possible to construct a range of cosmic numbers, and one of their fascinations is their hierarchical structure. This is shown by the following examples. The gravitational radius of the universe is R, and in the case of a nucleon the gravitational radius is $a_g \simeq G m_p/c^2$ (a nucleon of radius a_g produces curvature in the metric of equal radius), and therefore:

$$\frac{a_g}{a_0} \simeq \frac{G m_p m_e}{e^2} = F_1^{-1} \tag{4}$$

The actual or observable mass of the universe is $M \simeq Rc^2/G$, and its density ρ in terms of the nucleon density $\rho_0 \simeq m_p/a_0^3$ is

$$\frac{\rho}{\rho_0} \simeq \frac{M}{m_p}\left(\frac{a_0}{R}\right)^3 \simeq F_1\left(\frac{a_0}{R}\right)^2 \sim F_1^{-1} \tag{5}$$

Expressing M in nucleon masses we obtain Eddington's number $N = M/m_p$:

$$N = \frac{M}{m_p} \simeq \frac{Rc^2}{G m_p} = F_1 \frac{R}{a_0} \sim F_1^2 \tag{6}$$

and similarly, $R/a_g \simeq F_1^2$.

The numbers considered so far are of the form F_1^n, where n is either a positive or negative integer or zero The cosmic numbers, however, are not limited to only integral values of n. Thus, if a mass has as a radius R_s equal to its gravitional radius $2GM_s/c^2$, and a density $\rho_0 \simeq m_p/a_0^3$ equal to that of nuclear matter, the number of nucleons in the mass is $N_s = M_s/m_p$, or:

$$N_s \simeq (\tfrac{1}{2} F_1)^{3/2} \sim F_1^{3/2} \tag{7}$$

which is close to the number of nucleons in a massive star[4]. It is seen that a star at nuclear density has a radius given by:

$$\frac{R_s}{a_0} \simeq N_s^{1/3} \sim F_1^{1/2}, \quad \frac{R_s}{R} \sim F_1^{-1/2} \tag{8}$$

and is the geometric mean of the radii of a nucleon and the universe. Furthermore, if we use the natural length $\lambda = (\hbar G/c^3)^{1/2}$ for quantum fluctuations of the space-time metric[5], then:

$$\frac{\lambda}{a_0} = \left(F_1 \alpha \frac{m_p}{m_e}\right)^{-1/2} \sim F_1^{-1/2} \tag{9}$$

Using this result further numbers can be constructed. (The mass density of the fluctuations is F_1^2 times that of a nucleon; each cell of volume λ^3 has a mass of $F_1^{1/2}$ nucleons, and there are $F_1^{3/2}$ cells per nucleon.)

There is thus a second group of dimensionless numbers of the form F_1^n where n now has half-integral values. The two groups together form a hierarchy of numbers arranged at the discrete levels of the order F_1^n, where $n = 0$,

$\pm \tfrac{1}{2}, \pm 1, \pm \tfrac{3}{2} \ldots$ Whether there is a third group of quarter-integral values of n of any significance is a matter for speculation (for example, the number of stars per galaxy and the number of galaxies in the Universe is of the order $F_1^{1/4}$) and at the present stage of our knowledge the subject scarcely seems worth pursuing. The apparent coincidence of the numerical values of R/a_0 and F_1 has in the past been a reason for suggesting that the physics of the atom and the cosmos are in some way linked together. The hierarchy of numbers such as (1)–(9) with its simple and well-defined relationship gives further support to this point of view. According to Dirac's principle[6]: "any two of the very large dimensionless numbers occurring in Nature are connected by a simple mathematical relation, in which the coefficients are of the order of magnitude unity". Thus if N_i, N_j are any two of the dimensionless numbers we can state the principle in the form:

$$N_i = C_{ij} N_j F_1^{n_{ij}} \tag{10}$$

where $C_{ij} \sim 1$ is a constant and n_{ij} is a positive or negative integral or half-integral number.

Is there Antisymmetry in Atomic and Cosmic Interactions?

As it stands the force ratio F_1 is not a satisfactory expression of the relative magnitudes of the electromagnetic and gravitational interactions. In small-scale systems, as in atomic physics, it is true to say that the electromagnetic interactions predominate to the extent indicated by (2). But matter tends to be electrically neutral, and in large systems the situation is therefore reversed and it is the gravitational interactions which predominate. It can scarcely be said that F_1 is a universal force ratio for it is limited to either atomic-size systems or to a Universe that contains only a single electron and a single proton. For this reason it is interesting to inquire whether we can derive a second force ratio F_2. In the same way as F_1 expresses the predominance of electromagnetic interactions in atomic-size systems, so F_2 will express the predominance of gravitational interactions in cosmic-size systems.

Let us consider a four-particle universe containing two hydrogen atoms in the ground state. This highly simple universe allows a direct comparison between the interactions of atomic and cosmic systems, and has the advantage of acknowledging the basic electrical neutrality of systems larger than those of an atomic size. If the hydrogen atoms are separated by a distance equal to R, then F_1 applies to the atomic regions within the hydrogen atoms, and F_2 applies to the cosmic region containing the two atoms. As the Coulomb force between the atoms is zero we shall use their magnetic moments $\mu = e\hbar/2m_e c$, aligned parallel or anti-parallel in each other's field, for estimating the magnitude of the electromagnetic interaction. Comparing the relative magnitude of the electromagnetic and gravitational forces between the two atoms separated by the cosmic-like distance R, it follows:

$$F_2 = \frac{3\mu^2}{G m_H^2 R^2} = \frac{3m_e}{4\alpha^2 m_p} F_1 \frac{a_0^2}{R^2} \sim F_1^{-1} \tag{11}$$

where $m_H \simeq m_p$ is the mass of the hydrogen atom.

In this elementary universe the electromagnetic interactions predominate by a factor of F_1 in small systems of atomic dimensions, and the gravitational interaction predominates by a factor of F_1 in a large system of cosmic dimensions. These interactions are thus anti-symmetric in magnitude for the smallest and largest systems possible. Although anti-symmetry of interactions in this sense is interesting we must remember that it applies only to a rather artificial universe and that it does not shed any light on the physics underlying the cosmic numbers. However, the concept of symmetry or anti-symmetry is sufficiently satisfying to reinforce our belief that $R/a_0 \sim F_1$ is a fundamental relation in the physical world.

Cosmology and the Cosmic Numbers

If the atomic constants and also $\hbar$ and c are independent of the age of the Universe, then the relation $R/a_0 \sim F_1$ implies that $G \propto R^{-1}$. This dependence of the gravitational constant on the age and radius of the universe has various cosmological consequences and narrows down considerably the number of possible cosmological models.

Mach's principle[5] (inertia depends upon the distribution of mass in the universe), as formulated by Sciama[7] and Brans and Dicke[8], requires that $GM \propto R$ in a uniform universe of actual or observable mass M. Hence, in a non-static universe Mach's principle is only true if the mass varies as: $M \propto R^2$. In the Lyttleton–Bondi[9] charge-excess hypothesis the charge excess per particle is estimated to be 10^{-18} e. According to this hypothesis a cosmic-size system is characterized by $F_2 \sim 1$. This is not in agreement with a principle of anti-symmetry, for according to this principle any charge excess that might exist should be of the order 10^{-40} e.

Of the various cosmological models that have received attention there are three that conform with the relation: $G \propto R^{-1}$. The first is the steady-state universe[10] in which G and R are both constant. Hoyle[11] has shown that:

$$8\pi\rho G T^2 = 3 \tag{12}$$

where ρ is the mean density of matter. The radius of the observable universe is $R = cT$ and its total mass is given by:

$$\frac{M}{m_p} = \frac{Tc^3}{2Gm_p} = F_1 \frac{R}{2a_0} \tag{13}$$

The steady-state theory fulfils in the simplest and therefore perhaps the most satisfying way the conditions laid down by the cosmic numbers.

The second is Dirac's universe[6]. In this model G is constant in 'dynamic time' but varies in 'atomic time'. With GR constant in dynamic time and mass conserved the dynamic units of time and distance are $t^* = t^2/2t_0$, $l^* = lt/t_0$. Using dynamic units Dirac considers an Einstein–de Sitter[12] universe of zero cosmological and curvature constants. Transforming the results back into atomic units we find:

$$2\pi\rho G T^2 = 3 \tag{14}$$

with $R = \tfrac{1}{2}cT$, and:

$$\frac{M}{m_p} = F_1 \frac{R}{4a_0} \tag{15}$$

The age of this universe is $t = \tfrac{1}{3}T$, and it follows that $G \propto t^{-1}$, $R \propto t$, $\rho \propto t^{-1}$, $M \propto t^2$. The geophysical and astrophysical consequences of G varying inversely with atomic time have been discussed by several authors, particularly by Dicke[13], and at the present stage of the discussion the evidence in support of such an effect is not very convincing. Also, N_s given by expression (7) is approximately the number of nucleons in a star, and it is difficult to see how this number can vary as $t^{3/2}$ when mass is conserved.

A third type of universe has been considered by Jordon[14]. In this particular model the metric has a positive curvature constant, and in order that $G \propto t^{-1}$, $R \propto t$, $\rho \propto t^{-1}$, $M \propto t^2$ in a finite universe matter must be continuously created. This type of model has so far not received any great attention; this may be because there is no clear evidence that G has varied in the course of time. We notice that in the steady-state theory the number N_s is constant and this strongly suggests that the creation process operates uniformly throughout space independently of the stars; in Jordan's theory however $N_s \propto t^{3/2}$ and therefore the creation process must operate primarily within the stars.

All three of the foregoing models obey Mach's principle and yet none is in strict conformity with general relativity. The only existing cosmological model consistent with the equations of general relativity and the principles of Mach and Dirac is the original static universe of Einstein's which is now of historical interest only.

Dicke[15] has questioned Dirac's principle on the grounds that one cannot rule out entirely the possibility that the approximate equality of R/a_0 and F_1 might be nothing more than a characteristic of the present epoch. If F_1 is constant and R increases with time in an evolving Universe Dicke estimates that intelligent life appears when $R/a_0 \sim F_1$. But in this discussion we have seen that the relation $R/a_0 \sim F_1$ has associated with it a hierarchy of numbers $F_1{}^n$ which connects the properties of elementary particles with the metrical properties of space-time. Also $R/a_0 \sim F_1$ is equivalent to a form of anti-symmetry of interaction in atomic and cosmic systems. With only the coincidence of R/a_0 and F_1 to consider we might be willing to believe that Dicke is correct, but it is quite another matter to suppose that an ordered sequence of numbers and anti-symmetry (however feebly formulated) is a coincidence of the present epoch. It seems not unreasonable therefore to believe that the cosmic numbers are evidence of the existence of a fundamental unity of the atom and the cosmos.

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DIURNAL EFFECTS IN MAGNETIC CONJUGACY AT VERY HIGH LATITUDE

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FOLLOWING the general confirmation of the concept of conjugacy from $L \approx 2 \cdot 6$ to $L \approx 5$ by magnetic, Earth current, ionospheric, and auroral techniques it was decided to establish a pair of very high latitude stations to examine the phenomenon polewards of the auroral zone[1]. (L values are McIlwain's parameter[2]. In an undistorted dipole field these would be the geocentric distances to the field line in the equatorial plane, expressed in Earth radii.)

A magnetic station was set up in September 1962 at Shepherd Bay on the Boothia Peninsula of northern Canada (geog. co-ords. 68° 48′ N., 93° 24′ W.), which is approximately conjugate to the New Zealand station of Scott Base in Antarctica (geog. co-ords. 77° 51′ S., 166° 45′ E.). The geomagnetic latitudes are approximately 78·3° N. and 79·0° S. respectively, corresponding to $L \approx 26$.

Because of the inclination of the geomagnetic to the spin axis, the Shepherd Bay station leads Scott Base by approximately 6 h 40 min in rotation, with respect to the Sun.

The period September 15–30 has been examined, by visual comparison of the records, for correlation in H and D disturbances at the two stations. It is evident that a considerable degree of correlation persists in the night-time disturbance even at this geomagnetic latitude (upper two sets of magnetograms of Fig. 1). Disturbances had the same times and usually preserved a similar form, as already confirmed for the auroral zone and at lower latitudes.

However, these polar cap stations also exhibit the well-known day-time maximum[3] and for this the correlation is less apparent. To obtain a rough indication of the diurnal behaviour of the correlation, a number was allocated from 0 to 4, by visual comparison, corresponding to zero correlation, poor, moderate, good, and one-to-one, respectively. A number was allocated to each hour of record and the numbers averaged for each hour over all the days examined. The result is shown in Fig. 2, where the averages have been normalized so that unity corresponds to a one-to-one relationship between the two stations.

There is a broad night-time maximum of good correlation, and an inferior maximum of moderate correlation about midday. Points of poor correlation (often zero

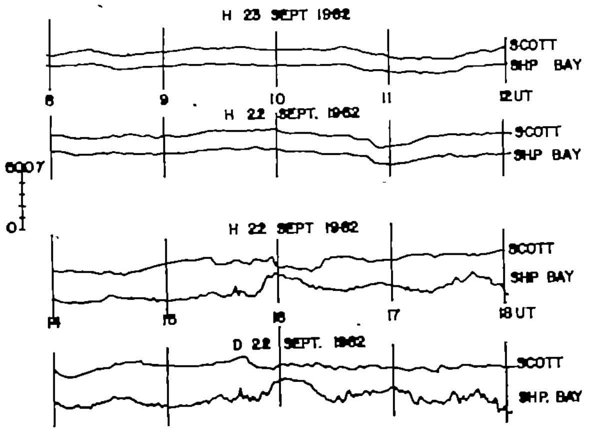

Fig. 1. Comparison of magnetograms from Shepherd Bay and Scott Base. The upper two sets typify good or one-to-one correlation. The lower two sets show poor or zero correlation

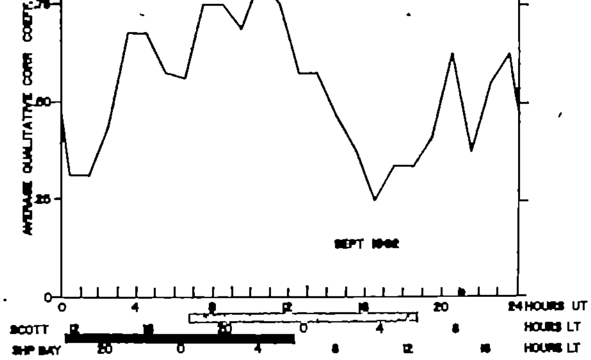

Fig. 2. Variation of correlation between Shepherd Bay and Scott Base magnetic disturbances; U.T. and both local times (L.T.) appear. The shading refers to local night at the two stations (taken as 1800–0600 L.T.)

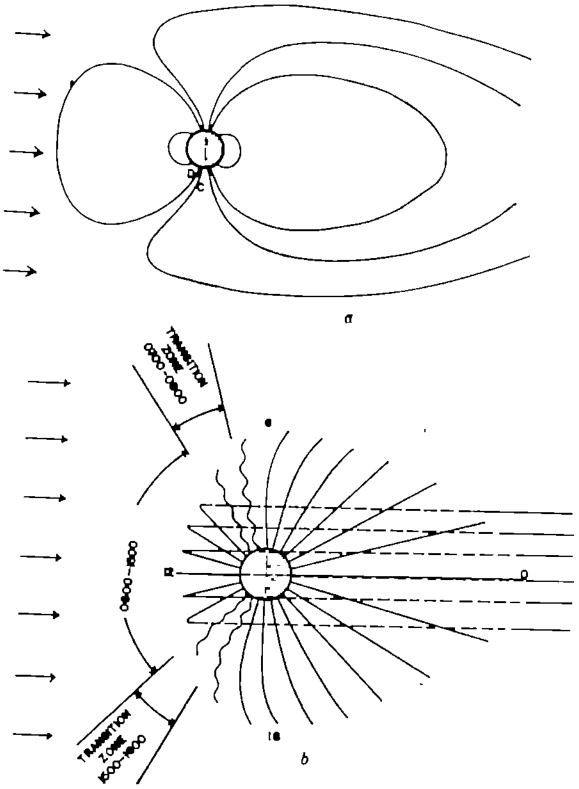

Fig. 3 Distortion of Earth's high-latitude field lines, viewed from the side (above) and from the North Pole (below), due to pressure of solar wind Times given below are 'average local time' (A.L.T.). It is suggested that 'transition zones' occur between alternative long-tail and over-the-pole field paths, and that these time-zones match the times of worst correlation between conjugate stations at very high latitude (Fig 2)

correlation on individual days) divide the two maxima at ~ 0100 U.T. and ~1700 U.T. (see lower two sets of magnetograms, Fig. 1).

One possible interpretation of the foregoing is illustrated in Fig. 3a and b. The night-time maximum corresponds to a field linkage of the conventional dipole form (rather elongated but not grossly distorted), for which conjugacy is well-established in the auroral zone and at lower latitudes. One would expect conjugacy to be maintained around this long field line, though less infallibly than for shorter lines (lower latitudes) where the terrestrial field is stronger. This accords with observation.

As the Earth rotates, the field line approaching the daylight side (0600 h, A.L.T.) encounters steadily increasing resistance from the solar wind[4] and at ~0700–0800 A.L.T. it prefers to link backwards over the polar caps. (A.L.T. here denotes 'average local time', that is, it represents the average orientation of that field plane with respect to the Sun–Earth line at the equinoctial period. 0600 A.L.T. means that Shepherd Bay is 0920 local time (L.T.) and Scott Base is 0240 L.T.) This mode of linkage, which has often been suggested in recent years[5], can be thought of as a 'relaxing' of the system to a more stable configuration of lower potential energy. In fact, the coupled system, magnetosphere plus solar stream, could on this model be treated as a 2-level relaxation oscillator.

From ~ 0800 to ~1500 A.L.T. some degree of conjugacy can again manifest itself via this highly distorted field path, giving rise to the inferior maximum of Fig. 2.

At 1500 or 1600 A.L.T. the field line is encountering sufficiently reduced solar pressure that the normal dipole-type field can be re-assumed. In terms of energy, this corresponds to the returning dominance of magnetic over kinetic energy.

According to the present picture the times 0700–0800 and 1500–1600 A.L.T. might be expected to show poorest correlation between disturbances registered at the two stations, these being the transition or 'relaxation' intervals when a direct field linkage between northern and southern conjugates would be non-existent or erratic. (These are the minima at 0100 and 1700 U.T. in Fig. 2.)

A recent paper by Lebeau and Schlich[6], while not an investigation of a conjugate pair, is relevant because it compares magnetograms from very high latitude stations at approximately the same geomagnetic longitude but differing by about 3° in geomagnetic latitude. The stations, Dumont d'Urville and Charcot (geomag. 75·6° S., 78·3° S., respectively), straddle the zone where a radical change of field linkage might be anticipated, for example, lines *D* and *C* in Fig. 3a.

Correlation coefficients were calculated, based not on the detailed forms of the disturbances but on the magnitude of the disturbance experienced in each hour. Lebeau and Schlich found that: (1) the maximum activity occurred at about midday at both stations; (2) d'Urville was relatively more disturbed than Charcot at all times, but especially so at about midday; (3) the minimum correlation (≈ 0·6) of average activities occurred at midday and the maximum (≈ 0·95) at midnight.

The significant point for us is that although both stations were, on the average, most disturbed about midday, there was least correlation between them at that time. While this might have various interpretations it does not contradict the view that the higher-latitude field line (line *C*, Fig. 3a) has been forced into a different configuration leading to the forward projecting 'horns'. It is then reasonable that lines *C* and *D* should be exposed to relatively independent disturbances.

Another interesting feature of Lebeau and Schlich's result is item (2). This is probably related to the recent finding by O'Brien[7], using *Injun I* data, that the high-latitude termination of electron trapping ($E > 40$ keV) is ~69° ($L \sim 8$) on the night side of the Earth but extends to ~ 75° ($L \sim 16$) on the day side. If the source of the disturbance is associated in some way with the outer 'surface' of the trapped radiation (the 'splash catcher' idea[8]) as appears to be the case for the polar (night-time) aurora, then d'Urville would lie almost under the daytime termination of this surface in the ionosphere, whereas Charcot would lie several degrees polewards.

Referring again to the work of Lebeau and Schlich, it would be helpful to know what correlation exists in the detailed forms of magnetic activity at their two stations, over the course of the day. This might give an indication of the size of the conjugate area at various levels of activity in very high latitudes[9].

Returning to the Scott–Shepherd Bay comparison, one further observation is of interest. Besides the diurnal variation of correlation between the respective magnetograms there is a diurnal variation in the relative intensity of magnetic disturbance, which is best discussed in terms of local time of each station. The amplitude is greater at Shepherd Bay at ~1000 h (Shepherd L.T.), and is greater at Scott at ~ 1300–1400 h (Scott L.T.).

This is explainable in terms of the 'precessional' motion of the Earth's magnetic axis about the spin axis. At 1000 Shepherd L.T. the field line through Shepherd Bay is tilted downwards, with respect to the Sun–Earth line, exposing a greater length of field line, which is therefore presumably more vulnerable to solar shocks at its northern end. At 1300–1400 Scott L.T. the tilt is the other way, the 'underside' of the field being more exposed to the Sun, so one might expect the Scott station to receive the stronger signal, as observed. In either case the enhanced effect is probably due to the combination of circumstances: (1) greater exposure of one 'horn'; (2) shielding of the other 'horn' by the intervening (stronger) magnetic field. But there may well be other factors.

The results presented here were obtained during an equinoctial study, and the argument used in the previous paragraph predicts that a seasonal effect on the relative magnitudes of conjugate disturbances at Shepherd and

Scott should be observed as the year proceeds. As the December solstice approaches the much greater extremes of tilt of the axis (23·50° ± 11° for a centred dipole) should further enhance the signal at Scott relative to Shepherd Bay. The reverse would apply in June.

We must emphasize that the results reported here are very preliminary. Considerably more data, and the calculation of mathematical correlation coefficients, are needed to place the information contained in Fig. 2 on a firm basis.

Data from Scott Base were obtained through the courtesy of Dr. A. L. Cullington, Superintendent of the Magnetic Survey, Department of Scientific and Industrial Research. New Zealand. Equipment at Shepherd Bay was operated by *DEW* line personnel with the co-operation of the Defence Research Board of Canada. This work was sponsored by the Air Force Cambridge Research Laboratories, Office of Aerospace Research, under contract *AF* 19(604)–6180.

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QUANTITATIVE DIFFERENTIAL THERMAL ANALYSIS: HEATS AND RATES OF SOLID-LIQUID TRANSITIONS

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THE reduction of differential thermal analysis to a firm quantitative basis appears of such potential usefulness that numerous attempts have been made to bring it about[1]. The scope of a solution to this problem is being examined. This solution essentially effects a temperature balance between sample and reference thermocouples while a thermal balance is maintained within the sample structure as follows:

(1) Temperature balance between sample and reference thermocouples. An auxiliary heater is inserted in the sample structure. So long as the sample undergoes no change in heat content, ΔH, the heater input is kept at zero or some low constant value. When such a change begins to occur, the input to the heater is increased or decreased for an endothermic or exothermic process respectively. This is done in such a manner that the temperature difference between the sample and reference thermocouples is maintained at the same value as before the change began (Fig. 1). It follows that the change in the electrical energy input, ΔQ, required to accomplish this (Fig. 2), is related to ΔH. However, unless the gains in and losses of heat by the sample structure from and to the surroundings are made to be equal, ΔQ will equal ΔH only fortuitously.

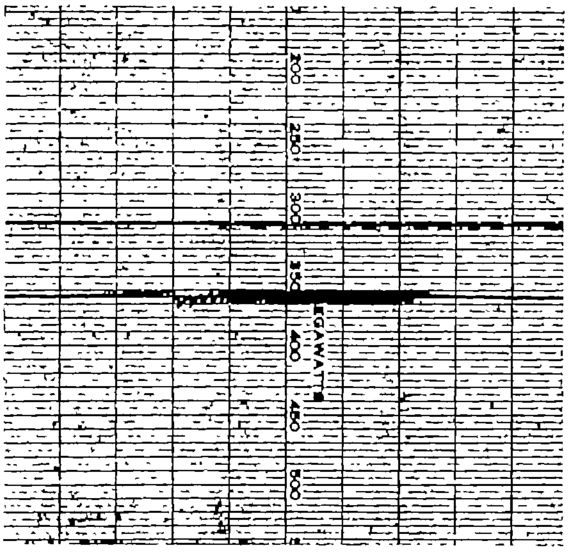

Fig. 1. The appearance of electrically compensated thermal process (fusion of aluminium) on otherwise normal differential thermal analysis record. Heating rate: 0·07° C/min; abscissa; 5 mm/division; ordinate; 0·03° C/division

(2) Thermal balance within the sample structure. In order to obtain a general idea of what the condition $\Delta Q = \Delta H$ entails, the arrangement of Fig. 3 is imagined in which dimensionless heat source H, heat sink S (such as a melting sample) and thermocouple T_s are embedded in a solid which is immersed in a gas. The thermal balance condition:

$$\frac{dH}{dt} = k(T_h - T_m) + \frac{dG}{dt} = k(T_h - T_m) + \frac{dL}{dt} = \frac{dQ}{dt} \qquad (1)$$

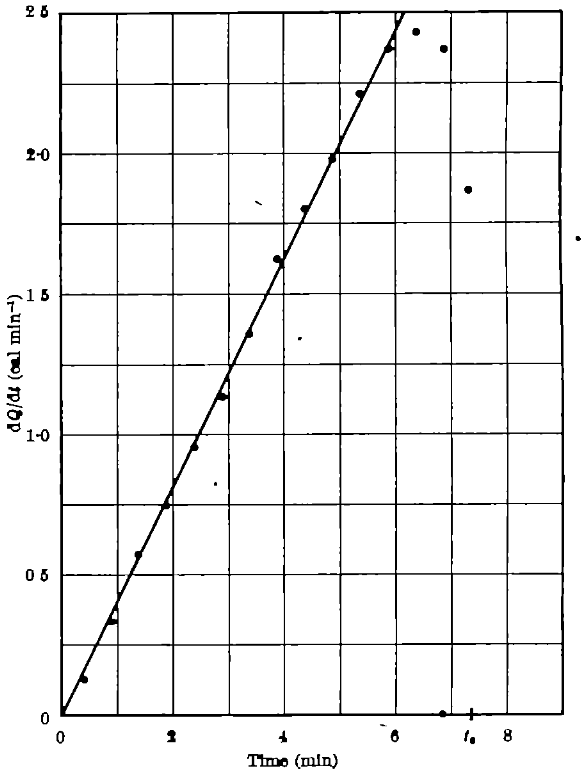

Fig. 2. The rate of electrical energy input into the sample heater as a function of time in order to compensate a thermal process (fusion of tin). Full line; theoretical; points; experimental

where T_m = melting point, T_h = heater temperature and $\frac{dG}{dt}$, $\frac{dL}{dt}$ the rates of extraneous gain in and loss of heat respectively, when considered in conjunction with the appropriate relations[1] of heat transfer, yields:

$$\frac{dG/dt}{dL/dt} = \Delta = \frac{T - T_m}{T_h - T} = \frac{R + rd}{R + r'd'} \qquad (2)$$

where T = gas temperature, d, d' = distances of sample and heater respectively from the adjacent walls of the solid and R, r, R', r' = heat resistance constants[1]. The condition of compatibility between equation (2) and the temperature balance condition, namely, $T = T_c = T_r$, where T_r = the temperature of the reference thermocouple, yields:

$$\frac{D}{D'} = \Delta \qquad (3)$$

where D, D' = distances sample – thermocouple and heater-thermocouple, and Δ is already defined by equation (2).

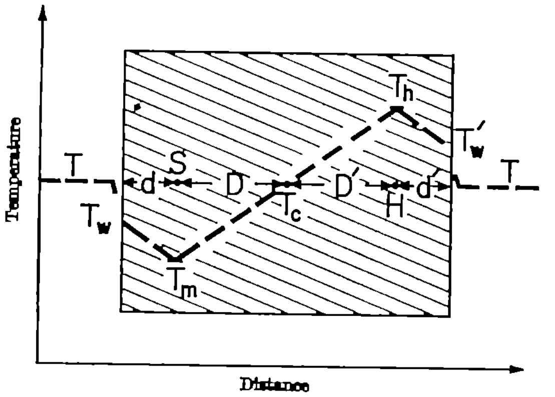

Fig. 3. Thermal balance within imaginary sample structure

For exothermic processes involving initially constant input to H, the foregoing are simplified in that the sum of $\frac{dL}{dt}$ from H and S is held constant before, during and after the process.

Equations (2) and (3) indicate that, even for the idealized model of Fig. 3, the requirements for equality between electrical and process energies are strict. If, for example, $\frac{D}{D'} \neq 1$, then $\Delta Q \neq \Delta H$ even if the result of Fig. 1 is obtained. However, these equations indicate that, in principle, it is possible to adjust or balance the sample structure until $\Delta = 1$ by varying the parameters d, d', D and D' and if necessary R, R', r and r'. The apparent alternative of operating with a known $\Delta \neq 1$ found by calibration is, as will be seen, normally unnecessary.

A practical arrangement that obeys or approximates the general ideas conveyed by equations (2) and (3) may possibly take a number of forms. The approach preferred here was to fold the arrangement of Fig. 3 on itself in the sense of surrounding the sample by the heater (Fig. 4). The outer surface temperature of the heater is kept at T or made to oscillate equally above and below T (Fig. 1), so that for the entire structure $\int dG \simeq \int dL \sim 0$. This is done either: (a) by manually switching the heater on and off at constant power input, the duration of each pulse being such (usually more than 1 sec) that T_c oscillates equally above and below T (Fig. 1); or (b) by adjusting the power either manually or automatically, as by utilizing an electronic controller (work in progress). For the former method the amplitude of oscillations is kept normally below 0·03° C = 1/16 in. pen travel on the recorder. The total 'power on' time is found by a stop-watch or a recorder.

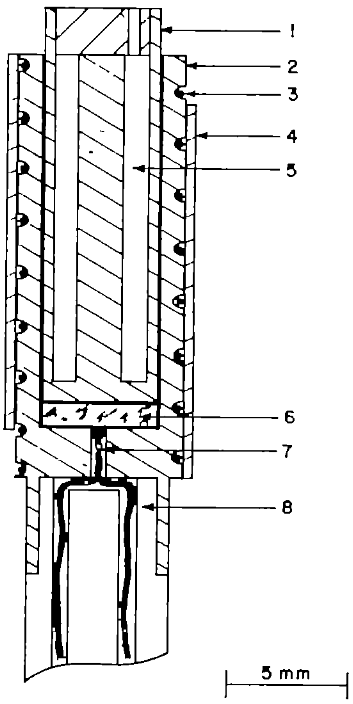

Fig. 4. Cross-section of sample structure: 1, sample container (material compatible with sample); 2, heater casing (nickel); 3, ceramic-coated heater wire (tungsten); 4, heater shield (nickel); 5, sample; 6, insulator (silica); 7, Pt-Pt: 13 Rh thermocouple; 8, ceramic conduit and support (porcelain)

The 'distance' between the thermocouple and the sample container (the concept conveyed by equation (3)) is adjusted until the correct heat of fusion of a substance such as lead is obtained. In order to have a compact design, this distance is increased, not by placing the sample far from the thermocouple, but by inserting a wafer of quartz 1·5 mm in thickness between the two. The bearing of equation (3) is illustrated by the fact that if no insulator is used at all the error becomes + 2 per cent; if the thermocouple is in direct contact with the sample container, but not with the heater casing, the error amounts to + 10 per cent or more depending on the intimacy of the contact; if the thickness of the quartz is increased the error becomes negative, amounting to − 8 per cent for a quartz thickness of 10 mm.

Complete lack of electrical interference between the thermocouple (μV) circuit and the heater circuit is of course necessary.

Once the sample structure was balanced, using lead, the correct heats of fusion were obtained without further adjustment of the sample structure for aluminium, tin and sodium nitrate, thus covering a useful range of temperature (Table 1). Sodium nitrate was chosen because of its anomalous melting behaviour[4]. Samples and container materials were of purity exceeding 99·999 per cent except in the case of sodium nitrate, which was 99·98 per cent pure. The materials were handled and weighed, when indicated, in a nitrogen dry box and the fusions performed in purified argon. Table 1 indicates that the results are independent of variations in gas pressure, etc., at least within the limits shown.

For exothermic processes (freezing), the initially constant input to the heater is the same as shown in Table 1. The E.M.F. of the reference thermocouple is then proportionately increased either by supplying an external constant E.M.F. or by utilizing a reference heater, which is normally present, since both sample and reference structures are used for measurements indiscriminately. All substances in Table 1 froze at rates too high for manual compensation because of supercooling. This tendency was arrested in the case of lead by using a container of

Table 1. DETERMINATION OF HEATS OF FUSION AND INTERCOMPARISON WITH CRITICAL LITERATURE VALUES

Substance and sample container(s)*	Number of determinations	Range of sample mass (g)	Range of compensation energy W	Range of compensation energy Sec	Mean ΔH_{fusion} and range cal/mole	'Best' critical values in literature: Kubaschewski and Evans[3]	'Best' critical values in literature: K. K. Kelley[4]
Sn-(*a*)	5	0·3411–0·7671	0·0563–0·1691	270·3–628·8	1,691 (1,686–1,701)	1,690 ± 30	1,720
Pb-(*a*)(*b*)	6	0·9673–1·2783	0·0759–0·1258	175·7–828·0	1,134 (1,128–1,140)	1,150 ± 30	1,140
Al-(*a*)	6	0·0991–0·2165	0·1278–0·2102	182·2–637·0	2,577 (2,561–2,604)	2,500 ± 30	2,570
NaNO$_3$-(*c*)	6	0·1861–0·2079	0·1196–0·1879	193·5–242·2	3,564 (3,545–3,575)	—	3,490

* (*a*) Spectroscopically pure graphite; (*b*) No. 446 stainless steel; (*c*) 99·999 per cent pure silver.

Range of heating rates by external furnace: 0·01–0·50° C/min. Range of pressures of argon gas: 378–1,010 mm mercury. Range of area under normal (uncompensated) differential thermal analysis peaks 0·8–1·5 cm^2/Joule.

99·999 per cent nickel. The heat of crystallization found for this sample was within ± 1 per cent of its heat of fusion.

For zero order kinetics, $dn/dt = (1/\Delta H)(dH/dt)$ where n = moles of molten substance, and dH/dt = the rate at which heat energy is actually absorbed by the substance. For a balanced sample structure, $dH/dt = k(T_h - T_m)$. If a linear heating rate is provided by the external furnace, $T_h - T_m = At$. Combining the last three equations results in $dn/dt = (kA/\Delta H)t$ and $dH/dt = kAt$. The last equation on integration yields $k = 2\Delta H/At_0^2$ or:

$$\frac{dH}{dt} = \frac{2\Delta H}{t_0^2} \cdot t$$

where t_0 is the total time required for the fusion. Accordingly, if $dH/dt = dQ/dt$, that is, if the kinetics of the process can be determined by the procedure described here, a plot of the experimental dQ/dt as a function of time should prove to be a straight line with origin at zero and slope equal to $2\Delta H/t_0^2$. Fig. 2 shows that this is so.

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ESTIMATION OF MASS-SCATTERING COEFFICIENT OF BIOLOGICAL SAMPLES

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DETERMINATION of mass thickness of biological samples, from the contrast in their electron microscope images, has been carried out by many workers[1-14]. Under normal operating conditions the contrast at a given point of the image is governed by the exponential relation[15]:

$$I = I_0 e^{-S\rho t} \tag{1}$$

where I_0 is the intensity of the electron beam incident on the object of mass thickness ρt, ρ being the density of the material and t the thickness of the object in the direction parallel to the incident beam, I is the intensity of the transmitted beam which reaches the image plane and S is the effective total electron-scattering cross-section per gram of the material. By using carbonaceous samples of known diameter and density and measuring their image contrast some authors[8,12] have estimated the values of S under various operating conditions of the electron microscope. For scattering by a single element, the total atomic cross-section σ is related to S by the expression:

$$S = \sigma \frac{N}{A} \tag{2}$$

where N is Avogadro's number and A the atomic weight of the element. Since biological objects consist mainly of carbon, hydrogen, nitrogen and oxygen, and if objects of thickness less than 900 Å are used, we need only be concerned with single scattered electrons[4].

Zeitler and Bahr[7] state that the effect of inelastic scattering can be disregarded in the aperture range of 10^{-3}–10^{-4} radians. Some recent approaches to this situation have been taken by Valentine[16] and Burge and Silvester[12]. The experimental values of S obtained by Burge and Silvester[12] show that the effect of inelastic scattering cannot be disregarded in the aperture range of 10^{-2}–10^{-3} radians. Moreover, inelastic scattering is more predominant than the elastic scattering in the aforementioned range. But, if one takes into account both the effects, the discrepancy between theoretical and Liesegang's experimental measurement of contrast[17] cannot be explained. However, only the effect of elastic scattering calculated from Lenz's[18] equation with R, the atomic radius, from the Wentzel model fit excellently the experimental results of Liesegang[17]. Table 1 shows the comparison of measured contrast at 60 kV with theory. Table 2 shows the comparison of our experimentally determined S values with those calculated from Lenz's expression for elastic scattering cross-section of carbon atom with R from the Wentzel model. The theoretical and experimental results are in good agreement.

The biological samples studied are the cell membranes of: (*a*) *Escherichia coli* (30); (*b*) human red blood cell (30); (*c*) *Leishmania donovani* (30); (*d*) *E. coli* bacteriophage tail (30); (*e*) tobacco mosaic virus (30); (*f*) yeast ribonucleo-protein (100); (*g*) polystyrene latex (30). The number against each specimen denotes the number of particles examined.

For the preparation of cell membranes, purified normal cells in each case were disrupted by chemical means to remove the cellular contents. The cell membranes, after separation, were collected on carbon-coated grids, dried up and thereby we obtained collapsed cell membranes. For the preparation of *E. coli* bacteriophage the modified technique of Hillier *et al.*[19] as developed by Guha[20] was

Table 1. THEORETICAL VALUES OF CONTRAST OF A 280 Å THICK CARBON FILM AT 60 KV WITH $\theta = 10^{-3}$ RADIANS, ASSUMING $\rho = 1·6$ G CM^{-3}

Atomic radius from:	Contrast in percentage taking into account. Elastic scattering cross-section	Inelastic scattering cross-section	Total scattering cross-section
Wentzel model	18·3*	11·9	28·0
Diamagnetic susceptibility measurement	13 6	9·9	22·2
Hartree model	25·0	14·3	35·7

* Liesegang's[17] measured value of contrast in percentage is 17 5

followed. TMV, yeast ribonucleo-protein and monodisperse polystyrene latex particles were supplied by the Rothamsted Experimental Station, the Distillers Co., Ltd., Edinburgh, and Dow Chemical Co., Midland, United States, respectively.

The electron microscopic observations and photographs were made under Siemens Elmiskop 1, with a 60-kV electron beam at an objective aperture angle of $9\cdot124 \times 10^{-3}$ radians, and afterwards photographed on Ilford process plates. After due processing of the plates, the photographic densities D and D_0 in the imaged region and in the background, respectively, were measured with a micro-densitometer. For the photographic negatives used it was found from a previous experiment that the γ-value under the foregoing conditions was 2·8 with the photographic density-range 1·5–3·0. It is well known that the measured densities D_0 and D are related to the corresponding electron densities by the relation:

$$D_0 - D = \log_{10} (I_0/I) \qquad (3)$$

From (1) and (3) with $\gamma = 2\cdot8$ we get:

$$S = 0\cdot82 \,.\, (D_0 - D)/\rho\, t \,.\, \text{cm}^2\ \text{g}^{-1} \qquad (4)$$

Thus, knowing mass thickness of the specimens, the corresponding S values for them are calculated as shown in Table 2. The $D_0 - D$ values of this table for the *E. coli*, red blood cells and *L. donovani* membranes correspond to twice the membrane thickness, as the two membranes collapse together during preparation and provide the photographic density. So half these $D_0 - D$ values correspond to thickness of a single membrane. Separate values of D_0 and D are not shown in Table 2, but the measured values of them are well within the range 1·5–3·0.

Table 2. VALUES OF S

Specimen	Thickness (t) (Å) from indirect measurements	Density (ρ) (g cm^{-3}) from indirect measurements	Measured $D_0 - D$	Experimental S* cm^2 g^{-1} × 10^{-4}
E. coli membrane	125 (21)†	1·30 (27)	0·197	4·96
RBC membrane	200 (22)	1·30 (27)	0·282	4·45
L. donovani membrane	250 (23)	1·30 (27)	0·342	4·32
TMV	150 (24)	1·37 (28)	0·103	4·12
Yeast RNP	200 (25)	1·50 (29)	0·162	4·42
E. coli bacteriophage tail	252 (20)	1·30 (27)	0·187	4·68
Polystyrene latex	880 (26)	1·05 (12)	0·501	4·45

* The theoretical S (elastic) value for the carbon atom in our experimental conditions is $4\cdot61 \times 10^4$ cm^2 g^{-1}.

† The number within brackets denotes the reference from which the corresponding value is taken

From the comparison of experimental and theoretical S values it is suggestive that only elastic scattering effect is responsible for contrast consideration, at least at the aforementioned operating condition of the microscope, though theoretically the inelastic scattering effect is 67 per cent to that of elastic effect. In Table 1 calculations of contrast in percentage have been made on different models and compared with the experimental value in order to show the merits of the different models for practical purposes. It is seen that Lenz's[18] formula with R from the Wentzel model is a suitable one. The foregoing experimental procedure is also useful for the determination of density of biological samples[13] of known thickness and vice versa. Here a knowledge of theoretical scattering cross-section is necessary, and thus, if the elastic scattering cross-section for a carbon atom is calculated from Lenz's formula with Wentzel radius, the thickness or density values can be found from equation (4) with some confidence, provided the microscope is operated at 60 kV and at an aperture angle near about 10^{-3} radians.

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BLOCK OF INSULIN SECRETION FROM THE PANCREAS BY D-MANNOHEPTULOSE

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A TRANSIENT diabetic state can be induced in the rat by a single injection of the seven carbon sugar D-mannoheptulose[1]. Kraicer, Simon and Shelesnyak[2] have suggested that mannoheptulose induces this diabetic state by interfering with the secretion of insulin by the pancreatic islets in response to glucose. An *in vitro* technique suitable for examining the effect of substances on the secretion of insulin utilizing pieces of rabbit pancreas has recently been described[3]. This technique has now been used for investigating the effect of mannoheptulose on insulin secretion induced by glucose. To justify the use of pancreatic tissue of the rabbit, evidence is presented that the rabbit exhibits the same physiological response to mannoheptulose as the rat.

The effect of mannoheptulose on blood glucose concentration was investigated in six male adult rabbits, body-

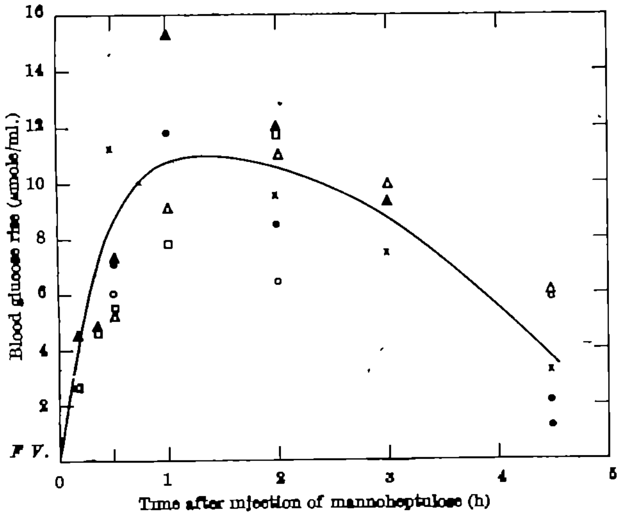

Fig. 1. Increase of glucose concentration in blood after administration of mannoheptulose (intravenously, 2 g/kg) to fasted rabbits. Each rabbit is represented by a different symbol. The curve joins the means (*F.V.*, fasting value)

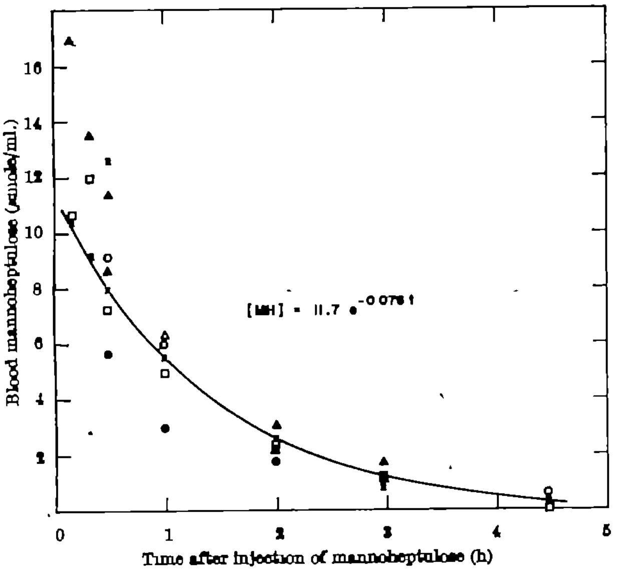

Fig. 2. Concentration of mannoheptulose in blood after administration of mannoheptulose (intravenously, 2 g/kg) to rabbits. The curve was calculated by regression analysis

weight (mean ± *S.D.*) 1·6 ± 0·2 kg. The animals were deprived of food for 24 h and injected intravenously with 2 g/kg of D-mannoheptulose in 1–7 ml. of isotonic sodium chloride. Blood samples, taken from the contralateral ear vein, were analysed for total reducing sugar[4] and mannoheptulose[5], glucose concentration being obtained by difference. Chromatography of concentrated blood filtrates by the method of Nordal and Benson[6] confirmed that mannoheptulose and glucose were the only sugars detectable.

The effect of D-mannoheptulose on insulin secretion was examined as follows. Adult male rabbits deprived of solid food (but with free access to 5 per cent aqueous glucose) for 15–18 h were rapidly anaesthetized with 300 mg hexobarbitone sodium by ear vein. The body and tail of the pancreas were quickly removed and dropped into Krebs supplemented medium[7] gassed with oxygen + carbon dioxide, 95 : 5 (physiological saline medium supplemented with 5 m.equiv./l. sodium L-glutamate, fumerate and pyruvate and also Armour bovine plasma albumin, fraction V, 2 mg/ml.). The pancreas was divided into pieces weighing 100–300 mg with exclusion of obvious non-pancreatic tissue and pre-incubated for 30 min in medium (see foregoing) containing glucose 0·6 mg/ml. After washing with the same medium each piece was transferred to a stoppered pot containing 3 ml. of medium and subjected to a sequence of five separate incubations of 15- or 30-min duration in media containing D-glucose, 0·6 mg/ml., 3 mg/ml., 0·6 mg/ml., 0·6 mg/ml., and 3 mg/ml. (referred to as phases 1–5). In experiments with D-mannoheptulose the sugar was added to a concentration of 3 mg/ml. in phases 4 and 5. Insulin was estimated in residual incubation media from all except the third phase of the sequence by immunoassay[8]. This experimental design was chosen because earlier experience[8] showed that the output of insulin in response to a high concentration of glucose (3 mg/ml.) measured by the difference in output in phases 1 and 2 can be repeated (phases 4 and 5) after a rest period (phase 3). This technique permits each piece of pancreas to be used as its own control and the effect of mannoheptulose to be ascertained by comparing output of insulin in phases 4 and 5 (when the sugar was present) with that in phases 1 and 2 (when the sugar was absent).

For the *in vivo* experiments the results were as follows. The blood glucose concentration following injection of mannoheptulose is shown in Fig. 1. The blood glucose rose to a maximal concentration of 300 ± 40 mg/100 ml. (mean ± *S.E.*) at 1 h after injection (mean fasting value, 100 mg/100 ml.) and remained elevated for more than 4½ h. The blood mannoheptulose fell exponentially, being halved every 55 min (Fig. 2).

For the *in vitro* experiments the average output of insulin in control experiments by six pieces of rabbit pancreas was enhanced by increasing the concentration of glucose in the medium (0·6–3 mg/ml.) from 4·1 to 18·3 mμg insulin/min/piece of pancreas in the first two phases of incubation and from 4·1 to 27 in the last two phases (1 mμg = 10^{-9} g). This confirms, as mentioned previously, that the response to glucose can be repeated a second time. In the presence of mannoheptulose (3 mg/ml.) the average output of insulin was not increased by raising the concentration of glucose in the medium from 0·6 to 3 mg/ml. (outputs of 3·5 and 3·5 mμg insulin/min/piece of pancreas in phases 4 and 5) in spite of the fact that the same pieces had shown a good response to glucose in phases 1 and 2 (outputs 3·5 and 12·5 mμg/min). The statistical significance of these changes has been shown by comparing the ratios (phases 1–2 and 4–5) of the parameter of the insulin assay method used (the radioactivity of the insulin-antibody complex which is inversely related to insulin concentration). In the control series the ratios (mean ± *S.E.*) were 2·45 ± 0·45 and 3·10 ± 0·31 (t for difference is 1·2 and $P > 0·3$). In the mannoheptulose series the ratios were 2·13 ± 0·20 and 1·03 ± 0·07 (t for difference is 5·2 and $P < 0·001$). We conclude that D-mannoheptulose completely suppressed the stimulating effect of D-glucose on insulin output by isolated rabbit pancreas in these experiments without affecting the small output of insulin at a low glucose concentration.

The conclusion of Kraicer, Simon and Schelesnyak[9] that mannoheptulose inhibited the secretion of insulin from the pancreatic β-cells was based on the apparent experimental exclusion of alternative explanations for the hyperglycaemia and ketonaemia following its injection into intact animals. Thus, mannoheptulose did not reduce the effect of injected insulin on blood glucose and ketone bodies of the intact rat[9] or further elevate these parameters in the pancreatectomized animal[9]. The absence of an effect on peripheral glucose uptake in the presence and absence of insulin was also shown with several tissues from the rat tested *in vitro*[10]. There was evidently no net glycogenolysis induced by mannoheptulose since the glycogen content of liver and to a lesser extent kidney and muscle was increased 4 h after its injection in the rat[1]. It was also not possible for conversion of mannoheptulose to glucose to account quantitatively for the hyperglycaemia.

The increment in blood glucose seemed to be due largely to gluconeogenesis since in the starved rats used in the earlier experiments liver glycogen would have been very low. Furthermore, mannoheptulose injection was accompanied by a rise of urinary non-protein nitrogen in intact fasted rats[11] and a further elevation of the blood non-protein nitrogen in nephrectomized animals[9]. The necessity for circulating corticosteroids in the experimental animals if the diabetogenic effects of mannoheptulose were to be exhibited[11] was interpreted as being a necessary condition for adequate gluconeogenesis. The present experiments with rabbits furnish direct evidence for the inhibitory effect of mannoheptulose on insulin secretion but leave open the possibility of an additional effect of the sugar on gluconeogenesis through some other mechanism.

Since conventional metabolic studies on mammalian β-cells are not feasible because of their relative scarcity in pancreatic tissue, the metabolic role of mannoheptulose can only be readily defined using other tissues and work along this line is in progress. If a pathway of metabolism or an enzyme system affected by mannoheptulose were defined this would provide a valuable clue to the mechanism whereby glucose stimulates insulin release from β-cells.

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INDUCED ARTERIAL OSCILLATIONS OF BLOOD PRESSURE AS A METHOD FOR INVESTIGATING REGULATION OF LEVEL OF BLOOD PRESSURE

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THE existence of homeostatic systems which control arterial blood pressure implies a stable equilibrium-level for a given physiological state; if the pressure is displaced from this value, the systems will oppose the effect of the imposed change.

In the method described here, the system was displaced from its original equilibrium state; its tendency to return to the original condition was then observed. This displacement was achieved by applying periodic trains of electrical stimuli to the central end of the rabbit's aortic depressor nerve, thus causing the mean value of the resulting arterial pressure oscillation to fall with respect to the original equilibrium value. The duration of the stimulus trains was controlled by the arterial pressure itself: when as a result of depressor stimulation the pressure fell, at a minimum pre-determined value the stimulus ceased; the pressure then rose, and at a maximum pre-determined value the stimulus recommenced. The cycle then repeated itself.

The arterial pressure was measured from a common carotid artery with a capacitance transducer coupled to a gated beam discriminator[1]. The output voltage of the latter was monitored by a Schmitt trigger circuit which changed state at the predetermined pressure values, thereby actuating a relay which connected or disconnected the stimulator in the required manner.

Simultaneous pressure, ECG, heart rate and stimulus records were made.

The experiments were carried out on rabbits anaesthetized with sodium pentobarbital (30 mg/kg body-weight), or under deep ether anaesthesia during the surgical phase, a local anaesthetic being applied to the wounds. In all experiments the animals were paralysed with gallamine triethiodide ('Flaxedil'), maintained with constant pulmonary ventilation, both depressor nerves sectioned, and both common carotid arteries tied.

The following results were obtained: on commencement of a series, the pattern of oscillation (henceforth referred to as 'Pattern *P*') was characterized by short stimulation periods and long non-stimulation periods. The first four oscillations shown in Fig. 1, or those in Fig. 2*A*, illustrate typical examples of this pattern.

Pattern *P* gradually shifted during a series in the direction of Pattern *S*, characterized by long stimulation periods and short non-stimulation periods. Pattern *S* can be observed in the second part of Fig. 1, and in part *C* of Fig. 2. Frequently the arterial blood pressure did not fall sufficiently to interrupt the stimulus, whereupon the oscillation ceased. This was regarded as a marked tendency of the system to move in the *P*–*S* direction (Fig. 1). On many occasions when this 'escape' occurred no further oscillations were produced; if the stimulus were then disconnected, the blood pressure rose transiently above its final normal resting value. After a rest period, oscillations could usually be restored on re-application of the stimulus.

Once the system had escaped from the oscillation induced by stimulation of one of the depressor nerves, immediate alternative stimulation of the other depressor did not renew the oscillation, although previously the stimulation of either depressor had caused similar arterial blood pressure oscillations.

'Spontaneous' escapes or *P*–*S* changes occurred frequently in non-anaesthetized animals, and generally coincided with insufficient curarization. These changes were rarely observed when the animals were anaesthetized with pentobarbital.

While oscillating, the system manifested an increased sensitivity to some stimuli: limb flexion, light tactile

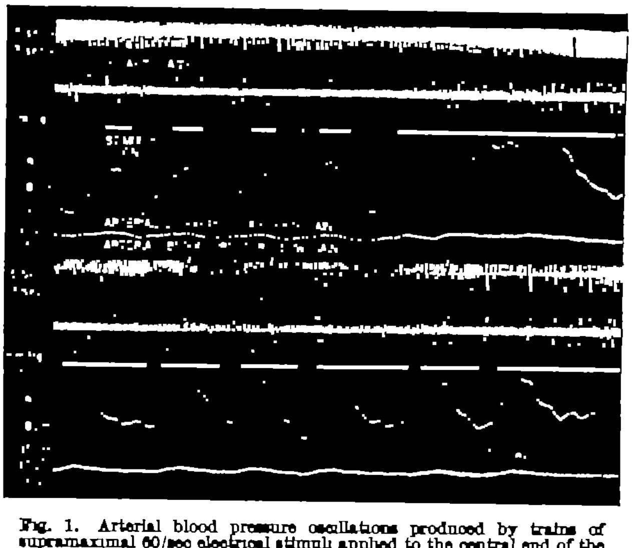

Fig. 1. Arterial blood pressure oscillations produced by trains of supramaximal 60/sec electrical stimuli applied to the central end of the left depressor. Rabbit (1·5 kg) anaesthetized initially with ether, atropinized. Respiration pump stroke volume 150 c.c., pumping rate 34/min. The series illustrated began 1 min before the last four (shown) typical oscillations. The original undisturbed mean arterial pressure was 90 mm mercury. Both parts of the figure are continuous

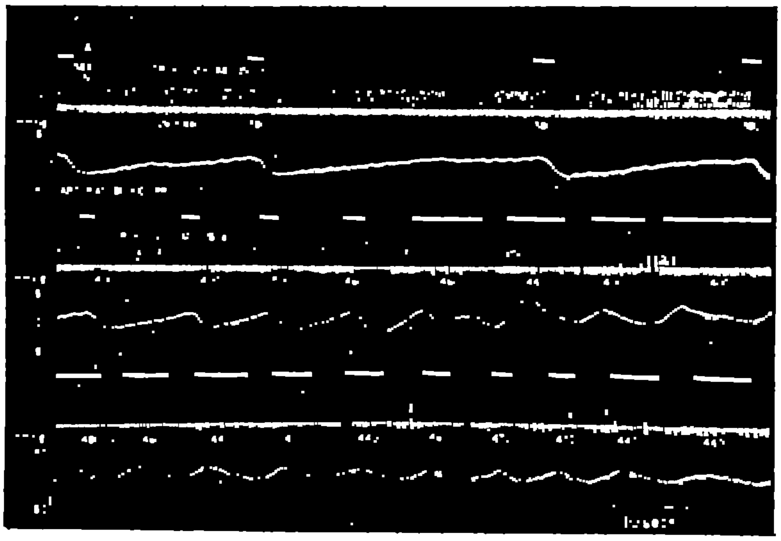

Fig. 2. Arterial blood pressure oscillations produced by trains of supramaximal electrical stimuli applied to the central end of both depressors. Rabbit (1·5 kg) anæsthetized initially with ether. In *A* the respiration pump stroke volume was 150 c.c., pumping-rate 34/min. In *B* at moment indicated by arrow, the stroke volume was reduced to 125 c.c. Between *A* and *B* a part of the ultimate cycle is not shown, identical in all respects to the preceding ones. *B* and *C* are continuous. The first number below the ECG record indicates the control duration of the cardiac cycle, and the remainder the maximum durations resulting from the stimulation of both depressors. The series illustrated began three minutes before the (shown) typical oscillations of part *A*. The original undisturbed mean arterial pressure was 130 mm mercury

stimuli or intravenous injections of 10–20 μg potassium cyanide produced a marked *P–S* pattern change (similar to the example in Fig. 1.) These stimuli did not produce observable changes in arterial pressure when the system was in repose.

As an example of a *P–S* change, Fig. 2 shows the result of hypoventilation on the mode of oscillation: part *A* of Fig. 2 illustrates the pattern obtained with a ventilation of 150 c.c. At the commencement of part *B* the ventilation was reduced to 125 c.c., a resulting shortened non-stimulation period was rapidly observed, as can be seen in the first three oscillations after ventilation reduction, whereas the maximum and minimum arterial blood pressure values have not yet changed appreciably.

Hyperventilation produced a change of the oscillation pattern in the opposite direction.

Besides the hypotension which resulted from depressor stimulation, a diminution of heart rate also took place. During escape, or a *P–S* change, reflex bradycardia did not decrease: on the contrary, it frequently increased. In atropinized and vagotomized animals, a similar change of pattern in the *P–S* direction was always obtained, thus showing that the *P–S* change is independent of the heart rate.

The *P–S* change cannot be satisfactorily explained as due to 'system fatigue'; a more probable interpretation might be that there is a 'build-up' of vasoconstrictor sympathetic activity which tends to suppress the oscillations. This is supported by the fact that any stimulus which is known to increase sympathetic activity also accelerates the *P–S* change (hypoventilation, injection of minute doses of potassium cyanide, painful and tactile stimuli, etc.).

The *P–S* change might also be regarded as indicating a specific tendency of the system to return to its original condition of greater stability[2].

Spontaneous *P–S* changes in animals anæsthetized with pentobarbital were infrequent and appeared as a prolongation of the stimulation period without the concomitant shortening of the non-stimulation periods. In addition, the system exhibits a reduced tendency to return towards its original equilibrium. This might be interpreted as a reduction in homeostatic efficiency. It is suggested that pentobarbital anæsthesia diminishes the 'build up' of sympathetic vasoconstrictor activity.

The 'build up' of sympathetic activity might be explained as due to:

(1) The existence of other unknown receptors besides those which were excluded in the present experiments (aortic, vagal and carotid sinus receptors).

(2) The rhythmic application of stimuli. (Alternating inhibitory and excitatory influences on sympathetic activity might produce a gradual increase in the latter as a result of post-inhibitory rebound.)

(3) The application of electrical stimuli to the depressor nerve in place of normal physiological activation.

We thank Drs. A. Rosenblueth and J. Garcia Ramos for their helpful criticism.

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SIALIC ACID IN FIBRINOGEN AND 'VULCANIZATION' OF THE FIBRIN CLOT

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According to our present knowledge, during the clotting of fibrinogen thrombin becomes involved in the process twice. First thrombin splits off 3 per cent of fibrinogen in the form of two peptides[1]. These altered fibrinogen molecules, now called fibrin molecules, aggregate to form the primary clot[2] (Fig. 1). In its second interaction thrombin activates a plasma component (the Laki–Lorand factor—various names have been given to this plasma component: fibrin stabilizing factor[17], fibrinase[18], Laki–Lorand factor[19]. Until the exact mode of action of this enzyme is elucidated, it is preferable to use a non-specific name. We refer to it in this article as LLF) into an enzyme[3], which 'vulcanizes' the clot by introducing strong bonds between the fibrin molecules; as a result the clot becomes insoluble in 5 M urea[4]. In normal plasma the insoluble clot is formed. If the LLF is missing from the plasma, the clotting process is incomplete, the 'vulcanization' of the clot does not occur and a tendency for bleeding[5] as well as an impairment of wound healing sets in[6]. It was found by Laki[7] and by others[8–10] that during interaction of thrombin with fibrinogen, not only peptides were liberated but also carbohydrate residues containing mannose and galactose were released. Chandrasekhar *et al.*[11] have recently observed that sialic acid is also liberated during clotting.

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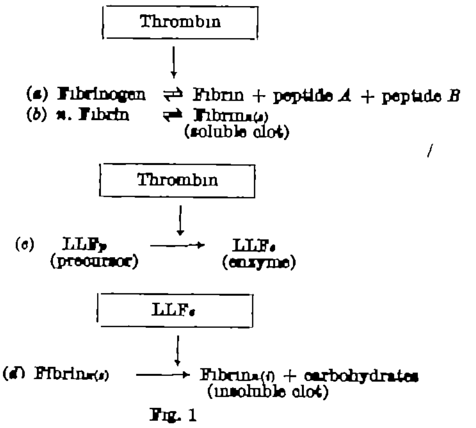
Thrombin
(a) Fibrinogen ⇌ Fibrin + peptide A + peptide B
(b) n. Fibrin ⇌ Fibrinn(s)
(soluble clot)
Thrombin
(c) LLFp (precursor) → LLFe (enzyme)
LLFe
(d) Fibrinn(s) → Fibrinn(i) + carbohydrates
(insoluble clot)

Fig. 1

These sugars originate from the carbohydrate moiety of fibrinogen which in the case of bovine fibrinogen amounts to about 3 per cent[7].

Szara and Bagdy[8] found that the release of carbohydrate material goes parallel with the action of thrombin in spite of the fact that thrombin is not a carbohydrate-splitting enzyme. Thus the action of thrombin in liberating carbohydrates can only be indirect. Since fibrinogen preparations, unless specially treated, are contaminated with LLF, it is likely that this enzyme (activated by thrombin) is responsible for the release of carbohydrates from fibrinogen. To test this assumption we modified the carbohydrate moiety of fibrinogen by selectively removing sialic acid with the enzyme sialidase (neuraminidase). Bovine fibrinogen (97 per cent clottable), prepared by the method of Laki[7] was treated with sialidase (from *Vibrio cholerae*) to split off sialic acid. The enzyme-treated fibrinogen was finally separated with ammonium sulphate precipitation. The assays of neutral carbohydrates by the orcinol method[13] and that of sialic acid by the thiobarbiturate method[14] showed that the enzyme removed only the sialic acid residues from fibrinogen. Ultracentrifugal examination revealed that no detectable change occurred in the sedimentation properties of fibrinogen (Fig. 2).

It is seen in Table 1 that clots obtained from sialic acid-free fibrinogen in the presence of fresh human plasma (as the source of LLF) were easily soluble, whereas those from untreated fibrinogen were only sparingly soluble in 5 M urea solution. The fibrin clots from treated fibrinogen were all in solution within 2 h, while much of the clots from the untreated fibrinogen remained insoluble after 24 h and even after 7 days. Experiments using purified LLF kindly supplied by A. G. Loewy also gave similar results. Apparently, the removal of sialic acid from bovine fibrinogen modified the substrate in such a manner that the LLF was no longer able to act on it.

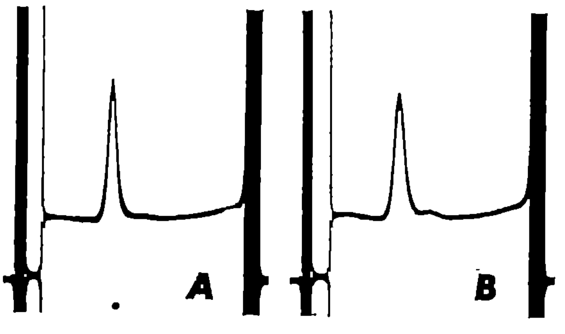

Fig. 2. Ultracentrifuge pattern of native and sialic acid-free fibrinogen. *A*, native fibrinogen, $S_{20,w} = 7.72$. *B*, sialic acid-free fibrinogen, $S_{20,w} = 7.52$.

Protein concentration, 5·0 mg/ml.; solvent, 0 3 M potassium chloride; rotor speed, 59,780 r.p.m. Pictures taken 34 min after reaching speed at bar angle of 50°.

Table 1. SOLUBILITY OF NORMAL AND SIALIC ACID-FREE FIBRIN CLOTS IN UREA

Duration of incubation of the clots in urea	Optical density at 280 mμ of the dissolved portion of the clots*	
Hours	Normal clots	Sialic acid-free clots
0·5	0·081	0·640
1·0	0·226	0·965
2·0	0·256	1·015

* Corrected for the optical density of the plasma blanks.

The reaction mixture consisted of 1·0 ml. fibrinogen, 1·0 ml. 0·3 M KCl, 0·4 ml. M/5 *tris*–maleate buffer of pH 6·5, 1·4 ml. distilled water, 0·2 ml. M/40 calcium chloride, 0·1 ml. human plasma and 0·02 ml. thrombin. Thrombin and plasma were added simultaneously. Suitable controls, in which plasma and calcium were left out from the reaction mixture, were also run. The clots were allowed to stand at room temperature for 3 h. After the incubation, 4·0 ml of a 10-M urea solution was added to each of the tubes (final urea concentration 5 M) and the clots were shaken uniformly and at definite intervals. 1·0 ml. each of the clear supernatant was taken from the respective tubes at different time intervals, mixed with 4·0 ml. of 0·3 M KCl and the optical density at 280 mμ was measured in a Beckman spectrophotometer. A solution compounded similarly, but without the fibrinogen and plasma in the reaction mixture was used as the blank. Another blank, the plasma blank, was also run, using human plasma in the original reaction mixture, but not the fibrinogen.

Our results reported in this article, coupled with the observation[15] that the activation of LLF goes parallel with an increased release of carbohydrates during clotting, strongly suggest that LLF is an oligosaccharide-splitting enzyme. (Recently, Lorand *et al.*[20] proposed that LLF is a transpeptidase. According to their assumption the peptides released from fibrinogen in the presence and absence of this factor should be different. Recent experiments of J. A. Gladner and A. J. Osbahr in this laboratory in co-operation with A. G. Loewy (Haverford, Pa.)[21] have shown that the peptides released from human fibrinogen using human thrombin and human LLF were identical irrespective of whether the clotting was carried out to produce the soluble or the insoluble clot. This crucial experiment suggests that LLF is not a transpeptidase.)

The introduction of firm bonds between the fibrin molecules as a result of LLF may be due to a Schiff-base like combination between amino groups and carbonyl groups of the carbohydrate residues liberated in the splitting. This possibility is all the more likely, since under certain conditions carbonyl reagents inhibit the clotting of fibrinogen[7]. The inhibition by the carbonyl reagents may be due to a competition for the same amino groups that otherwise would be involved in forming the Schiff-base type bonds between fibrin molecules.

The role of this carbohydrate-splitting enzyme, LLF, in wound healing is still not understood. It is worth while, however, to point out that another carbohydrate-splitting enzyme, β-glucuronidase, shows increased activity in healing wounds[16]. This enzyme acts on mucopolysaccharide, the ground substance of collagen.

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RELATIONSHIP OF PLASMA SALICYLATE CONCENTRATION TO URINARY SALICYLATE EXCRETION RATE

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CUMMINGS and Martin[1] have reported recently that maximum plasma salicylate-levels after oral doses of aspirin occurred much earlier than maximum urinary excretion rates of salicylate. They referred to similar observations by others and pointed out that this fact has received no special comment.

The colorimetric analysis of salicylate in the urine yields values that must be expressed in terms of apparent salicylate, because both salicylic acid and its metabolite salicyluric acid are measured. If salicyluric acid is excreted in significant amounts, a delayed apparent salicylate excretion peak should be expected, due to the time required for the biosynthesis of the metabolite. On the other hand, a specific assay for salicylic acid should yield a urinary excretion pattern in which the peak excretion rate coincides with plasma salicylate concentration peak. We wish to report the results of experiments which illustrate and elucidate these contentions.

Two adult male subjects ingested 1 g doses of aspirin (dissolved in about 240 ml. of water by the addition of 1 g of sodium bicarbonate) in the morning on empty stomach. Urine collections were made at appropriate times, and aliquots of these were assayed for salicylic acid and salicyluric acid by a combination of the methods of Brodie *et al.*[2] and Trinder[3]. The maximum excretion rate occurred about 2·5 h after administration of the drug (Fig. 1), and more than 90 per cent of the apparent salicylate excreted was in the form of salicyluric acid. The delayed excretion peak can therefore be attributed to the time required for metabolite formation.

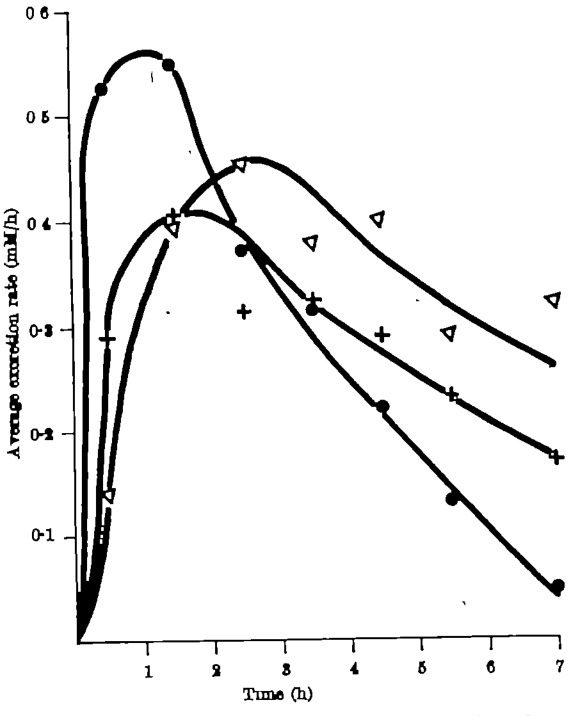

Fig. 1. Salicylate excretion rate after oral ingestion of 1-g doses of aspirin in aqueous solution

▽, salicyluric acid excretion; ●, salicylic acid excretion when urine was alkalized; +, salicyluric acid excretion when urine was alkalized

Unfortunately, the fraction of aspirin excreted as free salicylic acid was too small to determine the time of maximum excretion. This fraction may, however, be increased by making the urine slightly alkaline, because the excretion rate of salicylic acid is increased at higher *p*H. The time of maximum excretion is not affected. The experiment was repeated therefore under conditions in which the urinary *p*H was increased by administration of 21 g of sodium bicarbonate in divided doses over 6 h, beginning 3 h prior to aspirin ingestion. The administration of sodium bicarbonate resulted in urinary *p*H's of 7·6 in one subject and 7·9 in the other. A considerable amount of free salicylic acid was excreted under these conditions, and the maximum excretion rate occurred at about 1 h after administration of the drug (Fig. 1). It is known from other investigations that peak plasma salicylate concentrations also occur about 1 h after oral administration of aspirin in readily absorbable form, as has been pointed out by Cummings and Martin[1].

Maximum excretion of salicyluric acid occurred 1·5–2 h after administration of the drug, which is later than the time of maximum salicylic acid excretion, but somewhat earlier than maximum salicyluric acid excretion in the first experiment. While an increase in urinary *p*H should have no effect on the time of maximum salicylic acid excretion (because salicylic acid excretion rate is directly proportional to the salicylate plasma level), it will modify the time of maximum salicyluric acid excretion. The excretion of this metabolite is the terminal step in a two-step process (biosynthesis followed by excretion). In the dosage used, both these processes can be described by first order kinetics[4]. The rate of excretion of salicyluric acid can be represented, therefore, by the following expression, which gives the rate of accumulation of the product of two consecutive first-order reactions:

$$\frac{\mathrm{d}U}{\mathrm{d}t} = \frac{k_2\, k_1 f S^0}{k_1 - k_2}\left(\exp\,[-k_2 t] - \exp\,[-k_1 t]\right) \qquad (1)$$

In the equation, $\mathrm{d}U/\mathrm{d}t$ represents the salicyluric acid excretion rate, f is the fraction of the ingested dose which will be excreted eventually as salicyluric acid, S^0 is the amount of salicylate in the body after absorption of the dose, t is time, k_1 is the rate constant for biosynthesis of salicyluric acid, and k_2 is the rate constant for urinary excretion of salicyluric acid. The constants k_1 and k_2 have dimensions of reciprocal time. It should be pointed out that the value of fS^0 need not be known for the derivation that follows. If the derivative of equation (1) is obtained and the resulting expression is equated to zero and rearranged, the following equation results:

$$t_{\max} = \frac{\ln(k_2/k_1)}{k_2 - k_1} \qquad (2)$$

where $t_{\max}$ is the time of occurrence of the maximum excretion rate of salicyluric acid. The rate of biosynthesis of salicyluric acid, and therefore k_1, should be independent of sodium bicarbonate ingestion and urinary *p*H. However, the rate of excretion and the excretion rate constant k_2 increase at higher *p*H (ref. 5). Therefore, $t_{\max}$ will be less at higher *p*H's because the value of the right-hand side of equation (2) will decrease as k_2 increases. This is in agreement with the experimental data (Fig. 1).

Cummings and Martin[1] suggest that serum salicylate-levels are disproportionately high (and tissue salicylate-levels are disproportionately low) when the drug is absorbed very rapidly and that therefore 'the relative speed of absorption of aspirin formulations cannot be accurately assessed by comparison of respective plasma salicylate concentrations'. This contradicts commonly accepted principles governing the rate of transfer of drugs from the circulation to the tissues, and could imply the existence of an active and saturable process concerned with the movement of drugs across the membranes of blood vessels. The rate of transfer of salicylate from the circulation to the tissues is understood generally to be a function of concentration gradient (which is essentially equal to plasma salicylate concentration during the absorption phase). This may be modified by plasma protein binding, but in a manner opposite to that suggested by Cummings and Martin, because a greater fraction of salicylate exists in free (diffusible) form at higher concentrations[6,7]. Data are available in the literature in support of these statements. For example, Davison *et al.*[8] determined plasma and brain salicylate-levels after oral administration of aspirin and sodium salicylate to rats. Both drugs were given in rapidly absorbable and more slowly absorbed forms. The ratio of plasma to brain salicylate-levels was actually somewhat lower after administration of the drugs in rapidly absorbed form than after administration of these drugs in more slowly absorbed form. This is probably due to the plasma-protein binding effect already referred to.

Our experimental results and other evidence indicate that both plasma salicylate and urinary excretion data are satisfactory indicators of the relative absorption rate of orally administered salicylates.

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MECHANISM OF THE SUCCINIC DEHYDROGENASE CATALYSED REACTION

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IT has been recently demonstrated[1,2] that succinic dehydrogenase acts in a stereospecific fashion with respect to the substrates L-chlorosuccinic acid and L-methylsuccinic acid and it also has been shown that the dehydrogenation effected by the enzyme with succinate[3] and with L-chlorosuccinate[2] is *trans* in nature. The catalysis by the enzyme of the removal of *trans* hydrogens has suggested[3] the possibility that the hydrogens concerned are individually removed. This possibility has been investigated by means of anaerobic exchange experiments with L-chlorosuccinate and with succinate, and it is the province of this communication to report that one of the two hydrogens of a removal *trans* pair is initially exchanged.

Experimental conditions for the exchange of succinate hydrogens with proton of the medium have been defined by Englard and Colowick[4] and it is to be noted that the presence of fumarate is required for the exchange. In a similar manner exchange of the hydrogens of L-chlorosuccinate requires the presence of the oxidized substrate for extensive exchange. Fig. 1 demonstrates the anaerobic rates of exchange at *p*H 7·3, 34° of the hydrogens of L-chlorosuccinate in the presence and absence of chlorofumarate. It is to be noted that the initial rate of exchange catalysed by succinic dehydrogenase is markedly increased by the presence of chlorofumarate. In this connexion it is possible that the rate in the absence of chlorofumarate is affected by the slow dehydrohalogenation of chlorosuccinate in neutral solution, the product being fumarate and the fumarate catalysing the exchange. The true rate in the absence of chlorofumarate may thus be considerably lower than the observed rate. The average amount of deuterium incorporated in the 12- and 24-h samples in the presence of chlorofumarate amounts to 5·1 atom per cent deuterium and since complete exchange of one of the three carbon-bonded hydrogens under the conditions of the experiment, 27·0 per cent deuterium oxide, would yield L-chlorosuccinate with 5·4 atom per cent deuterium, it would seem that only one of the three carbon-bonded hydrogens is exchanged.

To substantiate this point, the distribution of deuterium in exchanged L-chlorosuccinic acid (Table 1) was ascertained by the mass spectrometer. The exchanged L-chlorosuccinic acid was converted to L-chlorosuccinic anhydride with phosphorus pentoxide[5] and the chloroanhydride was then thermally dehydrohalogenated[6] to yield maleic anhydride. The thermal dehydrohalogenation proceeded, essentially, with retention of deuterium and since those hydrogens of L-chlorosuccinic acid, removable by succinic dehydrogenase, are *trans*[2] (when the carboxyl groups are also *trans*) the thermal dehydrohalogenation of L-chlorosuccinic anhydride proceeds in a *cis* fashion, namely:

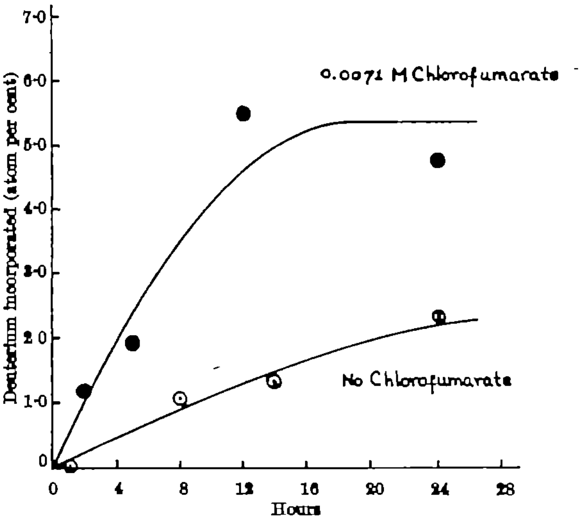


Fig. 1. Rate of incorporation of deuterium into L-chlorosuccinate by anaerobic exchange catalysed by succinic dehydrogenase at *p*H 7·3, 34°. Reaction mixtures were 0·05 M in phosphate buffer, 0·036 M in L-chlorosuccinate, 5·5 × 10^{-4} M in methylene blue and contained 7·5 ml. of the Slater modification of the Keilin–Hartree preparation from horse heart per 140 ml. For each point on the curve 140 ml. of reaction mixture was incubated under nitrogen for the appropriate time period and the reaction stopped by addition of 6 M sulphuric acid to *p*H 1·0. L-Chlorosuccinic acid was then isolated by ether extraction, purified by recrystallization from ethyl acetate and its deuterium content determined by the falling drop method on the water obtained by combustion

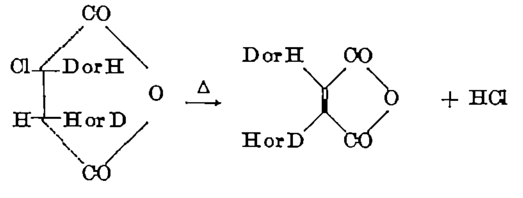


Table 1. DISTRIBUTION* OF DEUTERIUM IN PRODUCTS OBTAINED FROM EXCHANGED L-CHLOROSUCCINIC ACID†

Mass No.	L-Chlorosuccinic anhydride Normal	L-Chlorosuccinic anhydride Exchanged‡	L-Chlorosuccinic anhydride Excess§	Maleic anhydride Normal	Maleic anhydride Exchanged‖	Maleic anhydride Excess§
98	80·2	80·2	—	80·5	80·5	—
99	3·98	17·0	13·0	4·62	17·1	12·5
100	0·80	2·68	1·88	1·08	2·60	1·52

* Relative heights of maleic anhydride parent peaks, 98, 99, 100. L-Chlorosuccinic anhydride also yields these peaks.

† 500 ml. of a reaction mixture, 0·036 M in L-chlorosuccinate, 0·0068 M in chlorofumarate, 0·05 M in phosphate buffer, *p*H 7·6, containing 250 ml. of D_2O and 40 ml. of a Slater enzyme preparation, was incubated at 34° for 4 h. L-Chlorosuccinate, 960 mg, m.p. 179°–180° after lyophilization from water, containing 7·23 per cent deuterium, was obtained by acidification of the reaction mixture to *p*H 1, extraction with ether and recrystallization from ethyl acetate of the crude residue.

‡ Analysis gave 6·40 per cent deuterium, calculated from mass spectral results 5·60 per cent, calculated from deuterium content of exchanged acid 6·02 per cent.

§ To be multiplied by the dilution factor of two, since the exchanged L-chlorosuccinic acid was diluted with an equal weight of normal acid for conversion to L-chlorosuccinic anhydride and maleic anhydride.

‖ Analysis gave 8·22 per cent deuterium, calculated from mass spectral results 8·35 per cent and 7·75 per cent, calculated from deuterium content of exchanged acid 9·00 per cent. The normal maleic anhydride was prepared from normal chlorosuccinic anhydride and both maleic anhydrides contained a small amount of a chlorine-containing impurity.

and the maleic anhydride thus derived from exchanged L-chlorosuccinic acid is suitable for distribution studies. It was also found that L-chlorosuccinic anhydride underwent *cis*-dehydrohalogenation in the mass spectrometer, its 98, 99, 100 mass number pattern being the same (Table 1) as that for maleic anhydride. The results obtained (Table 1) with both L-chlorosuccinic anhydride and maleic anhydride derived from exchanged L-chlorosuccinic acid show that of 100 molecules, 25–26 have undergone exchange of one hydrogen for deuterium and 3–4 exchange of two hydrogens. These results, then, also indicate that succinic dehydrogenase catalysed exchange proceeds by exchange of one of the two hydrogens of a removable pair albeit the second hydrogen may exchange at a decidedly slower rate. From other, longer, exchange experiments, exchanged L-chlorosuccinic acid was converted to dimethyl L-chlorosuccinate (7·1 per cent deuterium found, 6·65 per cent deuterium calculated for complete exchange of one hydrogen), exchanged succinic acid was converted to dimethyl succinate (8·2 per cent deuterium found, 10 per cent deuterium calculated for complete exchange of two of the four hydrogens) and the distribution of deuterium containing molecules in each of these esters was investigated with the mass spectrometer. The distributions, so obtained, are qualitatively in line with the results given here. Quantitative calculation of distributions was considered to be approximate since parent peaks were not obtained and the cracking pattern of the normal esters which was considered as a basis for calculation is, in the case of dimethyl succinate, not consistent with the cracking pattern of the dideuterio-ester and the tetradeuterio-ester[6]. It is of interest to note in connexion with succinate exchange experiments that exchange of one hydrogen by deuterium should lead to observable[7] optical activity in the exchanged succinic acid.

It is clear from the observed distributions that one of the two hydrogens of a removal *trans* pair is exchanged at a much faster rate than the other and it would seem, therefore, that the processes resulting in exchange of both hydrogens are not chemically equivalent. Thus one of the hydrogens might be removed as a proton (rapid exchange) and the other, initially removed as a hydride ion, might afterwards undergo slow exchange.

The requirements for exchange and the distributions of deuterium in exchanged L-chlorosuccinate and exchanged succinate can be rationalized on the basis of either of the following reaction sequences:

$$SH_2 + E_{ox} \rightleftharpoons SH_2 \cdot\cdot E \rightleftharpoons SH^{(+)} + EH \qquad (1)$$

$$SH^{(+)} \rightleftharpoons S + H^+ \qquad (2)$$

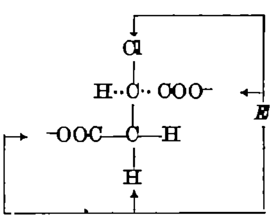
$$EH \rightleftharpoons EH' \rightleftharpoons EH'' \rightleftharpoons E_{red} + H^+ \qquad (3)$$

or:

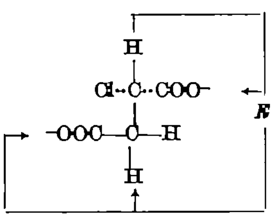
$$SH_2 + E_{ox} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} SH(-) \cdot\cdot E \rightleftharpoons S + EH \qquad (4)$$

$$EH \rightleftharpoons EH' \rightleftharpoons EH'' \rightleftharpoons E_{red} + H^+ \qquad (5)$$

In these sequences succinate and L-chlorosuccinate are represented by SH_2, fumarate and chlorofumarate are represented by S, oxidized succinic dehydrogenase is represented by E_{ox}, reduced enzyme by E_{red} and the reaction between reduced substrate and oxidized enzyme is written as a hydride ion transfer. In the first sequence, reactions 1, 2 and 3, the products of the initial transfer are written as SH^+, the carbonium derived from reduced substrate by loss of a hydride ion, and as *E*H, enzyme reduced by addition of a hydride ion. Both SH^+ and *E*H react further, SH^+ losing a proton with or without enzymatic intervention to give the oxidized product, S, and *E*H undergoing an internal oxidation, that is, the site originally reduced by the hydride ion undergoes oxidation at the expense of a neighbouring site and the hydrogen atom originally transferred may now exchange with protons of the medium. The second sequence (reactions 4 and 5) differs from the first only in that reaction is initiated by loss of a proton and then followed by hydride ion transfer. The described sequences are consistent with the conditions required for exchange since in the absence of added fumarate or chlorofumarate only the small amount of oxidized substrate produced by the stoichiometric amount of oxidized enzyme, *E*, would be available for the reverse reaction in which H^+ is incorporated, and it is to be expected that under these conditions incorporation of hydrogen would be minimal. Further, the presence of only reduced substrate would keep the enzyme substantially in the reduced state and relatively little catalyst would be avilable for the forward reaction. In the presence of added oxidized substrate, chlorofumarate or succinate, more substrate would be available for reversal of hydride transfer to enzyme and the ratio of oxidized to reduced enzyme would be increased. Since each effect results in an increased rate of reaction at an opposite side of the equilibrium, exchange with the system at equilibrium will be speeded up by the presence of added oxidized substrate. It should also be noted that exchange does not occur with D-chlorosuccinate, either in the presence or absence of chlorofumarate. Since D-chlorosuccinate is a competitive inhibitor of succinic dehydrogenase[1,2], it forms a complex with the enzyme and this complex and the enzyme complex with L-chlorosuccinate can be depicted[2] as:



and

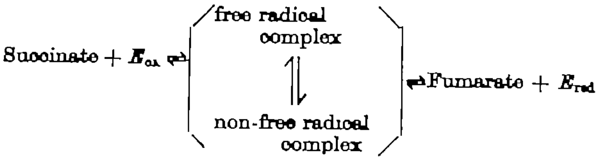


D-Chlorosuccinate complex

L-Chlorosuccinate complex

it is to be noted that the enzyme may attack the α-hydrogen of L-chlorosuccinate but not that of D-chlorosuccinate. On this basis only, experimental evidence not being available at present, it is considered that the α-hydrogen of L-chlorosuccinate is the hydrogen initially attacked by the enzyme. On the basis of the first sequence (initiation by hydride ion transfer) it follows that the *trans* β-hydrogen is initially exchanged and on the basis of the second sequence (initiation by proton transfer) it would follow that initially the α-hydrogen is exchanged. Again, the position of the initially exchanged hydrogen has not yet been experimentally demonstrated.

Hollocher and Commoner[8] have established that a free radical is formed in an anaerobic system consisting of succinic dehydrogenase, succinate and fumarate at equilibrium, all three components being required. From concentration effects of co-substrates on the amount of free radical formed, the following equilibrium reaction scheme was proposed:



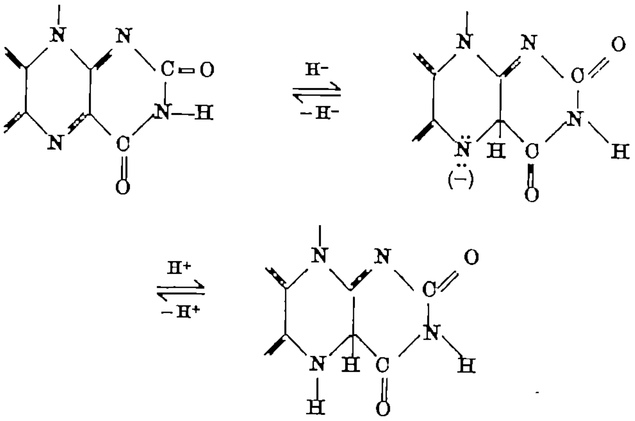
$$\text{Succinate} + E_{\text{ox}} \rightleftharpoons \left(\begin{array}{c} \text{free radical complex} \\ \upharpoonleft\downharpoonright \\ \text{non-free radical complex} \end{array} \right) \rightleftharpoons \text{Fumarate} + E_{\text{red}}$$

The experimental conditions required for the formation of free radical are the same as those required for exchange and the proposed schemes for free radical formation and exchange are similar in outline. It thus seems likely that hydrogen exchange and free radical formation occur by a common pathway and a hypothetical pathway utilizing F for the flavin moiety apparently covalently linked to apoenzyme[9] and Fe^{III} for the oxidation state[10] of iron in oxidized succinic dehydrogenase is given as follows:

$$\text{H}^- \text{ (from substrate)} + F\text{—}E\text{—}\text{Fe}_2^{\text{III}} \rightleftharpoons \text{H}F^{(-)} - E \cdots \text{Fe}_2^{\text{III}} \quad (1)$$

$$\text{H}F^{(-)} - E \cdots \text{Fe}_2^{\text{III}} \rightleftharpoons \text{H}F\cdot - E \cdots \text{Fe}^{\text{II}}, \text{Fe}^{\text{III}}$$
$$\text{H}F\cdot - E\cdot\cdot\text{Fe}^{\text{II}}, \text{Fe}^{\text{III}} \rightleftharpoons F\text{—}E\cdot\cdot\text{Fe}_2^{\text{II}} + \text{H}^+ \quad (2)$$

In this depiction, oxidized and reduced substrates and exchange with SH^+ have been omitted so as to direct attention on the enzyme. It is considered that hydride ion is transferred from substrate to the enzyme, the flavin moiety being the acceptor[11] and being reduced by the two electron transfer to an anion which in turn can reduce by two one-electron transfers two Fe^{III} ions of the four attached to enzyme[12], regenerating oxidized flavin and liberating transferred hydrogen as a proton. This scheme is not inconsistent with the interpretation of free radical data[8] and also provides for exchange of the second hydrogen of a removable *trans* pair at, presumably, a slower rate than exchange of the other (as a proton). This scheme also provides a direct pathway for the anaerobic reduction of DPN by succinate in mitochondria[13] since a hydride ion could be transferred from substrate to flavin to DPN. The intermediate role of flavin between hydrogen transfer and electron transfer also becomes apparent on the basis of the foregoing scheme. The site at which a hydride ion attacks riboflavin is not known and it may well be that hydride is not transferred to one of the two positions at which hydrogen is commonly considered to be attacked in reduced riboflavin[14]. By analogy with alloxan, it may be that riboflavin is attacked, as follows:



It should also be noted that iron–alkyl complexes have recently been reported[15] to undergo reversible removal of a hydride ion and it is possible that similar complexes are operative in the succinic dehydrogenase system.

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SOCIAL FORCES DETERMINING SPACING AND HEAD ORIENTATION IN A FLOCK OF DOMESTIC HENS

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It has been known for some time that animals do not space themselves at random under high density conditions; for example, Hediger's[1] concept of "specific individual distance" between animals in a group is based on the observation that there is a minimum distance below which animals do not approach one another.

Observations on domestic poultry suggested that the factor involved in this spacing tendency was visual and associated with the relative positions of other faces in the vicinity. Such a hypothesis could be tested by the analysis of head positions of poultry from photographs.

A stable flock of 750 Australorp × White Leghorn females 10 months of age housed intensively on deep litter in a shed 100 ft. × 30 ft. was used in this investigation. Photographs were taken from above by a hidden observer without regard to the content of the photographic field, which was 7·5 ft. × 5 ft., an area of about 38 sq. ft. To vary the number of birds per photograph the birds were then crowded into $\frac{1}{2}$ and then $\frac{1}{4}$ of the total area, and in all 24 usable photographs were obtained, with from 5–17 birds per photograph.

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Two hypotheses were tested from these photographs: (1) Is the distribution of head locations random? (2) Are the heads randomly oriented?

The method of Clark and Evans[2] was used to test randomness of head locations. This method uses the distance to nearest neighbour of each bird to calculate a coefficient, R, which varies from zero (extreme clumping) to 2·15 (perfectly uniform spacing) with an expectation of 1·0 when spacing is random.

The second hypothesis is that the orientation of the head of every bird depends on the proximity and orientation of the heads of its neighbours. Any neighbour may influence a bird directly or through its effect on another neighbour. A simple model for testing direct effects of neighbours is the partial regression equation:

$$Y = b_1X_1 + b_2X_2$$

where Y, X_1 and X_2 are as defined in Fig. 1, and b_1 and b_2 measure the independent effects on Y of X_1 and X_2 respectively.

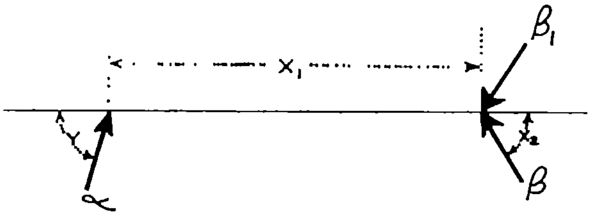

Fig. 1. The arrows represent the position of heads of fowls α, β and β_1. β is the nearest neighbour of α and their head angles to face each other are Y and X_2. The distance between them is X_1.

It can be seen from Fig. 1 that birds β and β_1 each have the same X_2 angle. Since these may be different social situations, β_1 is classed as 'opposite' and β as 'same' side angles. Separate analyses were made for each situation to determine whether they gave rise to different regressions.

The regressions were calculated for nearest, second nearest and third nearest neighbours, on the assumption that nearest neighbours would exert the strongest influence.

Birds around the edges of the photograph were included in the analyses although their correct ranking neighbours may not have appeared on the photograph. The errors introduced by this procedure are unknown, but would lower the apparent significance of the regressions obtained, if indeed real relationships were present.

(1) *Distribution of Head Locations.* Twenty-four photographs (239 birds) were analysed; an average R value of 1·259 ± 0·0110 was obtained. This represents a very highly significant ($P < 0\cdot001$) deviation from randomness, indicating that birds' heads tend to be somewhat evenly spaced throughout the pen.

(2) *Influences on Head Orientation.* (*a*) ***First, second and third nearest neighbours.*** Partial regression coefficients, b_1 and b_2 for first, second and third nearest neighbours were calculated for each of the 24 photographs, the results being summarized in Table 1.

Table 1. MEAN VALUES OF b_1, b_2 AND THE POOLED (WITHIN ANALYSIS) PROPORTION OF VARIANCE DUE TO REGRESSION (r^2) FOR FIRST, SECOND AND THIRD NEIGHBOURS

	Neighbours First	Second	Third
b_1 (degrees/ft.)	23·1 ± 7·8**	10·8 ± 7·9	1·9 ± 10·3
b_2 (degrees/degrees)	−0·326 ± 0·063***	−0·113 ± 0·079	−0·217 ± 0·109*
r^2	0·336 (F = 1·76*)	0·254 (F = 1·19)	0·224 (F = 1·01)

Table 1 shows clearly not only the magnitude of the effects found but also that the relationships are mainly concerned with nearest neighbour, though there may be some effect of other near neighbours.

(*b*) *Nearest Neighbour Analyses.* The mean b_1 and b_2 values for 'same' and 'opposite' sides are as follows:

b_1 (same) = 50·5 ± 30·8, b_1 (opposite) = 44·9 ± 41·32
b_2 (same) = −0·35 ± 0·12, b_2 (opposite) = −0·42 ± 0·27

The statistical differences between the two situations are not significant, so that social differences are probably also negligible, if present. The division into two classes reduced the degrees of freedom per analysis, and raised the standard errors very considerably.

The effect of the proximity of the nearest neighbour is clearly established (b_1, Table 1). For any constant head angle of its nearest neighbour, a bird turned 23° to face that neighbour as the latter approached one foot, and turned away as the neighbour retreated. The regression measures the linear or average component of this movement, but it is not possible to say whether in fact the effect is the same over all ranges of distance.

The correlation between X_1 and X_2 was calculated for each photograph and an average value of 0·239 ± 0·084** was found. Thus its nearest neighbour also turned towards each bird as the distance between them decreased. The general picture is, then, that as two birds approach each other, they also face each other more directly. This motion appears from observations to be defensive.

On the other hand, the average b_2 value for nearest neighbours was negative and highly significant (b_2, Table 1). This means that at any constant distance between neighbours they tend to avoid each other's frontal aspect. Once again this is an average effect.

When the pooled within photograph partial regression equation was calculated the following results were found: $b_1 = 22\cdot9 \pm 4\cdot8^{***}$; $b_2 = -0\cdot130 \pm 0\cdot063^{*}$. The agreement with the average value is good for b_1, but not nearly so good for b_2. This suggests that the b_2 values for separate photographs are heterogeneous. One factor likely to be responsible, density, was examined. The regression of b_2 on number of birds per photograph (N) was:

$$\hat{b}_2 = -1\cdot338 + 0\cdot103\,N \ (P \simeq 0\cdot10)$$

where $\hat{b}_2$ is the estimated value of b_2 from this regression. This regression, though not significant, may be a partial explanation of the heterogeneity of b_2 values. If genuine, this relation would mean that at high densities the hens show less tendency to avoid each other's frontal aspect, which is consistent with the finding earlier, that as the distance between neighbours diminishes, they tend to face each other. Alternatively, it could be suggested that at higher densities the presence of many birds in the immediate vicinity may make it impossible for the hen to avoid simultaneously the faces of its neighbours.

Discussion and Summary

Evidence has thus been presented to show that hens do not arrange themselves at random under normal intensive housing conditions. The spacing of the heads is more regular than it would be under random spacing. This regularity is probably based on visual interactions with neighbouring birds. As a neighbour approaches, a bird tends to turn to face it. Nevertheless, they tend to avoid each other's frontal aspect in so far as this is compatible with the tendency already mentioned. There was slight, but not significant evidence that this latter tendency decreased as density increased. Second and third nearest neighbours had less effect than did nearest neighbours.

It seems clear from this evidence that the presence of a bird's head constitutes a social force on its neighbours. The word 'force' is here used to mean that there is a measurable effect on behaviour of neighbours.

This force diminishes with distance and is maximum directly in front of the head. A possible analogy is to compare this force with the fields of magnetic force associated with a single pole of a magnet.

The movements of birds in such a field of forces are not at random—they maintain their heads at a regular pattern of spacing and orientate them to avoid the frontal aspects of other birds, yet turn, probably defensively, to face neighbours who approach closely.

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RIBONUCLEOPROTEINS IN LEAVES INFECTED WITH TOBACCO MOSAIC VIRUS

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THE presence of at least two proteins characteristic of tobacco mosaic virus (TMV) infection were demonstrated by introducing amino-acids labelled with carbon-14 to sections of diseased tobacco leaves in nutrient solutions. These proteins were detected using agar-gel electrophoresis[1,2]. Anomalous ribonucleoproteins in the supernatant fluids after ultracentrifuging extracts of diseased plants were noted by Bawden and Pirie in 1956 (ref. 3) and by Dupont-Mairesse and Jeener in 1960 (ref. 4). The former purified their preparations by hydrogen ion and salt fractionation and the latter by a precipitin reaction. This investigation reports on the separation of ribonucleoproteins related to TMV by agar-gel electrophoresis and describes some of their biochemical properties.

A common strain of TMV was purified by passage through the local lesion host *Nicotiana glutinosa* L. and then returned to seedlings of the systemic host *Nicotiana tabacum* L. var. Haronova from which leaf samples were later taken for this investigation. Components of extracts from the resulting diseased plants were separated by agar-gel electrophoresis. The infectivity of the virus preparations was assayed quantitatively using *N. glutinosa*.

Serologically related fractions were located within the agar gel by immunoelectrophoresis[5]. The following procedure was used to prepare antibody against TMV. Leaves of TMV diseased *N. tabacum* were frozen overnight, thawed and ground and the extracted juice adjusted to *p*H 7·2 with potassium phosphate. The juice was submitted to two cycles of differential centrifugation at 78,000*g* for 90 min and 2,000*g* for 15 min. The resulting pellet was dialysed against physiological saline. The antigen was injected into rabbits. (The TMV antiserum was supplied by Dr. N. S. Wright.)

Uniformly labelled L-leucine-^{14}C was obtained from the Biochemical Centre, Amersham, England, and radioactive orthophosphate was obtained from Atomic Energy of Canada, Ltd. Radioactivity was measured with a Nuclear-Chicago *D*47 gas flow Geiger tube. The relative amount of radioactive leucine and phosphate incorporated into the same sample was determined by measuring the total disintegration and the disintegration of phosphate. The radioactivity rate of the phosphate was obtained by placing a piece of celluloid at a defined distance between the Geiger tube and the sample. The celluloid absorber reduced the radioactive carbon detection by 99·8 per cent and the phosphate detection by 23·1 per cent.

When the agar was dried by flowing dry air over one side of the agar sheet, the particles within the gel diffused to the surface forming an infinitely thin layer. After removing the buffer salts the radioactivity of this thin layer was measured directly.

The term 'soluble antigen' as used in this investigation was that phosphorus-free soluble protein serologically related to TMV (ref. 6). Tentative identification of this zone was accomplished by co-electrophoresis with a nucleic acid-free preparation of TMV (ref. 7).

A description of the technique for sampling the agar gel for electron microscopy has been published[8]. Electron micrographs were kindly taken by a colleague, Dr. M. Weintraub.

(*a*) *Agar-gel fractionation of crude TMV plant extracts.* Earlier work on crude diseased plant extracts on agar gel revealed a number of fractions peculiar to the disease[2]. The so-called 'fast' band was probably the most readily detectable fraction apart from the band of non-aggregated TMV. Following the TMV band were other bands associated with the disease in addition to bands formed by aggregated TMV particles.

From the results of many experiments on crude TMV extracts at various intervals following infection of the host and on diseased hosts grown under a variety of conditions, it was concluded that the concentration of the fast band relative to the TMV band was variable. When the TMV diseased host, *N. tabacum*, was placed in unfavourable conditions of growth, the concentration of the fast band was greatly augmented. Concentration of the fast band was much greater from infected leaf disks than from an infected, intact, vigorous host plant.

(*b*) *Immunoelectrophoresis of crude TMV plant extracts.* After fractionation of the crude extracts by electrophoresis, at least four areas within the agar were serologically related to TMV (Fig. 1). Since serological activity in agar gels can be observed only by visible precipitin reactions, there may be other serologically related areas which were not detected. The precipitin reaction within the gel appeared to be the same for the area near the origin and the TMV band. These areas gave a narrow but strong reaction. The serologically related areas running before and behind the TMV band were rather diffuse faint broad areas extending out from the TMV band. No precipitin reaction could be detected by using the same TMV antiserum against healthy extracts nor extracts of plant tissue infected with potato *X* virus.

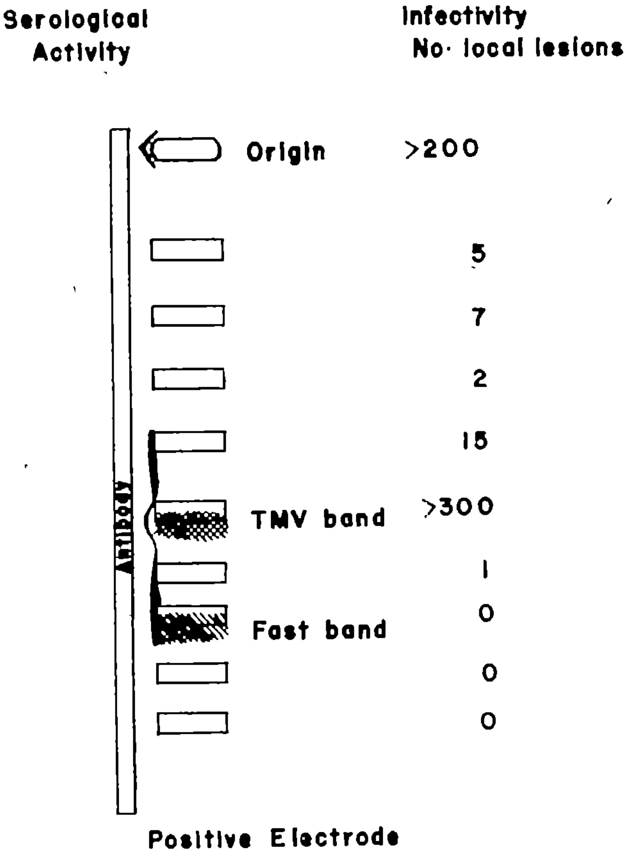

Fig. 1. Location of serological activity and infectivity of a crude TMV plant extract after agar gel electrophoresis

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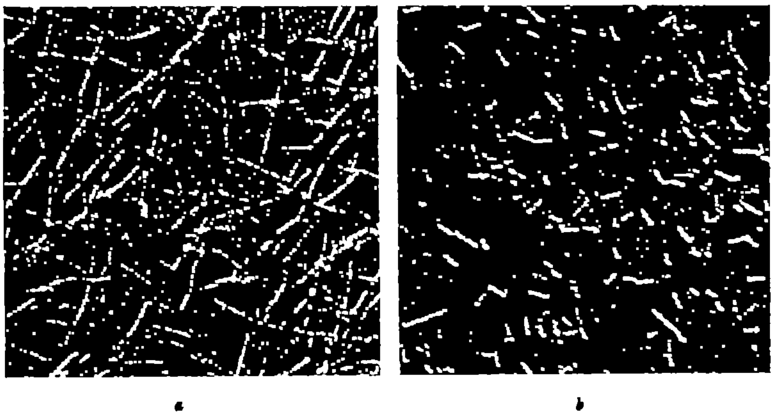

Fig. 2. Electron micrographs of material from the (*a*) TMV band and (*b*) fast band of a crude TMV plant extract after agar-gel electrophoresis (shadowed with palladium; × *c*. 13,300)

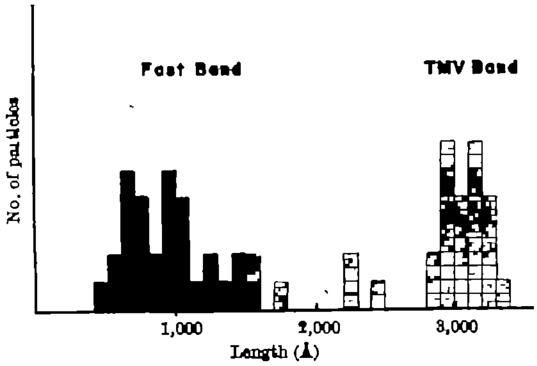

Fig. 3. Size distribution of particles from the TMV band and fast band from Fig. 2 (black and shaded) indicate the size of the particles found in the electron micrographs of the fast band and the TMV band respectively

(*c*) *Location of infectivity within agar gels*. The infectivity of various locations within the agar gels was determined by crushing samples of agar from these areas over the leaf surface of the local lesion host, *N. glutinosa*. The results of such an experiment are shown in combination with serological activity in Fig. 1.

Several experiments indicated that there may be comparatively low infectivity within the fast band in an area nearest to the TMV band. Infectivity was never observed in the distal areas of the fast band. A high proportion of the infectivity did not migrate from the origin and low infectivity was detected scattered between the origin and the TMV band.

(*d*) *Electron microscopy*. Electron microscopy of TMV preparations fractionated by agar-gel electrophoresis was rewarding. From a crude preparation, electrophoresis fractionated the sap according to size, charge on the particle, and adsorption on agar gel[8,9]. Thus, the preparations for electron microscopy were exceptionally free of debris, containing particles characteristic of a particular zone. Electron micrographs of the TMV band and the fast bands are shown in Fig. 2. Size distribution is shown in Fig. 3.

(*e*) *Radioactive isotope studies*. Labelled orthophosphate and amino-acids can be incorporated into TMV (refs. 2 and 10) in amounts that can be measured easily by determining the radioactivity in the dried gels. Fig. 4 shows the rate of incorporation of orthophosphate and leucine into TMV in 2- to 3-mm leaf squares. Two observations appear interesting: leucine-^{14}C is detected in the TMV particle earlier than orthophosphate; the curve for isotope incorporation into the TMV band is very similar to that of the fast band.

The ratio of leucine-^{14}C incorporated into TMV to leucine-^{14}C incorporated into the fast band increased with time. In the latter experiment the fast band has about half the total radioactivity of the TMV band, but 1/10 the protein as measured by optical density or by staining. (Optical density of the bands within the gel was measured by a continuous scanning apparatus devised by me.) Therefore, the specific radioactivity of the protein in the fast band exceeds that of the TMV band. However, since the newly synthesized virus is diluted with pre-formed virus, the specific activity does not indicate the sequence of formation of the virus nor the other fraction. Whereas the incorporation of leucine into the TMV band and the fast band is of the same type as judged by the curves, the amount of radioactive phosphorus taken up by these two fractions was quite different. This suggested that the nucleic acid in these two fractions was at least in part dynamically different. Probably a large

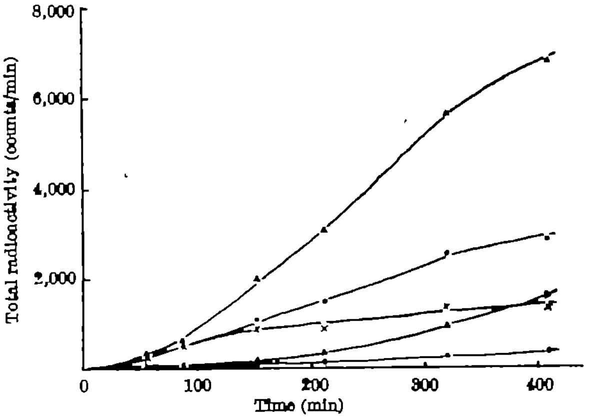

Fig. 4. Rate of orthophosphate and leucine incorporation into the TMV band, the fast band, and the soluble antigen. 2–3 mm leaf squares from plants suffering from severe mosaic and light green in colour (pot bound) were placed in Hoagland's solution (ref. 1) containing 10 μc. of uniformly labelled leucine-^{14}C and 100 μc. of orthophosphate-^{32}P. Samples were removed at time intervals and the plant juice separated by electrophoresis (ref. 1)

▲, TMV band leucine-^{14}C; ●, fast band leucine-^{14}C; ×, soluble antigen leucine-^{14}C; Δ, TMV band orthophosphate-^{32}P; ○, fast band orthophosphate-^{32}P

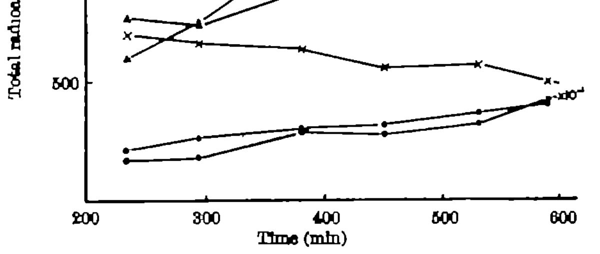

Fig. 5. Rate of orthophosphate and leucine depletion in the TMV band, the fast band and the 'soluble antigen'. 2–3-mm leaf squares from plants suffering from severe mosaic were placed in distilled water for 20 h under artificial light. The distilled water was replaced with Hoagland's solution containing 10 μc. of uniformly labelled leucine-^{14}C and 100 μc. of orthophosphate-^{32}P. After 60 min, the leaf tissue was washed four times with distilled water followed by three washings with Hoagland's solution containing the non-radioactive substrate (0·14 g/l. KH_2PO_4 and 0·1 g/l. of L-leucine). The leaf tissue was placed in the latter non-radioactive substrate and samples removed at time intervals for extraction and electrophoresis. The symbols are the same as in Fig. 4

part of the radioactive phosphorus of TMV was derived from *in vitro* incorporation as previously described[11]. The leucine-^{14}C content of the 'soluble antigen' behaved as if this fraction was transient. No radioactive phosphate was located in the 'soluble antigen' area.

In order to determine whether the radioactivity of some fractions was transitory, the diseased leaf sections were permitted to take up radioactivity for a short period, washed to remove extra-cellular radioactivity and then incubated in non-radioactive substrate for given times (Fig. 5). It was seen that the fraction corresponding to the soluble antigen was the only one which lost total radioactivity suggesting that the soluble antigen itself was transitory. Radioactivity in the fast band and the TMV band increased during the experiment. It was decided that leaf disks or sections were unsatisfactory for this type of experiment because of the long time required to deplete the tissue of radioactivity.

From these experiments it is readily seen that electrophoresis of TMV preparations in agar gels provides a valuable analytical tool for biochemical investigations. From a single small sample fractionated by agar-gel electrophoresis it was possible to obtain data on size, electrophoretic mobility, infectivity, ratio of incorporation, concentration and serological activity.

It has been shown by me[10] and recently by Dupont-Mairesse and Jeener[4] that nucleic acid may be incorporated into or associated with the virus or virus protein during extraction of the virus from the diseased leaf, but whether this is merely association or a demonstration of synthesis remains to be proved. The fast band described here is consistently found in TMV preparations isolated by centrifugation at 78,000*g* for 90 min. The band does not appear to be a degradation product of the virus because there is higher specific activity of the protein portion and the concentration of the fast band is independent of the concentration of the TMV band. If the particles of the fast band are on the path to synthesis of the virus then it could be assumed that the nucleic acid within this protein would also be found as a constituent of the typical virus particle. But the rate of isotopic enrichment in these particles is different from that of the finished virus particles. Therefore the mature virus particle could not be synthesized by linear polymerization of the material of the fast band unless the additional phosphate radiation found in the mature virus is added at this time. Thus it is conceivable but not proved here that the virus protein of the fast band could have the role of selectively gathering specific ribose polynucleotides of the cell the composition of which would not resemble the typical virus nucleic acid until a final synthetic step.

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ORIGIN OF METHYL KETONES FOUND IN STEAM DISTILLATES OF MILK FAT

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It has recently been suggested by Boldingh and Taylor[1] that the homologous series of methyl ketones with an odd number of carbon atoms from C_7 to C_{15}, obtained by steam distillation of milk fat at 180° C, is formed from β-keto-acids. Their experiments indicated that β-keto-acids are present in bound form as triglyceride components in milk fat. Investigations in progress when Boldingh and Taylor's report[1] appeared have shown that very similar quantities and relative proportions of the same homologous series of methyl ketones are formed as artefacts during the steam distillation of butterfat at 100° C. Exhaustive distillation indicated that only limited quantities of the methyl ketones could be obtained from milk fat and that the precursors were thus present in relatively small amounts[2].

In an attempt to confirm that the β-keto-acids (or other precursors of these methyl ketones) were intermediates in fatty acid synthesis, milk fat was obtained from a cow which had been milked 9 and 22 h after the intravenous injection of [carboxy-^{14}C]-acetate (5 mc.). 15 g of a composite sample of the milk fat from the two milkings were then exhaustively steam distilled at 100° C, the distillate (10 l.) being led directly into 2,4-dinitrophenylhydrazine reagent. The distillate and reagent mixture were held at room temperature for 48 h, the 2,4-dinitrophenylhydrazones extracted and separated by column partition chromatography[3]. The fractions were identified, and their purity established, by paper chromatography[4,5]. The concentrations of methyl ketones were determined spectrophotometrically as the 2,4-dinitrophenylhydrazones at 362 mμ. In addition to the homologous series of methyl ketones from C_3 to C_{15} with odd numbers of carbon atoms, small amounts of butan-2-one were also isolated (Table 1).

To determine specific activities, suitable aliquots of each of the 2,4-dinitrophenylhydrazones were dissolved in 5 ml. scintillation fluid (0·5 per cent 2,5 diphenyloxazole + 0·03 per cent 1,4-di-[2-(5-phenyloxazolyl)]-benzene in toluene) and counted at room temperature in a liquid scintillation system (*NE* 8301; Nuclear Enterprises (G.B.), Ltd., Edinburgh). Corrections for quenching by the 2,4-dinitrophenylhydrazones were established by the use of *n*-hexadecane labelled with carbon-14 as an internal standard. The specific activities of the isolated 2,4-dinitrophenylhydrazones, corrected for quenching, showed that C_9, C_{11}, C_{13} and C_{15} methyl ketones exhibited the highest activities (Table 1).

Table 1. DISTRIBUTION OF RADIOACTIVITY IN METHYL KETONES OBTAINED BY EXHAUSTIVE STEAM DISTILLATION AT 100° C OF MILK FAT FROM A COW AFTER INTRAVENOUS INJECTION OF [CARBOXY-^{14}C]-ACETATE

Methyl ketone	mg/kg milk fat	Moles as per cent of total	Specific activity/ mg C*	Per cent of total radioactivity
C_{15}	51	11·8	5,820	13·7
C_{13}	28	7·4	9,800	23·3
C_{11}	15	4·6	19,170	45·2
C_9	20	7·4	4,290	10·1
C_7	28	12·8	1,690	4·0
C_5	16	9·7	270	0·6
C_4	8	5·8	1,300	3·1
C_3	45	40·5	—	—

* Methyl ketones counted as 2,4-dinitrophenylhydrazones and corrected for quenching.

This was confirmed qualitatively by preparing autoradiographs of the paper chromatograms used to separate and identify the 2,4-dinitrophenylhydrazones. No radioactivity was detected in the 2,4-dinitrophenylhydrazone of acetone but the excessive quenching in this instance made it difficult to establish the accuracy of this observation. In an attempt to overcome quenching, the methyl ketones from C_7 to C_{15}, which had been shown to possess a large

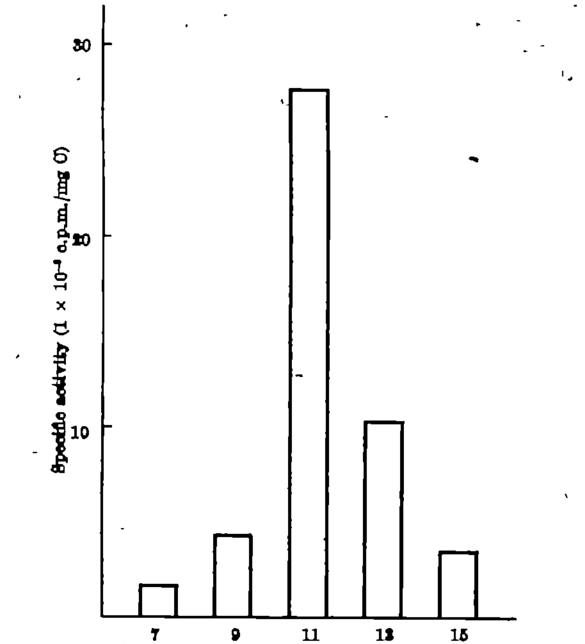

Fig. 1. Relationship between specific activity and chain length of methyl ketones found in steam distillates of milk fat from a cow intravenously injected with 5 mc. of [carboxy-^{14}C]-acetate. Methyl ketones were regenerated from the corresponding 2,4-dinitrophenylhydrazones

proportion of the total radioactivity, were regenerated from the corresponding 2,4-diphenylhydrazones with lævulinic acid[6] and counted as here. Much less quenching was encountered and the correction factors needed, as determined by the use of ^{14}C-*n*-hexadecane, were correspondingly smaller. The specific activities obtained by this method (Fig. 1) were, with the exception of the increased activity of C_{11}, almost identical to the activities determined from the 2,4-dinitrophenylhydrazones directly.

There is a marked similarity between the carbon-14 labelling pattern of these methyl ketones and the fatty acids isolated by Popják, French, Hunter and Martin[7] from a composite sample of milk fat obtained 1–12 h after the intravenous injection of a lactating goat with [carboxy-^{14}C]-acetate. Although the C_{11} methyl ketone and the C_{12} fatty acid possessed the maximum activities in the respective experiments, the activities fell away on either side of these maxima in like manner. Some differences in labelling pattern are to be expected since the milk fat in the present investigation was obtained from a cow milked 9 and 22 h after injection. In another experiment Gerson, Hawke, Shorland and Melhuish[8], using milk fat from a cow into which ^{14}C-valerate had been injected intravenously, found two maxima of specific activity, one at C_{12} for the fatty acids C_{10}–C_{16} and the other at C_4 for the range C_2–C_8. More significantly Popják *et al.*[7] found that fatty acids of higher molecular weight than palmitic acid possessed very little radioactivity and in the present investigation no heptadecan-2-one could be detected in any of the distillates, despite the relatively large amounts of C_{18} acids in milk fat.

The generally accepted view is that, in the biosynthesis of milk fat[9–11] and other lipids[12], the fatty acids up to and including palmitic are synthesized from an acetate pool whereas C_{18} acids and above are synthesized by less direct pathways or from the blood lipids. The agreement in the specific activity patterns of the methyl ketones found in steam distillates of milk fat and the corresponding fatty acids with one more carbon atom would appear to justify the conclusion that both have common precursors such as β-keto-acids. It is conceivable that trace amounts of such precursors, intermediates in fatty acid synthesis from acetate in the mammary gland, escape normal reduction to the corresponding fatty acid and become incorporated in the triglyceride molecule of milk fat.

We gratefully acknowledge the gift of radioactive butterfat from Mr. T. Gerson, Fats Research Laboratory, Wellington.

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EFFECT OF PUROMYCIN ON THE REPLICATION OF WESTERN EQUINE ENCEPHALITIS AND POLIOMYELITIS VIRUSES

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IN previous work we have described the effect of two amino-acid analogues on the replication of Western equine encephalitis virus (WEE)[1] and poliovirus[2]. *Para*-fluorophenylalanine (FPA) was shown to reduce cellular protein synthesis if used at sufficiently high concentrations[1]. Under these conditions FPA also inhibited the appearance of infectious viral RNA and viral antigens[3]. In contrast, FPA at low concentrations did not inhibit the synthesis of protein in the infected cell nor did it inhibit the formation of infectious RNA of WEE and poliovirus[2,3]. However, no infectious virus was formed, and it was found that this was most likely due to the incorporation of the amino-acid analogue into viral proteins (ref. 3, cf. ref. 5). Another amino-acid analogue—ethionine—which is always incorporated and even at high concentrations does not inhibit protein synthesis significantly produced effects on WEE virus formation similar to those of FPA at low concentrations[4]. Thus it was suggested that the synthesis of aberrant proteins due to the incorporation of amino-acid analogues is still compatible with the proper formation or preservation of infectious viral RNA (ref. 5). On the other hand, any substance which actually inhibits the mechanism of protein synthesis, such as FPA at high concentrations, might be expected to interfere with the appearance of infectious RNA in the cell.

Because puromycin is a powerful inhibitor of protein synthesis (cf. refs. 3 and 5) and has a mechanism of action supposedly quite different from that of amino-acid analogues[6,7], its effect on the appearance of infectious RNA of WEE and polio was examined. Fig. 1 shows the dose response curves to puromycin of virus maturation and appearance of infectious RNA in the case of WEE. Virus maturation is more sensitive to the inhibitor than the synthesis of infectious RNA, although the differences are somewhat less pronounced than with FPA (ref. 2).

Puromycin at 7·5 μg/ml. can also inhibit completely the appearance of infectious polio RNA in HeLa cells (Fig. 2). At this concentration the incorporation of lysine-^{14}C into cellular proteins is drastically inhibited (Fig. 3). By simply removing the inhibitor from the infected cultures, the infectious RNA accumulated in the cells at a rate which was identical to that of the controls. This is demon-

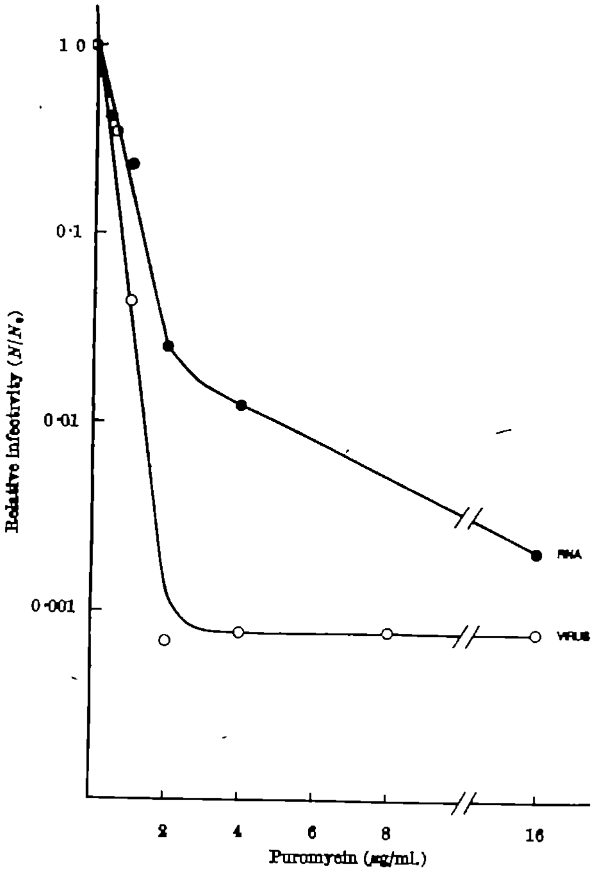

Fig. 1. Dose response of the formation of WHE virus and infectious RNA to puromycin. The infected cells were exposed to the indicated concentrations of puromycin for 5 h. Aliquots of cells were then tested for cell-associated virus (CAV) and cold phenol extractable RNA

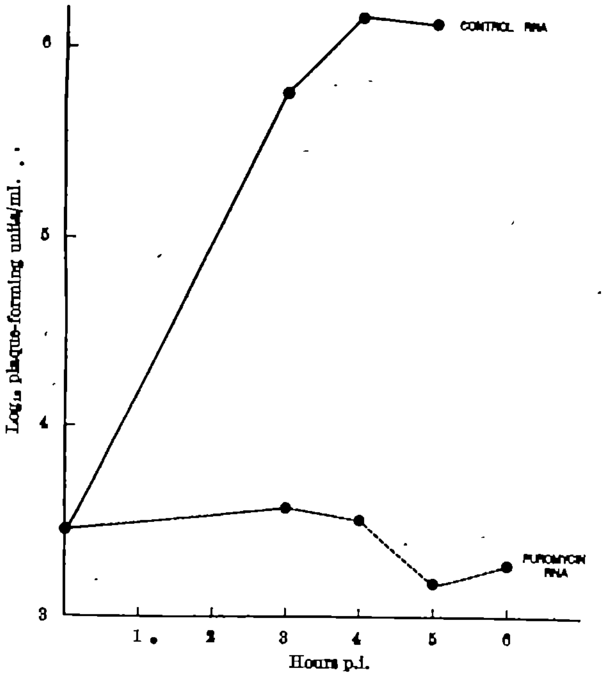

Fig. 2. Inhibition by puromycin of the demonstrable formation of infectious polio RNA. Infected HeLa cells were extracted for infectious polio RNA after various times of incubation. The inhibited cells received 7·5 μg/ml. of puromycin at time 0. Time 0 is the time of re-incubation following 1 h of incubation for virus adsorption

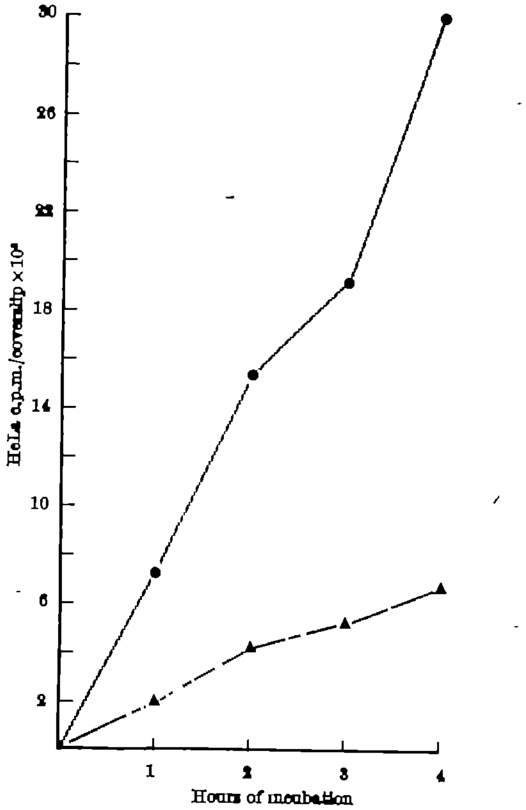

Fig. 3. Inhibition of ^{14}C-lysine into the proteins of HeLa cells in the absence and presence of puromycin (7·5 μg/ml.). ●, HeLa control, ▲, HeLa puromycin

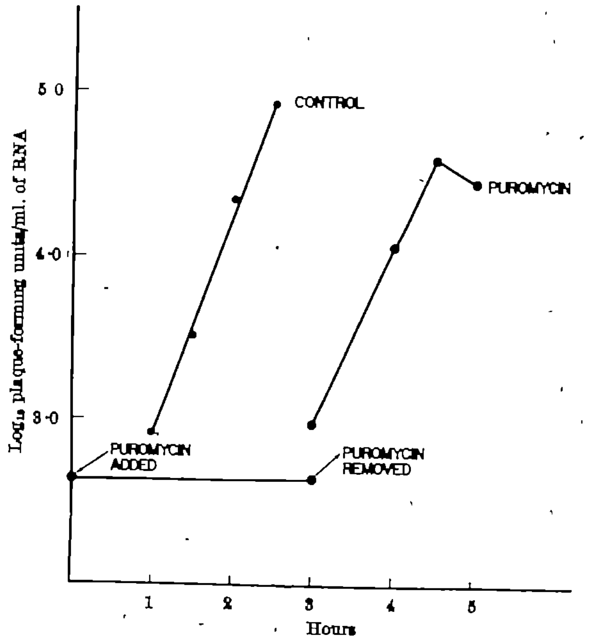

Fig. 4. Onset of polio RNA formation after removal of puromycin. The puromycin-inhibited cells were incubated for 3 h with 7·5 μg/ml. of puromycin following 1 h of virus adsorption. The inhibitor was removed by washing the cells twice with prewarmed phosphate-buffered saline. Prewarmed medium was added and the cells were further incubated for the indicated times

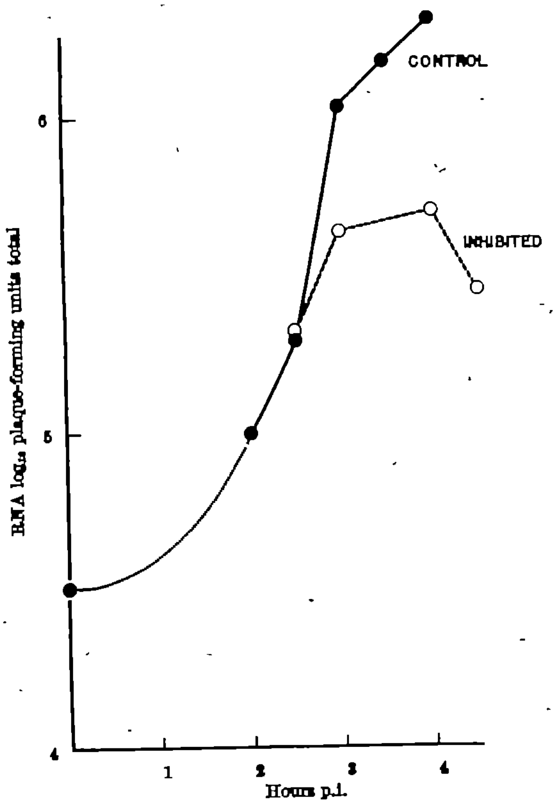

Fig. 5. Interruption of polio RNA formation by puromycin. An aliquot of the infected cells received 15 µg/ml. of puromycin at time 2½ h. Samples of controls and inhibited cells were extracted for infectious RNA at the times indicated

strated in Fig. 4, which also shows that there was no appreciable lag period between removal of the inhibitor and increase of infectious RNA. It would appear, therefore, that the lag period usually observed between infection and the rise of infectious polio RNA is due to mechanisms other than those which are inhibited by puromycin. This is of particular interest with respect to the problem of the requirement for an 'early protein synthesis' before the production of infectious RNA in the case of certain animal viruses. Our results suggest that HeLa cells may replicate polio RNA without the preceding synthesis of novel proteins, for example, enzymes. Such a possibility was already suggested by previous experiments with FPA (refs. 1 and 5) and more recently by results obtained by Wheelock using puromycin in HeLa cells infected with Newcastle disease virus[6]. Other alternatives to this conclusion are: (1) that early proteins are produced within a very short period following removal of the inhibitor; or (2) that the early proteins were formed despite the puromycin which at the concentrations used may still allow some protein synthesis to occur (Fig. 3).

The increase of infectious viral RNA cannot only be prevented but can also be interrupted by puromycin. This is shown in Fig. 5 in the case of poliovirus-infected HeLa cells. Thus, there is strong evidence that the continuing synthesis of a non-enzyme protein is also required for either formation or preservation of infectious polio RNA in HeLa cells. The fact that puromycin, like FPA, halts any additional increase of infectious RNA adds considerably to the previously advanced notion that the protein in question is non-enzymatic in function[1,5]. When FPA is used as an inhibitor, this amino-acid analogue could still permit the synthesis of a theoretical enzyme which is not enzymatically active but is nevertheless able to compete for the substrate sites with active enzymes formed before the addition of FPA. Such competition between active and inactive enzyme molecules of comparable affinity to a given substrate could, of course, drastically reduce or even abolish formation of the end-product of the reaction, that is, the infectious RNA. With puromycin as inhibitor such a mechanism is less feasible because it is likely to either permit the synthesis of functional enzymes or inhibit their synthesis completely. Both puromycin and FPA have now been shown to exert very similar effects on the appearance of infectious RNA. It might therefore be concluded that in either case the protein which is required for the appearance of infectious RNA is not an enzyme but a structural protein, that is, the viral protein[5].

The work was supported in part by a contract with the U.S. Army Chemical Corps Biological Laboratories, Fort Detrick, Maryland, and a grant from the Samuel S. Fels Fund.

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PRE-LUMIRHODOPSIN AND THE BLEACHING OF VISUAL PIGMENTS

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THE visual pigment rhodopsin is composed of the protein opsin to which is attached as chromophore a hindered *cis* isomer of vitamin A aldehyde, 11-*cis* retinal. (In accordance with recent recommendations of the Committee on Nomenclature of the International Union of Pure and Applied Chemistry, we shall use hereafter the following terminology: for vitamin A, 'retinol'; for vitamin A aldehyde (retinene), 'retinal' or 'retinaldehyde'; for vitamin A acid, 'retinoic acid'.) Rhodopsin bleaches in the light over a series of transient, highly coloured intermediates to an eventual mixture of opsin and all-*trans* retinal[1].

Some years ago, by irradiating rhodopsin at −50° to −100° C, we obtained what we thought to be the first such intermediate, called lumirhodopsin. On warming to about −20° in the dark, lumirhodopsin yielded a second intermediate, metarhodopsin; and on further warming and with access to water this hydrolysed to the final mixture of all-*trans* retinal and opsin[2].

Hubbard and Kropf analysed these processes further[3]. Free retinal in solution is isomerized by those wave-lengths of light that it absorbs to an eventual steady-state mixture of all possible *cis* and *trans* isomers[1]. Light acts in the same way on the retinal attached as chromophore in a visual pigment. The absorption of a first photon by rhodopsin, for example, activates the isomerization of its 11-*cis* chromophore to all-*trans*. The absorption of a second photon produces a new electronically excited state, from which the molecule may emerge all-*trans* or in any of its mono*cis* configurations. Long-continued irradiation at $-50°$ to $-100°$ consequently ends in a steady-state mixture of isomers, still attached to opsin. In this final mixture, the 11-*cis* fraction is rhodopsin; the 9-*cis* fraction is isorhodopsin[1]; the all-*trans* fraction, with perhaps a representation of other *cis* isomers, is lumirhodopsin. On warming in the dark, the lumirhodopsin bleaches over metarhodopsin to retinal and opsin; the rhodopsin and isorhodopsin, which are stable in the dark, remain as residues. The change from lumi- to meta-rhodopsin involves a rearrangement of the protein structure.

A few years ago Yoshizawa *et al.* at Osaka University began to obtain evidence that the irradiation of rhodopsin at liquid nitrogen temperature ($-195°$ C) yields an earlier product than lumirhodopsin[4]. Constraints of equipment limited these workers to indirect measurements, which showed, however, that the new intermediate is developed maximally by irradiating at $-195°$ with light of short wave-lengths (440 mμ), and that it reverts almost completely to rhodopsin on re-irradiating with light of wave-length 612 mμ at the same temperature[5]. The absorption spectrum of the new intermediate must therefore lie far toward the red from that of rhodopsin, which scarcely absorbs light at 612 mμ. This intermediate was shown to be stable to about $-140°$, above which it forms lumirhodopsin[6]. We shall call it pre-lumirhodopsin.

Grellmann, Livingston and Pratt at the University of Minnesota have recently examined the new intermediate in solutions and suspensions of rod outer segments, and have studied the kinetics of its decay to lumirhodopsin[7].

The present investigation confirms Yoshizawa's earlier observations by direct low-temperature spectrophotometry, and goes on to analyse the status of pre-lumirhodopsin in the chain of intermediates involved in the bleaching of rhodopsin and isorhodopsin.

Methods

All measurements were made with the Cary 11 recording spectrophotometer. The absorption vessel was modified from one for which we are indebted to Dr. W. B. Gratzer[8], in which a cold finger, filled with liquid nitrogen, ends in a copper block which holds the preparation. All this is enclosed within a glass jacket, the space between the cold finger holding the preparation and the jacket being continuously evacuated. For further insulation the cell is embedded in a block of 'Styrofoam', the light path being closed at the ends with plane quartz windows. The rhodopsin solutions, in 2 per cent aqueous digitonin, were mixed with various proportions of glycerol. In the absorption vessel they form a film about 0·8 mm thick between a front quartz plate and a back opal glass plate, held in the copper block with screw-on rings. A similar plate of opal glass is placed in the comparison beam. The temperature is measured continuously with an iron–constantan thermocouple inserted in the copper block. Pouring liquid nitrogen in the cold finger brings the temperature of the pigment solution close to $-195°$. Other temperatures are obtained by allowing the system slowly to warm. At temperatures below about $-50°$, the glycerol–water mixtures form a glass. Below about $-100°$, this cracks and crazes to various degrees. The resultant scattering of light is swamped by the opal glass window directly behind the pigment solution. Before measuring each spectrum the recording pen is arbitrarily zeroed at 700–750 mμ, a wave-length range in which none of these pigments absorbs appreciably.

Measurements

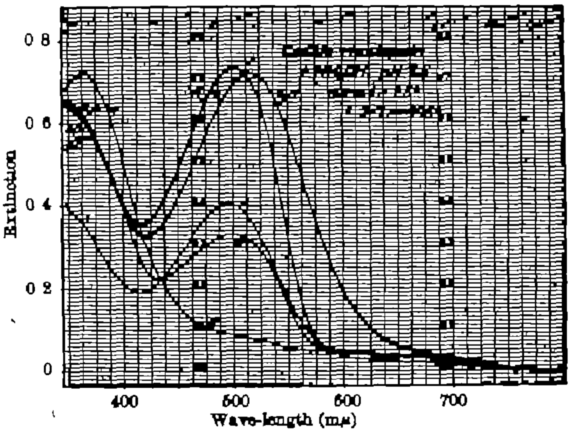

Fig. 1. Cattle rhodopsin in a glycerol–water mixture (55 : 45) containing 0·1 M hydroxylamine, at pH 7·5. The spectrum was recorded at room temperature (1), and again in the vitrified solvent at liquid nitrogen temperature (2). Long irradiation at 440 mμ displaces the spectrum to (3), representing a steady-state mixture of rhodopsin and pre-lumirhodopsin. Re-irradiation at 600 mμ brings the spectrum back to (4); the pre-lumirhodopsin has been reconverted to rhodopsin, accompanied by a trace of isorhodopsin. Re-irradiation at 440 mμ restores the steady-state mixture of rhodopsin and pre-lumirhodopsin (5). Pre-lumirhodopsin and rhodopsin are therefore perfectly interconvertible by light at this temperature. The preparation was warmed to 25°, its spectrum measured, and then re-cooled to $-195°$ (6). Then it was re-warmed to 25°, and completely bleached in the light to all-*trans* retinal and opsin, the retinal combining with hydroxylamine to yield retinaldehyde oxime (λ_{max} 367 mμ). On re-cooling to $-195°$ this yielded curve (7). Since retinaldehyde oxime does not absorb light above 450 mμ, curve (7) at higher wave-lengths represents impurities and scattering. Subtracting this from the other curves yields the corrected absorption spectra shown at the left in Fig. 2

Absorption spectrum. On cooling rhodopsin in glycerol–water mixtures from room temperature to $-195°$, its extinction rises 1·70 ± 0·11 times (average of 11 measurements) (Fig. 1, curves 1–2). On this degree of cooling the solvent contracts, by our estimate 7·7 per cent in 67 per cent glycerol and 6·5 per cent in 50 per cent glycerol; so that the rise of extinction corrected for volume contraction is about 1·58. Simultaneously λ_{max} moves from 498 to about 505 mμ.

The absorption band is also sharpened, its long-wave-length limb falling to zero more abruptly than in the warm (cf. rhodopsin, Fig. 2; isorhodopsin, Fig. 7). This is a general phenomenon, owing to the fact that at $-195°$ all the molecules are in the zero vibrational state, and all transitions to the first electronically excited state on absorbing a photon jump off from this single ground-level; whereas in the warm a small fraction of the molecules occupy higher vibrational states, from which transitions to the electronically excited state involve smaller energies, that is, longer wave-lengths of absorption. Hence the absorption bands in the warm end in an attenuated, long wave-length tail, which is lost at low temperatures.

The displacement of spectrum to longer wave-lengths on cooling, though characteristic of the visual pigments and free carotenoids[9], is opposite in direction to the shift observed in porphyrin derivatives, notably chlorophylls and hæm proteins, the spectra of which shift toward the blue on cooling[10]. The long wave-length absorption bands of all such molecules represent electronic transitions from the ground to the first excited state, on which are superimposed a variety of overlapping rotation-vibration changes. Cooling, by altering the relative probabilities of vibrational changes, might displace the 'centre of gravity' of the composite band in either direction; yet the likelier result is a displacement toward the blue, as observed with porphyrin derivatives, since cooling eliminates higher vibrational levels in the ground state, thus making for longer transitions. The contrary behaviour of the carotenoids and their derivatives is probably associated with the fact that carotenoid spectra in the warm lie at 'abnormally' short wave-lengths, owing to steric hindrance between the ring and side-chain[11] and—in various *cis* forms—also between —H and in some cases —H and —CH$_3$ groups along the chain[12]. Such hindrances interfere with conjugation and hence with resonance, and tend to displace the spectrum towards shorter wave-lengths. Cooling apparently relieves such steric hindrances, so permitting more effective conjugation, and shifting the spectrum towards the red.

It is tempting to relate the large rise of extinction of rhodopsin on cooling to the same cause. All-*trans* and the relatively unhindered *cis* isomers of retinal rise in extinction only about 10 per cent on cooling to − 195°; whereas the highly hindered 11-*cis* retinal, owing to the relief of steric hindrance on cooling, rises in extinction 1·63 times[9], as does rhodopsin in the present experiments. It should be noted, however, that isorhodopsin, which possesses as chromophore the relatively unhindered 9-*cis* retinal, rises in extinction about the same amount on cooling to − 195°, so that the large rise of extinction on cooling both these pigments cannot without qualification be ascribed to steric hindrances intrinsic to the free chromophore. It is possible that the attachment to opsin introduces distortions of chromophore configuration that are relieved by cooling, quite apart from what distortions may be present in the free retinals.

As already noted here, below −100° our glycerol–water solutions craze to various degrees, though when cooled as rapidly as in these experiments they seem to remain otherwise clear (that is, non-opalescent). The rises of extinction we observe therefore are not as reliable as if measured in wholly clear media, yet there is no present reason to believe that they involve large errors. If accepted tentatively, they mean that, since at 25° rhodopsin has a molar extinction in aqueous solution of 40,600 (ref. 13) and about the same extinction in glycerol–water mixtures, its molar extinction at −195° is about 64,000. Also, since the molar extinction of isorhodopsin at room temperature is 43,000, and rises as does that of rhodopsin on cooling, its molar extinction at − 195° is about 68,000.

Pre-lumirhodopsin. When rhodopsin is irradiated at −195° with blue light (436 or 440 mμ), λ_{max} shifts from 505 to about 517 mμ (Fig. 1, curve 3, 5). The maximum extinction falls slightly, but there is a broad rise of extinction at long wave-lengths, owing to the formation of pre-lumirhodopsin. The final result of this irradiation is a steady-state mixture of rhodopsin and pre-lumirhodopsin.

To evaluate this mixture, the preparation is warmed to room temperature in the dark. The rhodopsin remains intact, whereas the pre-lumirhodopsin bleaches to retinal and opsin, the retinal immediately combining with the hydroxylamine present to form retinaldehyde oxime. The spectrum of the residual rhodopsin was measured at room temperature, and again after re-cooling to −195° (Fig. 1, curve 6). Finally, the preparation was again warmed to room temperature, bleached completely and re-cooled to −195° (curve 7). This last spectrum displays the absorption band of retinaldehyde oxime (λ_{max} 367 mμ). Since retinaldehyde oxime does not absorb appreciably at wave-lengths longer than 450 mμ, above this wave-length curve 7 represents the effects of impurities and scattering. By subtracting this portion of the spectrum from the other curves, one obtains the true absorption spectra of the other components in the visible region.

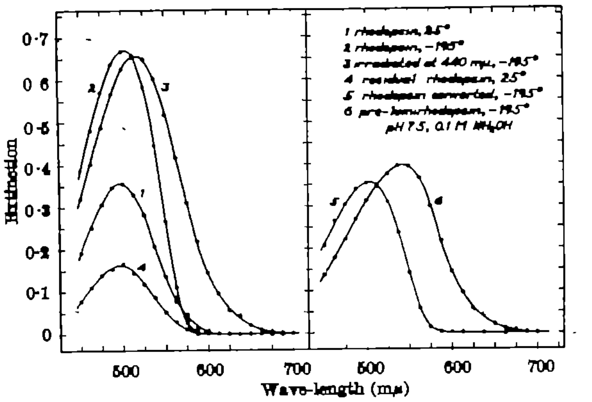

Fig. 2. The spectrum of pre-lumirhodopsin. At the left are shown corrected absorption spectra taken from Fig. 1, except for 4, which is the corrected spectrum of the residual rhodopsin, measured at 25°. The fraction 4/1 represents the proportion of the original rhodopsin remaining in 3; and this fraction of 2 represents its spectrum at −195°. Subtracting this from 3 yields the spectrum of pre-lumirhodopsin (curve 6; λ_{max} 543 mμ). The fraction (1−4)/1 represents the fraction of rhodopsin converted to pre-lumirhodopsin; and this fraction of curve 2 yields its spectrum at −195° (curve 5). Curves 5 and 6 therefore represent the spectra of equivalent concentrations of rhodopsin and pre-lumirhodopsin

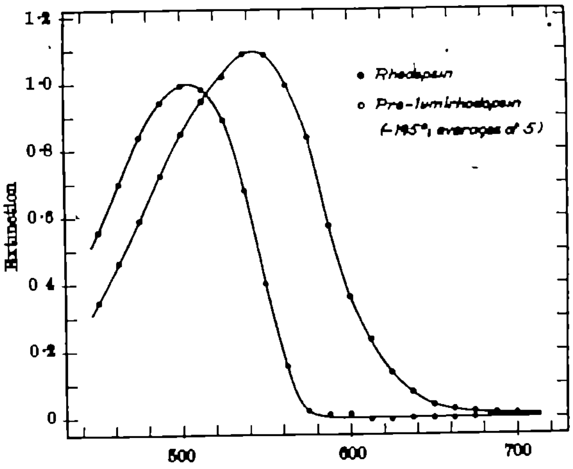

Fig. 3. Absorption spectra of equivalent concentrations of cattle rhodopsin and pre-lumirhodopsin in glycerol–water glasses at liquid nitrogen temperature. Averages from five experiments. Rhodopsin (λ_{max} 505 mμ) is here given arbitrarily the maximal extinction 1·0. Pre-lumirhodopsin (λ_{max} 543 mμ) then has the maximal extinction 1·13

Such corrected absorption spectra, taken from the results in Fig. 1, are shown in Fig. 2. Curve 1 is rhodopsin at room temperature, curve 2 the same preparation at −195°. Curve 3 is the product of irradiation at 440 mμ, a mixture of rhodopsin and pre-lumirhodopsin. Curve 4 is the rhodopsin in this mixture, left after warming the solution to allow the pre-lumirhodopsin to bleach. As already said, this residual rhodopsin was measured both at room temperature and at −195°. The latter spectrum (Fig. 1, curve 6), subtracted from curve 3, yields directly the absorption spectrum of pre-lumirhodopsin. Because, however, a second cooling involves a different pattern of cracking of the solvent, and might introduce an error, we have preferred to use the spectrum of the residual rhodopsin measured in the warm (Fig. 2, curve 4). The ratio of this to curve 1 represents the fraction of the original rhodopsin present in curve 3. This ratio—about 0·45—of curve 2 represents the spectrum of the residual rhodopsin at −195° present in curve 3. Subtracting this from curve 3 yields the spectrum of pre-lumirhodopsin (curve 6). Curve 4 subtracted from curve 1 represents the amount of rhodopsin converted to pre-lumirhodopsin; and this fraction (about 0·55) of curve 2 is shown as curve 5. Curves 5 and 6 therefore show the absorption spectra of equivalent amounts of rhodopsin and pre-lumirhodopsin at −195°.

Repeated experiments of this kind have yielded very similar results. The averages of 5 sets of such measurements are shown in Fig. 3. The average λ_{max} of pre-lumirhodopsin is 543 mμ, and its maximal extinction is on the average 1·13 times that of rhodopsin. We can therefore assign to it tentatively the molar extinction 72,000 at −195°.

Interconvertibility of rhodopsin, pre-lumirhodopsin and isorhodopsin. The conversion of rhodopsin to pre-lumirhodopsin appears to involve only the isomerization by light of the 11-*cis* chromophore to all-*trans*. We used to distinguish between photosensitive and light-stable components of visual systems; but in the sense that they are isomerized by light, all forms of retinal, free and bound, are photosensitive. Their irradiation in all circumstances ends in steady-state mixtures of isomers. The composition of such mixtures depends on the wave-length of the incident light: the steady-state mixture always consists predominantly of those isomers the

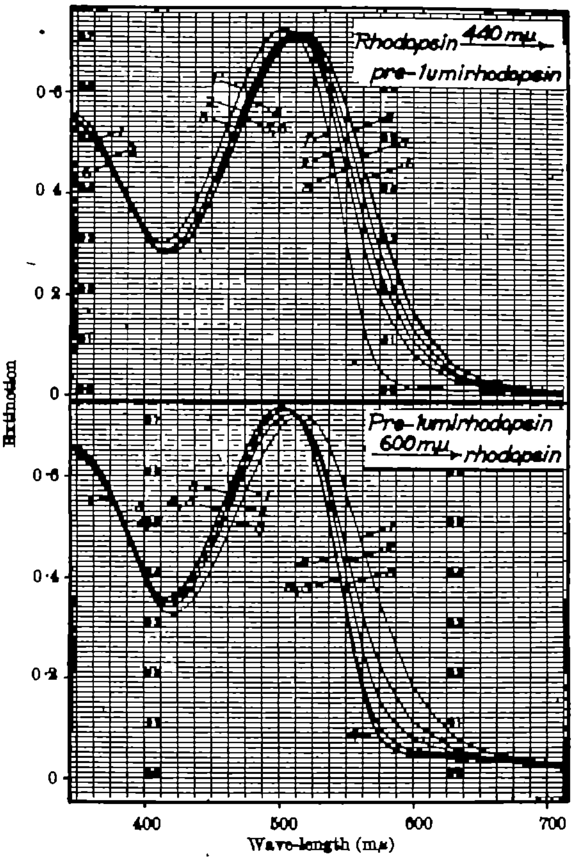

Fig. 4. Course of isomerization of rhodopsin by irradiation at 440 mμ to a steady-state mixture of rhodopsin and pre-lumirhodopsin (above); and the reversion of this mixture to rhodopsin by irradiation at 600 mμ (below). Above, curve 1: cattle rhodopsin in 55 per cent glycerol in water at −195°. Curves 2–6: products of irradiation at 440 mμ for successive periods of 5, 5, 10, 20 and 40 min. Below: the product of irradiating rhodopsin at −195° with 440 mμ (curve 1) is re-irradiated at 600 mμ for successive periods of 5, 5, 10 and 25 min (curves 2–5). Curve 5 almost coincides with *a*, the spectrum of the original rhodopsin, but lies at slightly shorter wave-lengths, owing to the formation of a trace of isorhodopsin. The main processes here are the conversion of a fraction of the rhodopsin (about 55 per cent) to pre-lumirhodopsin (above), and its reversion to rhodopsin (below). The presence of an isosbestic point at about 512·5 mμ in both transformations indicates that only these two components are involved

absorptions of which lie farthest away from the incident radiation. Thus, irradiation with blue light drives the mixture predominantly toward its red-absorbing components, whereas irradiation with red light drives it toward its blue-absorbing components[8]. If one could irradiate at a wave-length that all the isomers but one could absorb, the entire product would be trapped in the form of that transparent isomer. In the present instance, irradiation at 440 mμ drives the steady-state almost entirely toward rhodopsin and pre-lumirhodopsin, and away from the shorter wave-length isorhodopsin (9-*cis* chromophore).

Irradiation at 440 mμ having produced this steady-state mixture (Fig. 1, curve 3), re-irradiation with light of wave-length 600 mμ drives the spectrum almost exactly back to that of rhodopsin (curve 4)—actually just perceptibly to shorter wave-lengths, owing to the formation of a trace of isorhodopsin. More isorhodopsin is not formed because all-*trans* retinal, here the chromophore of pre-lumirhodopsin, isomerizes much more rapidly to the 11-*cis* configuration (that is, to rhodopsin) than to 9-*cis* (isorhodopsin)[8]; and the rhodopsin, once formed, is not isomerized further because at − 195° it scarcely absorbs at 600 mμ.

On re-irradiating at 440 mμ, the spectrum moves back to its earlier position, that of the mixture of rhodopsin and pre-lumirhodopsin (Fig. 1, curve 5). This experiment shows that rhodopsin and pre-lumirhodopsin are almost perfectly interconvertible by light at −195°.

Fig. 4 shows the kinetics of these interconversions: the transformation of rhodopsin by irradiation at 440 mμ into the steady-state mixture of rhodopsin and pre-lumirhodopsin (top); and the reversion of this mixture by irradiation at 600 mμ to rhodopsin (bottom). The presence of a sharp isosbestic point at about 512·5 mμ as the reaction proceeds in either direction is an assurance that it involves only these two components. The formation of a just distinguishable trace of isorhodopsin by irradiation at 600 mμ scarcely disturbs these relationships, for the reason already noted: that rhodopsin scarcely absorbs at this wave-length, and hence is not isomerized by it.

If, however, one irradiates with a wave-length that rhodopsin does absorb—say 546 mμ—the steady-state is shifted very largely toward isorhodopsin. This is shown in Fig. 5. Irradiation of the original rhodopsin at −195° with the mercury green line (546 mμ) shifts the spectrum from curve 2 to 3. Irradiation of this product at 436 mμ shifts it to curve 4—the familiar steady state mixture of rhodopsin and pre-lumirhodopsin. Re-irradiation at 546 mμ brings it back to curve 5, coincident with curve 3. Warming this product to room temperature in the dark reveals a residual light-sensitive pigment with λ_{max} at 489 mμ (curve 6), close to that of cattle isorhodopsin (485 mμ). The final product of irradiation at 546 mμ is therefore mainly isorhodopsin, with less rhodopsin and still less pre-lumirhodopsin, the latter principally accounting for the rise of extinction at long wave-lengths (curves 3 and 5, compared with 2).

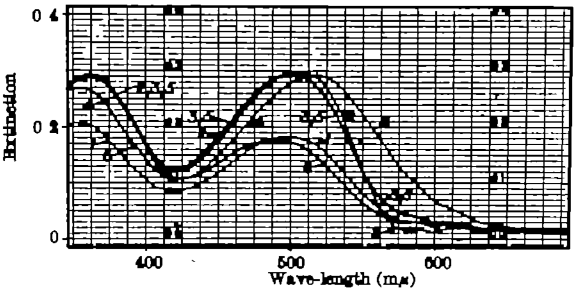

Fig. 5. Interconversions of rhodopsin, pre-lumirhodopsin and isorhodopsin at −195°. 1, Cattle rhodopsin in a glycerol–water mixture (2 : 1) at 24·5°. On cooling to −195° the spectrum went to 2. On long irradiation at 546 mμ it was displaced to 3, representing a steady-state mixture of isorhodopsin with lesser amounts of rhodopsin and pre-lumirhodopsin. On re-irradiation at 436 mμ it went to 4, representing a steady-state mixture of rhodopsin and pre-lumirhodopsin (about 45 : 55). On re-irradiation at 546 mμ the spectrum returned to 5, which coincides with 3. On warming to 24·5° it went to 6; the pre-lumirhodopsin in 5 had bleached to all-*trans* retinal and opsin, leaving a residue of isorhodopsin (λ_{max} 485 mμ) mixed with a lesser amount of rhodopsin

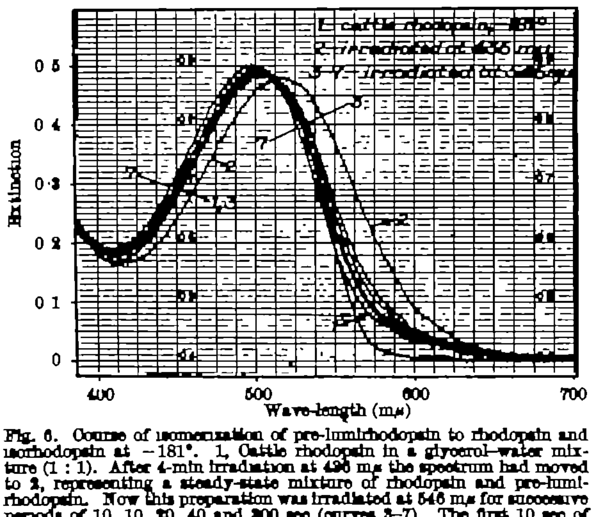

Fig. 6. Course of isomerization of pre-lumirhodopsin to rhodopsin and isorhodopsin at −181°. 1, Cattle rhodopsin in a glycerol–water mixture (1 : 1). After 4-min irradiation at 436 mμ the spectrum had moved to 2, representing a steady-state mixture of rhodopsin and pre-lumirhodopsin. Now this preparation was irradiated at 546 mμ for successive periods of 10, 10, 20, 40 and 300 sec (curves 3–7). The first 10 sec of irradiation brought the spectrum almost back to that of rhodopsin; further irradiation only slowly displaced the spectrum toward shorter wave-lengths. This behaviour is an expression of the fact that the all-*trans* chromophore of pre-lumirhodopsin isomerizes much more readily to 11-*cis* (rhodopsin) than to 9-*cis* (isorhodopsin), the principal component in the final steady-state

The steady-state product at 436 mμ is transformed into the steady-state product at 546 mμ by a peculiar route. This is shown in Fig. 6. The experiment begins with the irradiation of rhodopsin (curve 1) at 436 mμ to produce the usual steady-state mixture of rhodopsin and pre-lumirhodopsin (curve 2). This, then, is irradiated at 546 mμ, eventually to arrive at curve 7, representing mainly isorhodopsin, with small admixtures of rhodopsin and pre-lumirhodopsin. The significant point is that the first 10 sec of irradiation at 546 mμ has so much greater effect than the next 10 sec and all subsequent irradiations. The first such irradiation brings λ_{max} from about 517 to 506 mμ (curve 3), close to that of rhodopsin; whereas another 370 sec of irradiation are needed to bring λ_{max} to about 497 mμ (curve 7), close to that of isorhodopsin. It was shown some time ago that, at −20°, metarhodopsin isomerizes with a much higher quantum efficiency to rhodopsin than to isorhodopsin[8]. Apparently the same is true of pre-lumirhodopsin at liquid nitrogen temperature. The first 10 sec of irradiation convert pre-lumirhodopsin largely to rhodopsin, whereas further irradiation at this wave-length only slowly drives the steady-state over toward isorhodopsin. These mechanisms are discussed further later on.

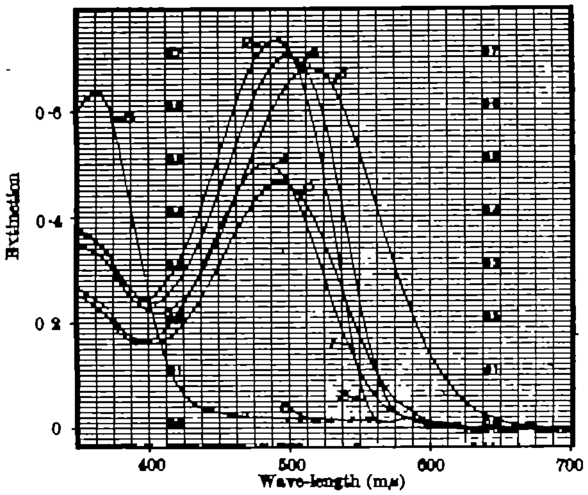

Fig. 7. Interconversions of cattle isorhodopsin, pre-lumirhodopsin and rhodopsin at −195°. 1, Isorhodopsin synthesized by incubating cattle opsin with 9-*cis* retinal, in glycerol–water (2 : 1) containing 0·1 M hydroxylamine, at *p*H 7·5, 22·5°. 2, The same at −195°. Irradiation at 436 mμ for 256 sec brings the spectrum to 3, the same steady-state mixture of rhodopsin and pre-lumirhodopsin (about 45 : 55) that is obtained by a similar irradiation of rhodopsin. Re-irradiation at 579 mμ for 32 sec brings the spectrum to 4; this is now a mixture of rhodopsin with a minor amount of isorhodopsin, as shown by warming in the dark to 20° (curve 5). Finally the preparation was bleached completely at 20° to yield curve 6, displaying the absorption band of retinene oxime at 367 mμ

Alternatively, one can begin such an experiment with isorhodopsin (Fig. 7). This was prepared by incubating cattle opsin with 9-*cis* retinal. On cooling to −195°, its spectrum rose from curve 1 to 2, λ_{max} being displaced from about 485 mμ at room temperature to 491 mμ in the cold. Irradiation at 436 mμ yields the same steady-state mixture of rhodopsin and pre-lumirhodopsin as one obtains by irradiating rhodopsin (curve 3). Re-irradiating this mixture at 579 mμ drives the spectrum back to curve 4 (λ_{max} 500 mμ); and warming the preparation in the dark to room temperature shows this to be rhodopsin, with a small admixture of isorhodopsin (λ_{max} 494 mμ). Other experiments have shown that if this last irradiation had been performed at 600 mμ, we would have obtained almost pure rhodopsin.

These experiments demonstrate the well-nigh perfect interconvertibility of rhodopsin, isorhodopsin and pre-lumirhodopsin by light at −195°. Apparently at this temperature light activates only the geometric isomerization of the chromophores, leaving the protein relatively unaltered.

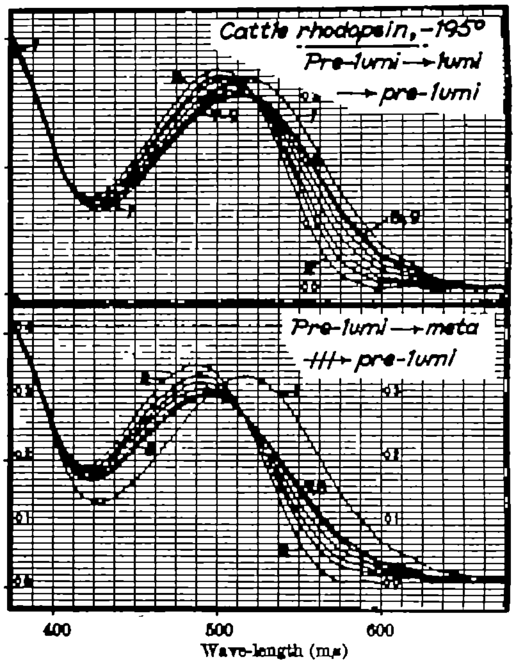

Fig. 8. Demonstration that irradiation at −195° can convert lumirhodopsin to pre-lumirhodopsin (above), but not metarhodopsin to pre-lumirhodopsin (below). Above, cattle rhodopsin in glycerol–water (2 : 1) containing M/10 hydroxylamine, at *p*H 7·5, was irradiated at 436 mμ to a steady-state mixture of rhodopsin and pre-lumirhodopsin curve 1). On warming to −90° in the dark, the pre-lumirhodopsin was converted to lumirhodopsin; and on re-cooling to −195° this—still mixed with rhodopsin—yielded curve 2. Now re-irradiation at 436 mμ brought the spectrum from 2 to 9, which possesses the same λ_{max} though a slightly lower extinction than 1, showing that this irradiation had re-converted lumi- to pre-lumirhodopsin. Below, irradiation of a similar preparation at 436 mμ and −195° produced a similar mixture of rhodopsin and pre-lumirhodopsin (curve 1). This was warmed to −15° to permit the pre-lumirhodopsin to be converted to metarhodopsin. On re-cooling to −195° the mixture of rhodopsin and metarhodopsin yielded curve 2. Long irradiation at 436 mμ brought the spectrum from 2 to 8: the rhodopsin in 2 had formed the usual steady-state mixture of rhodopsin and pre-lumirhodopsin, but the metarhodopsin was unchanged

Interconvertibility of pre-lumi- and lumi-rhodopsin. Hubbard, Brown and Kropf have shown that rhodopsin and lumirhodopsin are interconvertible by light at −65° (ref. 14). The experiments of Yoshizawa and Kitô implied that the irradiation of lumirhodopsin at liquid nitrogen temperature also causes a partial reversion to rhodopsin, and hence potentially to pre-lumirhodopsin[15].

Fig. 8 (top) shows the reversion of lumi- to pre-lumi-rhodopsin at −195°. Cattle rhodopsin was repeatedly brought back and forth between −195° and −90° until it had settled down to a fairly constant E_{max} at −195° (0·350). On irradiating at 436 mμ, its spectrum moved to curve 1, representing the usual steady-state mixture of rhodopsin and pre-lumirhodopsin. On warming this in the dark to −90°, then re-cooling to −195°, the spectrum had changed to curve 2: the pre-lumirhodopsin had changed to lumirhodopsin, still accompanied by rhodopsin. On re-irradiating at 436 mμ (−195°), the spectrum gradually shifted from (2) to (9), that is, back to the steady-state mixture of rhodopsin and pre-lumirhodopsin. The slight fall of E_{max} may represent a trivial discrepancy associated with the warming and re-cooling, or possibly some modification of pigment by the long irradiation. The sharp isosbestic point at about 523 mμ indicates the transformation of a single precursor into a product, in this case lumirhodopsin into pre-lumirhodopsin.

A specially designed experiment shows that this transformation requires light. Rhodopsin was cooled to −136° and irradiated with 436 mμ to the steady-state mixture of rhodopsin and pre-lumirhodopsin. Warming to −90° converted the latter to lumirhodopsin. This did not change on being re-cooled to −134° and held at that temperature for 1·5 h in the dark.

Failure of metarhodopsin to revert to pre-lumirhodopsin at −195°. At −20°, rhodopsin and metarhodopsin are freely interconvertible by irradiation[9]. At −65°, however, irradiating rhodopsin yields lumi- but not meta-rhodopsin; and conversely irradiating metarhodopsin yields no lumirhodopsin or rhodopsin. The change from lumi- to meta-rhodopsin appears to involve a rearrangement in the structure of opsin, apparently blocked in both directions at this low temperature[14].

The lower portion of Fig. 8 shows an experiment to test the possibility that metarhodopsin can revert to rhodopsin and pre-lumirhodopsin at −195°. Like the preceding experiment, this was begun by bringing the rhodopsin preparation back and forth repeatedly between −195° and −20° until a reasonably stable E_{max} was achieved at −195° (0·34). Then it was irradiated at 436 mμ to yield the usual steady-state mixture of about 45 per cent rhodopsin and 55 per cent pre-lumirhodopsin (curve 1). The preparation was now warmed to −15° in the dark to permit all the pre-lumirhodopsin to be converted to metarhodopsin, and was re-cooled to −195° (curve 2). The resultant mixture of metarhodopsin and rhodopsin (about 55 : 45) has λ_{max} 487 mμ. On re-irradiating at 436 mμ at this temperature, the spectrum changed gradually from (2) to (3). The source of this change is the conversion of the rhodopsin present in (2) to the usual steady-state mixture of rhodopsin and pre-lumirhodopsin. The composition of the final mixture, if no change has occurred in the metarhodopsin, is expected to be about 55 per cent metarhodopsin, 20 per cent rhodopsin (that is, 0·45 × 0·45), and 25 per cent pre-lumirhodopsin (that is, 0·55 × 0·45). The shape and position of curve 3 (λ_{max} 496 mμ) agree reasonably well with this prediction. It is concluded that metarhodopsin does not revert to rhodopsin (or pre-lumirhodopsin) at −195°.

Temperature-stability ranges. In Fig. 9 a preparation of rhodopsin, having been irradiated at −195° with light of wave-length 436 mμ to produce the steady-state mixture of rhodopsin and pre-lumirhodopsin with λ_{max} 517 mμ, was allowed to warm slowly in the absorption vessel in the dark. Periodically it was re-cooled to −195° and its spectrum re-measured. The figure shows the change of λ_{max} at −195°.

As the temperature rises, the pre-lumirhodopsin in the original steady-state mixture remains stable to about —140°. Then λ_{max} shifts to about 500 mμ, as pre-lumirhidopsin goes over to lumirhodopsin. This remains stable to about −40°; then λ_{max} shifts again, to about 487 mμ, as lumi- goes over to meta-rhodopsin. Above 0°, λ_{max} in the visible region rises to about 499 mμ, as metarhodopsin is hydrolysed to retinal and opsin, leaving a residue of rhodopsin mixed with a little isorhodopsin. Simultaneously a new absorption band appears in the ultra-violet (367 mμ), owing to the formation of retinaldehyde oxime. If this experiment is begun with isorhodopsin rather than rhodopsin, the results are the same.

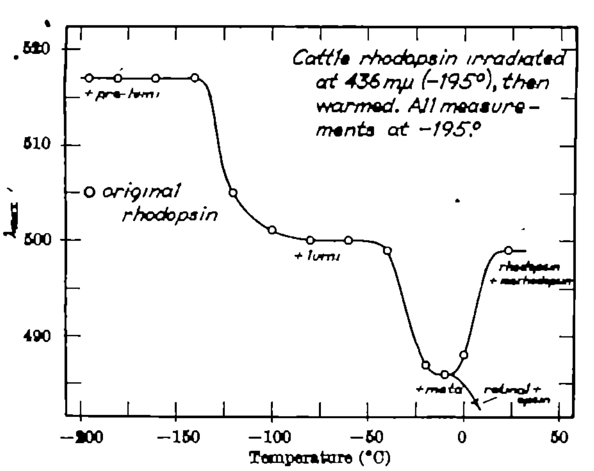

Fig. 9. Stepwise bleaching of rhodopsin by gradual warming. Cattle rhodopsin at −195° (λ_{max} 505 mμ) was irradiated at 436 mμ to the steady-state mixture of rhodopsin and pre-lumirhodopsin (λ_{max} 517 mμ). Then the preparation was allowed to warm to various temperatures, and each time was re-cooled to −195° and its spectrum re-measured. Its λ_{max} remained unchanged to about −140°. Then it fell to about 500 mμ, as pre-lumirhodopsin went over to lumirhodopsin. Above about −40° it fell again, to about 487 mμ, as lumirhodopsin formed metarhodopsin. Above 0° it rose again in the visible region, as metarhodopsin bleached to all-*trans* retinal and opsin (λ_{max} 387 mμ, not shown), leaving a residue of rhodopsin mixed with a small amount of isorhodopsin (λ_{max} 499 mμ)

Discussion

Throughout these experiments we have to deal with two kinds of change: the geometrical isomerization of retinaldehyde chromophores activated by light, and associated rearrangements of the protein, opsin. These two types of process stand in the relation of independent and dependent variable, and lead on the irradiation of rhodopsin or isorhodopsin to a succession of products, the interrelationships of which are shown in Fig. 10.

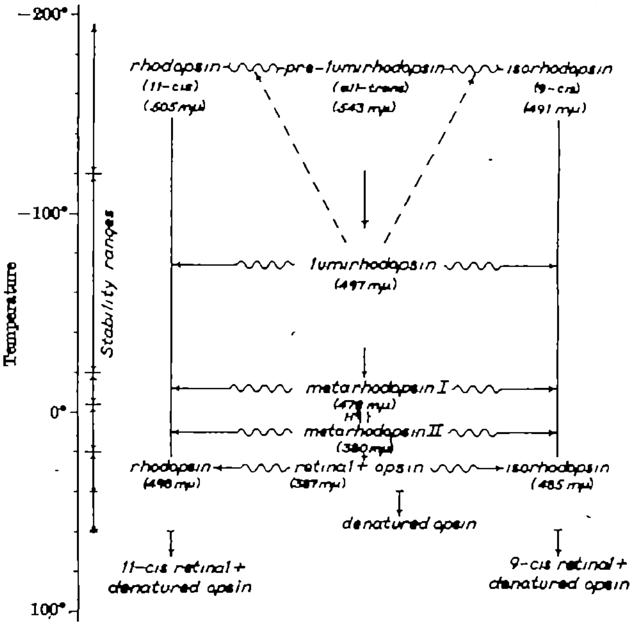

Fig. 10. Diagram showing inter-relationships of photochemical and thermal reactions of rhodopsin and isorhodopsin, arranged on a temperature scale. Photoreactions are symbolized by wavy lines, thermal reactions by straight lines, and sequences of both by both symbols in succession. The stability ranges of the various molecular species are bracketed by transitional temperatures at which the reactions are half completed in about 10 min (that is, fully completed in about 1 h). This diagram is extended to include also data on heat denaturation; heat bleaches rhodopsin and isorhodopsin by changing the configuration of opsin, whereas light does so by isomerizing retinal (ref. 16). Metarhodopsins I and II represent tautomeric forms of metarhodopsin in equilibrium with each other, higher temperatures, acidity or a higher proportion of glycerol in the medium favouring metarhodopsin II (ref. 17)

The geometrical isomerization of the chromophores is a typical photochemical reaction which can be completed in a rigid solvent at −195° C. It is responsible for instituting at that temperature perfectly reversible steady-states among rhodopsin, pre-lumirhodopsin and isorhodopsin, the final composition of which depends on the wave-length of irradiation, tending always to absorb the incident radiation minimally.

Certain circumstances govern the pathways by which these steady-states are achieved in the cold. Each geometric isomer of retinal, whether free or the chromophore of a visual pigment, is raised by the absorption of a photon to its own excited state, different from that of other geometric isomers; and each double bond isomerizes independently of the others[18]. (If the geometric isomers of retinal possessed a common excited state, *cis* isomers should require less energy to reach this than the all-*trans* isomer, since the ground states of *cis* isomers lie at higher energy-levels than that of all-*trans* retinal. Hence the absorption spectra of *cis* isomers should lie at *longer* wave-lengths than that of all-*trans* retinal; whereas in fact they lie at *shorter* wave-lengths. One can conclude not only that there is no common excited state but also that the energy-levels of *cis* isomers must lie even farther above all-*trans* in the excited than in the ground states.) Thus 11-*cis* retinal, for example, on absorbing a photon, may isomerize to all-*trans*; and this, on absorbing another photon, may isomerize to another *cis* configuration. For this reason interconversions between rhodopsin and isorhodopsin must involve multiple light absorptions, and must go through pre-lumirhodopsin at −195°, just as they must go through lumirhodopsin, or metarhodopsin, or all-*trans* retinal and opsin at higher temperatures.

A second condition involves the quantum efficiencies of these isomerizations. Hubbard and Kropf[8] have shown that the quantum efficiencies for interconversions among rhodopsin, metarhodopsin and isorhodopsin at −20° stand in the ratio.

$$\text{rhodopsin} \underset{0\cdot5}{\overset{1}{\rightleftharpoons}} \text{metarhodopsin} \underset{0\cdot3}{\overset{0\cdot1}{\rightleftharpoons}} \text{isorhodopsin}$$

Since photoisomerizations play the primary part in these reactions, probably about the same relationships obtain at −195°, when pre-lumirhodopsin takes the place of metarhodopsin.

This array of quantum efficiencies explains why the irradiation of a mixture of rhodopsin and pre-lumirhodopsin (about 45 : 55) at 600 mμ produces only a trace of isorhodopsin (cf. Fig. 1, 4). At −195°, neither rhodopsin nor isorhodopsin absorbs the 600 mμ radiation, which acts therefore only on pre-lumirhodopsin, converting it to rhodopsin and isorhodopsin in the proportion 5 : 1, set by the ratio of quantum efficiencies. The steady-state is therefore expected to contain the original rhodopsin (45 parts), rhodopsin newly formed from 5/6 of the pre-lumirhodopsin (55 × 5/6 = 46 parts), and the remainder —9 per cent—isorhodopsin. This estimate is reasonably consistent with the small displacement of spectrum that is observed (cf. Fig. 1, curve 4, compared with 2). (It should be conceded that owing to the slow formation of isorhodopsin under the conditions of these experiments, we may not have wholly reached the steady-state by curve 4 in Fig. 1 or curve 5 in Fig. 4 (bottom).)

These relative quantum efficiencies also explain the peculiar approach to the steady-state shown in Fig. 6, in which irradiation at 546 mμ very rapidly institutes a transitory steady-state between pre-lumirhodopsin and rhodopsin, but only slowly brings these into a final steady-state with isorhodopsin. These transformations involve a considerable conversion of rhodopsin to isorhodopsin, which for reasons already stated must go through pre-lumirhodopsin.

On gradually warming pre-lumirhodopsin in the dark, it undergoes the stepwise series of thermal rearrangements shown on a temperature scale in Fig. 10. An accumulation of aberrations in our own and other workers' observations over the years have led us lately to re-examine metarhodopsin. We find it to involve two tautomeric forms in equilibrium with each other, metarhodopsin I (λ_{max} about 478 mμ in cattle preparations), which forms first, entering into an equilibrium with metarhodopsin II (380 mμ), in which raising the temperature, acidity or the proportion of glycerol in the medium favours metarhodopsin II (ref. 17). It will be noted that all our references to metarhodopsin here involve metarhodopsin I. Finally, all-*trans* retinal hydrolyses from opsin, to which it had apparently been bound in all these chromophores as a Schiff base:

$$C_{19}H_{27}CH{=}N\text{—opsin} + H_2O \rightarrow$$
$$C_{19}H_{27}CHO\ (\text{retinal}) + \text{opsin—}NH_2$$

These changes—except perhaps the last—involve progressive rearrangements in the structure of opsin. One consequence is to expose new reactive groups, not available to reagents in rhodopsin itself. When bleaching is completed, two sulphydryl groups and one proton-binding group with pK about 6·6 (imidazole ?) have been exposed; and preliminary experiments show that the latter group is available by the time metarhodopsin II has formed[19]. It seems probable that this opening up of opsin structure is a decisive event in visual excitation[20].

The isomerization of retinal to the 11-*cis* or 9-*cis* configuration makes possible the regeneration of rhodopsin or isorhodopsin. In forming these pigments, opsin adjusts its structure to these configurations of retinal. Perhaps better said, opsin and the chromophores assume geometries that fit together, and are stabilized in the combination by mutual interactions. No other configuration of retinal can produce this effect; so that the isomerization of the 11-*cis* or 9-*cis* chromophore to all-*trans* promptly makes the combination unstable.

For opsin to adapt its structure to 11-*cis* or 9-*cis* retinal, temperatures are needed at which the requisite re-arrangements can occur. At room temperature, all of them can occur, and opsin combines spontaneously with either isomer. Opsin as it exists in metarhodopsin can still re-arrange at −20° to form rhodopsin or isorhodopsin, but can no longer do so below −50° (ref. 14; Fig. 8, bottom). Opsin as it exists in lumirhodopsin can re-arrange to form rhodopsin or isorhodopsin even at −195° (upper part of Fig. 8). To the degree that the configuration of opsin is moulded specifically by its combination with 11-*cis* or 9-*cis* retinal, lumirhodopsin can be transformed to pre-lumirhodopsin only over rhodopsin or isorhodopsin. Light probably must first isomerize the all-*trans* chromophore of lumirhodopsin to 11-*cis* or 9-*cis*, so inducing the rearrangements of opsin needed to form rhodopsin or isorhodopsin. Then, by the absorption of another photon at −195°, the chromophores of these pigments are isomerized back to all-*trans*, forming pre-lumirhodopsin.

It is possible that light has another kind of effect in such reactions. None of the reactions being discussed is highly endergonic, so that whether or not the absorption of a photon causes isomerization of the chromophore, a large residue of the absorbed energy should remain available. This might be important, particularly at low temperatures, in activating protein rearrangements, either through a localized excitation of protein groups, or, the residual energy having been degraded into heat, through a more general 'melting' effect on the protein in the neighbourhood of the chromophore. Light could scarcely have such effects, however, without simultaneously isomerizing retinal; so that one should assume the photo-isomerization mechanism in any event, perhaps aided secondarily by excitation or 'melting' of the protein.

A final problem involves the spectrum of pre-lumirhodopsin. Kropf and Hubbard[8,21] have attempted to account for the spectrum of rhodopsin as follows: the combination with opsin in a Schiff base linkage ($C_{19}H_{27}CH{=}N$—opsin) should shift the λ_{max} of 11-*cis* retinal (380 mμ) to about 367 mμ. Protonation of the Schiff base (that is, $C_{19}H_{27}CH{=}NH^{+}$—opsin) is expected to raise λ_{max} to about 440 mμ (ref. 22). It was suggested that the farther shift of spectrum to the λ_{max} of rhodopsin (498 mμ) is associated with the lowering of the energy-level of the excited state, owing to interactions between the conjugated side-chain of 11-*cis* retinal and opsin, for which closeness of fit is prerequisite. That is, 11-*cis* retinal, and to a lesser extent the 9-*cis* isomer, are good chromophores because they *fit* opsin—after the necessary rearrangements—better than do other isomers. By the same token, any loosening of fit between opsin and its chromophore should weaken their mutual interactions and shift the spectrum toward the blue.

How then do we account for the λ_{max} of pre-lumirhodopsin ? Since it lies far toward the red from rhodopsin, does that imply that its all-*trans* chromophore fits opsin

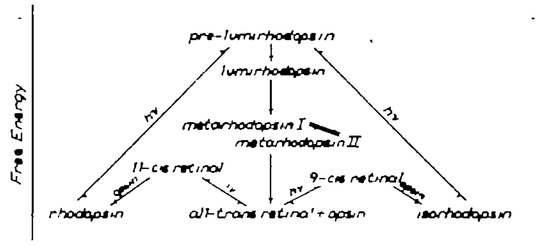

Fig. 11. Diagram showing the order in which intermediates in the bleaching and regeneration of rhodopsin and isorhodopsin lie on a scale of relative free energies (chemical potentials). Relative distances on the energy scale mean little in this diagram; only the order is important

even more closely, and interacts with it more strongly, than the 11-*cis* chromophore? But why then is pre-lumirhodopsin unstable?

The same problem has arisen earlier with regard to lumirhodopsin. The spectra of some vertebrate lumirhodopsins lie at longer wave-lengths than the corresponding rhodopsins (bull-frog, 7 mμ; cusk, 19 mμ longer[14]). Such displacements of spectrum might at least in part be explained simply, for the change of configuration from 11-*cis* to all-*trans* in itself involves a shift of $\lambda_{\max}$ toward the red, about 7 mμ in free retinal, and perhaps much more in the chromophores of visual pigments. This red shift might perhaps more than counterbalance the opposed tendency toward a blue shift owing to loosening of fit. But in the squid, lumirhodopsin lies about 50 mμ toward the red from rhodopsin[14]; and here as in cattle pre-lumirhodopsin such an explanation seems inadequate.

A consideration of the order of rhodopsin, isorhodopsin and the intermediates of their bleaching on a free energy (chemical potential) scale offers the way out of this dilemma (Fig. 11). The arrangement here is dictated by the direction of spontaneous change in the case of the thermal reactions. The existence of thermal equilibria also permits us to assign a ΔF_s of about 2 kcal for the isomerization of all-*trans* retinal to 11-*cis* (ref. 23); and about −0·5 kcal for the change from metarhodopsin I to II in neutral 33 per cent glycerol at 20° (ref. 17). No other numerical values are yet available, and the figure shows otherwise only qualitative relationships.

What emerges is that the ground-state of pre-lumirhodopsin lies at a considerably higher energy than those of rhodopsin or isorhodopsin—sufficiently higher to encompass all the intervening states. This large rise in the energy-level of the ground state of pre-lumirhodopsin, if not accompanied by a comparable rise in the level of the first electronically excited state, would mean shorter transitions to the excited state, so explaining the shift of absorption spectrum toward the red. Indeed, the shift of spectrum offers a suggestion of how high the ground state of pre-lumirhodopsin may lie above those of its precursors, if one assumes tentatively that all these molecules differ relatively little in the levels of their first excited states. At −195° the shift of the $\lambda_{\max}$ of pre-lumirhodopsin toward the red implies on this basis that its free energy-level in the ground state may lie 3·9 kcal higher than that of rhodopsin, and about 5·5 kcal higher than that of isorhodopsin. (These estimates are based on the formula, $\Delta E = 28{,}540 \left(\frac{1}{\lambda_1} - \frac{1}{\lambda_2} \right)$, in which λ_1 and λ_2 are the shorter and longer wave-length maxima in mμ, and ΔE is the difference in energy of the absorbed quanta in kcal per mole. They involve sufficient uncertainty so that we have not tried to express implied differences in the free energies of rhodopsin and isorhodopsin in Fig. 11.)

We would suggest therefore that this rise in the energy-level of the ground state of pre-lumirhodopsin, by shortening the transition to its first electronically excited state, accounts for its long $\lambda_{\max}$. The effects of light on rhodopsin and isorhodopsin are probably best envisaged as follows: These pigments, as suggested by Hubbard and Kropf, owe their long wave-length spectra in part to the lowering of their first excited states by interactions between their chromophores and opsin dependent on closeness of fit. When the absorption of a photon isomerizes the chromophore of rhodopsin or isorhodopsin to all-*trans*, the side-chain interactions already established with opsin continue to hold both chromophore and opsin in what is now a highly constrained and distorted geometry. This is then pre-lumirhodopsin. The essential point is not that in pre-lumirhodopsin the all-*trans* chromophore fits opsin better—it probably fits less well; but that the strained condition of this molecule represents a large rise in the energy of its ground state, making it on one hand highly unstable, and also shortening the transition to the first electronically excited state, so displacing its spectrum toward the red.

Lumirhodopsin and metarhodopsin I then represent stages in the progressive relaxation of these constraints, opsin approaching more and more closely the configuration of the free protein, and retinal its normal all-*trans* geometry. As the fit between these components grows increasingly poorer, the interactions between them are correspondingly weaker and the energy-level of the ground state falls. Transitions to the first excited state therefore grow longer, and the spectrum shifts increasingly toward the blue. By metarhodopsin II, mutual interactions between retinal and opsin are at a minimum and the chromophore has lost its proton; the spectrum, as should be true of a Schiff base, lies at shorter wave-lengths than that of free all-*trans* retinal. All that remains is to hydrolyse retinal from opsin.

One of us (T. Y.), on leave of absence from Osaka University, occupies a research fellowship at Harvard University with funds provided by the National Science Foundation. This investigation was further supported with funds granted to the other (G. W.) by the National Science Foundation and the Office of Naval Research. We thank Mr. Paul K. Brown of this laboratory for help with designing and building the absorption vessel.

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LETTERS TO THE EDITOR

PHYSICS

Special Theory of Relativity

Prof. H. Dingle has sent me a reprint of his communication published under the above title on p. 985 of *Nature*, September 8, 1962, with the handwritten remark: "With kindest regards. Test case for the integrity of scientists".

Though former experience has taught me that discussing relativity with Dingle leads to no agreement I have to answer a challenge which is directed against the "scientific integrity" of myself and of others.

I use Dingle's notation. He restricts himself to the case of a linear motion along the x- or ξ-axis.

$K(x,t)$ is a 'stationary' system of reference, $k(\xi,\tau)$ a 'moving' one. Dingle writes down half the Lorentz transformation expressing τ by t and x, and half its inverse expressing t by τ and ξ. Then he quotes a passage from Einstein's paper, the first paragraph of which ends with the question: "*What is the rate of this clock, when viewed from the stationary system?*". Then follows the well-known derivation of the formula

$$\tau = t\sqrt{(1 - v^2/c^2)} \qquad (1)$$

Dingle now proceeds in this way: "And here is the passage leading to the opposite conclusion". The first paragraph of this new passage is completely identical with that of the original including the last sentence just quoted (in italics). Then he exchanges the co-ordinate systems and so obtains the result:

$$t = \tau\sqrt{(1 - v^2/c^2)} \qquad (2)$$

which he regards as a contradiction to formula (1).

The mistake is in the first paragraph quoted above (in italics); it should read, in the two cases:

1st case, clock at rest in k: *What is the rate of the clock in k, when viewed from the 'stationary' system K ?*

2nd case, clock at rest in K: *What is the rate of the clock in K, when viewed from the 'moving' system k ?*

In other words: formulæ (1) and (2) refer to different physical situations; t and τ have not the same meaning in (1) and (2).

If the clock is at rest in k and in motion relative to K, the reading of the hands of the clock at the beginning of the time-interval t in k is taken at the same place in k as the reading at the end of the time-interval. But the reading of the hands of this clock taken in K at the beginning and at the end of the time-interval are at different positions of the clock in K, as the clock is moving relative to K.

If, on the other hand, the clock is at rest in K and in motion relative to k, the situation is exactly reversed: the beginning and the end position of the clock are read at the same place in K, but at different places in k.

The two cases are therefore different, the symbols t and τ in expressions (1) and (2) referring to different physical situations; these are inverse and must of course correspond to an exchange of the symbols t and τ. This is exactly what expressions (1) and (2) express. There is no contradiction.

Dingle's objections are just a matter of superficial formulation and confusion. The simple fact that all relations between space co-ordinates and time expressed by the Lorentz transformation can be represented geometrically by Minkowski diagrams should suffice to show that there can be no logical contradiction in the theory. The use of such diagrams is explained, for example, in my book *Einstein's Theory of Relativity* of which a new cheap edition (Dover Publications, Inc., New York) is now available. I give here the diagram for the time dilation with a detailed legend (Fig. 1); it shows why the slowing down of a clock at rest in one system when observed from another system in relative motion is reciprocal in the two systems. Further explanations seem to be superfluous.

Max Born

Bad Pyrmont,
West Germany.

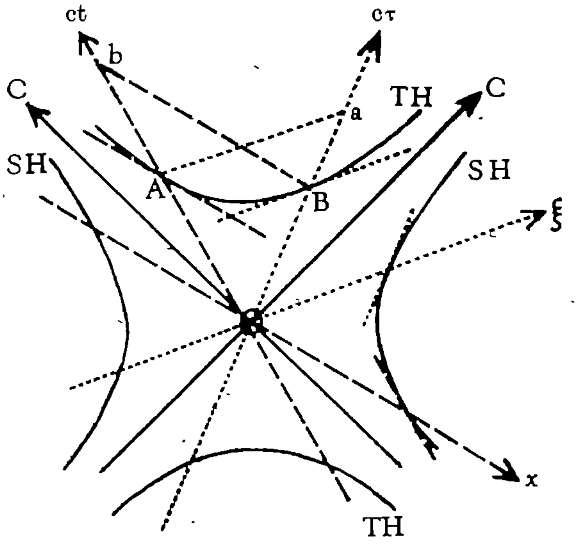

Fig. 1. C,C, Cross-section of light cone; SH, space calibration hyperbola; TH, time calibration hyperpola; x,ct, conjugate diameters = axes in K; $(\xi,\sigma\tau)$, conjugate diameters = axes in k; OA, represents the same time-interval in K as OB in k:

$$OA \sim OB$$

$$\begin{array}{ll} \text{Clock at rest in } K \left\{ \begin{array}{l} OA \sim ct \\ Oa \sim \sigma\tau \end{array} \right\} & \begin{array}{c} Oa > OB \sim OA \\ \tau > t \end{array} \\ \text{Clock at rest in } k \left\{ \begin{array}{l} OB \sim \sigma\tau \\ Ob \sim ct \end{array} \right\} & \begin{array}{c} Ob > OA \sim OB \\ t > \tau \end{array} \end{array}$$

I am grateful to Prof. Born for replying to my communication, and am especially pleased that one scientist of outstanding eminence has passed the integrity test: would there were others.

I must maintain, however, that he has not met the point I raised: this is shown by the following passage in his letter: "The mistake is in the first paragraph . . . the same meaning in (1) and (2)". In making this statement Prof. Born is clearly exceeding his rights: a question cannot be dismissed as a "mistake". It is my question, and I meant it exactly as I put it. Prof. Born substitutes a different question, which he assumes I should have asked, and gives an answer that does not contradict Einstein's conclusion. I agree, but the answer to my question does.

Prof. Born is wrong in saying that my expressions (1) and (2) refer to different physical situations and that t and τ have not the same meaning in (1) and (2). This is not a matter of opinion. I constructed the second statement, and I say categorically that if, in Einstein's statement, A is a clock fixed at the origin of what he calls the 'stationary' system, and X is a clock fixed at the origin of what he calls the 'moving' system, then in my statement also, A is fixed at the origin of that same 'stationary' system and X at the origin of that same 'moving' system. Furthermore, in both statements t is the time of an event according to clock A, and τ the time of the same event according to clock X.

What Einstein's statement shows is that, on his theory, between the two events, (1) the coincidence of the clocks, and (2) a later event occurring on X, the time-interval according to A is greater than that according to X, so that X is running slower than A. What my statement shows is that, in the identical physical situation, between the two events (1) the coincidence of the clocks, and (2) a later event occurring on A, the time-interval according to A is smaller than that according to X, so that X is running faster than A. The only difference between the two cases is that the intervals compared are those between different pairs of events; and the only conceivable way of avoiding the contradiction is by showing (consistently with the relativity postulate, that is, without assuming an ether in which A is uniquely stationary and X uniquely moving) that Einstein's interval between two events on X is valid for comparing the rates of the clocks, and that my interval between two events on A is not.

I repeat that there is only one physical situation—two clocks, A and X, in uniform relative motion; that there is no change of co-ordinate system in going from Einstein's statement to mine (A 'rests' and X 'moves' in both); and that there is no change of meaning of t or τ (t refers in both to readings of A, and τ to readings of X). Hence equations (1) and (2) show that A goes steadily both faster and slower than X.

The effective criterion of truth in these matters still resides in the pronouncements of mathematical physicists, none of whom, of stature comparable with that of Prof. Born, appears to share his sense of obligation on this point; and as I have been informed, with various degrees of emphasis, that my utterances on such things are imponderable, there is an urgent need for the weight of his unquestioned authority to reinforce a clear demonstration of the truth of the matter, whatever it may be.

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[See also p. 1248 of this issue.]

Fading of Latent Image in Japanese Fuji *ET-7A* and *ET-6B* Nuclear Emulsions

THE various parameters on fading[1-6] indicated experimentally are due to: the action of some constituents of the atmosphere, surrounding temperature, time of storage, and emulsion composition. Based on the available experimental evidence, the mechanism of fading is thought to be dependent on the oxidation of the development specks by atmospheric oxygen in the presence of water[6].

In order to examine the latent image fading effects, the plates were exposed to mono-energetic α-rays, which were prepared from thorium nitrate by 'electrolytic displacement' method[7]. The separated ThC by emitting β-rays transforms to ThC′, which is a pure mono-energetic α-emitter with energy 8·78 MeV; only a few of them originate from ThC (ref. 8).

The plates were irradiated by α-particles from ThC′ and stored in the vessels, under different conditions, for the purpose of examining the various parameters on fading. After insertion of the irradiated plates, the vessels were evacuated to a pressure less than 0·1 mm mercury and the vapour pressure in the vessel was adjusted to what was desired by opening the cork to concentrated sulphuric acid or distilled water, which was stored in the system, then filled with the gas. The vessels containing the exposed plates under the conditions described were stored in a constant-temperature bath which was maintained at 30° C for the assigned duration of times as indicated in Tables 1 and 2 and Figs. 1 and 2. They and the plates just

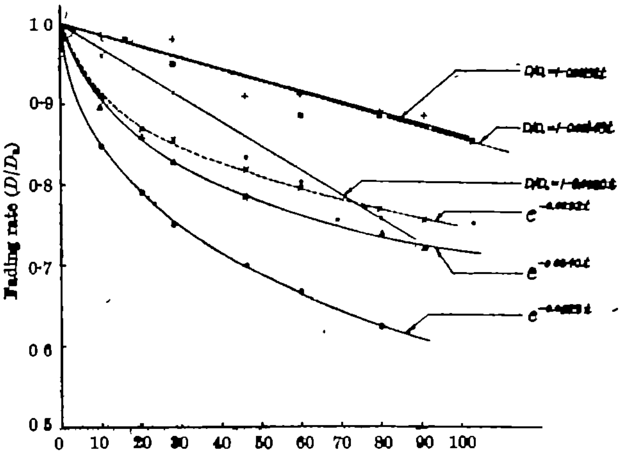

Fig. 1. Fading rate D/D_0 versus storage period t (days) in nitrogen, argon and oxygen gas at 30° C and 60 per cent relative humidity. +, *ET-7A* in nitrogen; □, *ET-7A* in argon; ●, *ET-7A* in oxygen; ×, *ET-6B* in argon; △, *ET-6B* in nitrogen; ○, *ET-6B* in oxygen. The statistical uncertainties are shown in Table 1

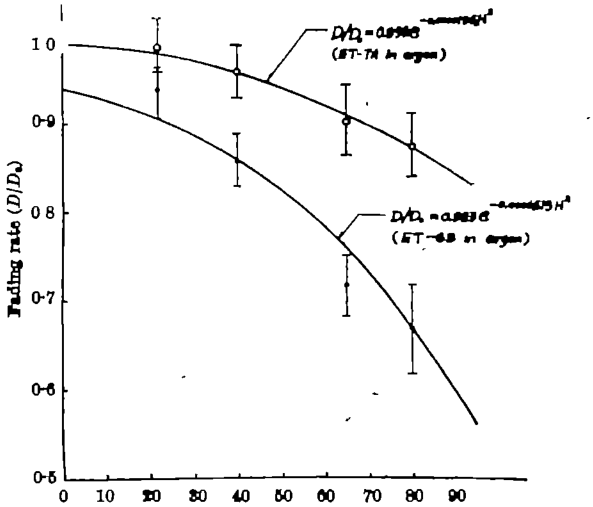

Fig. 2. Fading rate (D/D_0) versus relative humidity (H per cent) in argon at 30° C for 93 days' storage

Table 1. FADING RATE D/D_0 IN NITROGEN, ARGON AND OXYGEN GAS. D_0 IS THE GRAIN DENSITY OF α-PARTICLE TRACKS PRODUCED UPON IMMEDIATE DEVELOPMENT, D IS THE DENSITY AFTER t DAYS' STORAGE. THE STORAGE TEMPERATURE IS 30° C AND RELATIVE HUMIDITY, 60 PER CENT

Type of emulsion	*ET-6B*			*ET-7A*		
Atmosphere / Storage period (days)	Nitrogen	Argon	Oxygen	Nitrogen	Argon	Oxygen
0	1·000	1·000	1·000	1·000	1·000	1·000
10	0·894 ± 0·027	0·912 ± 0·026	0·849 ± 0·019	0·985 ± 0·040	—	0·960 ± 0·032
20	0·860 ± 0·029	0·878 ± 0·031	0·791 ± 0·021	—	—	—
28	0·830 ± 0·028	0·858 ± 0·040	0·752 ± 0·017	0·980 ± 0·041	0·950 ± 0·035	0·915 ± 0·050
46	0·787 ± 0·042	0·820 ± 0·036	0·702 ± 0·036	0·910 ± 0·033	—	0·885 ± 0·045
60	—	0·798 ± 0·032	0·668 ± 0·018	0·911 ± 0·047	0·886 ± 0·036	0·804 ± 0·041
80	0·740 ± 0·033	0·771 ± 0·039	0·626 ± 0·035	0·891 ± 0·049	0·885 ± 0·039	0·761 ± 0·030
91	0·723 ± 0·032	0·757 ± 0·030	—	0·885 ± 0·035	—	—
103	—	—	—	—	0·853 ± 0·041	0·752 ± 0·031

Table 2. FADING RATE D/D_0 AFTER 93 DAYS' STORAGE IN ARGON WITH VARIOUS HUMIDITIES AT 30° C

Vapour pressure (mm mercury)	7·00	12·73	20·68	25·46
Relative humidity (per cent)	22	40	65	80
D/D_0 for *ET-7A*	0·989 ± 0·041	0·961 ± 0·030	0·905 ± 0·038	0·876 ± 0·036
D/D_0 for *ET-6B*	0·941 ± 0·32	0·861 ± 0·031	0·721 ± 0·035	0·672 ± 0·053

irradiated were developed simultaneously with one part *ID*–19 stock solution to 10 parts distilled water at 20° C; special precautions were taken in plate processing aiming at easier grain counting of the developed α-tracks.

To examine the relation between fading rate and storage time, the plates were irradiated at a certain interval several times and processed at the end of the last exposure. Thus the emulsion plates were stored under constant conditions for different periods of time. The fading rate D/D_0 (ratio of the grain density after storage for a time t before development and density after immediate development) and the storage time t in days are given in Table 1 and plotted in Fig. 1. The atmospheres surrounding the plates were nitrogen, oxygen or argon and the vapour pressure of the atmospheres was 19·09 mm mercury corresponding to the relative humidity of 60 per cent at 30° C.

Relation between the fading rate and storage period for emulsion of type *ET–6B* can be expressed approximately by an empirical equation of the form $D/D_0 = \exp(-c\sqrt{t})$. For type *ET–7A*, an empirical equation expressing a straight line such as $D/D_0 = 1 - kt$ was found adequate for all cases. However, they can also be expressed by a formula $D/D_0 = \exp(-c'\sqrt{t})$, which is the form suited for emulsions of type *ET–6B*. The advantage of expressing D/D_0 by a straight line is the proper fitting of the line to the experimental data through a wide range of storage times. Conversely, if expressed by the form of $\exp(-c'\sqrt{t})$, a better fitting of the curve to the experimental data could be obtained in a region of the longer storage time (greater than 40 days), but a worse deviation at the shorter storage time. This does not agree with LaPalme *et al.*[9]. A formula $D/D_0 = \exp(-c''t)$ has been tested, and it was found that it did not fit so well as the other two foregoing formulæ.

In investigating the relation between fading rate and relative humidities, the exposed plates were sealed in vessels of assigned vapour pressures of 7·00, 12·73, 20·68 and 25·46 mm mercury corresponding to relative humidities of 22, 40, 65 and 80 per cent respectively at 30° C. These plates were then stored under a constant temperature of 30° C for 93 days before processing. The processed plates, after counting grain densities, were found to have a relationship between the fading rate and relative humidities expressed by $D/D_0 = a\exp(-bH^2)$ (Fig. 2 and Table 2). In the formula expressing D/D_0, H is the relative humidity under which the plate was stored, a and b are constants.

In extrapolating the curves to zero humidities, values of $D/D_0 = 0\cdot94$ for type *ET–6B*, and 0·99 for type *ET–7A* were obtained. It can then be assumed that the main parameter on fading is due to oxidation of the development specks by atmospheric oxygen in the presence of water[5].

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GEOPHYSICS

Origin of the Earth's Magnetic Field

FROM a knowledge of the mass of the Earth and its moment of inertia and from an analysis of seismic data, Bullen[1] has reconstructed a pressure-density curve for the Earth's interior. The outer core of the Earth is believed to be liquid since it does not transmit transverse seismic waves while the structure of the inner core is still in doubt. The entire core is believed to be predominantly iron.

According to Bullen, the interface between the inner and outer cores occurs at a depth of ~ 5,000 km corresponding to a pressure of $\sim 3\cdot3 \times 10^{12}$ dynes/cm², a temperature of ~4,000° K and a density of ~ 11·5 g/cm³ in the outer core and ~ 15·2 g/cm³ in the inner core. It is the purpose of this communication to suggest that the inner core may well be a new phase of iron in which two of the 3*p* 'argon core' electrons are transferred to the '3*d*–4*s*' band and that such a phase may account for the origin of the Earth's magnetic field.

That the foregoing values of density, pressure and temperature for the outer core are reasonable for iron comes from a knowledge of the compressibility of iron and its pressure dependence[2] $K = -(1/V)(\mathrm{d}V/\mathrm{d}P) = 5\cdot8 \times 10^{-13}\,(\mathrm{cm}^2/\mathrm{dyne}) \times (1 - 2 \times 10^{-13}\,P\,\mathrm{cm}^2/\mathrm{dyne}) = a(1 - bP)$ and an approximate value of the coefficient of volume expansion $\alpha = (1/V)(\mathrm{d}V/\mathrm{d}T) \simeq 80 \times 10^{-6}$/deg. This gives the density, ρ:

$$\rho(P,T) \sim \rho(0,0)\exp\{aP(1 - bP/2)\}\exp\{-\alpha T\}$$

where ρ(0) is the extrapolated density of liquid iron at $P = 0$, $T = 0$ leading to a value.

$$\rho(P,T) \simeq 11\cdot2\ \mathrm{g/cm^3}\ \text{(at the inner–outer core interface)}$$

in good agreement with Bullen's estimate. However, the density change in iron is only 3·5 per cent on melting so that the ~ 30 per cent change at the interface suggested by Bullen (11·5 g/cm³ versus 15·2 g/cm³) appears too large to attribute to normal solidification of iron in the inner core. On the other hand, the transfer of two of the 3*p* electrons to the '3*d*–4*s*' band would add two more electrons to the six bonding electrons (the two magnetic electrons are in anti-bonding states), and in analogy to the cases of the rare earth metals, cerium, europium and ytterbium, this would change the atomic volume approximately in proportion to the number of overlapping electrons. In iron this new high-pressure phase would correspond to a density change of ~ 25 per cent.

That the transfer of one or two electrons from the 3*p* shell to the overlapping '3*d*–4*s*' band is energetically feasible comes from an estimate of the band-width and the one electron energy eigen values of the 3*p* electrons. Because of the large overlap of the '3*d*–4*s*' electrons at these high pressures we can use free electron theory to give an approximate value of the band-width and this gives ~ 55 eV for the inner core as compared to 69 eV for the Hartree–Fock one electron 3*p* energy[3]. (The X-ray absorption edge in metallic iron corresponding to a transition from the 3*p* level to the Fermi level occurs at 53 eV.) (In analogy to the rare earth metals, thulium, ytterbium and lutecium, I suggest that two rather than one electron will be transferred from the inner closed shell although this point is not critical to my argument.)

Because of the very strong spin orbit coupling, the remaining four electrons in the 3*p* shell will probably couple according to Hund's rule leaving two unpaired electrons with $L = 1$, $S = 1$, $J = 2$. In order to estimate the Curie temperature of this new phase we need to estimate the exchange energy. While such a straightforward calculation is beyond the present capabilities of solid state physics, an approximate value can be obtained relative to the ordinary form of ferromagnetic iron for which we have experimental values of the exchange energy[4]. Since the exchange energy arises from the overlap of the band electrons with the unpaired atomic-like electrons we wish to estimate the exchange integral:

$$J \alpha \left| \int \psi_A(r_1)\psi_B(r_2) \frac{e^2}{r_{12}} \psi_A(r_2)\psi_B(r_1) d\tau \right|^2 / E_F \qquad (1)$$

where the subscript A denotes the unpaired atomic-like electron wave function and the subscript B the overlapping band electron wave function and E_F is the Fermi energy. For the normal ferromagnetic form of iron these are the unpaired $3d$ and the '$3d$–$4s$' band electrons respectively, while for our postulated high-pressure form of iron these are the $3p$ and the '$3d$–$4s$' band electrons respectively. Since the overlapping electrons at the bottom of the band in iron are extended quite far radially (Wood, J. W., unpublished results) we can assume ψ_B is constant throughout the atomic cell for both the normal and high-pressure phases. This term alone would lead to a ratio of ~ 6·5 for the values of J in the high-pressure and normal phases while the Fermi energy in the denominator (55 eV versus ~ 10 eV) reduces this to ~ 1·2. This leaves the evaluation of the r_{12} term for the unpaired $3p$ and $3d$ electrons respectively, and this must surely be greater for the former since they are more compressed radially. It is estimated that their contribution to J would lead to a ratio approximately of the order of their one electron energies (– 5·3 Rydbergs versus – 1·3 Rydbergs) or about a factor of 4. This roughly gives an overall factor of 4 for the ratio of J in the high-pressure and normal phases or a value of 0·04 eV for the high-pressure phase. (If we apply equation 1 to the normal cases of ferromagnetic iron and gadolinium, where in the latter case ψ_A refers to the $4f$ electrons, one obtains a ratio of ~ 40 for the J values as compared with the observed value of ~ 50.)

From statistical mechanics a close-packed structure with nearest neighbour exchange interactions[4] ($S = 1$) has a value of $J/kT_c = 0·08$, leading to an estimated value of the Curie temperature of our high-pressure form:

$$T_c \simeq J/0·08k = 5{,}500° \text{ K}$$

which is well above the inner–outer core interface temperature of ~ 4,000° K.

If this crude order of magnitude estimate is correct there are several consequences of note:

(1) If the planets contain cores similar in composition to the Earth only those of mass approximately equal to or greater than the Earth would develop sufficient gravitational pressure to create the high-pressure magnetic phase. Thus we would expect no magnetic field on Mercury, Mars, Venus and the Moon, but would expect magnetic fields on Uranus, Neptune, Saturn and Jupiter.

(2) If the inner core were saturated (a single magnetic domain) the magnetic field at the Earth's surface would be about ~ 150 gauss. The observed value of ~ 1 gauss is thus typical of the residual magnetization of an unmagnetized piece of iron.

(3) Magnetostrictive effects (spin-orbit coupling) can cause variations in the magnitude and direction of the Earth's magnetic field depending on the variation of the distribution of pressure on the surface of the inner core. Because the anisotropy energy is small compared with the compressional energy only small changes in pressure are necessary to effect changes in magnetic domain orientation.

(4) Inasmuch as static pressures of ~ 0·5 × 10^{12} dynes/cm^2 have been reached in the laboratory[5], it is probable that materials and techniques will improve sufficiently to reach this suggested critical pressure of ~ 3·5 × 10^{12} dynes/cm^2 for the onset of the high-pressure magnetic phase of iron. Thus this hypothesis is probably capable of laboratory refutation.

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GEOCHEMISTRY

Evidence of an Extraterrestrial Origin for Some Organic Constituents of Meteorites

The presence of organic matter within samples of carbonaceous chondrites is now well established[1–3]. However, it has been suggested from several sources that considerable terrestrial contamination of these meteorites may have occurred and that the organic matter is not indigenous. The experiments reported in this communication were conducted to determine whether the isotopic composition of the meteorite organic matter afforded any evidence regarding the origin of the compounds.

Samples of Orgueil, Murray, Mokoia and Haripura meteorites were extracted separately for 6 h in a Soxhlet apparatus with a 9 : 1 benzene–methanol mixture. The solvent was evaporated from the extract and the isotopic composition determined of the hydrogen, carbon and sulphur of residues from each meteorite by mass spectrograph. Results of these determinations are shown in Table 1.

Table 1. Isotopic Composition of Meteorite Organic Matter

Meteorite	Deuterium (%)	Carbon-13 ($^0/_{00}$)	Sulphur-34 ($^0/_{00}$)
Orgueil	+ 27·5	– 10·1	+ 3·14
Murray	+ 8·4	– 4·0	+ 2·86
Mokoia	+ 21·2	– 17·9	+ 1 09
Haripura	– 1·5	– 3·1	+ 2·51

Standards. Deuterium and carbon, Craig (ref. 6); sulphur, Thode *et al.* (ref. 7).

A comparison of these isotopic values with those for non-carbonaceous meteorites and for various terrestrial materials[8] reveals that the organic matter from three of the carbonaceous chondrites shows a considerable deuterium enrichment, while the carbon shows a lack of carbon-13 similar to terrestrial biogenic material. The small enrichment of sulphur-34 is the opposite fractionation to that occurring in sulphur bacteria and terrestrial inorganic processes. It is apparent that the isotopic composition of meteorite organic matter is quite unlike that of any terrestrial substances.

The evidence presented here is indicative of an extraterrestrial origin for a major fraction of the organic constituents of carbonaceous chondrites.

I thank the Curator of the Wanganui Museum, Dr. W. A. Watters, of the New Zealand Geological Survey, Dr. E. P. Henderson, of the Smithsonian Institution, and the Director of the Geological Survey of India for gifts of meteorite samples.

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METALLURGY

Destruction of the Widmanstatten Structure in Iron Meteorites by Laboratory Heat Treatment

A KNOWLEDGE of the response of iron meteorites to heat treatment is necessary for the understanding of such specimens as Kamkas[1] which appear to have suffered partial destruction of their Widmanstatten structures by cosmic heat treatment. Kasé[2] has published evidence to show that for the medium octahedrite Sacramento Mountains the nickel-rich taenite is still clearly visible after a heat treatment of 20 min at 1,000° C or 3 min at 1,200° C. Even after 4 min at 1,300° C there remains a faint trace of nickel enrichment which marks the previous location of a taenite area.

In the work recorded here more lengthy heat treatments were conducted on small (0·5 × 0·5 × 1·0 cm) fragments taken from a specimen of Canyon Diablo which is known to contain cohenite. In each case duplicate specimens were sealed in evacuated silica capsules which were heated to the maximum temperature over a period of three days and afterwards cooled to room temperature over three days. The period of holding at maximum temperature was 2 days at 1,000° C and 3 days at 1,100° C or 1,180° C. Experiments with specimens of this size do not correctly reproduce the conditions in a massive meteorite since at 1,100° C and 1,180° C it was found that all the schreibersite melted to eutectic and exuded along the original kamacite phase boundaries to produce a layer of phosphide-rich liquid on the outer surface of the specimens. At 1,000° C exudation was incomplete and the resolidification of liquid veins within the specimen produced structures similar to that illustrated by Perry[3]. With increasing temperature the phosphide eutectic, which had left the solid by way of grain boundaries in the original structure, showed an increased tendency to re-enter the solid by way of the new γ-grain boundaries which were formed by heat treatment of the kamacite. Macro- and micro-scopic observations were made on etched sections at locations away from the regions of grain boundary penetration. Macroscopic examination of the etched sections with the aid of a hand-lens revealed the recognizable remains of taenite ribbons after all heat treatments. Microscopic examination showed that the original kamacite regions had changed to a duplex arrangement of ragged ferrite and small carbide crystals. It was possible to detect the boundaries of the γ-grains from which the ferrite-carbide aggregate formed on cooling. The microstructure of the taenite regions showed a greater variation with heat treatment temperature. After the 1,000° C heat treatment the taenite still maintained a distinct boundary and was at no place crossed by the new generation of γ-grain boundaries; suggesting that the taenite had maintained its complete integrity at the heat treatment temperature. The taenite itself was characterized by a fine precipitate of non-metallics at the centre and an indistinct acicular structure at the edge.

After the 1,100° C heat treatment the taenite boundary was less sharp and was demarked by acicular (bainitic) transformation product. No precipitate was observed within the taenite and the presence of new generation γ-grain boundaries within the taenite was rare.

The 1,180° C heat treatment resulted in a coarse pattern of acicular transformation product within which the pattern of new generation γ-grain boundaries was continuous with that in the kamacite, indicating that at 1,180° C the taenite had lost its physical identity as a separate crystal but had retained a sufficiently different chemical composition to reveal itself by the production of acicular transformation product on cooling.

Less detailed examination of carbide-free specimens heated to 1,000° C and 1,100° C revealed a 'watery' appearance in the taenite without acicular transformation product. The acicular product may thus be tentatively identified as a carbon containing bainite.

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Topography of Kish Crystals and the Effect of Oxidation in Air

DETAILED studies have been reported recently on the production of etch pits in purified Ticonderoga natural graphite crystals as a result of oxidation in oxygen in the temperature range 700°–870° C[1,2]. Others have also been concerned with the effect of oxidation on the topography of natural graphite[3–6]. In this communication, the effect of oxidation on the topography of purified kish crystals is considered.

Kish is a waste product which forms on the surface of molten iron of high carbon content during the manufacture of steel. It is carbon which has been taken into solid solution with the iron and is afterwards evolved when the iron cools. Kish particles are large and flaky, resembling large particles of natural graphite. Walker and Imperial[7] have previously shown that purified kish has the same interlayer spacing as Ceylon natural graphite, indicative that it has a large crystallite size and essentially complete three-dimensional ordering.

The kish used in this investigation was purified by boiling alternately in hydrochloric acid and hydrofluoric acid (or in some cases just boiling in hydrochloric acid), followed by repeated washings in distilled water, and drying in a vacuum oven. Its structure has been further studied by transmission electron diffraction, using 50-kV electrons having a beam width of 10μ. The diffraction pattern (Fig. 1) is characteristic of a material of large crystallite size and a high degree of preferred crystallite orientation (essentially a mosaic single crystal).

The basal plane (000*l*) surfaces of the kish were studied by light microscopy. Typical micrographs of the unoxidized kish are shown in Figs. 2 and 3. In Fig. 2, the twin planes are readily distinguished as the striations running parallel to one another or intersecting at 60° (ref. 8). The presence of impurities on the basal plane, following treatment with hydrochloric acid only, is clearly in evidence. On a very significant portion of the surface, large hexag-

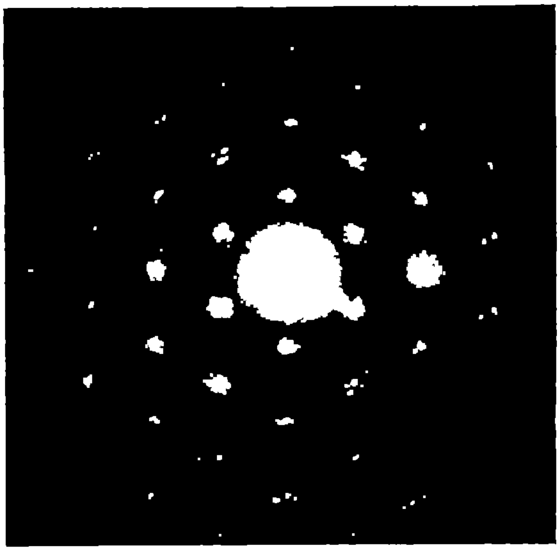


Fig. 1. Transmission electron diffraction pattern of purified kish

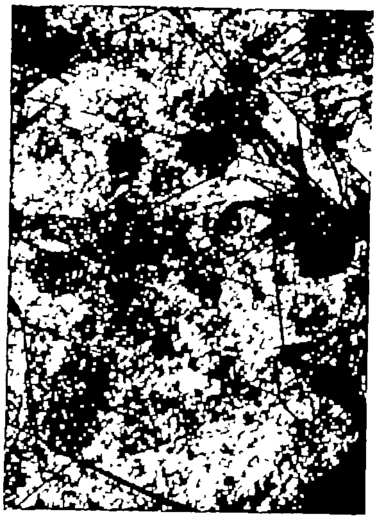


Fig. 2 Basal plane (0001) surface of kish purified in hydrochloric acid. Twin bands run parallel or intersect at 60° (× c. 200)

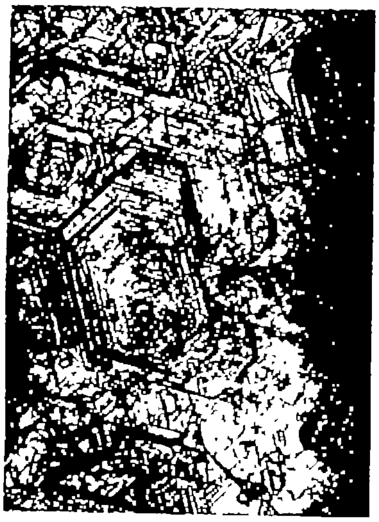


Fig. 3. Basal plane surface of purified kish showing large hexagonal stepped growth features. (× c. 200)

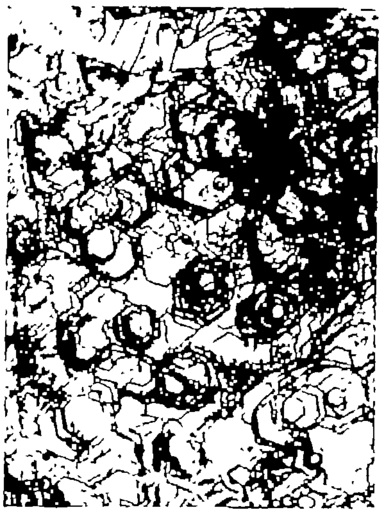
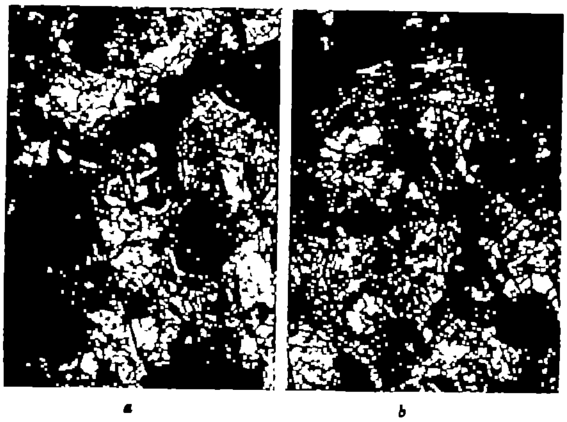


Fig. 4. Development of hexagonal pits upon air oxidation of purified kish at 850° C. (× c. 200)



a b

Figs. 5a and b. Coalescence of pits during a more advanced stage of air oxidation of kish purified in hydrochloric acid at 850° C. (× c. 150)

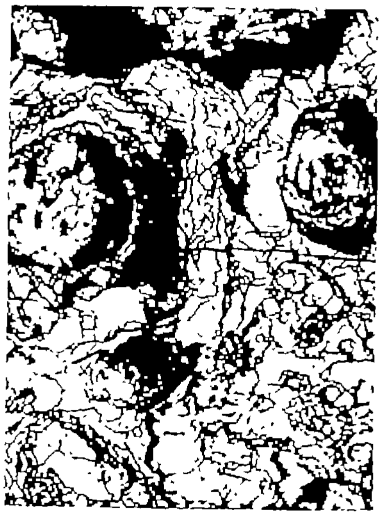


Fig. 6. Roughly hexagonal holes produced by rapid attack of air at 850° C on hexagonal stepped growths of purified kish. (× c. 200)

onal stepped growth features are in evidence (Fig. 3). It is suggested that the growth steps within these features arise from a piling-up of a large number of clockwise and counterclockwise spiral growth steps of molecular dimensions[9,10] (the spirals being invisible on our photographs). A substantial concentration of impurity nucleating centres from which such screw dislocations and spiral growth of graphite could occur might well be expected, considering the origin of kish.

Kish crystals were oxidized in air (1 atm.) at 850° C. Fig. 4 shows the development of a large number of hexagonal pits oriented parallel to each other and parallel to the twin lines, as previously discussed[1]. Figs. 5a and b show the results of coalescence of pits during a more advanced stage of oxidation. The shapes of the holes are reminiscent of those suggested by Ubbelohde, when considering defects in graphite[11].

In general, it is found that the large hexagonal stepped growths are attacked at an enhanced rate. Fig. 6 shows the result of exposure of such growths to air at 850° C for only about 5 min. Large, roughly hexagonal holes are produced, in contrast to the relatively minor attack on other portions of the surface. However, it is not possible to decide on the basis of this result whether the presence of dislocations or the possible concentration of impurities at dislocations is of primary importance in the production of pits in the basal plane. As discussed previously[8], uncertainty will remain until oxidation studies are carried out on ultra-high purity graphite of known dislocation content.

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CHEMISTRY

Reaction of Platinum and Boron under Pressure

DURING some high-temperature–high-pressure experiments involving boron, a new phase appeared in the X-ray diffraction patterns of the samples. This phase was traced to the reaction of boron and the platinum foil used to encapsulate the sample between the chrome carbide pistons of a Griggs and Kennedy simple squeezer[1]. Since platinum foil is a standard technique of sample enclosure for this type of experiment, it was decided to investigate the *P–T* phase diagram of this reaction so that it would not cause difficulty when encountered again.

At about the time this work was finished two papers came to our attention bearing on this problem and have prompted us to publish our results. The first was a paper by Jamieson and Lawson[2] in which they referred to the formation of a new phase from boron at 600° C and 40 kilobars (kb). No mention was made of platinum and, in reply to a letter of ours, Prof. Jamieson confirmed our suspicion that platinum foil was in contact with the boron. He was also kind enough to send us X-ray photographs of his samples. It will be seen that this new phase lies just to the right of the boundary between boron and platinum and platinum boride (Fig. 1).

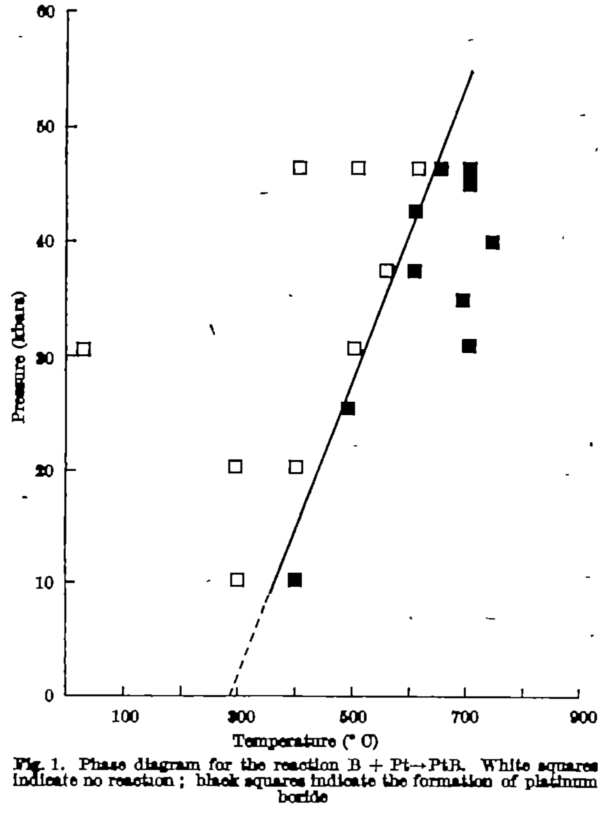

Fig. 1. Phase diagram for the reaction B + Pt→PtB. White squares indicate no reaction; black squares indicate the formation of platinum boride

Table 1. COMPARISON OF X-RAY DATA FOR PLATINUM BORIDE

	Aronsson		Jamieson		Whitney and Giese		
hkl	*d*	*I*	*d*	*I*	*d*	*I*	*d*, *calc.*
100	2·903	st	2·896	st	2·906	6	2·908
101	2·360	v st	2·364	v st	2·370	10	2·364
002	2·025	m	2·041	m	2·049	2	2·029
102	1·663	st	1·671	st	1·675	8	1·664
110	1·676	st					1·679
200	1·453	w	1·450	w	1·451	1	1·454
201	1·368	st	1·367	st	1·367	5	1·369
112	1·291	st+	1·293	st+	1·296	6	1·294
103	1·225	m+	1·232	m+	1·234	4	1·227
202	1·180	m	1·182	m	1·184	1	1·182
210			1·097	v w	1·096	1/2	1·099
211			1·063	m	1·059	4	1·061
203			0·9940	w	0·9928	2	0·9904
212					0·9661	2	0·9665
302					0·8735	4	0·8747
213					0·8540	4	0·8520
220					0·8373	1/2	0·8395
303					0·7889	3	0·7879

A recent paper by Aronsson *et al.*[3] has allowed us to identify the platinum boride as PtB (space group $P6_3/mmc$, $a = 3{\cdot}36$ Å, $c = 4{\cdot}06$ Å), and to confirm the identity of Jamieson's material with our platinum boride. There are a few weak X-ray diffraction lines not due to PtB which can be attributed to B_2O_3 and the original boron.

The slope of the curve shown in Fig. 1 was found to have a value of $dP/dT = 133$ bars degree^{-1}. It is of interest to note that Strong and Bundy[4] found the value of dP/dT for the melting point of platinum to be 140 bars degree^{-1}.

The X-ray diffraction results are listed in Table 1. We are grateful to Prof. Jamieson for permission to examine his films and publish the results.

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Electrical Storage and Hydrogen Transfer between Electrodes of Palladium and Palladium Alloys

ELECTRODES of palladium and palladium alloys can absorb large volumes of hydrogen. In general, the solid hydrides retain excellent electrical conductivity and are not severely embrittled or disrupted. For pure palladium, and for many alloys, the relationships between electrode potential *E* (with respect to a hydrogen reference electrode in the same solution) and hydrogen content (written here, Fig. 1, as the ratio, H/Me, of hydrogen atoms to the total number of metal atoms) exhibit 'plateau' regions—over which α- and β-phase hydrides co-exist—where *E* is relatively invariant ($E_{\alpha\beta}$). Over such 'plateau' regions, hydrogen transfer should, in principle, occur at a constant rate when connexion is made between an alloy and palladium or between two alloys. However, polarization[1] would be expected to occur rapidly if the diffusion of hydrogen within the electrodes were not sufficiently fast for the concentration of hydrogen at the surfaces to be continuously representative of the ranges of H/Me corresponding to the plateaux. The following experiments serve to illustrate what may be observed in practice.

The *E*–H/Me relationships, at 25° C, for palladium and two selected alloys[1–3] are shown in Fig. 1, where full lines indicate relationships which apply when hydrogen is being absorbed and broken lines approximate to relationships when hydrogen is desorbed relatively slowly. Each electrode was about 15 cm of 0·0274-cm diam. wire (about 0·1 g). The electrolyte was 1·0 N hydrochloric acid, thermostatted at 25° C and saturated with argon. All electrodes had been palladized to confer a low surface impedance[1,4] and prior to an experiment each had been caused[1] to absorb hydrogen until $E \sim 50$ mV (A, B and C in Fig. 1).

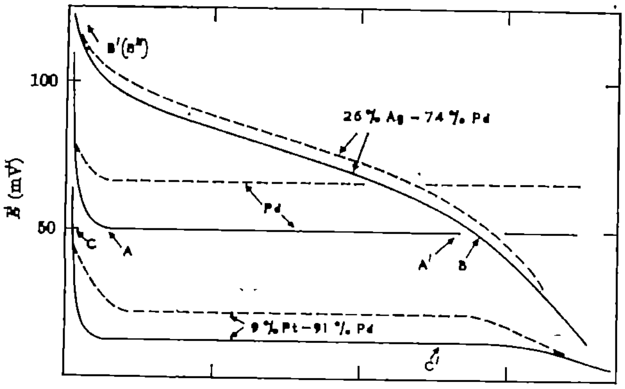

Fig. 1

In the first experiment (I) an E.M.F. of 200 mV was first applied between palladium and the palladium/silver alloy so that hydrogen was transferred (Fig. 1) from Pd/Ag/H ($B \rightarrow B'$) to Pd/H ($A \rightarrow A'$). The resultant current flow was measured (Fig. 2) by recording the potential drop across a 10 Ω resistor in series. On development of a polarizing potential of about 140 mV, the discharge of the cell, through the 10 Ω resistor, was then also recorded. The palladium/platinum alloy was combined with the palladium/silver alloy in the second experiment (II), which was carried out with the same procedure, so that, when charging, the hydrogen was likewise transferred from Pd/Ag/H ($B \rightarrow B''$) to Pd/Pt/H ($C \rightarrow C'$). Throughout both experiments the apparent internal resistance of the cells was approximately 20–30 Ω.

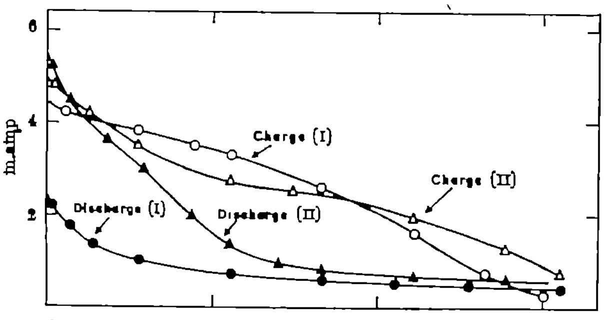

Fig. 2

During discharge over the plateau regions, the E.M.F. of the cells only corresponds, even ideally, to the differences between the 'absorption' value of $E_{\alpha\beta}$ for the electrode absorbing hydrogen and the 'desorption' value of $E_{\alpha\beta}$ for the desorbing electrode. However, Fig. 2 illustrates an improvement in terms of rapid discharge, which follows as a consequence of increasing this difference. Unfortunately, the rather restricted and imperfectly invariant plateau, illustrated in Fig. 1, for the 26 per cent silver – 74 per cent palladium alloy represents close to the highest 'plateau' values which have been reported at 25° C for the alloy systems so far investigated[1]. On the other hand, palladium alloys are available with lower values of $E_{\alpha\beta}$ (25° C); and somewhat higher rates of discharge than illustrated in Fig. 2 have been obtained from a similar cell composed of a 12 per cent platinum – 88 per cent palladium alloy[1] and the palladium/silver alloy. However, as E decreases the loss of stored hydrogen by diffusion of molecules away from the electrode becomes increasingly significant[5-7] since, for specimens with low surface impedance, E is related to the hydride vapour pressure, p by the relationship E (volts) $= -0{\cdot}029 \log p$.

We thank the International Nickel Co. (Mond), Ltd., for providing the palladium alloys.

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A Carbon Isotope Effect in the Decarboxylation of the Anisoyloxy Radical

There have been several reports of carbon isotope effects during the decarboxylation of dibasic carboxylic acids such as malonic acid[1]. Similar effects would be expected in the radical reaction:

$$R.CO.O. \rightarrow R. + CO_2$$

The rate at which the radical $R.^{14}CO.O$ dissociates is expected to be less than that for the corresponding reaction of the radical $R.^{12}CO.O$.

If aroyloxy radicals, for example the anisoyloxy radical, are generated in a medium containing reactive substances, there is competition between the processes:

$$CH_3.O.C_6H_4.CO.O. \rightarrow CH_3.O.C_6H_4. + CO_2 \quad \text{velocity constant} = k_1 \quad (1)$$

$$CH_3.O.C_6H_4.CO.O. + X \rightarrow \text{stable products} \quad \text{velocity constant} = k_2 \quad (2)$$

X may be a molecule with a labile hydrogen atom, in which case the stable product is anisic acid, or an unsaturated compound which captures the anisoyloxy radical to give another radical such as $CH_3.O.C_6H_4.CO.O.CH_2.CHX$. The fraction ($f$) of radicals losing carbon dioxide is equal[2] to $k_1/(k_1 + k_2[X])$. The velocity constant k_2 cannot be affected by introducing a heavy atom of carbon into the anisoyloxy radical. Any change in k_1, resulting from isotopic substitution, would alter the value of f; this would result in a change in the amount of carbon dioxide produced in a system in which a certain number of anisoyloxy radicals are generated.

Anisoyloxy radicals can be generated at a convenient rate by the decomposition of di-anisoyl peroxide in benzene at 60° C; under these conditions, about 50 per cent of the radicals dissociate to give carbon dioxide. The production of carbon dioxide by reaction (1) can be followed[3] by the method of isotope dilution analysis using peroxide labelled with carbon-14 at its carboxyl groups as the source of anisoyloxy radicals. The specific activity of the carbon dioxide produced during the decomposition is calculated from that of the original peroxide. If the expected isotope effect operates in reaction (1), the specific activity of the carbon dioxide must be less than the calculated value, and consequently low values must be obtained for the yield of carbon dioxide.

The weight of carbon dioxide formed during the decomposition of unlabelled di-anisoyl peroxide can be found by isotope dilution analysis; in this case, however, labelled carbon dioxide is added to the system after completion of the reaction. This procedure avoids interference from isotope effects and should lead, therefore, to true values for the yields of carbon dioxide.

Full details of the experimental procedure and the results will be published later; the first results show clearly, however, that the expected carbon isotope effect does operate during the dissociation of the anisoyloxy radical. Solutions of the peroxide in benzene (2·5 g/l.) were degassed and kept at 60° C for 48 h; during this time about 73 per cent of the peroxide decomposed. Yields of carbon dioxide were determined by both methods of isotope dilution analysis. By the technique involving ^{14}C-di-anisoyl peroxide, the weights of carbon dioxide produced from 1 mg of peroxide present originally were 0·0846, 0·0847, 0·0856 and 0·0855 mg; the mean value is 0·0851 mg. From experiments involving unlabelled peroxide, the weights of carbon dioxide were found to be 0·1024, 0·0940, 0·0851, 0·1084 and 0·1230 mg with a mean value of 0·1026 mg.

One of us (T. H. B.) is grateful to the U.K. Atomic Energy Authority for an award partly enabling this work to be carried out.

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Polarizability Theories of Polynucleotide Hypochromism

Bolton and Weiss have recently given[1] expressions for the decrease in optical absorption (hypochromism) of a classical oscillator caused by the polarizability of identical neighbouring oscillators, and suggest their theory explains the hypochromism of helical polynucleotides. Their result is surprising, since it violates the sum rule for oscillator strengths.

Bolton and Weiss consider the classical interaction of each oscillator with the incident electric field and the additional field caused by the induced moments of the other oscillators. For their dimer model with two parallel one-dimensional oscillators coupled in this way, the complex polarizability of one of the oscillators along its polarization direction can readily be verified (by insertion in the equations of motion) to be:

$$\alpha = [3e^2f/m(x^2 + z^2\omega^2)]\,[x - iz\omega] \qquad (1)$$

where e and m are the charge and mass of an electron, f is the oscillator strength, ω is the circular frequency of the incident light, z is an intrinsic damping frequency and x is related to the resonance frequency ω_0 of an isolated oscillator by:

$$x = \omega_0^2 - \omega^2 - 3ke^2f/m \qquad (2)$$

Here, k is a coupling parameter dependent on the intergroup distance d; k equals $-1/d^3$ for transversely oriented oscillators and $2/d^3$ for longitudinally oriented oscillators. When k equals zero, (1) gives the polarizability of an isolated oscillator.

The molar extinction coefficient ε at circular frequency ω is given by[2]:

$$\varepsilon = -\frac{4\pi N\omega}{2303\,c}(Im\ \alpha/3) = \frac{4\pi Ne^2f}{2303cm}\ \frac{z\omega^2}{(x^2 + z^2\omega^2)} \qquad (3)$$

(N = Avogadro's number; c = velocity of light.) Thus the absorption curve for any value of the coupling parameter k has the same maximum value and the same width (z) at half maximum absorption, but the circular frequency of maximum absorption is shifted from ω_0 by approximately $-3ke^2f/2\omega_0m$. (A quantum mechanical analogue is the shift in the spectrum of parallel degenerate transitions due to exciton strong coupling[3].) It is clear that the integrated intensity (the area under the ε versus ω curve) cannot change, and thus there is no hypochromism. Bolton and Weiss's expressions give the absorption at frequency ω_0, which is not the frequency of maximum absorption when the absorption curve has shifted.

Hypochromism can be explained by a similar local field treatment, however, when the polarizability of non-absorbing oscillators is taken into account. As will be shown, the results are exactly equivalent to the quantum mechanical perturbation theory derivations of Tinoco[4,5] and Rhodes[6], which obey the oscillator strength sum rule[6] and which (contrary to statements of Bolton and Weiss[1], and also of Nesbet[7]) can account for the observed hypochromism of polynucleotides without a change in the shape or position of the absorption band (through exciton weak coupling[3]).

Briefly, the treatment consists of calculating the net local field $\mathbf{E}$ at a monomer group i which contains the one-dimensional oscillator under investigation; the oscillator is polarized along the monomer-fixed unit vector e_{ia} and has resonance frequency ν_a. An oscillator in a nearby group j (which can be another monomer or a solvent molecule), having a resonance frequency ν_b (different from ν_a) and oscillator strength f_b and polarized along unit vector e_{jb}, has an in-phase polarizability at frequencies close to ν_a given from (1) by:

$$\alpha_{jb} = 3e^2f_b/4\pi^2m(\nu_b^2 - \nu_a^2) \qquad (4)$$

If the incident field is $\mathbf{E}_0 = Ae\cos(2\pi\nu t)$, where ν is close to ν_a, then the field at monomer i due to the induced point dipoles of all nearby groups j is:

$$\mathbf{E}' = -\sum_{j\neq i}\sum_{b} \alpha_{jb}r_{ij}^{-3}\,[e_{jb} - 3(e_{ij}.e_{jb})e_{ij}]e.e_{jb}A\cos(2\pi\nu t) \qquad (5)$$

where r_{ij} is the intergroup distance and e_{ij} is a unit vector along the line joining the two groups.

The rate of energy absorption by monomer i at a given frequency ν is proportional to[8]:

$$[\,(\mathbf{E}.e_{ia})/\cos(2\pi\nu t)\,]^2$$

This expression must be averaged over random directions of e relative to e_{ia}. Using the relations:

$$[\,(e.e_{ia})^2]_{av} = 1/3$$
$$[\,(e.e_{ia})\,(e.e_{jb})\,]_{av} = (1/3)e_{ia}.e_{jb} \qquad (6)$$

one finds that the ratio C of the integrated intensities for the rigid polymer and for the randomly oriented monomers is:

$$C = \{[\,(\mathbf{E}_0 + \mathbf{E}').e_{ia}]^2\}_{av}/\,[(\mathbf{E}_0.e_{ia})^2]_{av}$$
$$\approx 1 - 2\sum_{j\neq i}\sum_{b}\alpha_{jb}r_{ij}^{-3}\,[e_{ia}.e_{jb} - 3(e_{ij}.e_{ia})\,(e_{ij}.e_{jb})\,] \times e_{ia}.e_{jb} \qquad (7)$$

Finally, inserting (4) in (7), one obtains:

$$C = 1 - \frac{3e^2}{2\pi^2m}\sum_{j\neq i}\sum_{b}\frac{f_b}{(\nu_b^2-\nu_a^2)r_{ij}^3}$$
$$\times\ [e_{ia}.e_{jb} - 3(e_{ij}.e_{ia})\,(e_{ij}.e_{jb})\,]e_{ia}.e_{jb} \qquad (8)$$

which is identical to the expressions given by Tinoco[4] and Rhodes[6].

Quantitative applications of (8) to the hypochromism of polynucleotides have been made[4,6,9,10]. If the absorbing oscillators are of low frequency ($\nu_a < \nu_b$) and are longitudinally oriented (for example, $n \rightarrow \pi^*$ transitions in helical polynucleotides), the spectrum is expected to be hyperchromic[9], not hypochromic as predicted by Bolton and Weiss.

A more detailed description of this treatment, with applications to solvent effects on solute spectra, is being prepared for submission elsewhere.

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We have read with interest the preceding communication but we have the impression that DeVoe has misunderstood to some extent both Nesbet's and our work. In the first part of his communication, DeVoe refers to the well-known classical equation for a pair of coupled oscillators with regard to a given direction of the electric field. However, in our treatment of the dimer, it is explicitly stated that our equation refers to isotropic oscillators and averaged over all directions of the electric field vector. We have, in fact, calculated the absorption coefficient for the frequency corresponding to the maximum absorption in the monomer. However, in the case under consideration, this also corresponds to the frequency of maximum absorption in the dimer. In fact, the absorption due to the longitudinal dipole oscillations in the dimer is shifted in frequency (without change of intensity or width) and that due to the transverse oscillations is shifted in the opposite sense, but the mean frequency for the isotropic case (one longitudinal plus two transverse polarizations) is not changed. In the hypothetical case of a very narrow band, this would appear as a splitting, or if the monomer band is already visibly split this would result in opposite displacements of the components. In the cases under consideration we were concerned with a situation corresponding to nucleic acid, where the absorption bands would be too broad (half-width ~ 300 Å) for this splitting to be observed. Thus the net effect here is a reduction of the absorption at the peak, which, however, should be compensated by some additional broadening elsewhere so as to preserve the total oscillator strength; there is therefore no violation of the sum rule.

In the nucleic acids there are four different monomer units, the light absorption of which varies quite significantly around 2600 Å, each unit having two transitions polarized perpendicular to each other. It is clear, therefore, that any theory which is based on very few assumptions can only be regarded as a first approximation for such a complex structure.

The importance of the sum rule in these situations has been emphasized on a previous occasion[1], although in the later paper it was suggested that it might possibly not hold in this case. However, I am reassured now that it is valid and remains so even if the small retardation effects due to the finite distance between the monomer units are taken into account. In any event, neither our calculations nor Nesbet's treatment do, in fact, imply a violation of the sum rule for the oscillator strength. Nesbet[2] has given a quantum mechanical treatment for the hypochromic effect. This takes as its basis that in quantum mechanics the ground-state of the dimer contains an admixture of the state in which two units are excited due to the interaction between the virtual dipole moments of the units, which is also responsible for the van der Waals force. This can be interpreted in such a way that as a result of this there exists a radiative transition from the ground state to the state in which three units are excited, corresponding to the frequency 3ν, if ν is the frequency of maximum absorption of the single unit. This frequency, 3ν, is likely to be outside the region normally accessible to spectroscopic observations (in the case of the nucleic acids it would be around 900 Å) and it would, in general, be masked by absorption processes of a different kind situated in this region. This transition would use up some of the oscillator strength of the polymer, so that the fundamental absorption in the ultra-violet is reduced; there is, therefore, here again, no violation of the sum rule.

In the second part of his communication, DeVoe suggests a theory of hypochromism on the basis of our treatment but taking into account the non-absorbing oscillators present in the system. Such a treatment, although formally correct, raises doubts as to its significance both on theoretical and experimental grounds. DeVoe's statement that such an interaction is without change in position of the absorption band is not correct; in fact, the reduction in oscillator strength to compensate for that which would appear near the resonance frequency of the particular (non-absorbing) oscillator is always associated with a shift in frequency. Moreover, taking, for example, a transition at 2000 Å, as has been suggested, there is no obvious reason why this should only be significant in the polymer and not in the monomer, as the monomer unit is itself a relatively very large and complex unit which absorbs quite strongly at 2000 Å.

Therefore, it is not at all obvious, without making very special assumptions, why such a transition in the polymer should be more effective compared with a transition in the same spectral region within the same monomer unit. Moreover, as the interaction between oscillators decreases relatively rapidly with increasing distance, one should expect that an interaction within the same monomer would be more effective. This model would not therefore explain, without very special assumptions, the difference between the monomer and the polymer in any convincing way. DeVoe's treatment of this effect is purely qualitative, as indeed it has to be because the effect must depend on some definite assumptions regarding the number and frequencies of the non-absorbing oscillators which are taken into account, so that the whole treatment would be based on more or less arbitrarily chosen parameters.

Finally, it should be pointed out that in undenatured nucleic acid any hyperchromism at 2000 Å, or for that matter at any other wave-length in the ultra-violet region, as required by this theory, is not supported by experimental evidence.

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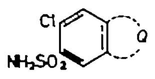
BIOCHEMISTRY

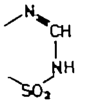
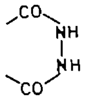
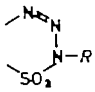
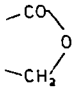
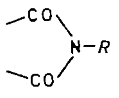
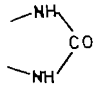
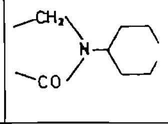
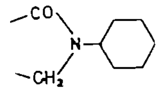
5-Chloro-2-*cyclo*hexyl-1-oxo-6-sulphamoyl Isoindoline : a New Diuretic

The introduction of chlorothiazide (Table 1, I) in 1957 marked a major advance[1] in the search for improved non-mercurial diuretics. Its advent was followed by a succession of more potent derivatives[2]. Clinically, however, apart from the size of dose, all these derivatives produced a similar therapeutic response[3]. One of the few disadvantages of these compounds is the loss of potassium which occurs concurrently with the excretion of sodium ions and can necessitate the administration of potassium supplements.

The initial object of the present research was therefore to find a compound which would cause relatively less loss of potassium than chlorothiazide. On the available evidence it seemed to us that such a compound was more likely to be found among analogues which represented major structural modifications to the heterocyclic ring of chlorothiazide (I), rather than among further substitution derivatives of chlorothiazide itself. Accordingly, we synthesized the structures (III)–(VIII) (Table 1), in which the endocyclic sulphonamide group of (I) was replaced by a number of markedly different groupings.

Table 1



Compounds with diuretic activity in the rat		Compounds inactive as diuretics in the rat	
Compound	Value of *Q*	Compound	Value of *Q*
(I) Chloro- thiazide		(V)	
(II)		(VI)	
(III)		(VII)	
(IV) M and B 8430		(VIII)	

Diuretic activity was first found in the benzothiatriazine analogues (II) of chlorothiazide, but it soon became evident that this new class was unlikely to provide the compound we sought. In the rat, diuretic activity of the compound (II; $R = CH_3$) equalled that of chlorothiazide, but the ion excretion pattern showed no relative advantage in regard to potassium loss. Changing the substituent R to benzyl[4] diminished activity. Reduction of the 3 : 4-double bond in (II; $R = CH_3$) to give analogues of hydrochlorothiazide was not chemically feasible.

The phthalimides[5] (III), on the other hand, were more promising chemically. Modification of the substituent R presented few difficulties, and of 27 compounds prepared the most active were those in which R = *cyclo*alkyl, particularly (III; R = *cyclo*hexyl), a compound found to be 6 times as active as chlorothiazide in the rat.

The related compounds (V), (VI), (VII) and (VIII) were inactive as diuretics. However, reduction of (III; R = *cyclo*hexyl) with tin and hydrochloric acid afforded the corresponding isoindoline[6] (IV), which proved to be markedly more potent than the starting material.

This compound (IV; M and B 8430), which formed non-hygroscopic colourless prisms, m.p. 266°–268°, soluble in water only to the extent of 16 mg/l. at 25°, was made the subject of detailed pharmacological study.

Assays in the rat and dog showed that M and B 8430 was about 100 times as active as chlorothiazide on oral administration. The onset and duration of the diuresis, and the excretion of both sodium and potassium ions, induced by equipotent doses of M and B 8430 and chlorothiazide, were very similar.

From experiments on the excretion of water, sodium, potassium and chloride ions in the rat and dog, it appeared that M and B 8430 inhibited tubular reabsorption of sodium since it caused an increased concentration of sodium in the urine, which was correlated with increase in dose, whereas water excretion, though dose dependent, was very much more variable. There was no change in glomerular filtration rate as measured by creatinine clearance in the dog. The compound was not a potent carbonic anhydrase inhibitor *in vitro*.

In chronic toxicity experiments in rats, dogs and monkeys over a period of 3 months, no gross abnormalities were found, nor was there any evidence of marked changes in the tissues at post-mortem examination. Further detailed, comparative toxicity investigations are being carried out.

The results of some preliminary clinical trials[7,8] have confirmed the diuretic effect of M and B 8430, the therapeutic dose being about 50 mg a day. The compound appears to have a potency and duration of action similar to those of hydrochlorothiazide. The compound was well tolerated. Relative potassium excretion was similar to that produced by an equipotent dose of chlorothiazide.

We thank Dr. A. K. Armitage and Mrs. J. E. Boswood for their collaboration in some of the early pharmacological experiments, and Mr. K. F. Rivott for the results of toxicity experiments.

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Constitution of the Cross-linkages in Elastin

Elastin is insoluble in all reagents except those which break peptide bonds, and when wet the protein behaves as a typical rubber-like solid[1]. These characteristics, together with the swelling properties, are consistent with the view that elastin can be regarded as a cross-linked polymer gel containing long peptide chains randomly crumpled and held laterally at intervals by strong chemical bonds[2]. In an attempt to isolate small regions of the peptide network containing the cross-links, Thomas and Partridge[3] degraded the protein by use of a succession of proteolytic enzymes followed by amino- and carboxypeptidases. The product consisted of amino-acids and small peptides together with a fraction of higher molecular weight. This fraction was isolated and was found to be bright yellow in colour with the blue-white fluorescence characteristic of elastin fibres.

Amino-acid analysis, after acid hydrolysis, showed that the purified fluorescent compound contained about 40 per cent of its weight as glycine, proline and alanine approximately in the molar ratio 1 : 1 : 2, but small quantities of other amino-acids were also present. From the content of proline and glycine the minimum molecular weight of the fluorescent peptide was near 1,000. Titration and end-group assays showed the presence of approximately 2 terminal α-carboxyl groups and approximately 2 terminal α-amino-groups per 1,000 g. One α-amino terminal was recovered as DNP-alanine, but although the second N-terminal residue reacted with fluorodinitrobenzene, the resulting DNP label could not be released from the chromophoric group by acid hydrolysis. It was concluded that, in the native protein, at least three and possibly four peptide chains spring from two carboxyl groups and two amino groups in the chromophore, thus generating the cross-linked structure.

To obtain more information about the structure of the chromophoric group, a preliminary examination of the products of acid hydrolysis of the coloured peptide has now been made. After hydrolysis with 5·5 N hydrochloric acid at 100° two major ninhydrin-reacting components other than the known amino-acids were isolated. For preparative purposes, the two compounds were separated together as a single fraction by adsorption on alumina from 50 per cent v/v aqueous methanol solution followed by elution with water. The mixture was then further fractionated by use of a 30-cm column of sulphonated polystyrene resin (8 per cent divinyl-benzene) with 0·2 N citrate buffer of *p*H 4·25 at 50° C. Two sharp, well-separated peaks (compounds *A* and *M*) of almost equal ninhydrin colour value were obtained. Both compounds were hygroscopic, colourless solids, soluble in aqueous solvents, but not in dry methanol; attempts to induce them to crystallize were unsuccessful. The compounds both gave heavy ninhydrin spots of zero mobility on paper chromatograms using butanol/acetic acid/water (4 : 1 : 5). With ethyl acetate/water/pyridine (2 : 2 : 1) both substances moved as discrete spots with closely similar R_F values and both behaved as homogeneous substances on paper ionophoresis at 40 V/cm.

Compound *A* showed ultra-violet absorption at λ_{max}^{NaOH} 237 and 270 mμ and λ_{max}^{HCl} 235 and 269 mμ; in both solvents there were inflexions at 275 mμ. The infra-red spectrum showed the typical amino-acid pattern. The absorption maxima for ionized carboxyl showed at 1,600 cm^{-1} (asymmetrical) and 1,395 cm^{-1} (symmetrical) and the amino-acid I and II bands (deformation frequencies) at 1,630 and 1,530 cm^{-1}. An ionized amino-group (stretching frequency) appeared at 3,020 cm^{-1}. The compound after drying at 100° analysed for $C_{26}H_{44}N_5O_8$ (found: C, 56·5; H, 8·1; N, 12·8: formula requires C, 56·2; H, 8·1; N, 12·6).

The titration curves showed 7·2 basic groups per 1,000 g titrating between *p*H 7 and *p*H 11·5 (3·6 groups per 1,000 g titrated with midpoint at *p*H 8·80 and 3·6 groups with midpoint at *p*H 9·85). The titre from *p*H 5·0 to *p*H 1·4 was 5·8 groups, but the titration was incomplete at *p*H 1·4 and showed an anomalous deflexion between *p*H 1·4 and *p*H 1·2. The best fit to the titration curve was given by a minimum molecular weight of 278 with groups at pK_1 1·70; pK_2 2·40; pK_3 8·80; pK_4 9·85; the curve suggested the possibility of a further group titrating acid to *p*H 1·60. Since the minimum molecular weight from elementary analysis is 555 the compound contains four charged amino- or imino-groups and four charged carboxyl groups, with the suggestion of a weakly basic group titrating in the extreme acid range. The compound thus reacts as a tetracarboxylic–tetra-amino-acid. This formulation accounts for the eight oxygen atoms of the empirical formula and four of the five nitrogen atoms. The ultra-violet spectrum suggests that the remaining nitrogen atom may be part of a heterocyclic ring.

Compound *M* showed ultra-violet absorption at λ_{max}^{NaOH} 282 mμ (inflexion, 290 mμ) and λ_{max}^{HCl} 280 mμ (inflexions at 285 mμ and 217 mμ). The infra-red spectrum showed the same typical amino-acid pattern as compound *A* and was closely similar in other features. The substance, dried at 100°, analysed for $C_{26}H_{41}N_5O_8$ (found: C, 56·6; H, 7·5; N, 12·8: formula requires C, 56·6; H, 7·4; N, 12·7). The titration curve was almost identical with that of compound *A* in the alkaline region (pK_3 8·80, pK_4 9·85) but the amount titrating between *p*H 7·0 and *p*H 11·5 was somewhat greater (7·5 groups per 1,000 g). The acid titre was identical for both compounds (pK_1 1·70, pK_2 2·40). On the basis of the titration of the two groups in the alkaline region the minimum molecular weight for compound *M* was 267. The minimum molecular weight from elementary analysis was 551 and the compound must again be formulatd as a tetra-carboxylic–tetra-amino-acid.

Compounds *A* and *M* are thus closely similar in composition, but differ in degree of unsaturation. The molecular weights of the compounds are sufficient to account for the greater part of the estimated residue weight of the chromophoric group in the coloured cross-linked peptide isolated from elastin, and the compounds may be degradation products of a single chromophore or may be derived from two such chromophores of similar but not identical compositions.

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Changes in Liver D-Glucuronolactone Dehydrogenase Activity

D-GLUCURONOLACTONE dehydrogenase occurs in the non-particulate fraction of liver homogenates, and in the presence of nicotinamide adenine dinucleotide (NAD) converts D-glucuronolactone to D-glucaric acid[1]. The enzyme is probably responsible for the D-glucaric acid excreted in normal mammalian urine[2]; its activity is conveniently measured by the specific inhibitory effect of D-glucarate, after heating at acid *p*H, on β-glucuronidase.

The dehydrogenase activity in mouse liver was found to be negligible in infant animals, and increased to a maximum value in 8-weeks old animals (Table 1). Similar results have been obtained with rats, and values for human fœtal (3–4 month) liver were about 50 per cent those of adult specimens.

Table 1. CHANGES IN MOUSE-LIVER D-GLUCURONOLACTONE DEHYDROGENASE ACTIVITY WITH AGE AND PREGNANCY

Strain was Tyler's Original (Mill Hill colony). Results are expressed in μmole D-glucaric acid produced in 1 h from 5 mM-D-glucuronolactone and 2 5 mM NAD at 37° and *p*H 6·5 (ref. 1), and values given as mean ± *S.E.*

Age (weeks)	Sex and No. of animals	Enzyme activity (μmole D-glucaric acid) Per g liver	Per whole liver
2	Male (3)	0 14 ± 0 04	—
4	Male (5)	5·3 ± 0 11	—
8	Male (5)	24 6 ± 2·1	—
11	Male (3)	19 9 ± 8 0	—
11	Female (4)	17 5 ± 2·4	20 1 ± 3 0
11	Female (7) (18 days pregnant)	24 2 ± 1 0	41·5 ± 2·3

Table 2. CHANGES IN FEMALE MOUSE-LIVER D-GLUCURONOLACTONE DEHYDROGENASE ACTIVITY IN ANIMALS CARRYING TUMOURS

All animals (*SAS* strain) were of the same age before treatment; groups 1–4 were killed 16 days after treatment and groups 5–8 were killed 7 days after treatment. Results are expressed as in Table 1

Group and No. of animals	Tumour and route of administration	Enzyme activity (μmole D-glucaric acid/g liver)
1 (3)	None	14·0 ± 1·5
2 (6)	Ehrlich subcutaneous	8·2 ± 0·9
3 (5)	*T*2146 subcutaneous	10 3 ± 1·2
4 (3)	*S*37 subcutaneous	7·5 ± 1·6
5 (3)	None	18·2 ± 1·2
6 (4)	Ehrlich intraperitoneal	9·0 ± 1·0
7 (4)	*T*2146 intraperitoneal	5·4 ± 1·5
8 (3)	*S*37 intraperitoneal	5 0 ± 1 4

The increased excretion of D-glucaric acid during human pregnancy[2] was reflected by a rise in the liver dehydrogenase activity of pregnant mice, also shown in Table 1. This change was more marked when comparing total liver enzyme activity, due to the increased organ weight of the pregnant animals. A decrease in the enzyme activity of uninvolved liver tissue occurred in mice bearing experimental tumours produced by the subcutaneous or intraperitoneal injection of tumour-cell suspensions (Table 2); autopsy specimens of histologically tumour-free liver from human subjects with cancer have also shown considerably reduced dehydrogenase activity.

Speculation on the significance of these changes must await investigation of the possible physiological action of the D-glucarate produced, for example, as a potential inhibitor of endogenous β-glucuronide hydrolysis.

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Enzymatic Cyclization of L-Glutamine and L-Glutaminyl Peptides

The following observations suggest that papaya latex contains an enzyme which catalyses the cyclization of L-glutamine and of L-glutaminyl peptides.

When commercial crude papain or dried papaya latex was added to a solution of L-glutamine and the *p*H adjusted to 7–12, free ammonia was formed at the expense of amide ammonia. At the optimal *p*H of 10, 1 mg of the soluble fraction of crude papain (Merck) caused the liberation of 0·057 μmole ammonia from 0·025 M L-glutamine solution per minute at 30° C. Paper chromatography showed that the glutamine was simultaneously converted to a compound which did not stain with ninhydrin, but which stained with starch-iodide following chlorination[1], and which had the same R_F in *n*-butanol/acetic acid/water (4 : 1 : 1) as pyrrolidone carboxylic acid.

These changes were not observed with D-glutamine, or with crude papain solution which had been heated at 90° C for 20 min. They were not substantially affected by dialysing the papain or by pretreating it with iodoacetate (0·01 M) or ethylenediamine-tetraacetic acid (0·01 M). Pure papain and chymopapain were inactive. Unlike the non-enzymatic cyclization of glutamine[2,3], the foregoing reaction was not accelerated by phosphate or bicarbonate.

Glutamine derivatives such as L-glutaminyl–L-leucine and L-glutaminyl–L-asparagine were de-amidated and converted to ninhydrin-negative compounds by crude papain considerably more rapidly and at a lower *p*H than L-glutamine.

Damodaran and Ananta-Narayanan[4] examined the formation of ammonia during enzymatic proteolysis and concluded, *inter alia*, that crude papain contains a de-amidase. The possibility that this de-amidase is identical with the foregoing enzyme is at present being investigated.

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Iron Metabolism in Avian Erythroblastosis

Earlier work[1,2] has indicated a disturbance in iron metabolism in avian erythroblastosis, a virus disease of chickens in which there is an erythroblastic hyperplasia of the bone marrow with the entry of primitive red cells into the circulating blood. In normal birds injected intravenously with iron-59, the radioisotope disappears from the plasma and reappears in 24–48 h. In birds with erythroblastosis the activity reappears more quickly[1], autoradiographic investigations in blood smears suggesting that most of the activity is associated with the primitive cells[2].

Combined electrophoretic and autoradiographic studies, made on a limited scale, have shown that most of the radioactive iron in the cellular elements of leukæmic blood is not in the form of hæmoglobin. Similar *in vitro* studies were made. Blood samples from five normal and eight leukæmic birds were incubated with iron-59 and aliquots were removed at 6 and 24 h. The samples were separated into plasma and red cells, and hæmolysates were made of the latter. Both plasma and hæmolysates were subjected to electrophoresis and autoradiography. As incubation of the normal blood proceeded, radioactivity in the plasma β- and γ-globulin bands declined parallel with its appearance in the hæmoglobin bands. In two of the five normal blood hæmolysates, a small amount of activity was present in a component other than hæmoglobin. This component migrated with about half the speed of the slowest hæmoglobins, comparable with the speed of fowl 'ferritin'[3]. Autoradiographs of electropherograms prepared in the same way from the eight *in vitro* incubated leukæmic bloods showed marked radioactivity in a position corresponding to this slow band with only slight activity in the hæmoglobin region.

Two alternative explanations can be offered: either 'blocking' of iron occurs at an early stage in the synthesis of hæmoglobin, or diversion of iron occurs to sites other than hæmoglobin. In the former the intermediate would accumulate in the primitive cells, with elevation of non-hæm iron; in the latter such an increase would not occur. It was thought that determination of non-hæm iron in the liver and whole blood would help point to the correct alternative. Kennedy's thiocyanate method for total iron[4] was used with normal and affected livers; a micro-modification was used with whole blood[5]. Non-hæm iron was determined by these methods after its extraction by pyrophosphate[6]. All figures obtained are based on the average of duplicates. Adult birds (6 normal and 10 leukæmic) were used for the liver determinations and five-week-old chicks (17 normal and 32 leukæmic) for the blood determinations. The non-hæm iron was markedly elevated in the liver but not in the whole blood (Table 1). Blood smears stained for iron by Perl's method also failed to show evidence of free iron in the mature or primitive cells. The absence of a major elevation of non-hæm iron in the blood could point to a cycling of iron through the cells without it reaching hæmoglobin and, instead, reaching a non-hæm iron pool in the liver (and possibly the spleen[1]). The non-hæm iron in the liver is bound to a protein with some of the properties of a ferritin. The protein is heat resistant, contains non-hæm iron and is precipitable with 35 per cent ammonium sulphate and 4 per cent cadmium sulphate[7].

These observations raise many questions, which include the following: Is the non-hæm iron complex appearing in the *in vitro* cultivated blood 'ferritin'? Is the disturbance

Table 1. TOTAL AND NON-HÆM IRON IN THE LIVER AND BLOOD OF NORMAL BIRDS AND BIRDS WITH ERYTHROBLASTOSIS

	Normal	Erythroblastosis
Total iron		
Liver (mg/g tissue)	0·87 ± 0·07	2·98 ± 0·44
Blood (mg/100 ml.)	28·37 ± 0·66	23·44 ± 0·70
Non-hæm iron		
Liver (mg/g tissue)	0·58 ± 0·05	2·79 ± 0·49
Blood (mg/100 ml.)	1·85 ± 0·20	2·14 ± 0·20

in iron metabolism the result of virus-induced alterations in the red cells, or plasma, or both, or is it the indirect result of increased cell destruction ? Finally, is the disturbed iron metabolism responsible for the hæmatological changes which occur in erythroblastosis[8] ? Work on these problems is proceeding.

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Enzymatic Investigations of Partially Purified Hog Kallikrein

KALLIKREIN, discovered by Frey in 1925 [1], was observed to be a hypotensive agent. Later work by many investigators has led to the present hypothesis that kallikrein is an enzyme, formed from a precursor kallikreininoken, which acts on protein substrates to liberate the biologically active polypeptides, substance *P*, kallidin II, and bradykinin. It is these substances which manifest the hypotensive activity[2,3]. Because trypsin possesses the same ability to release active polypeptides, it becomes important for future investigations to differentiate clearly the two enzymes, trypsin and kallikrein.

It has been noted in the literature that kallikrein has activity on synthetic substrates[4,5]. We have used these substrates to differentiate trypsin from kallikrein. The compound benzoyl arginine ethyl ester (BAEE) is a specific substrate for trypsin-like enzymes. Hydrolysis of BAEE yields benzoyl arginine and ethyl alcohol and can be measured directly by following the increase in optical density at 253 mμ on a Beckman *DU* spectrophotometer. This increase is directly proportional to the amount of enzyme present[6]. Utilizing 10^{-3} M BAEE in 0·05 M phosphate buffer *p*H 7·0 we have derived activity curves for kallikrein (Fig. 1).

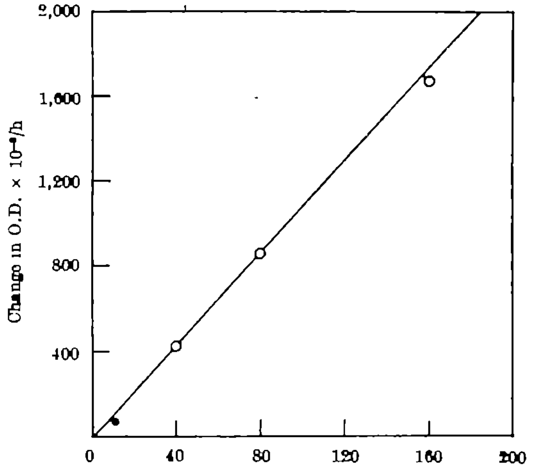

Fig. 1. Indicated amounts of enzyme incubated with 3·0 ml 10^{-3} M BAEE in total volume of 3·4 ml.

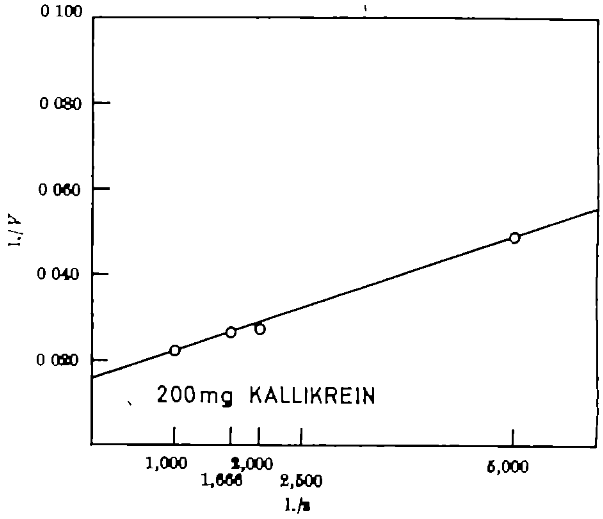

Fig. 2. Relationship of BAEE concentration to reaction velocity. $Km = 3·7 \times 10^{-4}$

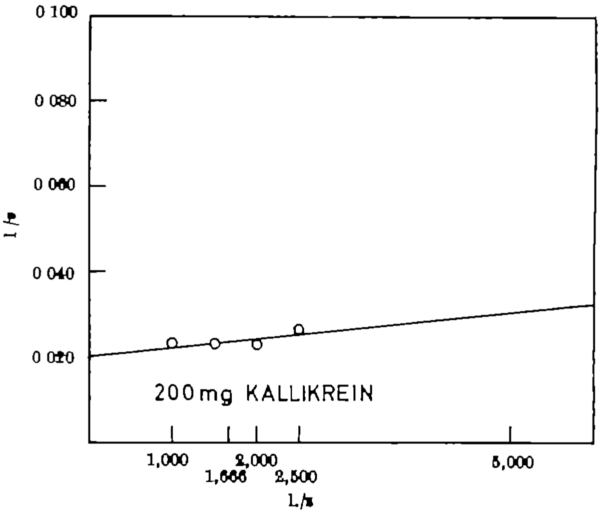

Fig. 3. Relationship of BAME concentration to reaction velocity $Km = 1·8 \times 10^{-4}$

By varying the substrate concentration, it is possible to deduce Michaelis constants for kallikrein. Using the equations of Lineweaver and Burke[7]: $\frac{1}{v} = \frac{Km}{V} + \frac{1}{V}$, we have found the *Km* of kallikrein on BAEE to be $3·7 \times 10^{-4}$ (Fig. 2).

We have also measured the *Km* of kallikrein for benzoyl arginine methyl ester and find it to be $1·8 \times 10^{-4}$ (BAME) (Fig. 3). Both *Km*'s differ greatly from the *Km* of trypsin, which is $8·0 \times 10^{-6}$ (ref. 8). The similarity of the two *Km*'s for kallikrein is to be expected since the stereochemistry of the two substrates BAEE and BAME is similar.

Addition of 10^{-2} M calcium chloride results in a 1·25 increase in activity of trypsin as reported by Neurath[9]. We have been able to show the increase in activity with respect to trypsin but 10^{-2} M Ca^{++} has no stimulatory effect on kallikrein.

The synthetic ester toluene sulphonyl arginine methyl ester (TAME) is active with trypsin and its activity can be measured by increase in optical density of 253 mμ. Kallikrein has no activity with TAME (Table 1). To rule out the contamination of kallikrein by chymotrypsin, the enzyme was assayed for chymotryptic activity with the synthetic substrate acetyl tyrosine ethyl ester (ATEE) by the method of Schwert and Takenaka[6]. No activity for chymotrypsin was detected.

Table 1. COMPARISON OF KALLIKREIN AND TRYPSIN ON TAME

Enzyme	Δ O.D. with BAEE × 10^{-3}/h	Δ O.D. with TAME × 10^{-3}/h
200 μg Kallikrein	2,700	0
20 μg Trypsin	10,000	2,600

Kallikrein was found to lyse fibrin clots by the method of Astrup and Mullertz and Lassen[10] and also to have caseinolytic activity. No plasminogen activating activity could be determined. Thus, kallikrein is unable to convert plasminogen to plasmin. (We would like to thank Dr. Edwin O. Salzman, who performed these assays.)

The enzyme loses its activity above 55° C. It shows the same pH optimal curve as reported by Webster[5].

Differences in the action of trypsin inhibitors on kallikrein and trypsin were also found. These differences will be the subject of another publication. This evidence strongly suggests that kallikrein is a distinct enzyme. Further evidence must await greater purification of kallikrein.

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A Water-soluble Lipid Complex obtained in the Macromolecular Phase by Gel-filtration of Human Bile

THE manner in which cholesterol remains soluble in the bile is of interest since it may be of importance for the formation of gall stones.

That cholesterol is present in the bile in some form of macromolecular complex has been suggested by some authors. Isaksson[1] isolated a complex from freeze-dried bile consisting of lecithin and bile acids which *in vitro* dissolved cholesterol. By electrophoresis of bile, Verschure *et al.*[2] and later Juniper[3] found a lipoprotein complex which also contained bile pigment.

The aim of the work recorded here was to separate a presumptive macromolecular complex in native human bile from other bile components. For this purpose, gel-filtration was used since it permits the separation of large molecular components such as proteins from other components of lower molecular weight.

The material was human gall-bladder bile. The gel used was 'Sephadex' (Pharmacia) type G–25 medium and G–75 medium (limits for complete exclusion are for G–25 in the molecular weight range of 3,500–4,500 and for G–75 in the range 40,000–50,000).

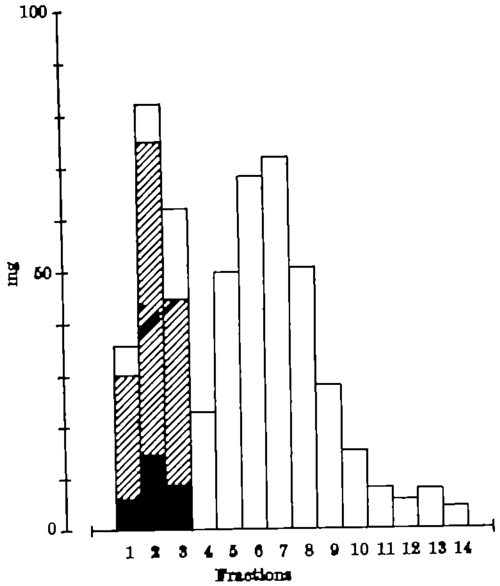

Fig. 1. Amount of solids, cholesterol and phospholipids in the 25-ml. fractions collected during gel-filtration of 3 ml. bile. Double hatching, cholesterol; single hatching, phospholipids; white, other components

An aliquot of 3 ml. of the bile sample was carefully pipetted on to the top of a 'Sephadex' column (diam. 2·5 cm, length 40 cm) and eluted with distilled water at a constant pressure of 50 cm of water. Fourteen fractions of 25 ml. were collected and freeze-dried. The solids in each fraction were extracted with diethyl ether or first with ether and then with chloroform. Analyses of the cholesterol in the lipid extracts of the fractions were performed according to the method of Theorell as modified by Cramér and Isaksson[4]. The amount of phospholipids was determined by analysis of the phosphorus content according to Fiske and Subbarow[5].

The fractions were grouped into two parts. Part I corresponded to the volume in which the large components of high molecular weight such as protein are to be found, and part II comprised the fractions where small components of low molecular weight are to be found.

The results show that the cholesterol and phospholipids were only found in part I (Fig. 1), and that this was the total amount of these two components in the sample may be seen in Table 1. Fractions 1–3 were green and slightly opalescent. The other fractions comprising part II were slightly green or yellow-brown and clear.

Table 1. CONTENT OF SOLIDS, CHOLESTEROL AND PHOSPHOLIPIDS IN HIGH AND LOW MOLECULAR FRACTIONS OBTAINED BY GEL-FILTRATIONS ('SEPHADEX' G–25) OF A 3-ML. SAMPLE OF HUMAN BILE COMPARED WITH A CONTROL SAMPLE FROM THE SAME SPECIMEN

	3 ml. bile separated by gel filtration Part I	Part II	3 ml. freeze-dried bile
Solids (mg)	183	332	475
Cholesterol (mg)	28·5	0	30·0
Phospholipids (mg)	119·8	0	124 0

Bile from another patient was eluated on two different gels (G–25 and G–75) and only part I of each filtration was analysed (Fig. 2 and Table 2). Essentially the total amounts of cholesterol and phospholipids were recovered in part I independent of the type of the gel used. The solutions were green and slightly opalescent.

Table 2. CONTENT OF SOLIDS, CHOLESTEROL AND PHOSPHOLIPIDS IN THE HIGH MOLECULAR FRACTIONS AFTER GEL-FILTRATION ('SEPHADEX' G–25 AND G–75) OF BILE SAMPLES COMPARED WITH THE TOTAL CONTENT IN A CONTROL FROM THE SAME SPECIMEN

	3 ml. bile Part I G–25	3 ml. bile Part I G–75	3 ml. bile Total
Solids (mg)	28·5	26·1	95
Cholesterol (mg)	3·7	3·7	3·6
Phospholipids (mg)	16·2	16·8	19·6

Thus by gel-filtration of native bile all the cholesterol and phospholipids were eluated in an opalescent green water solution in the macromolecular phase, in which proteins and other substances of molecular weights of

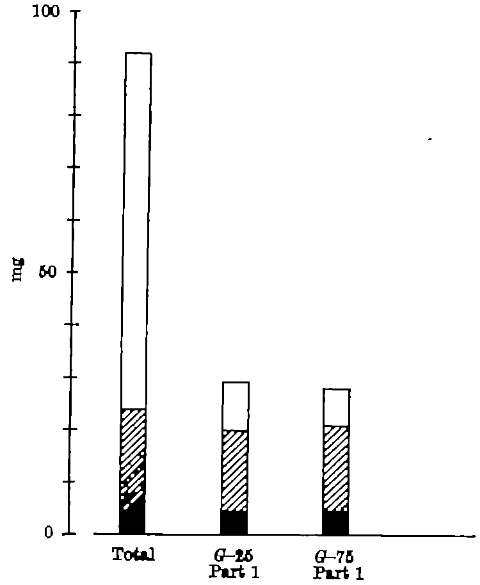

Fig. 2. Amount of solids, cholesterol and phospholipids in the 3 ml. bile compared with the amounts found in the high molecular fractions of the same quantities of bile after gel filtration on 'Sephadex' *G*–25 and *G*–75. Double hatching, cholesterol; single hatching, phospholipids, white, other components

> 40,000–50,000 are presumed to be found. The bile lipids, cholesterol and phospholipids comprise more than 2/3 of the solids, in this phase. The rest probably consists of the bile pigment, biliverdin and protein.

The bile lipids, cholesterol and phospholipids, may be present in some form of macromolecular water-soluble complex which can be separated from native bile with the large components such as proteins. Some of the bile pigment (biliverdin) accompanies these substances.

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Accumulation of Hæmatins by Polychætes

MANY polychæte annelids appear to darken with age; the lugworm *Arenicola marina* does so, and the terebellid *Amphitrite johnstoni* is also brown when old. In terebellids the heart-body becomes bulkier and in lugworms dark tissue around the intestinal vessels increases with age. This darkening is partly due to an accumulation of hæmatin; in *Amphitrite johnstoni* the dark colour is wholly due to hæmatin, the pigment accumulating in the cœlomic cells as well as in the heart-body. In *Arenicola marina* the hæmatin in the skin and that in the tissue surrounding the intestinal vessels both show pyridine hæmochromogen bands at 547·5 and 517 mμ. In *Amphitrite johnstoni* the hæmatin in the cœlomic cells to which, owing to the transparency of the body wall, the hue of the worm is due shows pyridine hæmochromogen bands at 554 and 520 mμ; that from the heart-body at 547 and 516 mμ, reminiscent of meso- rather than proto-porphyrin and similar to the malarial pigment. The hæmoglobin in the blood of both worms, and the hæmoglobin in the cœlomic cells of *Amphitrite johnstoni* show pyridine hæmochromogen bands at 557 and 525 mμ characteristic of hæms with protoporphyrin nuclei.

The accumulation of hæmatin with age in tissues which are known to be also hæmatopoietic[1] suggests that hæmatin may be a normal product of hæmoglobin breakdown in these worms. It may be that the reason why bilins of chlorocruorin have not been identified is that normal breakdown of chlorocruorin also leads to equivalent hæmatins. The accumulation of bilins in some other polychætes[2–4] suggests that there may be some variety in the ways in which hæmoglobin is catabolized in these worms, but following the work of hEocha *et al.*[5] it is not clear whether these are always derived from the worms' own hæms or from algal pigments in the food.

An investigation of these problems is in progress.

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ANATOMY

Effect of Total Thyroidectomy in the Rabbit Fœtus on Fœtal Cholesterol Metabolism

IN earlier communications[1,2] it was shown that the fœtal thyroid gland influenced the cholesterol metabolism of the rabbit fœtus. Total thyroidectomy in the 22–23 day fœtus was found to result in a highly significant increase in blood cholesterol and the results are shown in Table 1.

Table 1. SERUM CHOLESTEROL IN THYROIDECTOMIZED AND CONTROL RABBIT FŒTUSES

Fœtuses	Age at investigation	No.	Mean serum cholesterol (mg/100 ml. ± *S.E.*)
Thyroidectomized (at 22–23 days)	29–30 days	7	129* ± 7·7
Litter-mate controls	29–30 days	12	86* ± 3·5

* These differences are significant, $P < 0.001$.

The method used for fœtal thyroidectomy was similar to that described by Jost[3] and involves the removal of the lower half of the larynx and the upper rings of the trachea together with the thyroid gland. It was noted that the fœtuses which had undergone this operation were unable to swallow during intra-uterine life, the stomach of each operated fœtus containing a negligible amount of fluid whereas the control fœtuses all had full stomachs, presumably from intra-uterine swallowing of amniotic fluid. This raises the possibility that the mechanical interference to the neck of the fœtus, by preventing swallowing, may have been responsible for raising the cholesterol-level in the fœtal blood.

In order to investigate this point further a new technique has been developed for removing the thyroid of the rabbit fœtus. The strap muscles overlying the thyroid are divided, exposing the gland, which is then divided into two parts by a verticle incision through the isthmus. Each lobe is dissected off the trachea and larynx under a dissecting microscope, the laryngeal nerves being left intact. The skin incision is closed with 3–4 fine sutures of Barraquer silk on a 4-mm corneal needle. When examined 6 days later just before term, these fœtuses had well-healed operation wounds, the trachea and œsophagus were free, but the stomach was filled with fluid as in the litter-mate control fœtuses. This new technique did not interfere in any way with fœtal swallowing.

Using this technique, ten fœtuses were subjected to thyroidectomy on the 23rd day of intra-uterine life, and then allowed to continue developing within the uterine cavity until just before term. The fœtuses were recovered by Cæsarean section on the 29th day of pregnancy after a further six days of development.

Blood was taken from these ten fœtuses and also from 25 litter mates for controls, and the serum cholesterol was estimated by the method of Abell *et al.*[4]. The results (Table 2) show a highly significant increase in serum cholesterol following fœtal thyroidectomy.

Table 2. SERUM CHOLESTEROL IN RABBIT FŒTUSES SUBJECTED TO TOTAL THYROIDECTOMY WITH PRESERVATION OF THE LARYNX AND TRACHEA

Fœtuses	Age at investigation	No.	Mean serum cholestero (mg/100 ml. ± *S.E.*)
Thyroidectomized (at 23 days)	29 days	10	118·5* ± 11 7
Litter-mate controls	29 days	25	71·12* ± 5·9

* These differences are significant, $P < 0{\cdot}001$.

This experiment therefore confirms that the fœtal thyroid of the rabbit is essential for the normal regulation of cholesterol in the fœtal blood. Further, maternal thyroid secretions are unable to compensate for the loss of the fœtal thyroid, although maternal hormones have been shown to cross the placenta in increasing quantities towards the end of pregnancy[5].

These results further confirm that the effects reported in the earlier communication[1] were due to the removal of the thyroid and not the interference with the swallowing mechanism of the fœtus resulting from the removal of the larynx.

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PHYSIOLOGY

A Model System for Transcellular Active Transport

Oxender and Christensen[1] have proposed that the achievement of transcellular transport of amino-acids and sugars against concentration differences is a consequence of intracellular accumulation. Intestinal mucosal cells will accumulate certain sugars during absorption *in vitro*. McDougal, Little and Crane[2] have shown that, during the absorption of D-galactose by hamster intestine, the sugar concentration is greatest in the epithelial layer. Similar conclusions were also drawn for galactose and 3-O-methyl-glucose by Kinter and Wilson[3,4] from autoradiographic studies of hamster intestine. On the basis of their findings that the cells in the crypts of Lieberkühn also exhibit an intracellular accumulation of sugar, Kinter and Wilson suggest that, in the intestine, an intracellularly directed sugar pump "is present along the entire cell membrane, the net movement from lumen to blood being due to the far greater surface area because of the microvilli at the luminal border of the cell"[4].

The properties of a simple model system based on the hypothesis of Oxender and Christensen will now be described.

Consider an absorbing cell operating on material here called substrate. The cell is an element of a tissue composed of a single sheet of identical cells which separates two fluid compartments of infinite capacity (sides 1 and 2), each compartment containing substrate (sugar, amino-acid) at the same constant concentration a. It is supposed that the membranes composing the faces bordering either side of the cell possess both the following independent properties: (1) A substrate pump operating to move substrate at a rate p moles/sec from the external compartment into the interior of the cell. For simplicity the rate of pumping is considered to be constant and independent of both the internal (x) and external (a) substrate concentrations. (2) A diffusion leak of magnitude $K(x-a)$ moles/sec. Let the values for face 1 be p_1 and K_1 and for face 2, p_2 and K_2. It is assumed that substrate is conserved in the system, that is, that it is neither synthesized nor utilized by the cells.

For a unit volume of cell, with the internal concentration $x=0$ at zero time, the internal concentration of substrate after time, t, is given by:

$$x=[a+(p_1+p_2)/(K_1+K_2)]\,[1-\exp-(K_1+K_2)t] \qquad (1)$$

the uptake from side 1, across face 1:

$$A_1 = p_1 + K_1 a \exp - (K_1 + K_2)t - \frac{K_1}{K_1 + K_2}(p_1 + p_2)\,[1-\exp - (K_1 + K_2)t] \qquad (2)$$

and the output from the cell across face 2:

$$A_2 = \frac{K_2}{K_1 + K_2}(p_1 + p_2)\,[1 - \exp - (K_1 + K_2)t] - K_2 a \exp - (K_1 + K_2)t - p_2 \qquad (3)$$

In steady-state conditions, that is, when $(K_1+K_2)t$ has values large enough to reduce the exponential terms to insignificant levels, the limiting values are:

$$x_s \rightarrow a+(p_1+p_2)/(K_1+K_2) \qquad (4)$$

and

$$A_s=A_1=A_2\rightarrow(p_1K_2-K_1p_2)/(K_1+K_2) \qquad (5)$$

Under steady-state conditions provided $p_1/p_2 > K_1/K_2$, a transcellular transport of substrate will occur from compartment 1, across the cell, into compartment 2. It then follows that the rate of inward pumping into the cell may be greater at side 2 than side 1, yet, provided that $p_1/p_2 > K_1/K_2$, transcellular transport will still occur into compartment 2. If K_1 is zero, that is, there is no substrate leak across face 1, then $A_s = p_1$ and is independent of p_2 and K_2. If p_1 is zero, for finite positive values of p_2 the sign of A_s is negative, that is, the direction of the transport will be into side 1 from side 2. Hence in the model system, the ability to be pumped into the cell from side 1 by the process p_1 is a necessary but not a sufficient condition for substrate to be subjected to transcellular transport from side 1 to side 2. In general, for given positive values of p_1 and p_2, the absorption rate, A_s, in the system described is independent of the external concentration a and is determined by the value K_2/K_1, while the internal concentration, x, is determined by K_1+K_2 as well as by the external concentration, a.

The application of model systems of the sort described to active transport phenomena in tissues raises questions which appear to be capable of being resolved experimentally. It is also clear that any one particular property of even a simple transcellular transport system is not necessarily a property of the pumping mechanism on which the activity of the system is based. For example, in the absence of other evidence, the finding that pyridoxal or pyridoxal phosphate stimulates net amino-acid transfer across layers of tumour cells arranged as a membrane[1] may be explained as the result of effects of these substances either on an exit process or on an entrance pump for amino-acids or by a combination of both. The fact that 'active' sugar transport across the mucosal layers of the small intestine requires the presence of oxygen may not necessarily mean that aerobic conditions are essential for the activity of a sugar pump localized in the cell membranes; the effects are equally explicable if anoxia increases the relative leakiness of the mucosal faces of the absorbing cells ($K_1/K_2 \succ p_1/p_2$). In a similar fashion, differences in the relative leakiness of the faces of the two poles of mucosal cells from different regions of rat intestine may underly the differences in the capacity of these regions to absorb either glucose[5,6] or galactose[7]. Now a substrate may possess physical and chemical properties such that it can be operated on by the pumping process p, but these properties need not be the same as those which determine the magnitudes of K_1 and K_2. For example, in a given system, the former might depend on a particular stereochemical configuration of a substrate, while the K-values might depend on the molecular weight. D-xylose has not been shown to be actively transported against a concentration gradient in small intestine, but there is recent evidence suggesting an interaction between the pentose and glucose during absorption[8]. Xylose may share an inwardly directed sugar pump with glucose, but intracellular pumping, even at high rates, does not necessarily mean that transcellular active transport will occur.

In other similar model systems, but in which the processes underlying the substrate leak across the cell faces are more elaborate than those of simple diffusion (for example, facilitated diffusion), competition between substrate molecules for exit from the cell could have important effects. For example, the overall specificity of the whole system for transcellular transport of a particular class of substrates, including such effects as those of one substrate on another, will be the resultant of the specificity of the intracellularly directed pump for substrate and the specificity of the leak.

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Stimulation of Insulin Secretion *In vitro* by Adenosine Triphosphate

WE have already shown that the metabolites (glucose, citrate, β-hydroxybutyrate), the final function of which is the production of energy in the $\sim P$ form[1,2], give place to release of insulin *in vitro* when they are added to the incubation buffer surrounding slices of pancreas. For this reason, we considered it interesting to determine if ATP when added to the incubation buffer produces any effect on the release of insulin, although what is generally accepted is that the metabolic processes in which this nucleotide takes part are intracellular.

A piece of rabbit pancreas weighing 200 mg was incubated for 3 min at 37° C in Krebs–Henseleit buffer containing ATP 1×10^{-3} M and another piece of the same weight (± 5 mg) was incubated in the same buffer but containing hydrolysed ATP (1×10^{-3} M–N/10 hydrochloric acid for 15 min at 100° C) as control (final pH 7). The insulin of the buffer was extracted by the method of Grodsky and Tarver[3] and evaluated by the technique using adipose tissue of the rat epididymis. The results are expressed in uptake of glucose and the statistical study was performed using the paired observations method[4].

Table 1. EVALUATION OF THE INSULIN (GLUCOSE UPTAKE) EXTRACTED FROM THE INCUBATION BUFFER (CONTAINING ATP OR HYDROLYSED ATP) SURROUNDING PIECES OF RABBIT PANCREAS

Metabolites		Mean glucose uptake mg/100 of tissue/4 h	
ATP (1×10^{-3} M)	(20)	1·082	± 0·066* P=0 01
ATP hydrolysed (1×10^{-3} M)	(20)	0·781	

**S.E.* The values in parentheses represent the number of experiments.

As can be seen from Table 1 the insulin extracted from the buffer of incubation containing adenosine triphosphate is higher than that obtained from the control. The difference is highly significant. We think that this fact supports our hypothesis about the role of the ATP in release of insulin.

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Increased Survival-time in Dystrophic Mice treated with Methylandrostenediol Dienanthoylacetate

ANABOLIC steroids have been noted to increase survival time in dystrophic mice, *Jax*/129 (*dydy*), but the disease syndrome was not otherwise altered. Testosterone propionate did not possess this ability[1,2]. It was of interest to evaluate an anabolic steroid, 17-α-methylandrostene-5-diol-3β-17β-dienanthoylacetate (MADD), which has a prolonged period of activity.

Mice with hereditary muscular dystrophy (*dydy*) were obtained by mating *Jax*/129 *mice* (*Dydy*) from the Jackson Memorial Laboratory, Bar Harbor, Maine. The quarters were controlled to room temperature and Purina laboratory chow and water were available *ad libitum* in containers accessible to the dystrophic mice. Mice were weaned at 3–4 weeks with no special transitional diet, and experimental groups formed by dividing litters. A portion of the dystrophic mice were treated with 5 mg MADD in sesame oil per mouse in a single intramuscular dose per week, the dose being equivalent to the methylandrostenediol. Body-weights and mortalities were recorded to the nearest week, and post-mortem examinations were made on mortalities.

Survival time was increased approximately two-fold in the MADD-treated dystrophic mice as compared with untreated controls (Table 1), with no difference apparent between the sexes. Growth was approximately the same in the treated and untreated dystrophic mice with females

Table 1. RESULTS OF MICE TREATED WITH MADD

Group	No. of mice	Median survival-time and range (weeks)	Av. weight at 3 weeks (g)	Av. weight at 13–27 weeks (g)
Normal males	94	not detn.	10	32
Normal females	79	not detn.	9	26
Dystrophic males	13	5 (4–7)	7	dead
Dystrophic females	12	6 (4–9)	6	dead
Dystrophic males receiving MADD	22	13 (7–23)	7	21
Dystrophic females receiving MADD	25	18 (4–27)	7	19

generally weighing less than males. Histological examinations revealed degeneration in the skeletal muscle of all the dystrophic mice, and the treatment with MADD did not appear to alter the disease syndrome except to increase survival-time.

The survival-time of the untreated dystrophic mice was less than reported by some workers[1,2], possibly because a special diet was not employed during the weaning period. The survival data of this experiment approximated that of other workers employing conditions similar to those here[4,5]. The difficulties discussed by Miller *et al.*[6] in evaluating survival data with dystrophic mice are to be considered and necessitated the use of sufficient mice to allow for litter differences. Larger litters generally have dystrophic mice with small body-weights and poor survival.

It may be concluded that the treatment of mice with hereditary muscular dystrophy by an anabolic steroid, methylandrostenediol dienanthoylacetate, lengthened survival time but did not otherwise alter the disease syndrome.

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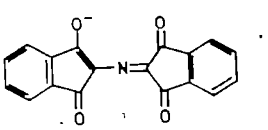
HISTOCHEMISTRY

Use of the Ninhydrin Reaction for Quantitative Estimation of Amino Groups in Insoluble Specimens

It is well known that the development of a purple colour (Ruhemann's purple) on reaction of ninhydrin with primary amino (NH_2) groups of proteins occurs even when the protein is not in solution. Thus, Pearse[1] describes attempts to use the ninhydrin reaction for histochemical staining of protein, which failed due to the ease of diffusion of the coloured product. In fact, the soluble purple compound, to which formula (I) is usually assigned, is not in any way attached to the protein; it results from deamination of the protein (left otherwise unchanged), the abstracted nitrogen being incorporated in (I)[2]. Under suitable conditions, the coloured product is released completely from the solid into the solution, and the quantitative ninhydrin estimation, usually carried out on dissolved amino-acids and proteins, then becomes equally applicable to insoluble material. All primary NH_2 groups react, ε-NH_2 groups (lysine residues) giving, for reasons which are not certain, 65–70 per cent of the colour yield of α-NH_2 groups, in almost all cases[3]. Where the former far outnumber the latter, as in tissue preparations and very many proteins, comparison with a standard gives the actual or relative amino content with fair accuracy. This appears to be the simplest method at present available for obtaining such information.



(I)

This technique for analysing insoluble and denatured proteins, and protein-containing material, is of particular value where it is desired to know the amino content of biological specimens before and after treatment with various cytochemical reagents. For this purpose, the test material is fixed, and subjected to reactions under cytochemical conditions, small weighed samples being analysed at each stage. (An alternative approach, not as yet pursued, would be to use serial microtome sections, comparing similar sections after different treatments. Weighing is thereby avoided, and since large numbers of sections will normally be required in each reaction tube to give a measurable colour, errors due to inherent differences between sections are minimized.)

To check the validity of the method, it was thought advisable to apply it to an insoluble protein of precisely known constitution. Lysozyme was therefore denatured by maintaining the dissolved protein at *p*H 9–10 and 37° C for several days, and then precipitating with ethanol, and its ninhydrin colour yield was compared with that of an amino-acid standard, under identical reaction conditions. 1 mole of lysozyme was found to be equivalent to 4·5 moles of leucine, in close agreement with the figure of 4·35 equivalents obtained by Slobodian, Mechanic and Levy[3] for the native protein (Tallan and Stein[4] earlier obtained the figure of 4·9). Assuming a contribution of 1·1 leucine equivalents from the *N*-terminal lysine residue, as is usual[3,5], colour due to the other five ε-NH_2 groups is, in the present case, 3·4 equivalents. This corresponds to an average yield from each of these ε-NH_2 groups of 68 per cent of the colour from an α-NH_2 group—that is, within the range 65–70 per cent normally expected.

On theoretical grounds, two factors could limit the accuracy of analysis of insoluble material, relative to that of the usual reaction in solution, namely, possible inaccessibility of some NH_2 groups to ninhydrin, and possible occlusion of some Ruhemann's purple by the solid. In practice, the second source of error is avoided, and the first minimized, so far as possible, by ensuring that each solid particle is very small in at least one of its dimensions, by limiting the quantity of material in each reaction tube, and by providing adequate agitation of the suspension while the colour is developing. Experience has shown that populations of single cells, such as sperm and amœbæ, may be used directly, provided that any clumps of the dried fixed material are broken up before analysing; mammalian tissues are normally finely powdered after fixation. It is advisable to restrict analysis to samples smaller than 5 mg, since variable results have sometimes been obtained with larger quantities. When reaction conditions, in a given case, have been satisfactory: (1) optical density values are reproducible, and increase more or less linearly with sample size; (2) no coloration occurs when the ninhydrin-reacted solid is washed with 50 per cent ethanol, or further reacted with ninhydrin; (3) colour yield does not increase when the material is more finely ground; (4) the colour of the ninhydrin-reacted specimen is not usually appreciably darker than before reaction (but is occasionally so). Where there has been any chemical treatment before analysis, it must be ensured that any products left bound or deposited in the solid do not interfere with the ninhydrin estimation.

The experimental procedure is essentially an extension of that of Moore and Stein[5]. Dried samples (1–4 mg) are accurately weighed into 15 ml. centrifuge tubes, and 1 ml. of buffered ninhydrin-hydrindantin reagent is added to each. The tubes are covered with aluminium foil, and shaken at about 200–500 oscillations per minute in a boiling water bath for 15 min, using a flask shaker. The tubes are then removed to a cold water bath, where 5 ml. of 50 per cent ethanol are added, and are shaken to mix before spinning down the solid. (Due to the shaking, the hydrindantin disappears at the time of boiling.) For tissue specimens, further six-fold dilution of a portion of the supernatant will normally bring the colour into the range where its optical density can be measured on a colorimeter or spectrophotometer. A different final dilution ratio may be required for some other materials, such as denatured proteins exceptionally rich or poor in lysine. A blank is included with every group of analyses.

The method as described is at present being utilized in a programme of development of specific electron stains for protein[6], as a guide to the extent and specificity of reactions, and has been applied to rat liver, ram sperm and *Amoeba proteus*, with ethanol, osmium tetroxide and formalin as fixatives. While results of these investigations lie outside the scope of this communication, attention may be directed here to the need for caution in interpreting ninhydrin data, in certain instances. In particular, there is some indication that, after osmium fixation, many more NH_2 groups may be available for reaction than are revealed by ninhydrin. This apparent 'blocking' has not yet been explained, but the observation underlines the need to apply a multiplicity of analytical techniques when attempting to interpret cytochemical reactions.

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The Periodic Acid – Schiff Reaction

THE periodic acid–Schiff (PAS) reaction—the term was introduced by McManus[1,2]—is one of the most widely used histochemical methods. In a recent review of "Periodate Oxidation Techniques" McManus[2] states that the first publication, describing the demonstration of mucin and some other structures by the Schiff reagent after treatment with periodic acid, appeared in *Nature* in 1946[3]. In 1948 Hotchkiss[4] published his description of the PAS reaction, which had been applied already in 1947 by Gersh[5] and by Catchpole[6]. Also in 1947 Marchese[7] used the PAS reaction for the demonstration of glycogen, but independently Lillie[8] used acidified periodate for the same purpose. Since other histochemical texts[9–11] give essentially the same version of the development of our knowledge of the PAS reaction, we should like to direct attention to the work of A. L. Shabadash, whose publications on the PAS reaction apparently are not widely known outside the U.S.S.R.

At a meeting of the Institute for Biology and Biochemistry of the Academy of the Medical Sciences of the U.S.S.R., held on February 14, 1946, Shabadash presented a paper on "A Rational Method for the Histochemical Determination of Glycogen, and its Theoretical Basis". This paper was published in 1947 (ref. 12). After surveying critically the methods in use for the fixation and demonstration of glycogen Shabadash discusses Bauer's method[13], and goes on to say that he experimented with several other oxidants, among them potassium permanganate, but that he was unable to produce the reactive aldehyde groups necessary for the interaction with leukofuchsin. ". . . After a whole row of experiments and investigations I settled on periodic acid which was proposed by Malaprade (1927) and used by Jackson and Hudson (1937) for the study of the structure of starches. . . . I investigated the action of dilute (0·01 mol.) solutions of periodic acid on glycogen *in vitro* and in control preparations (sections of liver) with subsequent treatment with leukofuchsin. The results proved the correctness of the theoretical assumptions. The brightness and intensity of the colour reaction was superior to that of any of the methods published in the literature or known to me; it differed from them not only in the degree of sensitivity, but also in its chemical basis . . ." Shabadash realized that this method was applicable not only to the identification of glycogen, but also of polysaccharides in general. He used both periodic acid and sodium or potassium periodate in water in dilutions which, depending on the amount of glycogen present in the tissues, varied from 0·01 mol. (0·23 gm. per cent) periodic acid to 0·002 mol. periodate. The time of exposure varied from 15 to 25 min. No reducing rinse, only distilled water, was used as a wash. After 20–25 min in leukofuchsin the sections were rinsed in a sulphite wash.

Several points are of interest in this publication.

(1) Shabadash discusses the nature of the PAS reaction, its specificity, the necessity of enzymatic digestion for control purposes, and the possibility of substances other than complex carbohydrates responding to this treatment, at length, so that his paper is unquestionably the most detailed account of the PAS reaction published at that time.

(2) Although Shabadash extensively quotes the work of earlier writers who have investigated the histochemical demonstration of glycogen or other carbohydrates, he does not mention the first publication by McManus[3] of whose work he, presumably, did not know.

(3) Shabadash's paper appears to be the result of a prolonged, systematic search for a specific histochemical method for the detection of glycogen.

(4) In the paper summarized here Shabadash states that the colour obtained is stable and permits the preservation of histological sections under ordinary conditions for years. He then adds: "I have preparations which have preserved their original brightness for seven years". He makes a similar claim in another publication, an extensive monograph on the histochemistry of the normal central nervous system, which appeared in 1949 (ref. 14). In a footnote on p. 104 he states "My preparations have retained their brightness over a period of five to seven years". According to these claims, then, Shabadash had used the PAS reaction as a histochemical tool some time between 1939 to 1944. It should be pointed out that the work of Hotchkiss, started in 1945, remained unpublished for three years, so that Shabadash cannot have known of it.

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IMMUNOLOGY

Loss of Immunological Reactivity of an Alpha-2 Globulin after Prolonged Freezing of Serum

Most investigators who preserve sera for future analysis take it for granted that the serum proteins are stable at temperatures around −20° C. Some evidence supports this assumption: the titres of certain antibodies or of rheumatoid factor, for example, remain unaltered after even several years in a deep freeze. This communication shows that most human sera, from both healthy and sick people, contain an α-2 globulin which loses its antigenic (precipitin) characteristics after prolonged storage at −20° C.

An antihuman serum was made by injecting a rabbit intravenously with 1 ml. of pooled human serum three times weekly for eighteen months. This pool was made by combining equal amounts of serum from 200 patients in a State hospital. Immunoelectrophoresis using the antihuman serum and several human sera showed antibodies to various proteins including about 10 α-globulins. The antihuman serum was absorbed with another serum pool made by combining equal amounts of serum from 20 blood donors. 1 ml. of the antiserum was mixed with 25 μl. of the blood-donors' pooled serum, left at room temperature for 30 min and then centrifuged at 3,500 r.p.m. for 10 min. Using a small-scale double-diffusion-in-agar technique[1], 0·5 μl. amounts of each reactant were placed in wells the centres of which were 8 mm apart and the edges 4 mm apart. The preparations were left in a moist chamber at room temperature for 48 h.

When a recently drawn human serum and the absorbed antiserum were used as reactants, two precipitin lines could usually be seen. The first (line 1) was a dense line, occasionally closely split, present about 1 mm from the edge of the well containing the human serum. The second (line 2) was a hazy, faint line present about 1 mm from the edge of the well containing the absorbed antiserum. There was no reaction between the absorbed antiserum and saline, nor between human serum and rabbit serum taken before immunization had been started.

When the human serum had been kept for a long time in a deep freeze at −20° C, line 2 tended to disappear, an observation made in more than 1,100 sera taken from both healthy and ill persons. Table 1 shows the findings in 763 different sera for which the dates of drawing were precisely known. Line 2 was seen in all freshly drawn sera; in about 30 per cent of sera stored for 9 months; and in only 5 per cent of sera stored for 30 months. Among 36 freshly drawn sera, line 2 was present in all; on retesting 14 weeks later, 5 of them had entirely lost line 2. The disappearance of line 2 depended more on the length of time the serum had remained frozen than on either mere freezing or the number of times the serum had been frozen and thawed, because there was no diminution in the intensity of line 2 in 20 freshly drawn sera which had been 20 times rapidly brought to the temperature of dry ice and then thawed. Nor did heating the serum at 56° C for 30 min cause line 2 to change intensity.

Both line 1 and line 2 were shown by immunoelectrophoresis on cellulose acetate to be α-2 globulins. Line 1 was immunologically identical with that produced by the S_f 0–400 lipoproteins (isolated in an analytical ultracentrifuge, and provided by the Institute of Medical Physics). Line 2 has not been identified. Use of commercially available serum fractions and antisera showed it to be neither cœruloplasmin nor α-2 macroglobulin. The absorbed antiserum did not react with fibrinogen, and addition of neither heparin nor thrombin to fresh sera abolished line 2—so that line 2 is probably neither a cryofibrinogen nor the heparin-precipitable protein described by Thomas, Smith and Von Korff[2]. Siderophyllin, Cohn's fractions II, III and IV 5 + 6, and an isolated cryoglobulin did not react with the absorbed antiserum.

Table 1. Presence or Absence of Line 2 in 763 Different Sera for which the Dates of Drawing were known

Age of serum	Total No.	Line 2 present	Line 2 absent
3 days	79	79 (100%)	0
2 weeks	80	80 (100%)	0
1–3 months	91	89 (98%)	2 (2%)
3–6 months	88	71 (81%)	17 (19%)
6–9 months	86	59 (69%)	27 (31%)
9–12 months	68	18 (26%)	50 (74%)
12–15 months	40	13 (32%)	27 (68%)
30–36 months	231	12 (5%)	219 (95%)

These investigations show that most human sera contain an α-2 globulin which loses its immunological reactivity after a long period in the deep freeze. Although stored sera are used all over the world for numerous investigations, systematic investigations have not been made of the effects of lengthy storage of the serum on the various proteins; nor has a comparison been made of the various methods of preservation to determine the least deleterious. The results of such inquiries would be valuable

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Development of the Fœtus in Female Rats Injected with Homologous Heart Tissue

Recent observation of the occurrence in human beings of auto-antibodies specific to heart tissue has given support to the hypothesis of an auto-immune ætiology in rheumatic fever, following myocardial infarction and postcardiotomy state[1,2]. In the rabbit, heart-specific antibodies have been produced readily against heterologous heart tissue and much less readily against homologous heart tissue[3-5]. While there is good evidence from clinical and experimental sources of the existence of antiheart antibodies whether from autologous, homologous or heterologous stimuli, there is considerable confusion as to the part they play in cytotoxic action on the heart. If antiheart antibodies are cytotoxic then they could readily influence developing embryonic heart tissue to produce either arrested growth with persistent anomalies or death. The possibility that such an immune (auto-immune) mechanism may play a part in the development of congenital heart disease seemed worthy of investigation.

Mature Sprague–Dawley female rats were immunized with homologous rat heart tissue one week before mating, approximately at the time of mating, and one week following mating. The number of new-born, their weight, and the gross and histological changes in the heart of the new-born were evaluated. Antigen was prepared from adult rat hearts of Sprague–Dawley rats removed shortly after death. The hearts were trimmed of connective tissue, minced with scissors and rinsed with cold saline to remove visible traces of blood. Extracts were made by homogenation with 4 times the tissue volume of saline carried out for 3 days at 4° C. The antigen was mixed with equal parts of complete Freund's adjuvant and emulsified. Female rats were divided into 3 experimental groups and 3 corresponding controls. Group I consisted of 5 female rats, 4 injected 7 days and 1 injected 12 days before mating (1 ml. of the antigen emulsion, 0·5 ml. subcutaneously and 0·5 ml. intraperitoneally). Group II consisted of 4 females immunized the morning following mating, and Group III consisted of 3 females immunized 7 days following mating. Control animals were mated at the same time as the experimental counterpart but were

Table 1. FŒTAL DEVELOPMENT IN FEMALE RATS INJECTED WITH HOMOLOGOUS HEART ANTIGEN AND IN NON-IMMUNIZED CONTROL RATS

Group	Rat No.	Experimental			Control		
		Days immunized referable to mating	Size of litter	Average weight of new-born rats (g)	Rat No.	Size of litter	Average weight of new-born rats (g)
I	1 2 5 8 16	+7 (+10)† +7 (+10) +7 (+10) +7 (−12) +12	0 0 7 (5) 7 (5) 0 0 9	8·2 (6·85) 8·0 (6·4) 6·0	19 21 22	0 0 9	7·9
II	9 10 18 26	−1 (+12) −1 (+12) −1 −1	8 (0) 0 (0) 0	7·2	7 13 14	8 (6) 9 0	7·5 (6·9) 6·6
III	3 4 6	−7 (+10) −7 (+10) −7	0 (9) 0 (0) 0	(8·3)	11 15 25 27	0 (9) 0 11 0	(7·4) 8·8
Total	12		50 (Av. 7·1)	7·27	10	52 (Av. 8·5)	7·52
Ratio new-born to matings			2·5		4·33		

Controls mated on same day as experimental counterpart. † Parentheses indicate re-immunized and re-mated

not immunized. Some of the rats were re-immunized and re-mated. In Table 1 the results of immunization of the 12 female rats of the experimental animals are summarized. The figures in parentheses represent re-immunization and re-mating. The corresponding findings of 10 control animals are also shown in Table 1.

Comparison between experimental and control shows little difference, although there is a slight decrease in the number of new-born in the immunized animal, and the average weight of each new-born rat is slightly less. No attempt was made at this time to evaluate the effect of prolonged immunization although some rats in both groups were re-immunized and re-mated, and in those that did conceive the new-born rats were not affected. Morphological and histological evaluation of the hearts, livers, and spleens of the new-born rats showed no evidence of abnormal development, or tissue injury. It appears from these preliminary observations that immunization on a short-term basis of adult female rats with homologous rat heart tissue is ineffective in interfering with normal cardiac formation or development of the fœtus. To establish whether or not immunization had occurred in the adult female rats, re-immunization at the end of the experiment (6 months) was carried out and 3 weeks post-re-immunization the animals were killed and their hearts, livers and spleens were removed, washed free of blood, blotted and the hearts and spleens weighed. A portion of each tissue was fixed for histological sections and the remainder minced and extracted for immunological examination against serum of each immunized animal. The control animals were treated in the same manner. The mean weights of the hearts were: experimental 1·0913 g (range 0·920–1·2672); control 1·0811 g (range 0·9613–1·4065) with a standard deviation of 0·123 g. The average weights of the spleens were: experimental 0·6841 g (range 0·5328–1·0008); control 0·6942 g (range 0·5617–0·9205) with a standard deviation of 0·135 g. No evidence of tissue change differing from control was found by gross or microscopic examination. Serological investigations with gel-diffusion technique of Ouchterlony[4] in which extracts of heart, spleen, and liver of each animal was tested against serum from experimental animals and controls gave negative results. A more sensitive system, consisting of kaolin particles, coated with the tissue proteins and reacted with serum of experimental and control animals also gave negative reactions. (Kaolin utilized in a particle agglutinating system developed in the authors' laboratory for detection of thyroid antibodies is a sensitive indicator of serological reaction. Kaolin actively absorbs protein at *p*H 5–7, and when washed and preserved with albumin forms a stable particle suspension the agglutination of which is readily apparent both macroscopically and microscopically.) In both systems rabbit anti-rat heart antibody gave strong positive reactions.

No detrimental effect of short-term immunization was apparent either on the mature female rats or her offspring. Gross and microscopic examination of heart, spleen and liver of the immunized mature rats and their offspring gave no evidence of changes attributable to the process of immunization. The short-term immunization procedure was selected with the thought in mind that a very minimal antibody production would be sufficient to disturb the embryological development of the heart. It is quite possible that the antigens of the embryonic heart are not susceptible to antibodies prepared against mature adult hearts. Failure to demonstrate serological evidence of immunization may explain the failure of impaired cardiac and fœtal development subsequent to short-term immunization of pregnant rats. Glueckssohn-Waelsch[7] produced abnormalities of nervous system development in embryonic mice from *DBA*/1 *Jax* strain mothers injected with mouse-brain and adjuvant prior to mating. Mothers of the same strain of mice injected with mouse heart and adjuvant in contrast to brain did not mate readily, and many matings were sterile but no cardiac abnormalities of embryos were found. Fertility appeared to be impaired in our experimental animals, but the series is too short to substantiate Glueckssohn-Waelsch's observation. The mothers, in her investigations, in contrast to ours, received several weeks of antigen injection prior to mating, but serological evidence of immunization was not studied. In later work[8] she did show positive precipitin reaction in 7 of 10 mothers immunized with brain tissue. Ebert[9] demonstrated preliminary indications of specific effects of antiheart and antispleen sera on differentiation of mesodermal elements of the chick blastoderm. The question of action of organ specific antibodies against fœtal tissue during development requires further investigation. The problem of antiheart antibodies is being re-investigated with prolonged immunization and utilization of antisera prepared against rat-heart in the rabbit. Evidence of antibody formation utilizing these systems may facilitate understanding of specific heart antiheart antibodies and clarify the effects such antiserum might have on the development of the heart as well as general embryonic development.

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PATHOLOGY

Quantitative Technique for Uniform Tumour Implantation by Trocar in Gel-foam

Tumour transplantation in animals by injection of tumour fragments or tissue mince does not offer a quantitative method of implanting a known number of viable cells. Cell suspension techniques give the quantitative data but do not afford a uniform growth in a single implantation site. Often the injected fluid suspension spreads in the tissue and yields more than one tumour or a 'spread-out' tumour, the size of which is not a reflexion of cell count only.

The following method was devised to yield both the known cell count as well as single site of implantation with resulting uniformity of tumour growth.

Sterile methods are used in all procedures. Solid or ascites tumours are obtained from donor mice. Solid tumours are gently homogenized in a glass Dounce homogenizer or a cyto-sieve[1] with a 10-fold volume of sterile Locke's or other physiological diluent (1 g tumour plus 10 ml. diluent) containing 500 units penicillin and 50 units streptomycin per ml. The mechanical homogenization is accomplished by hand, using only six full strokes of the plunger with a coarse fitting Dounce homogenizer with a clearance of about 0·005 in. The resulting homogenate is allowed to sediment by gravity in an ice water bath for 10 min. The supernatant is removed with a syringe and 13-gauge trocar and transferred to a sterile vial. Aliquots are removed and treated with an equal volume of 0·5 per cent solution of eosin according to the method of Schrek[2]. Counts of the viable (non-stained) cells are made on a hæmocytometer. A minimum of 3 aliquots are counted, and if uniform, subsequent dilutions of the homogena[illegible] are prepared to yield the desired viable cell [illegible] The final dilution is recounted to [illegible] precision.

Sterile absorbable gelatine sp[illegible] the Upjohn Co., Kalamazoo, [illegible] tumour fragments. Microscopic examination of a fixed stained preparation of the cell suspension impregnated gel-foam fragment reveals the presence of tumour cells in the interstices of the gel-foam.

This technique is at present used by us for tumour propagation and has been used in several experiments in which a uniform single site implantation of a quantitated tumour cell inoculum was desired.

An experiment adopting the foregoing method was performed with 144 *C3H* mice using the mammary adenocarcinoma *H*2712. A cell suspension containing 50,000 viable cells per 0·1 ml. was prepared as here, absorbed on to the sterile gel-foam cubes and implanted by trocar subcutaneously in the supra-scapular area. A uniform tumour growth pattern resulted in the implanted mice. The latent period was 12–15 days from time of implant and the tumours, uniform in contour, achieved approximately equivalent sizes in all mice as tumour growth progressed.

This method adopting gel-foam impregnation has also been used by us for implantation of chemical carcinogens, as well as tumour cells, subcutaneously, intra-vaginally and in other anatomical sites.

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Suppression of Experimental Allergic Encephalomyelitis by Cytotoxic Drugs

The action of cytotoxic drugs in experimental allergic encephalomyelitis is of interest in the examination of the mechanism of this disease in animals and may have therapeutic implications for auto-immune disease in man. Hoyer *et al.*[1] have shown that 6-mercaptopurine can suppress experimental allergic encephalomyelitis in rabbits and guinea pigs, but Field[2] has reported negative findings in the latter species.

In the observations described here, two compounds were tested for activity against experimental allergic encephalomyelitis and hæmolysin formation in the guinea pig—*cyclo*phosphamide ('Endoxana'), an alkylating agent, and 4-amino-*N*-methyl-pteroylglutamic acid ('Methotrexate'), a folic acid antimetabolite.

Guinea pigs weighing 200–500 g were sensitized with a single intradermal injection of homologous whole brain suspension, emulsified with an equal volume of complete Freund adjuvant (Difco). Each injection (0·1 ml.) contained 1 mg guinea pig brain (dry weight) and 0·1 mg M butyricum. *Cyclo*phosphamide 20 mg/kg/day and 'Methotrexate' 5 mg/kg/day were given intraperitoneally, while control animals received 1 ml. isotonic saline each day by [illegible] The interval between sensitization and [illegible]tion of drug administration, were [illegible] 1. In addition, those animals [illegible](1–24 days) were given 1 ml. [illegible]heep red cells subcutan-[illegible]olysin production.

Table 1. Effect of Cytotoxic Drugs on Experimental Allergic Encephalomyelitis

Days of treatment	No. of animals in group	Paralysed by day 17	Paralysed by day 22	Animals with histological lesions	
	5	5	5	5	Controls
	5	3	3	5	
	4	4	4	4	
	14	12	12	14	Total
1–24	5	0	0	0	*Cyclo*phosphamide
1–16	5	0	0	0	
1–8	5	0	2	4	
9–15	5	0	0	5	
	20	0	2	9	Total
1–24	5	0	0	0	'Methotrexate'
1–16	5	0	0	5	
1–8	5	1	4	5	
9–15	5	0	3	4	
	20	1	7	14	Total

the survivors killed 21–24 days after sensitization. The central nervous system was sampled by a complete sagittal section of the brain and spinal cord, and examined for histological evidence of experimental allergic encephalomyelitis—perivascular cuffing, meningitis, and subependymal infiltration. The hæmolysin titre of the serum was estimated using 4 *MHD* complement and an end-point of 50 per cent hæmolysis.

*Cyclo*phosphamide and 'Methotrexate' each suppressed the development of experimental allergic encephalomyelitis completely when given for the full 24 days of the experiment (Table 1). Following shorter periods of administration the disease was either totally inhibited (*cyclo*phosphamide 1–16 days), or the clinical manifestations were reduced and the onset of paralysis significantly delayed ($P < 0{\cdot}05$). Twelve of the 14 control animals were paralysed by the seventeenth day, compared with only 1 of the 40 treated animals. There was no apparent difference between those animals in which treatment was started on the day of sensitization, and those in which it was delayed until the eighth day.

The formation of hæmolysin to sheep cells was also suppressed by each drug (Table 2).

Table 2. Effect of *Cyclo*phosphamide and 'Methotrexate' on Hæmolysin Titres

Controls	*Cyclo*phosphamide	'Methotrexate'
512	<8	<8
512	0	0
256	0	0
128	0	0
128	0	0

All animals were submitted to post-mortem examination. Only one, on *cyclo*phosphamide, showed evidence of drug toxicity—multiple hæmorrhages into the viscera.

*Cyclo*phosphamide and 'Methotrexate' are known to inhibit a variety of immune responses in the guinea pig[3,4] and rabbit. The suppression of experimental allergic encephalomyelitis by these compounds may therefore be due to an effect on the immune mechanism itself, or alternatively to some non-specific anti-inflammatory action similar to that described for 6-mercaptopurine[5].

We thank Dr. Ruth Porter, of Lederle Laboratories, for the 'Methotrexate', and Dr. J. M. Simister, of Ward Blenkinsop and Co., for the 'Endoxana'.

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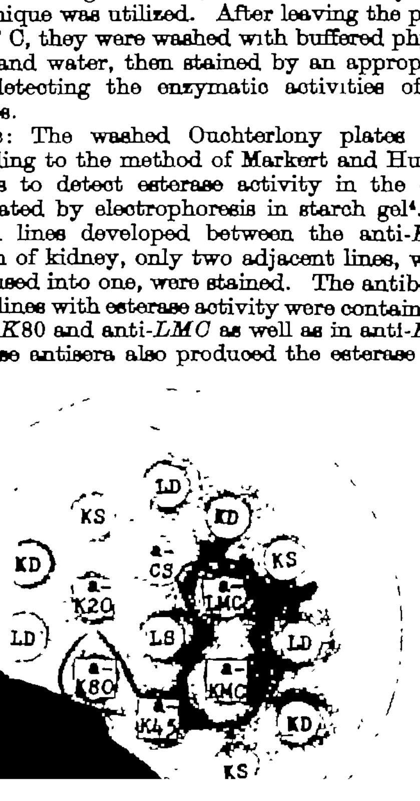
Enzymatic Activities of the Soluble Antigens in Kidney Microsomes

As revealed in the experiments using rat liver and chicken kidney, the microsomes consist immunologically of a number of different antigenic components[1]. On the other hand, it has become well known that the microsomal fraction contains many kinds of enzymes[2]. The question thus arises whether the immunologically resolved components may also differ from each other in enzymatic activity. In this communication we report that the activities of esterase and phosphatases are respectively localized in antigenically different components of the microsomes.

Adult chickens were used as the source of antigens for both injection and testing. Seven different types of antisera were prepared against rabbits. These were: (1) antiserum against liver microsomes (anti-*LMC*); (2) antiserum against kidney microsomes (anti-*KMC*); (3) antiserum against the proteins precipitated from deoxycholate-extract of kidney microsomes between 10 and 20 per cent saturation of ammonium sulphate (anti-*K*20); (4) antiserum against proteins precipitated between 25 and 45 per cent saturation (anti-*K*45); (5) anti-serum against proteins precipitated between 60 and 80 per cent saturation (anti-*K*80); (6) antiserum against unsedimentable post-microsomal fraction of kidney homogenates (anti-*KS*); (7) antiserum against chicken serum (anti-*CS*). The antibody patterns of these antisera, together with the procedures for separating the deoxycholate-extract of microsomes (*D*-fraction) into three immunologically distinct sub-fractions (*K*20, *K*45 and *K*80), have been communicated previously[3]. As the test antigens, *D*- and *S*-fractions were prepared from the livers and kidneys of freshly killed chickens.

For the immunological tests, the Ouchterlony agar diffusion technique was utilized. After leaving the plates for 36 h at 37° C, they were washed with buffered physiological saline and water, then stained by an appropriate method for detecting the enzymatic activities of the precipitin lines.

(1) *Esterase*: The washed Ouchterlony plates were treated according to the method of Markert and Hunter, who used this to detect esterase activity in the components separated by electrophoresis in starch gel[4]. Of 5–6 precipitin lines developed between the anti-*KMC* and *D*-fraction of kidney, only two adjacent lines, which occasionally fused into one, were stained. The antibodies to cause these lines with esterase activity were contained in anti-*KS*, anti-*K*80 and anti-*LMC* as well as in anti-*KMC* (Fig. 1). These antisera also produced the esterase lines



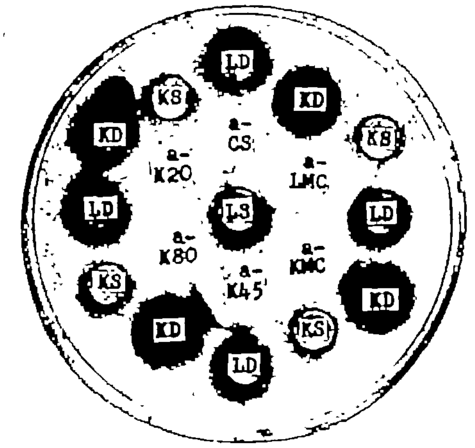


Fig. 2. Patterns of precipitin lines in Ouchterlony plate stained for detecting alkaline phosphatase activity. *KD*, *D*-fraction of kidney; *KS*, *S*-fraction of kidney; *LD*, *D*-fraction of liver; *LS*, *S*-fraction of liver; cf. text for symbols of antisera

in the reaction with the *S*-fraction of kidney or liver. Anti-*K*20 and anti-*K*45, however, contained few antibodies to give the precipitin lines with esterase activity. As previously reported, these antisera are highly '*D*-fraction specific'; that is, most of the antibodies which they contain are directed against the components predominantly distributed only in the *D*-fraction. Therefore, esterase activity should reside in the antigenic components identified to be 'non-specific' in the sense that they are found in common in both *D*- and *S*-fractions prepared from various organs. Anti-*CS* did not contain any antibodies to react with these components.

An aliquot of *D*-fraction of kidney was used for electrophoretic analysis with Smithies's starch gel method[5]. The run was made in borate buffer (0·01 M, *p*H 8·6) at a field strength of 11 V/cm for 7 h at 4° C. Following electrophoresis, the gel was longitudinally divided into two strips and one of them was stained to detect the component having esterase activity. A component with high mobility was stained. The other half of the gel was frozen, thawed and transversely cut into six small pieces. The deoxycholate-eluates from each piece were introduced into the respective peripheral wells of an Ouchterlony plate and allowed to diffuse against the antiserum filling the central well. Only the eluate of the piece which was taken from the locus corresponding to the site of esterase activity in the stained half gave rise to a stainable precipitin line in the reaction with anti-*KMC*, anti-*K*80, anti-*KS* and anti-*LMC*.

(2) *Phosphatases*. The washed Ouchterlony plates were stained with a solution containing α-naphthylphosphate and fast blue *BB* salt at *p*H 9 (with 0·05 M barbital buffer) for alkaline phosphatase and at *p*H 5 (with 0·2 M acetate-buffer) for acid-phosphatase[6]. Of the antisera used, only anti-*KMC* and anti-*K*45 produced the line which stained for alkaline phosphatase (Fig. 2). As shown in the earlier paper[3], anti-*K*45 is not only '*D*-fraction specific' but also highly 'kidney-specific', and this antiserum produced several lines, with the *D*-fraction of kidney only, in the unstained plates. The result does not directly imply that the component with alkaline phosphatase is 'kidney specific', because, after histochemical treatment, an identical stained line became detectable, though very faintly, between anti-*K*45 and the *D*- and *S*-fractions of liver. Faint lines were also detectable between anti-*KMC* and the *S*-fraction of kidney and liver. This fact suggests that the application of histochemical methods might make visible some of the precipitin lines otherwise left undetectable.

The lines positive for acid phosphatase were always very broad, and this made it difficult to identify precisely the stained components in the Ouchterlony plate, but it is fairly certain that the same lines positive for alkaline phosphatase were also stained at *p*H 5. Two stained lines sometimes appeared between anti-*KMC* and the *D*-fraction of kidney.

In the electrophoresis of the kidney *D*-fraction with starch gel, a component with low mobility is found to be positive for phosphatases staining, but elution of this component from the gel to permit testing it with the Ouchterlony plate has so far been unsuccessful.

The results described here indicate that the activities of esterase and the phosphatases reside respectively in immunologically different components of the microsomes, but they do not immediately prove that each immunologically resolved component corresponds to a different enzyme species. It might occur that multiple antigenic components display the same enzyme activity. Reciprocally, one antigenic component could display the activities of multiple enzymes, as was suggested in the case of acid and alkaline phosphatases.

It is doubtful whether the antibodies utilized here are directed against the enzymatically active groups of the molecules in the microsomes. Judging from the fact that the precipitin lines could be well stained, it seems to be conceivable that the antigenically determinant groups for producing the present antisera do not always coincide with the enzymatically active sites of esterase or phosphatases.

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Proteolytic Activation of Glucose-6-phosphatase in Normal, Diabetic and Hepatomic Rat Livers

Phosphorylation of glucose, the hexokinase reaction, is an irreversible one which, however, is reversible by a specific phosphatase, the glucose-6-phosphatase (G-6-pase). Therefore the velocity of the phosphorylation of glucose is not only dependent on the activity of the hexokinase, but is also a result of the contrary action of both the enzymes. It was shown by some authors[1,2] that the activity of G-6-pase is different in varying conditions of metabolism. Tissues or cells with a high catabolism of carbohydrates show a significantly decreased activity of G-6-pase, besides an increased activity of hexokinase[2]. Highly glycolysing tumours represent tissues with a remarkable catabolism of carbohydrates and also show a decreased activity of G-6-pase[3,4]. Hitherto no tumour has been found with a noticeable activity of this enzyme. On the other hand, it will be expected that tissues with a diminished consumption of glucose or increased synthesis of glucose from non-carbohydrate sources via the Embden–Meyerhof pathway exhibit an enhanced activity of G-6-pase. Accordingly Mokrasch[1] found increased activity of phosphatase in diabetic liver.

Freedland and Waisman[5] showed that *in vitro* the activity of G-6-pase can be increased by incubation with papain. The authors suppose this process to be a result of a proteolytic activation of the enzyme in consequence of the splitting of proteins or polypeptides as mascating substances from the molecule of the phosphatase. We found that activity of G-6-pase could be enhanced also by incubation of the enzyme with trypsin and for this reason we suppose that the proteolytic activation is an unspecific process[6]. Based on these theoretical considerations we were interested in examining the proteolytic activation of the G-6-pase on the different conditions of metabolism and to compare the rate of alteration of its activity. Also we hoped to consider whether the rapid raising of the activity of the enzyme during the development of a diabetes mellitus is based on a 'demascation' similar to the proteolytic activation of the G-6-pase *in vitro*. In this case, a lower proteolytic activation should be expected in the G-6-pase from diabetic liver than in the enzyme from normal liver.

On the other hand, the diminished activity of the enzyme in tumours could be explained by 'mascating' processes during the carcinogenesis. Accordingly in this case one can expect an increased proteolytic activation of the enzyme from tumours in comparison with corresponding normal tissues.

Therefore we examined the possibility of a proteolytic activation with trypsin of the G-6-pase from normal and diabetic liver and from hepatoma. Diabetes mellitus was produced in white Wistar rats by injection of 7 mg alloxan in saline. In order to control the diabetogenic action of alloxan we determined the blood sugar of the rats by means of enzymatic tests with glucose oxidase. Hepatoma was produced by feeding with 5 mg dimethylaminoazobenzene (butter yellow) daily for 200 days.

The G-6-pase is bound to the microsomes and therefore we carried out proteolytic activation in a homogenate of liver and also in isolated microsomes[7]. Proteolytic activation was carried out by incubation of the samples (homogenate or microsomes) with trypsin and inhibition of the action of trypsin by 'trasylol' (trypsin–kallikrein inhibitor, Bayer-Leverkusen) in the described manner[8]. We determined the activity of the G-6-pase by the method of Freedland and Harper[9] and the proteins by the biuret method. Absolute activity is defined as micromoles phosphate liberated/h in 1-ml. samples; specific activity as micromoles phosphate liberated/h and 1 mg protein at 37° C. Comparing the proteolytic activations we used the specific activity because in this case the occasional effects of dilutions were eliminated.

The results are summarized in Table 1: they are mean values of 4–6 experiments.

Table 1. COMPARISON OF THE PROTEOLYTIC ACTIVATION OF THE GLUCOSE-6-PHOSPHATASE IN NORMAL AND DIABETIC LIVERS AND HEPATOMA

	Normal liver	Diabetic liver	Hepatomic liver
Homogenate			
Initial absolute activity	187 ± 6·08	218 ± 7·00	57 ± 18·1
Absolute activity after proteolytic activation	312 ± 10·5	349 ± 11·5	52 ± 11·0
Increase (per cent)	67	60	−10
Initial specific activity	1·01 ± 0·05	1·67 ± 0·31	0·55 ± 0·24
Specific activity after proteolytic activation	3·42 ± 0·75	5·61 ± 0·10	0·41 ± 0·12
Increase (per cent)	268	238	−30
Microsomes			
Initial absolute activity	179 ± 4·12	240 ± 8·42	53 ± 15·0
Absolute activity after proteolytic activation	266 ± 15·1	334 ± 44	53 ± 18·3
Increase (per cent)	49	40	+0
Initial specific activity	2·43 ± 0·20	3·21 ± 0·45	1·12 ± 0·23
Specific activity after proteolytic activation	6·68 ± 0·39	7·67 ± 0·69	0·39 ± 0·07
Increase (per cent)	175	140	−65

Absolute activity, μmoles phosphate liberated/ml. h. Specific activity• μmoles phosphate liberated/mg protein h.

It can be seen that proteolytic activation of G-6-pase from diabetic liver compared with normal liver is not increased significantly. Also these results exclude a partial 'demascation' of the enzyme during the developments of the diabetic metabolism. On the other hand, similar experiments carried out by us[10] with fructose-1,6-diphosphatase (FDPase), which also acts irreversibly in the catabolism of carbohydrates, showed a partial 'demascation' of FDPase during the development of a diabetes. For this reason increase of activity of G-6-pase could be explained either by a two-step-mechanism of activation or by the existence of two isoenzymes of G-6-pase or by a *de novo* synthesis of the enzyme after the application of alloxan.

Also the results of a diminished or absent proteolytic activation in the hepatoma exclude a possible 'mascation' of the phosphatase during carcinogenesis. Since the carcinogenesis represents a long-acting process, the destruction of the enzymatic activity and an inhibition of the *de novo* synthesis during the development of a hepatoma is more probable. We consider the very low absolute and specific activity of G-6-pase in hepatoma as evidence of this.

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Enzymatic Dedifferentiation of Neoplastic Cells

VARIOUS authors have suggested that the enzymatic equipment of neoplastic cells need not necessarily be immutable and that it may show variations as the result of a number of completely unknown influences. This communication describes a striking example of enzymatic dedifferentiation recently observed in a chemically induced squamous cell carcinoma subjected to serial transplantation by various routes. The logical consequence of such an observation is to make us aware of how fragile are the grounds accepted as bases for the procedures at present in use for screening compounds as anti-cancer agents.

In this investigation squamous cell carcinoma was induced in the cervix and upper vagina of adult virgin *C3H/N* mice by twice-weekly painting of the ectocervix with a 1 per cent suspension of 3,4-benzpyrene in acetone. After fewer than 40 treatments all the animals showed invasive neoplasms. A squamous cell carcinoma, provoked by this method and serially transplanted under the skin of mice of isologous strain, was studied histochemically from the 79th to the 102nd transplantation generation. An ascites line, adapted from a benzpyrene-induced cervico-vaginal squamous cell carcinoma[1], was examined in its 280th to 287th transfer generations. The tumour was maintained by injecting ± 13 × 10[6] freely growing neoplastic cells into the peritoneal cavity of adult virgin *C3H/N* mice. The animals were killed from 7–9 days after inoculation. Solid transplants were obtained by subcutaneous injection of inocula (from passages 282–285) of the same magnitude. All the animals were killed by breaking their necks. The genital tracts of painted mice and the solid subcutaneous nodules were carefully dissected out, frozen with carbon dioxide, and cut serially (10μ) in a cryostat.

Smears were prepared from ascites fluid and kept in a refrigerator at −20° C. Smears and tissue sections were treated for the demonstration of cellular activity of 5-nucleotidase[2]. Although in normal cervico-vaginal epithelium of the mouse slight cyclic variations in the activity of 5-nucleotidase were observed, the over-all tissue activity of this enzyme is weak. Pathological, non-invasive (hyperkeratosis, dysplasia, *in situ* carcinoma) epithelium shows the same weak enzymatic activity except for its cornified layers. Invasive squamous cell carcinoma (early stromal invasion, micro- and macro-carcinoma), on the other hand, shows a constantly high activity of 5-nucleotidase in all its component cells[3]. Necrotic portions of the tumour, horny material, and poly-nuclear leucocytes show some degree of enzyme activity, whereas macrophages are inactive. The constantly high 5-nucleotidase activity typical of malignant squamous cells and also found in vaginal washings from animals bearing benzpyrene-induced cervico-vaginal carcinoma has been considered to be of definite diagnostic value[3].

The pattern of reactivity of the solid transplantable squamous cell carcinoma is strikingly similar to that found in the mother tumour, and does not vary with prolonged transfer.

The situation in the ascites line of such chemically induced cervico-vaginal carcinoma is entirely different. There is a complete absence of 5-nucleotidase activity in the tumour cells floating freely in the ascites fluid as well as in those so closely packed as to form solid subcutaneous tumours. Clusters and/or sheets of tumour cells, equally devoid of enzyme activity, were found infiltrating the subcutaneous muscle layer, the lymph nodes, and the liver sinuses.

Thus we observe a truly remarkable sequence of events. In the course of chemical carcinogenesis the activity of 5-nucleotidase suddenly increases in the squamous genital epithelium of the mouse, a fact which in itself is in distinct contrast to the activity of most of the enzyme systems studied. Moreover, this phenomenon is stable when the chemically induced carcinoma is transferred serially to the subcutaneous tissues, and remains unaltered notwithstanding long-continued transferring. On the other hand, ascites tumour cells derived from these structures show an equally remarkable drop in their enzymatic content: 5-nucleotidase activity is entirely absent and no rebound effect is found when such cells are grown as solid subcutaneous nodules. Thus it is clear that these two transplantation routes generate neoplastic cells the enzymatic equipment of which is of a different nature. Screening procedures used in cancer chemotherapy often make use of ascites tumour forms.

The example presented here makes it obvious that care must be taken when interpreting data based on such material, that is, observations based on an ascites line should not always be extrapolated to the tumour from which this line was adapted. Although solid transplantable carcinoma of the mouse cervix may prove a valuable tool for such screening purposes, the ascites line derived from it, which for reasons all too obvious was thought to be even more valuable, has not fulfilled its promise. However, by illustrating the process of enzymatic dedifferentiation, the ascites tumour has nevertheless proved to be worth while studying.

We thank Dr. I. Koprowska, Philadelphia, who kindly provided us with the original solid transplantable tumour and its ascites form *MT*–890.

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BIOLOGY

Activity of Gibberellin '*D*' on the Germination of Photosensitive Lettuce Seeds

Recent discoveries in the gibberellin series have brought to light a large number of new compounds the activity of which varies widely in different tests[1]. In particular, Hashimoto and Yamaki[2] with tobacco seeds, and Toole and Cathey[3] with lettuce seeds, reported that GA_4 was much more active than GA_3, while Brian *et al.*[1] indicate that GA_4 and GA_7, which had about equal activity on lettuce seeds, were both more active than GA_3. However, the latter group stated that they were "not able to establish a simple relationship between dose and response" in their test. Borriss and Schmidt[4] crystallized a gibberellin preparation from cultures of *Gibberella fujikuroi* which was 30 times as active as GA_3 on the germination of *Diplotaxus* seeds and up to 2,000 times more active than GA_3 on seeds of *Lepidium ruderale*. Later this preparation, first called gibberellin A_8 or '*D*' (ref. 4), was found to be a mixture of GA_4 and GA_7.

Through the courtesy of Prof. H. Borriss we have been able to test a sample of his purified material on Grand Rapids lettuce seeds under our conditions[5] and find it indeed to be of extremely high activity. This communication reports relative activity determinations and the interaction with the red/far-red system.

The tests were carried out with the shaking method previously described, duplicate samples of 50 seeds each in 2 ml. of solution in a 5-cm Petri dish at 25° C being used[5]. All manipulations were carried out rapidly under green light, but otherwise the seeds remained in absolute darkness throughout. The seeds for the present experiments unfortunately gave 30–40 per cent germination in darkness, as against less than 5 per cent in those previously used. However, their response to red and far-red light was the same as in the earlier lot, that is, 1-min exposure to either light produced the maximum effect. Gibberellins were prepared as stock solutions of 50 p.p.m. ('*D*') or 100 p.p.m. (A_3) in distilled water, and dilutions made from these just before use.

Fig. 1 shows that in comparative tests the activity of gibberellin '*D*' $(=GA_4+GA_7)$ is almost exactly 100 times as great as that of GA_3. The differences between the two curves, apart from the 100 times difference in absolute values, are probably not significant. The curve for GA_3 confirms in detail our previous data (Fig. 3 of ref. 5). The very high ratio of activities is remarkable, for although GA_7 is more active than GA_3 in several tests, it is usually so only by a factor of 2–3. It is reported as being only twice as active as GA_3 on the lettuce hypocotyl[1]. All in all, the unusually high ratio suggests a synergism between the two gibberellins.

These findings raise again the problem of whether light might act by releasing a gibberellin in the seed. It had been concluded that this is not the case[5], because: (*a*) the action of GA_3 could not be reversed by far-red; (*b*) light-treated seeds yielded no detectable increase in gibberellin. The latter tests were carried out on dwarfs 1 and 5 of corn, which fortunately are highly sensitive not only to GA_3 but also, and about equally so, to GA_4 and GA_7 (ref. 1). If light had acted to release appreciable amounts of GA_4 or GA_7, these substances should therefore have been detected in the tests, so that the doubts raised by Toole and Cathey[3] are not justified. On the other hand, the very high relative activity of the combination of GA_4+GA_7 found here might reopen the possibility of a liberation of both these in amounts below the threshold of the corn response, though adequate for germination of lettuce seed.

The influence of far-red on the germination caused by gibberellin '*D*' was, therefore, examined as follows. The seeds were soaked in a series of concentrations of '*D*' or GA_3 in the dark, and subjected to 5-min far-red light (about 30 ft.-candles) at 1·5 h after the beginning of soak-

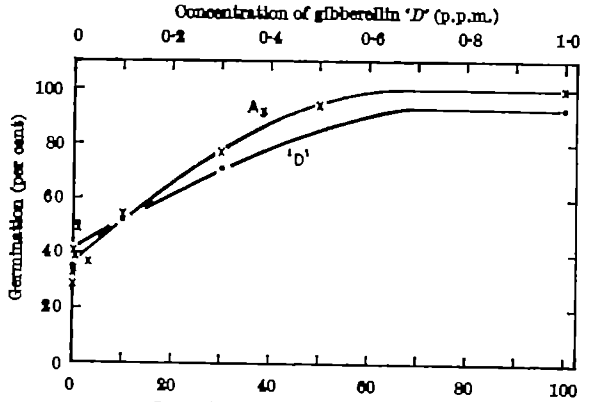

Fig. 1. Effect of gibberellin '*D*' or A_3 at various concentrations on the germination of Grand Rapids lettuce seeds in darkness

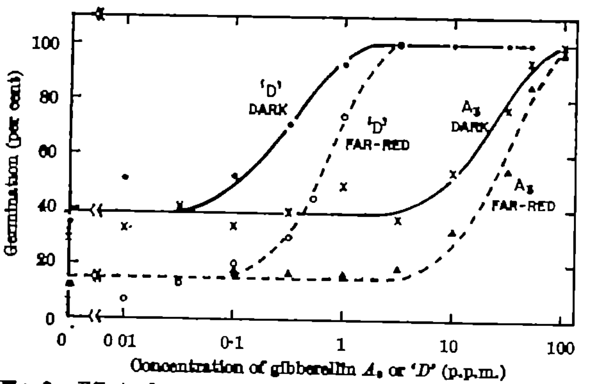

Fig. 2. Effect of 5-min far-red light on the germination of Grand Rapids lettuce seeds soaked in various concentrations of gibberellin '*D*' or A_3. Far-red light given at 1·5 h after the beginning of imbibition. Solid lines, far-red treatment: dotted lines, dark control (same as Fig. 1)

ing. This was previously shown to be the most sensitive time[5]. Germination was then determined 2 days later. Fig. 2 (in which the concentrations are plotted on a logarithmic scale) compares the germination with that obtained in complete darkness. It is seen that the far-red decreased the germination of the dark controls by almost 20 per cent. It decreases the germination of the seeds treated with low concentrations of either of the gibberellins by essentially the same amount. The curve for GA_3 extends to lower concentrations the data previously given for 100 p.p.m. (Tables 2 and 3 of ref. 5). At higher levels of both gibberellins the effect of the far-red disappears. At 3 p.p.m. gibberellin '*D*', indeed, the germination was 100 per cent with or without far-red exposure.

The conclusion that the action of gibberellin is not in the least reversed by far-red light is open to the reservation that the gibberellin might continue to act after the far-red exposure[6]. This difficulty was previously dealt with by limiting the GA_3 treatment to 2 h or less[5]. It was dealt with in another way by Haber and Tolbert[7], who transferred the seeds to 37° after exposure to far-red, since GA_3 does not promote germination at 37°. They found no reversal by far-red, but unfortunately their seeds germinated incompletely (only 39 per cent). In the present experiments the seeds were soaked in gibberellin '*D*' for 2 h, beginning 2 h after the onset of soaking. Thereafter the gibberellin was washed off and the seeds returned to water. To ensure maximum possible effect of the far-red the exposure was lengthened to 1 h, given either just before or just after the gibberellin '*D*'.

Table 1 shows that the far-red again decreased the control germination by 17 and 24 per cent respectively. The germination expected in gibberellin '*D*', calculated from this effect on the water controls, is shown in the last column. The agreement is good. It may be concluded, therefore, that *the action of gibberellin 'D' is not reversed by far-red*.

Table 1. Effect of 1-h exposure to far-red light on germination of Grand Rapids lettuce seeds treated with gibberellin '*D*' (3 p.p.m.) for 2 h. Gibberellin '*D*' treatment for 2 h begun 2 h after the beginning of soaking. 1-h exposure to far-red light given immediately before or after the gibberellin treatment, while seeds were soaked in water. Germination determined at 48 h after gibberellin treatment

Treatments				Germination (per cent)	
←Water→			←Water→		
1 h	1 h	2 h	1 h	Experimental	Expected
	—	Water	—	31	—
	FR	Water	—	14	—
	—	Water	FR	7	—
	—	Gibberellin '*D*'	—	62	—
	FR	Gibberellin '*D*'	—	42	45
	—	Gibberellin '*D*'	FR	32	38

Is there any other way in which the action of red light could be accounted for in terms of the release of gibberellin? In general, the effect of red light on germination can be reversed by far-red up to at least 8 h following exposure. The gradual onset of irreversibility is often referred to as the 'escape curve'. If red light were to liberate gibberellin, this curve would be in reality the curve showing gibberellin release. However, such an interpretation is exceedingly improbable because: (*a*) the gibberellin so released would as noted here have to be even more active than either constituent of gibberellin '*D*' to have escaped detection on corn dwarfs 1 and 5; (*b*) it would only be beginning to appear in the seed more than 3·5 h after soaking, which is a time of greatly lowered sensitivity[5]. It may be noted, too, that in stem elongation and the flowering of short day plants the effects of red light could scarcely be due to the liberation of gibberellin, since they act in the opposite sense.

Thus we conclude that gibberellin, instead of being a product of red light, must initiate one of the chemical reactions which result from the primary light reaction.

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Influence of Crop Residue Decomposition Products on the Germination of *Fusarium solani* f. *phaseoli* Chlamydospores in Soil

Fusarium solani f. *phaseoli*, the cause of stem and root rot of bean, has been shown to exist in nature primarily in the form of chlamydospores[1]. Such chlamydospores germinate on the addition of various sugars or amino-acids but not ordinarily when water alone is used[1]. The chlamydospores have also been shown to germinate in the presence of exudates of the host and various other plants[2,3]. Evidence is presented here to show that substances produced during the decomposition of crop residues under natural field conditions can also influence the germination of chlamydospores in the absence of exudates from living plants.

In these studies, chlamydospores were obtained by adding nutrient-media-free macroconidia to non-sterile soil[1]. Details of the procedure in obtaining the substances from decomposing plant residues are published elsewhere[4].

Briefly, the substances were obtained as cold water extracts from the plant residues which had been freed of the surrounding field soil prior to the extractions. Plant debris, soil particles and micro-organisms were removed from the extracts by ultra-centrifugation and tests were carried out immediately after the extraction to minimize possible changes in the activity of the extracts. Aliquots of about 3 g of the air-dried soil containing chlamydospores were saturated with the test extracts and extent of germination was determined after 24 h by microscopic examination of acid-fuchsin-lactophenol-stained soil smears. In the controls, distilled water was used in the place of the extracts. The results of a typical series of observations are given in Table 1.

Table 1. EFFECT OF DECOMPOSITION PRODUCTS ON GERMINATION OF CHLAMYDOSPORES OF *F. solani* f. *phaseoli* IN NON-STERILE SOIL

Source of test extract (days)	Germination rating	Source of test extract	Germination rating
Rye 0*	+ + †	Broccoli 0	–
10	+	7	+ +
18	0	16	+ +
25	0	24	+ +
Barley 0	+ +	Broad bean 0	+ +
10	+	10	+ +
17	+	18	+ +
24	+	25	+ +

* Type of plant residue and number of days it had been decomposing in the field prior to extraction. Rye and barley turned under when crop was heading out, broccoli and broad bean after harvest.

† Amount of germination rated as 0, +, + +; none, fair and good, respectively; see Fig. 1, which shows a '+ +' rating. No germination of chlamydospores was obtained in the controls in which distilled water was used in place of the various test extracts.

Extracts from all four crop residues used stimulated germination of the chlamydospores. Differences were noted between the various extracts, however, in the intensity of stimulation of the chlamydospore germination that was obtained and the subsequent vigour of growth of the germ-tube hyphæ. In general, two types of response were obtained. Some extracts induced vigorous germination of the chlamydospores followed by extensive growth of the germ-tube hyphæ (Figs. 1 and 2). The germ-tube hyphæ were large, stained well and appeared vigorous. The second, and perhaps most interesting, response was a stimulation of chlamydospore germination which was soon followed by lysis of the germ-tube hyphæ (Fig. 3). Some of the extracts did not appear to have any effect on chlamydospore germination since no stimulation was obtained. The aforementioned differences in response appeared to depend on the types of residues used and their decomposition periods in the soil. Highly active extracts were most frequently obtained from the residues during early stages of decomposition. The stimulatory effect of the rye and barley extracts appeared to be weaker and of shorter duration than in the case of broccoli and broad bean, and extracts from the latter two crops continued to stimulate the germination of chlamydospores even after the residues had been decomposing in the soil for up to 25 days. The extracts from rye and barley residues also gave more instances of germ-tube lysis. Since only a limited number of extracts were tested, however, the precise relationship between type of residue, decomposition period and chlamydospore germination could not be determined at this time.

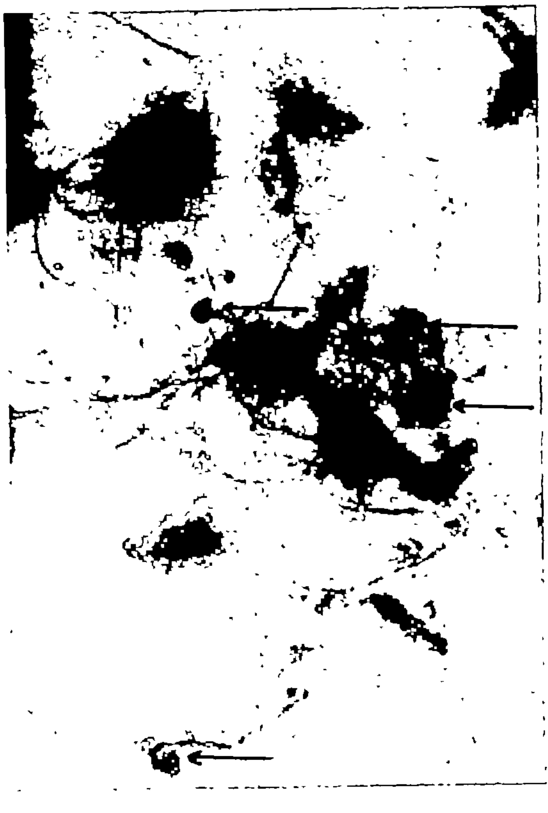

Fig. 1. Extent of chlamydospore germination of *F. solani* f. *phaseoli* in soil placed in contact with broccoli residues which had been decomposing for 7 days in the field. This amount of germination would have a + + rating. Arrows indicate germinated chlamydospores

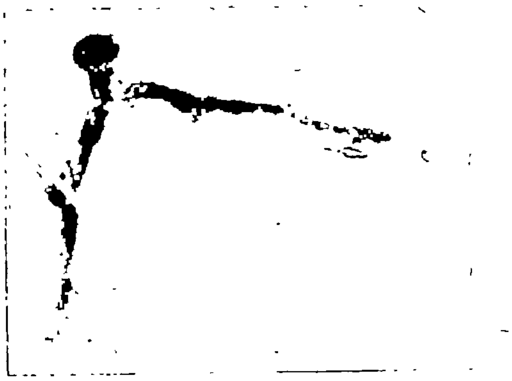

Fig. 2. Vigorously growing hyphæ arising from chlamydospore in contact with the broccoli residue; compare with Fig. 3

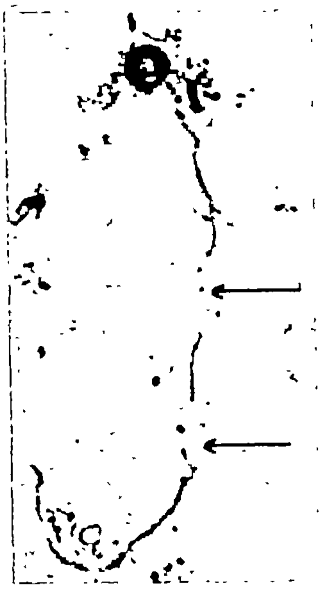

Fig. 3. Germination of chlamydospore in contact with barley residues which had been decomposing for 10 days in the field. The germ tube is thin and lysis of the contents has occurred in two areas indicated by arrows

To approach more closely conditions in Nature, partially to obviate the dilution problem and the possibility that results obtained with extracts could be due to artefacts in the extraction procedure, tests were also carried out with fragments of intact plant residue that had been decomposing in the field for various periods of time. In these tests, fragments of decomposing residue were covered with the previously mentioned soil containing the chlamydospores and moistened with water. Soil smears were prepared 24 h later of the soil in direct contact with the plant residue. Staining and extent of chlamydospore germination were evaluated in the same manner as with the extracts. The results obtained in these tests were in close agreement with those obtained with the extracts. Germination of chlamydospores, however, was stimulated only in close proximity to the residue fragment and no germination could be detected in the soil not in contact with the residue.

The results described have interesting implications with respect to factors in the soil which can influence the germination and survival of infecting propagules of the soil-borne pathogen, *Fusarium solani* f. *phaseoli*. It is evident, too, that substances produced during the decomposition of plant residues can be active in the support of a biosphere in the soil which in some instances and under certain conditions may rival in importance that around the roots of living plants in maintaining, increasing or decreasing the population of active propagules of a pathogen such as *F. solani* f. *phaseoli* and perhaps other soil-borne plant pathogens.

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Tissue Hydroxy-cinnamic Acids and Soil Moisture Stress

The stem tissue of pineapple plants (*Ananas comosus*, var. Cayenne) contains appreciable amounts of hydroxy-cinnamic acid derivatives ('phenolics'), largely as a quinic acid ester of *p*-coumaric acid[1,2] and an ester of ferulic acid. These phenolics are implicated in the metabolism of the plant growth hormone, indolyl-3-acetic acid[3,4].

This communication will present evidence that the amount of these phenolics in pineapple stem tissue is inversely related to the soil moisture content. This observation has obvious implications in connexion with various growth effects, including flowering, that may be affected by moisture stress.

The phenolics were extracted from 1–10 g of meristematic tissue from the stems of several plants by blending with water. In one experiment, they were then taken up in ethyl acetate after acidification of the aqueous extract. In the other experiments, the filtered aqueous extract was used directly. Similar results were obtained when the extractant was 2 per cent sodium dihydrogen phosphate or when the aqueous extract was boiled and filtered before spectrophotometry. These extracts showed a typical hydroxy-cinnamic acid spectrum with a maximum near 314 mμ (ref. 3); the absorbance per gram of tissue determined at this wave-length in the Beckman *DU* or *DK*-2 spectrophotometer quantitatively measured the stem phenolics.

The close inverse relationship between soil moisture sampled at the butt of the plant and stem tissue phenolics is evident from Fig. 1. The phenolics content fluctuated from week to week as the soil moisture-level varied with drought or rainfall. It is especially interesting to note that the relationship held even when the plant was presumably under only minor soil moisture stress. (A tension of 4 bar is equivalent to 28·0 per cent moisture in this soil; the wilting point of 15 bar is at 26·0 per cent soil moisture. Soil at the root zone has about 2 per cent less moisture than at the plant butt.) The results in Fig. 1 were for unirrigated plots, but even in adjoining plots receiving weekly irrigations to keep the soil moisture in the range 29–38 per cent, the phenolics curve showed a marked tendency to mirror the changes in the soil moisture curve from week to week.

It will be seen that the relationship is not merely a reflexion of tissue hydration under varying moisture stress. The phenolics calculated to a dry weight basis also show an inverse correlation with soil moisture (Fig. 1).

Table 1. CORRELATION OF SOIL MOISTURE WITH PHENOLICS IN STEM TISSUE OF PINEAPPLE PLANTS OR CROWNS (OAHU, HAWAII)

Sampling period	Field	r*	r†	No. of observations
Days 211–343, 1953	*A*	−0 41	−0·93	9
Days 204–338, 1957	*A*	−0 40	−0·76	10‡
Days 126–301, 1958	*B*	−0 87	−0·69	24
Days 126–301, 1958	*C*	−0 64	−0·54	24
Days 118–216, 1958 (crown stems)	*A*	−0 61	−0·02	15

* Correlation coefficient, phenolics versus soil moisture. The Field *B* results are those shown in Fig. 1.

† Correlation coefficient using soil moisture results two weeks previous to plant harvest.

‡ Using an ethyl acetate extract; other phenolics analyses were on aqueous extracts.

This observation of a close relationship between soil moisture and hydroxy-cinnamic acids in the growing point of the pineapple has been confirmed in experiments over several years (Table 1). In the first two experiments, covering a later period of the year than the 1958 tests, there seemed to be a lag of approximately two weeks before a change in soil moisture was reflected in the tissue phenolics content. When the correlation was for phenolics versus the soil moisture two weeks previously, the correlation coefficient was found to be −0·93 and −0·76 for the 1953 and 1957 experiments. In 1958, the best correlations were without the lag period in the soil moisture effect. In one test (Field *C*), an early wave of flowering occurred in early September. A good inverse correlation of −0·80 prior to flowering (17 samples) failed to hold once flowering was observed; the final seven observations showed a correlation coefficient of only

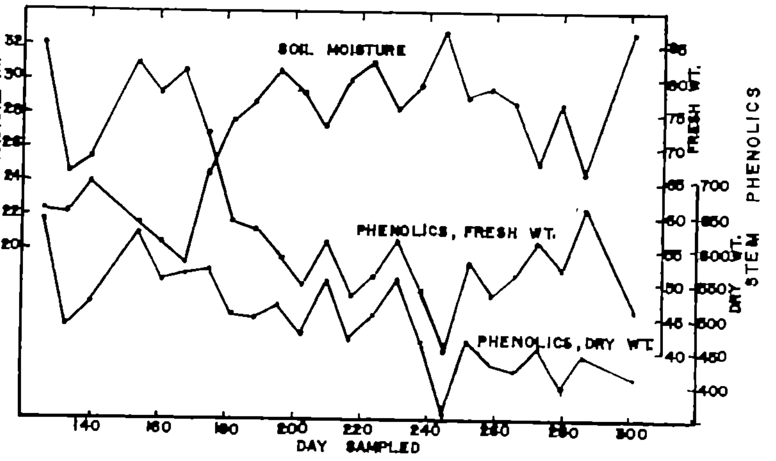

Fig. 1. Relationship of soil moisture and of phenolics in the stem tip of vegetative pineapple plants (Oahu, Hawaii, 1958). The phenolics values are absorbances at 313 mμ/g of tissue

+ 0·16 for stem phenolics versus soil moisture at the time of plant harvest.

Table 1 also gives the results of an investigation conducted by Dr. V. L. Singleton of these laboratories in which phenolics in the stem tissue of crowns of fruiting plants were determined. Even here we find the same inverse correlation with soil moisture which was observed for stem tissue of vegetative plants.

A similar correlation with other variables in the environment of the plant was not obtained. For example, radiation will bring about changes in soil moisture and might be suspected as being more directly involved, particularly in the phenolics variations in the fruit crowns. Yet the correlation coefficient for crown phenolics versus soil moisture was a significant − 0·61 (Table 1), whereas versus 'pan evaporation' it was an insignificant + 0·09. (Evaporation from a standard Weather Bureau pan is closely related to sunlight in pineapple areas.)

Owing to a change in research programme, I shall not be able to extend these observations to ascertain their significances in plant growth. It is hoped that reporting the available results at this time may interest other workers in determining whether tissue phenolics are responsive to soil moisture-levels in other plants, and whether growth effects such as flowering may be associated with the interrelationship reported here.

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Occurrence of Dormant Ascospores in Soil

WARCUP[1] reported that steaming was a simple selective method for isolating Ascomycetes from soil. The method used, however, gave little indication of the temperature at which most soil fungi are killed and at which Ascomycetes survive. With the development of equipment by which steam–air mixtures at various temperatures may be obtained[2], it was decided to reinvestigate the heat tolerance of Ascomycetes in soil.

Initially several soils from tropical Australia known to contain high populations of Ascomycetes were used. When soil plates[3] were prepared from samples of these soils steamed at temperatures between 50° and 75° C for 30 min, it was noted that the number of colonies of some Ascomycetes was markedly higher than in plates prepared from unheated soil. Since the numbers of colonies on different soil plates are not strictly comparable the experiments were repeated using the soil dilution method.

An aliquot of soil (2·5–5·0 g) was added to a water blank to give a dilution of 1 : 100 and, after shaking, 1-ml. portions were placed in a series of Petri dishes. The bulk soil suspension was then heated in a water bath at 60° C for 30 min; immediately after its removal further dilution plates were made. All plates were poured with 'Dox' + yeast agar containing 30 μg/ml. streptomycin and 2 μg/ml. aureomycin to prevent growth of bacteria.

The results of an experiment with a grey-brown loam (soil *A*) from Koolpinyah, Northern Territory, Australia, are given in Table 1. The fact that more colonies are obtained after heating suggests that this treatment stimulates dormant spores in soil to germinate. Here we follow Gottlieb's definition[4] of a dormant spore as one which "does not germinate under the same nutritive and environmental influences which later allow production of germ tubes". Examination of the propagules[5] of young colonies developing on plates prepared from heat-treated soil showed that in many cases the previously dormant fungi were growing from ascospores. While dormancy in ascospores, particularly of certain coprophilous Ascomycetes, is well known[6], so far as we are aware dormancy of this type has not previously been considered in relation to soil fungi.

Since ageing is known to induce dormancy[6] and the soils examined had been stored dry for 10–20 years it is possible that the observed dormancy was a result of drying and storage, and might not occur in spores in normal, moist field soil.

Tests were made on moist soil immediately after removal from the field but here dormancy could not be so simply demonstrated. The number of propagules of many Ascomycetes in soil is low. This means that plates prepared at dilutions appropriate to record Ascomycetes are crowded with colonies of other fungi. While the heat-treatment used kills many fungi, allowing Ascomycetes to be easily seen, plates prepared from unheated soil are so crowded that it is difficult to decide whether lack of colonies of an Ascomycete implies its absence or only its suppression by the numerous other organisms present.

In attempts to overcome this problem various selective media, including an Actidione medium[7], were tried. None, however, proved of use for the organisms being studied. It was then decided to investigate the use of ethyl alcohol as a selective agent. Many Ascomycetes survive when soil is immersed in ethyl alcohol, whereas most other fungi are killed by this treatment[8]. In subsequent experiments 2·5 g of soil was immersed in 60 per cent alcohol for 6–8 min, then soil and alcohol added to a water blank to give a dilution of 1 : 100 (and an alcohol concentration of less than 1 per cent). Preparation of dilution plates and heat-treatment were then carried out as previously.

The results of a test with moist soil from a forest nursery at Belair, South Australia (Soil *B*), are given in Table 1. Alcohol-treatment reduced the number of colonies in this soil from 300,000 to 2,000–3,000/g; many of the alcohol-resistant fungi were Ascomycetes. The results in Table 1 show that although certain fungi were killed by the subsequent heating, this treatment stimulated others to germinate and increased the total number of colonies recorded.

Fungi, the spores of which have given evidence of heat-activated germination, include some members of the Sordariaceae, *Aspergillus fischeri*, *A. fischeri* var. *glaber*, *A. quadricinctus*, *Penicillium parvum*, *P. avellaneum*, *Anthracobia* sp., besides other unidentified Ascomycetes (including further Discomycetes). The Discomycetes isolated do not fruit on agar media, but fructifications were obtained on blocks of plaster of Paris. While most heat-activated spores are ascospores, in a few cases the propagule would appear to be some other unit (chlamydospore).

Most experiments on heat-activation of spores in soil have been made at temperatures of 60°–65° C for a standard time of 30 min. Temperatures as high as 85° C have been shown to activate spores but usually, depending on the fungus, such higher temperatures also kill some spores.

Table 1. EFFECT OF HEAT TREATMENT OF SOIL ON NUMBER OF COLONIES OF CERTAIN FUNGI ON SIX SOIL DILUTION PLATES

	No. of colonies			
	Sample 1		Sample 2	
Treatment	Nil	Heat	Nil	Heat
Fungus	Soil *A* (dry, stored)			
Thielavia (a)	15	0	14	0
Penicillium spp.	15	0	21	0
Aspergillus fischeri var. *glaber*	2	63	2	51
Total No. of colonies	41	78	50	62
	Soil *B* (moist, fresh)			
Chaetomium sp.	34	0	25	0
Thielavia (a)	5	0	6	0
Penicillium spp.	33	13	20	8
Humicola sp.	7	25	10	19
2/4*H*15	15	25	12	22
Aspergillus fischeri var. *glaber*	2	41	2	46
Aspergillus fischeri	0	9	0	15
Anthracobia sp.	0	5	0	7
3/4*H*117 (Pyrenomycete)	0	4	0	7
Eladia sp.	0	5	0	5
Gelasinospora sp.	0	8	0	3
Total No. of colonies	165	202	136	176

About half the soil samples so far examined have given some evidence of the presence of heat-activated dormant spores.

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Determination of Distribution of Water in Wheat Grains by Interference Microscopy

It is of interest, especially in milling, to know the water content of different parts of grains, such as wheat. This communication describes a new method which permits the measurement of the water content with relatively high accuracy in every point of a microscopic section. The method is based on changes in refractive index of the tissue by water. The section is immersed in indane, a liquid with refractive index ($n_D = 1{\cdot}537$) similar to that of the dry tissue ($n_D = 1{\cdot}53$) and photographed in an interference microscope (Leitz) adjusted to give fringes in the field. Afterwards, the preparation is completely dried *in vacuo* (0·1 mm mercury, 96° C, 3 h), re-immersed in the liquid and photographed again in the interference microscope. The shift of the interference fringes (Fig. 1) between the two photographs, $\varphi_1 - \varphi_2$ (where φ, the optical retardation, = fringe distance × wave-length), enables the calculation of the water content in the following way: the amount of water per unit area m_w is given by $m_w = d \cdot \rho$, where d is the optical path length of water and ρ its density (1·00), so that $m_w = d$. Since the shift of the fringes:

$$\varphi_1 - \varphi_2 = d \cdot (n_i - n_w), \quad m_w = \frac{\varphi_1 - \varphi_2}{n_i - n_w}$$

where n_i is the refractive index of the immersion liquid and n_w that of water.

Since the value m_w depends on the section thickness, it is, in general, advantageous to relate the water content to the dry weight of the tissue. Dry weight m per unit area may be expressed in the form $m = \frac{\varphi}{\chi}$ (ref. 1), where φ is the optical retardation produced by the tissue. χ_w for grain sections containing mainly starch in water is 0·14 cm³/g (ref. 1). In our experiments the sections are immersed in indane with a refractive index of $n_D = 1{\cdot}537$. The corresponding χ_i is obtained by measuring the optical retardation of a section in water (φ_3) and in indane (φ_2) (dried section); χ_i is then $\frac{\varphi_2 \cdot \chi_w}{\varphi_3} = 0{\cdot}20$ cm³/g.[1] Thus the water content as a percentage of the dry weight can be represented by:

$$\frac{m_w}{m} \cdot 100 = \frac{\varphi_1 - \varphi_2}{n_i - n_w} \cdot \frac{\chi_i}{\varphi_2} \cdot 100 = 22 \cdot \frac{\varphi_1 - \varphi_2}{\varphi_2}$$

Best results can be obtained with sections 20–40µ thick made on a sliding microtome with an inclined knife at about 30° from the cutting direction. During sectioning the grains and the sections are covered with indane. Since this liquid is lipophilic, it prevents loss of water during the cutting and measuring processes. In order to obtain good penetration and efficient drying of the sections a volatile liquid has been chosen. Of all liquids tested indane has been most successful. Experiments have been performed to assess movement of water during sectioning and measuring. If the whole procedure is carried out within 10 min, no such movements can be detected.

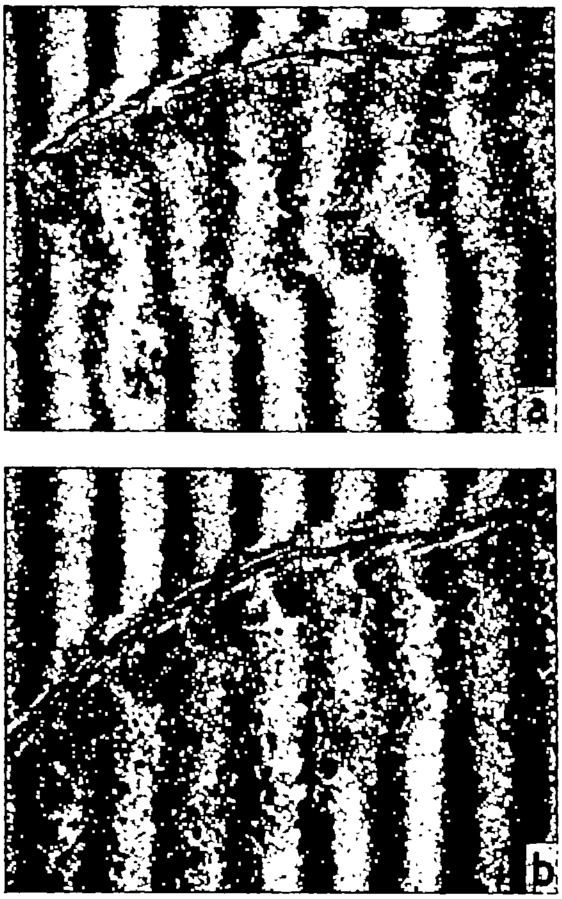

Fig. 1. Section of wheat grain, 30µ thick, with interference fringes. *a*, Before, *b*, after, drying

With experiments, in which sections of wheat and wheat meal are exposed to air containing different amounts of

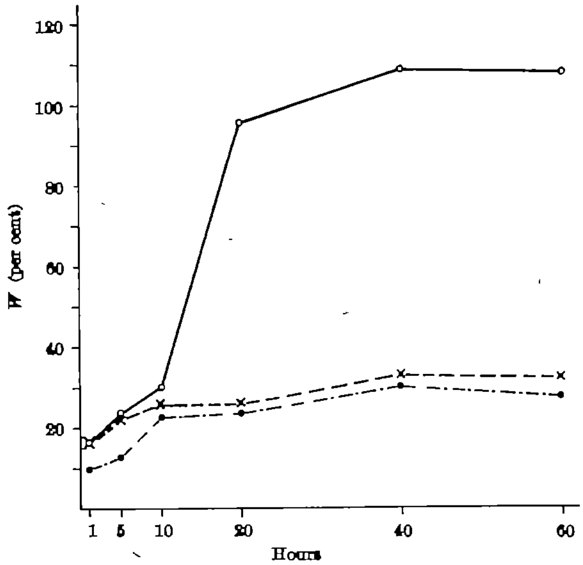

Fig. 2. Uptake of water in embryo (scutellum (○), aleuron layer (×) and starchy endosperm (●)) of Ramona wheat grains. Water content W as percentage of dry weight. Each point represents the average value of determinations in 36 sections

moisture and then checked on water content with the interference microscope or by weighing, respectively, a good proportionality between $\varphi_1-\varphi_2$ and m_w could be shown.

The accuracy of the method depends mainly on the accuracy in determination of the shift of the interference fringes, which in turn depends on the evenness of the sections. In good sections the fringes can be estimated to 0·1–0·2 fringes distance, corresponding to a water content of 1–2 per cent, nearly independent of the water content up to 30 per cent in the endosperm and 120 per cent in the germ. This fringe distance also determines the lower limit of detectable water.

In Fig. 2 the rate of uptake of water of different tissues in Ramona wheat grains is shown. The grains have been exposed to air saturated with water at 20° C. The water content is always highest in the germ (measured in the scutellum). Until about 10 h the water content in the aleuron layer is higher than in the starchy endosperm. After that time the water content of the aleuron and starchy parts approach the same asymptote. The distribution of water within this tissue is nearly equal throughout the whole grain.

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Possible Relationship between Photosynthesis and the Rise in Respiration in Diseased Leaves

Several investigations, most recently reviewed by Millerd and Scott[1], have established that the respiratory rate is increased in the leaves of green plants after their infection with parasitic fungi. The pathway of this increased respiration is not definitely established, but experiments[2,3] have shown that the C_6/C_1 ratio is decreased in infected leaves, indicating that the hexose monophosphate shunt is enhanced. The inadequacies of the C_6/C_1 ratio for determining the magnitude of operation of the shunt have recently been stressed[4].

The cause of this increase in respiration is unknown. However, it was observed[5] that there was little or no increase in the respiration of etiolated barley leaves after their infection with *Erysiphe graminis* var. *hordei* (powdery mildew) although respiration increased as much as 300–400 per cent in infected green barley leaves. A possible explanation of this observation is that the increased respiratory rate is related to changes in photosynthesis.

Table 1. Rates of Photosynthesis in Leaf Segments, Chlorophyll Content and Activities of Aldolase and NADPH-diaphorase in Homogenates of Non-infected and Infected Green Leaves of Barley 144 h after Inoculation with *E. graminis* var. *hordei*

Tissue	Photosynthesis (μl. O_2 evolved/100 mg fresh weight/h)	Chlorophyll content (mg chlorophyll/g fresh weight)	Enzymatic activities (μmoles substrate utilized/min/g fresh weight)	
			Aldolase	NADPH-diaphorase
Non-infected	1,092	1·96	5·00	4·0
Infected	480	0·72	0·96	2·5

To determine whether the rate of photosynthesis as well as the rate of respiration changes in infected green leaves, the rates of both these processes were measured in infected and non-infected leaves of a barley variety highly susceptible to *E. graminis* var. *hordei*. At the same time, the activities of various enzymes catalysing both photosynthetic and respiratory reactions were compared in homogenates of green leaves. Further, the activities of various respiratory enzymes were measured in homogenates of infected and non-infected etiolated barley leaves.

Seeds of barley, variety Manchuria, highly susceptible to infection by *E. graminis* var. *hordei* were sown in pots of soil in a controlled growth room. The temperature was 70 ± 2° F. The green plants were illuminated with 20,000 lux of white light for 16 h/day. Five days after sowing when the first leaf was about 2 in. high some of the plants were inoculated with spores of *E. graminis* var. *hordei*[6]. At various times thereafter both inoculated and non-inoculated leaves were harvested for determination of photosynthetic and respiratory rates and enzymatic activities. Photosynthesis by leaf segments was determined manometrically by measuring oxygen production during illumination at 25° C. The concentration of carbon dioxide was maintained at 1 per cent by the use of a 'carbon dioxide-buffer'[7]. Respiratory rates of leaf segments were determined manometrically by measuring oxygen consumption in the dark. Leaf extracts for enzymatic assays were prepared as described elsewhere[8]. The following enzymes were assayed in extracts of barley leaves by continuously recording the progress of the reaction using a Cary spectrophotometer, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, enolase, aldolase, NADPH-diaphorase, NADH-cytochrome c reductase.

Measurements of photosynthetic rates, chlorophyll contents, aldolase and NADPH-diaphorase activities in non-infected and infected barley leaves 144 h after inoculation are given in Table 1. At this time not only is the over-all rate of photosynthesis in infected leaf segments decreased when compared with non-infected leaves, but also the chlorophyll content, and the activities of aldolase and NADPH-diaphorase. In photosynthetic cells most of the aldolase and NADPH-diaphorase activities are localized in the chloroplasts[9,10]. These observations show that there is a destruction of the chloroplast in infected leaves.

Table 2 gives results obtained for the rates of respiration and the activities of various respiratory enzymes of green barley leaves 144 h after inoculation. In the infected leaves the respiratory rate is about three times higher than in non-infected leaves. The activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are considerably higher in homogenates of

Table 2. Rates of Respiration in Leaf Segments, and Enzymatic Activities in Homogenates of Non-infected and Infected Green Leaves of Barley 144 h after Inoculation with *E. graminis* var. *hordei*

Tissue	Respiration (μl. O_2 consumed/100 mg fresh weight/h)	Enzymatic activities (μmoles substrate utilized/min/g fresh weight)				
		Glucose-6-phosphate dehydrogenase	6-phosphogluconate dehydrogenase	Enolase	Isocitrate dehydrogenase	NADH-cytochrome c reductase
Non-infected	20	0·46	0·28	0·84	1·02	0·65
Infected	73	1·00	0·42	0·87	1·01	0·51

Table 3. Rates of Respiration in Leaf Segments, and Enzymatic Activities in Homogenates of Non-infected and Infected Etiolated Barley Leaves 144 h after Inoculation with *E. graminis* var. *hordei*

Tissue	Respiration (μl. O_2 consumed/100 mg fresh weight/h)	Enzymatic activities in leaf homogenates (μmoles substrate utilized/min/g fresh weight)					
		Glucose-6-phosphate dehydrogenase	6-phosphogluconate dehydrogenase	Aldolase	Enolase	Isocitrate dehydrogenase	NADH-cytochrome c reductase
Non-infected	12	0·290	0·21	1·68	0·69	0·31	0·021
Infected	14	0·270	0·18	1·36	0·65	0·30	0·027

infected leaves compared with non-infected ones. On the other hand, there is no change in enolase, isocitrate dehydrogenase and NADH-cytochrome *c* reductase activities in the infected leaves. These findings indicate that the increased respiration in these diseased leaves is mediated through the hexose monophosphate shunt, rather than through the Krebs cycle. Time-course experiments disclosed that the onset of these respiratory changes coincided with the decrease in photosynthetic activity.

The results with green leaves may be contrasted with those obtained with barley leaves which were kept in continuous darkness to suppress the development of a photosynthetic system. Although the growth of the infecting fungus on these leaves appears to be normal, the infected leaves do not show a significant rise in respiration nor an increase in the activities of enzymes of the hexose monophosphate shunt when compared with non-infected leaves (Table 3).

These experiments are consistent with the hypothesis that the hexose monophosphate shunt plays a major part in mediating the increased respiratory rate of green barley leaves infected with *E. graminis* var. *hordei*. The fact that this increase in respiration is evident only when photosynthesis is decreasing, coupled with the observation that respiration is not increased in infected etiolated leaves, suggests that in this highly susceptible variety the decrease in photosynthesis and respiratory rise are related. Since both photosynthesis and the hexose monophosphate shunt are major metabolic sources of NADPH, one possibility is that the increase in respiratory activity is tied to a loss of capacity to photoreduce NADP+.

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Concentration of Carbon Dioxide inside Leaves

DURING photosynthesis, the concentration of carbon dioxide in the intercellular spaces of a leaf, $[CO_2]_{int}$, determines the flux of carbon dioxide into the leaf if stomatal apertures and external concentration remain constant. Previously $[CO_2]_{int}$ has not been estimated during photosynthesis but has been equated with the external concentration at which photosynthesis ceased. Our object was to estimate $[CO_2]_{int}$ during active photosynthesis. Gabrielsen[1] found the minimum to which many plants would reduce the concentration of carbon dioxide in a closed system to be 100 p.p.m. by volume and concluded that $[CO_2]_{int}$ was also 100 p.p.m. Heath[2] arrived at the same conclusion. He drew air through leaves with porometers, varied the flow-rate and carbon dioxide concentration in the incoming air, and measured the carbon dioxide in the air leaving the leaf. Regardless of the rate of flow of air or concentration of carbon dioxide, the exhausted air contained about 100 p.p.m. carbon dioxide. Similar results were obtained by passing air over leaves at slow flow-rates.

The minimum concentration of carbon dioxide in a closed system or in a porometer system as used by Heath gives the concentration inside the leaf only when there is no net photosynthesis. This seldom occurs in normal outdoor conditions.

Carbon dioxide moves from the atmosphere to the surface of cells inside the leaf by turbulent transfer and diffusion to the leaf surface and by diffusion alone through the stomata and substomatal cavities to the internal cell surface. The process can be described by the equation:

$$P = \frac{[CO_2]_e - [CO_2]_{int}}{r_{a,CO_2} + r_{s,CO_2}} \qquad (1)$$

where P is the net photosynthesis or uptake of carbon dioxide, $[CO_2]_e$ and $[CO_2]_{int}$ are the concentrations of carbon dioxide (g/cm^3) in the external air and at the internal cell surface, r_{a,CO_2} is the resistance of external air, and r_{s,CO_2} is the resistance of the stomata and internal air to flow of carbon dioxide. We emphasize that $[CO_2]_{int}$ is at the surface of the cells inside the leaves, not at the site of carbon dioxide utilization.

Water is transpired from cell surfaces inside the leaf and escapes along the same path which carbon dioxide travels as it enters. Transpiration can be described by a similar equation:

$$T = \frac{[H_2O]_{int} - [H_2O]_e}{r_{a,H_2O} + r_{s,H_2O}} \qquad (2)$$

where T is transpiration, $[H_2O]_{int}$ and $[H_2O]_e$ are concentrations of water vapour (g/cm^3) in the air at the internal cell surface and in the external atmosphere, and r_{a,H_2O} and r_{s,H_2O} are the resistances of air and stomata to water vapour movement. Gaastra[3] found the ratio of $\frac{r_{a,CO_2}}{r_{a,H_2O}}$ in a system that included turbulent air to be about the same as $\frac{D_{H_2O}}{D_{CO_2}}$, the ratio of the diffusion coefficients for water and carbon dioxide in air. Since the diffusion path-lengths through the stomata are identical for water and carbon dioxide it can be shown that $\frac{r_{a,CO_2} + r_{s,CO_2}}{r_{a,H_2O} + r_{s,H_2O}}$ also equals $\frac{D_{H_2O}}{D_{CO_2}}$. Therefore, solving equations 1 and 2 simultaneously for $[CO_2]_{int}$ yields:

$$[CO_2]_{int} = [CO_2]_e - \left(\frac{P}{T}\right)\left(\frac{D_{H_2O}}{D_{CO_2}}\right)([H_2O]_{int} - [H_2O]_e) \qquad (3)$$

The right-hand terms of equation 3 were measured with corn plants actively assimilating carbon dioxide. Thus we could estimate $[CO_2]_{int}$ during active photosynthesis. The plants were in a 40 cm × 40 cm × 50 cm transparent chamber. This chamber was illuminated by seven 300-W incandescent spot lamps, partially immersed in water to remove sensible heat and infra-red radiation. The $[CO_2]_e$ was measured by means of an infra-red gas analyser coupled with an adjustable range, adjustable zero recording potentiometer. Whenever $[CO_2]_e$ fell to a pre-set level, measured quantities of carbon dioxide were automatically metered into the chamber to maintain the desired concentration ± 10 p.p.m. The meter values gave P. The $[H_2O]_e$ was measured by means of an electrical hygrometer and controlled at $6 \cdot 2 \times 10^{-6}$ g/cm^3 within $\pm 0 \cdot 1 \times 10^{-6}$ g/cm^3 by condensing water on a cold coil. The condensed water was measured and gave T. The temperature of the leaf was measured by means of a small thermocouple inserted in the leaf and a temperature of 20° ± 0·1° C was controlled indirectly by heating the

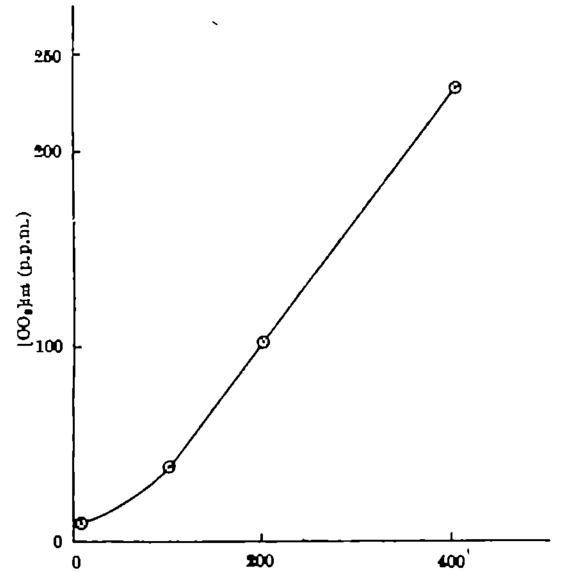

Fig. 1. Concentration of carbon dioxide at the internal cell surface, $[CO_2]_{int}$, as a function of the concentration in the atmosphere, $[CO_2]_a$

air. The $[H_2O]_{int}$ was assumed equal to the saturation vapour concentration at leaf temperature[3].

The illumination on a horizontal plane was 10,000 ft.-candles at the top and 6,500 ft.-candles at the bottom of the corn shoots. The concentration of carbon dioxide in the atmosphere, $[CO_2]_a$, was maintained at 8, 100, 200 and 400 p.p.m. and T and P were measured at each concentration. $[CO_2]_{int}$ was calculated and is shown in Fig. 1. Each point in Fig. 1 was calculated from the average of six consecutive 10-min determinations of T and P after a steady-state was reached.

As expected, the concentration of carbon dioxide inside the leaf increased as the concentration in the atmosphere increased. However, the increase in $[CO_2]_{int}$ was not directly proportional with increasing $[CO_2]_a$ because stomatal resistance changed with changes in carbon dioxide concentration. Measurements of the stomatal apertures were taken by means of silicone rubber replicas[4]. At 8 p.p.m., 100 per cent of the stomata had an aperture-width greater than 1μ. At 100 p.p.m., 70 per cent; at 200 p.p.m., 26 per cent; and at 400 p.p.m., only 12 per cent had apertures that large. In Fig. 1, $[CO_2]_{int}$ varied from 8 to 240 p.p.m. as $[CO_2]_a$ changed from 8 to 400 p.p.m.

Thus the concentration of carbon dioxide inside leaves is determined by the concentration difference and a series of resistances in the pathway from the atmosphere to the point of enzymatic fixation. Since both concentrations and resistances at different points in the pathway change, it is unrealistic to speak of a single concentration of carbon dioxide inside a leaf during photosynthesis, except under carefully specified conditions. In a steady-state, however, the concentration at the internal cell surfaces can be determined experimentally by a simultaneous measurement of photosynthesis and transpiration; or in the special case, when photosynthesis ceases because of low concentration of carbon dioxide, the concentration inside and outside the leaf will be the same.

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Niacin Content of Coffee

In a recent communication[1] A. Carvalho quoted some results of determinations of niacin in samples of coffee beans, which indicated variations in niacin content according to whether the coffee was grown with or without shade, and according to whether the 'cherries' were sun-dried or de-pulped before drying.

These results, which vary from 0·160 to 0·400 mg/g, are of the same order as the previously published values for roasted coffee[2-5], and Carvalho has since informed me that they were in fact made after the samples had been roasted in a small electric roaster, to an intensity a little less than that of a type called Italian roast (namely, a high roast). Due to an omission this point was not made clear in his letter.

The production of nicotinic acid (namely, niacin) by the decomposition of trigonelline during the roasting of coffee has been demonstrated[3], the nicotinic acid content thereby increasing to up to 10 times the amount present in the raw beans. The effects of roasting conditions on niacin content of roasted coffee therefore far outweigh variations in niacin content of the raw beans, and the trigonelline content of the raw beans appears to be a deciding factor. It would therefore be interesting to know of the effects of growing and processing conditions on the trigonelline content of coffee beans.

Similar considerations might also apply to the production of pyridine (one of the constituents of coffee aroma), which also is formed by the decomposition of trigonelline during roasting[6]. The effects of growing and processing conditions on trigonelline content would be expected to have a bearing on flavour differences in roasted coffee.

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Fluorescent Lighting in Biological Research

In view of the prevalent use of fluorescent lighting in many types of biological research (for example, refs. 1 and 2), it is felt that the following observations may be of general interest.

Fluorescent tubes contain mercury vapour; the short-wave-length discharge induces a visible radiation from the phosphor coating of the tube walls. During research on another topic[3], we observed emission from a typical daylight tube at 313, 334 and 366 mμ as well as in the visible spectrum. It was further shown that the glass of these tubes, 1 mm in thickness, transmitted over 50 per cent of the incident radiation at 310 mμ.

It has been shown that ultra-violet of 313 mμ is mutagenic in *Drosophila*[4] and it is generally known that light of this wave-length causes damage to tissue: while the ultra-violet incident on a surface more than 1 m from a 40-W tube is far less than that from summer noon sunlight, it is worth noting that it is common to place the fluorescent tube inside the cage or incubator and to leave it running continuously. This may seriously affect experimental results.

Most of the tubes also radiate strongly at 405 and 436 mμ and while this radiation may not be dangerous to living material, it may accelerate degradation and bleaching of various of the more labile animal pigments; this may be of significance when it is considered that the radiation from most types of daylight fluorescent tubes is at least five times and may be over ten times that from a 100 W tungsten lamp at 400 mμ for the same radiation at 540 mμ. Museum curators may find this to be of interest.

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MICROBIOLOGY

Bacterial Growth at Low Population Densities (II)

In a previous communication[1], the possible influence of low population densities on the growth of *Spirillum serpens* under steady-state conditions (lactate growth limiting) was reported. Qualitative evidence was given by the fact that the inhibition of growth at low concentrations of the limiting nutrient entering the chemostat was partly removed by lowering the concentration of dissolved O_2 or the addition of ascorbic acid to the medium. It was suggested that the reducing power of the cells becomes growth limiting in the system when the population density falls below a certain value. Additional data have been collected to substantiate this suggestion.

During the steady-state, the population density (x) is a function of the concentration of the limiting nutrient entering the chemostat (s_r): $x = y\,(s_r - s)$, where y is the yield coefficient and s the concentration of the limiting nutrient in the chemostat[2]. After direct determination of x (ref. 2) and s (with DPN and lactate dehydrogenase[3]), the decrease of growth observed in the presence of a decreasing s_r was calculated at the expense of the yield coefficient: $y = \frac{x}{s_r - s}$. The maximum growth rate (μ_{max}) and the saturation constant (K_s) were the same as given in ref. 2. The dilution rate (D) was 0·5 μ_{max}.

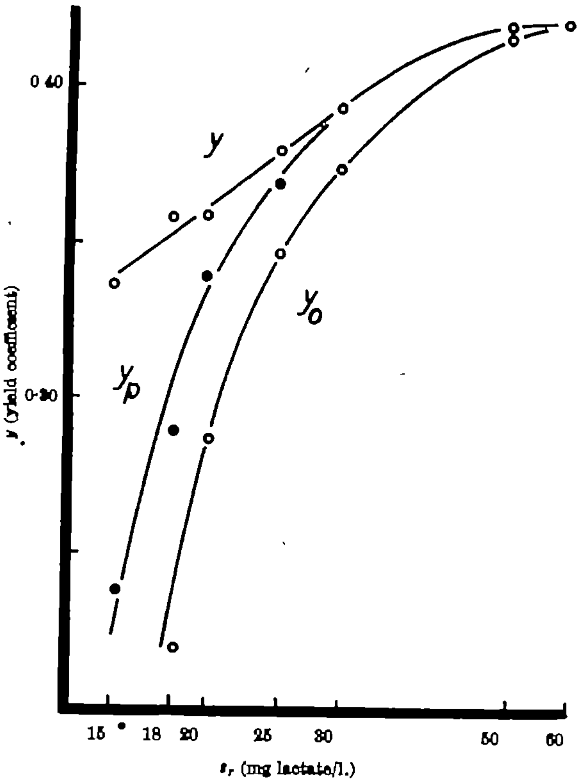

Fig. 1. Yield coefficient (y, y_o, y_p) plotted against the concentration of the growth-limiting nutrient entering a steady-state culture (chemostat) of *Spirillum serpens*

In Fig. 1, y_0 has been calculated on assumption that s stays constant at the given dilution rate ($s = 2{\cdot}2$ mg lactate/l.). Direct determination, however, showed a definite increase of s when s_r was decreasing below 50 mg lactate/l. This apparent waste of the limiting nutrient suggests an inhibiting effect upon the uptake and invalidates the definition of $s = K_s \frac{D}{\mu_{max} - D}$ (ref. 1) at low values of s_r in the present case. If the actual data of s are used in the calculation, y shows a slower decline as compared with y_0 (Fig. 1).

Repeating the experiment after the addition of 0·002 per cent ascorbic acid to the medium flowing into the chemostat, s was observed to increase more slowly. In other words, by supplying a reducing agent, the apparent waste of the limiting nutrient was partly prevented. Calculation of the yield coefficient in this case (y_p in Fig 1) gave a line almost parallel to y_0. Lowering the concentration of oxygen in the aerating gas mixture had a similar effect.

That part of the growth-inhibiting effect which is attributed to a decreasing reduction power of the population at low densities does not occur before s_r decreased below 30 mg lactate/l. The difference between the curves of y_0 and y_p demonstrates the remaining part of the growth-inhibiting effect which is not explained by a population phenomenon. In general, sub-optimal growth conditions result in a decrease of the yield coefficient at low population densities due to bacterial action on the environment. This effect is independent of a yield-affecting endogenous respiration or maintenance metabolism.

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Lysogeny in *Alcaligenes faecalis* and the Host-range of *A. faecalis* Bacteriophages

Eighty-six strains of *Alcaligenes faecalis* were investigated. Five of these were N.C.T.C. cultures. The remaining strains were isolated from faeces and correspond to the description in Bergey's *Manual of Determinative Bacteriology*[1]. Media adopted were those previously[2] used. Lysogeny was investigated by growing organisms singly and in mixtures in broth for 10 days and by ultra-violet induction. These methods have been described[2]. Thirty of the organisms proved lysogenic for one or more of the 86 strains. Thirteen strains were inducible by ultra-violet light. Moore and Pickett[3] investigated 40 strains of *A. faecalis*-like organisms and found 3 of them to be lysogenic for other members of the group.

Attempts were made to isolate phages active on *A. faecalis* from sewage by the enrichment technique of Adams[4]. Twenty-three phages were isolated in this manner. Phages were purified by repeated single plaque isolations. Lysates were stable when stored above 0·1 vol. of chloroform.

The host ranges of the 53 phages were investigated by spotting drops of lysates (plaque-forming titres about 10^8/ml.) on freshly poured lawns of the 86 strains of

A. faecalis and 110 Providence and *Proteus* cultures previously[5] used. A large number of *Escherichia coli* and *Pseudomonas aeruginosa* as well as *Shigella* and *Salmonella* spp. were also used in these experiments. Apart from their action on *A. faecalis* which enabled all 53 phages to be differentiated from one another, 5 of these phages productively lysed one or more of 4 *Proteus vulgaris* strains, 14 *P. mirabilis*, 2 *P. rettgeri* and 6 *Providence* strains. Two of these 5 phages were isolated from lysogenic strains of *A. faecalis* and lyse *P. rettgeri* N.C.T.C. 7480 and 7481 and *Providence* strain N.C.T.C. 9269. The remaining 3 phages (isolated from sewage) each lyse at least one strain each of *P. rettgeri*, *P. vulgaris*, *P. mirabilis* and *Providence*.

Organisms able to support the growth of a particular bacteriophage are considered[4] to be closely related and these results support previous findings[6] of relatedness between members of the *Proteus* group and between the latter and *Providence* organisms. With certain *Pasteurella pestis* phages[7] these 5 *A. faecalis* phages share the distinction of being able to attack members of two families.

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Interspecific Recombination in *Streptomyces*

GENETIC recombination of Streptomycetes was first reported by Sermonti and Spada-Sermonti in 1955[1]. By crossing different nutritional mutants of *Streptomyces coelicolor* various auxotrophic and prototrophic types have been obtained.

Several authors have obtained true recombination by crossing different mutant strains of *S. coelicolor*[2]. Moreover, recombination of different nutritional mutants of the same species have been reported for *S. rimosus*[3], *S. fradiae*[4], and *S. griseoflavus*[5].

In this work recombination experiments have been carried out with mutants of the following species of *Streptomyces*: *S. rimosus* A.T.C.C. 10910, *S. aureofaciens* A.T.C.C. 10762 and *S. coelicolor* I.S.S.

Nutritional mutants of *S. rimosus* and *S. aureofaciens* were obtained by ultra-violet radiation using the replica plating technique. Mutants of *S. coelicolor* were kindly supplied by Prof. G. Sermonti, Istituto Superiore di Sanità, Rome. Nutritional mutants were used because of their stability and suitability for recombination.

Two parental strains have been inoculated together on slants of complete medium, and incubated four days at 30° C. Collected conidia were suspended in sterile water, filtered through a cotton filter in order to remove hyphal fragments and clusters of conidia, and seeded on selective media.

The number of recombinant colonies recovered in various experiments is reported in Table 1.

Recombination has been obtained in all the interspecific crosses examined, namely, *S. rimosus* × *S. coelicolor*, *S. rimosus* × *S. aureofaciens* and *S. aureofaciens* × *S. coelicolor*. Recombination rates ranged from about 10^{-6} to 10^{-4} according to the various strains used and the selective plating media.

Considerable changes in pigmentations of recombinants were observed in the progeny of crosses *S. rimosus* × *S. coelicolor*, ranging from cream-white colour (*S. rimosus*) through grey, pink and red, to strong blue pigment (*S. coelicolor*), more intense than the normal colour of this species.

Table 1. INTERSPECIFIC RECOMBINATION IN *Streptomyces*

Crosses	No. of spores plated per dish	Supplements to minimal medium	No. of recombinants recovered*
S. rimosus × *S. coelicolor*			
*M*1 arg × *M*10 his	12×10^6	—	(12)
		HIS, STR	(17)
*M*1 arg × *M*15 arg	4×10^6	—	9 (45)
*M*1 arg × *M*16 arg	5×10^6	—	7 (80)
*M*3 nic × *M*13 met phe str	2×10^6	—	(5)
		MET	(6)
*M*8 arg his × *M*13 met phe str	5×10^6	—	20
		HIS	21
		HIS, PHE	24
		ARG	528
		ARG, MET	532
*M*8 arg × *M*17 met arg ade	$4{\cdot}5 \times 10^6$	—	1 (114)
		MET, ARG	1 (128)
		HIS, ADE	2 (140)
S. rimosus × *S. aureofaciens*			
*M*4 his × *M*6 met	5×10^6	—	0
*M*9 his × *M*6 met	$2{\cdot}5 \times 10^6$	—	6
S. aureofaciens × *S. coelicolor*			
*M*5 his × *M*10 his	$4{\cdot}5 \times 10^6$	—	0
*M*5 his × *M*13 met phe str	1×10^6	—	20
		PHE	24
		MET	28

ARG, arginine; HIS, histidine; ADE, adenine; PHE, phenylalanine; MET, methionine; NIC, nicotinic acid; STR, streptomycin. The same symbols in small letters indicate mutant alleles for requirement of the growth factor, or for resistance to the drug (str).

* Mean values from 10 consecutive assays, each on 10 plates. The numbers in parentheses are small colonies.

Arginineless recombinants were isolated from the combination *S. rimosus* *M*8 arg his × *S. coelicolor* *M*13 met phe str. Prototrophic recombinants were obtained from one of three isolated strains crossed with parent *M*13.

The relevance of the interspecific crosses in *Streptomyces* from the taxonomic, as well as from the biochemical point of view, needs no emphasis.

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A Medium for the Isolation of *Streptococcus faecalis, sensu strictu*

DURING the course of an ecological survey of the fæcal streptococci, the need has arisen for a medium which is more highly selective for *Streptococcus faecalis* than media devised for the enumeration of enterococci in general. A number of workers[1–4] have indicated that *Strep. faecalis* is the predominant streptococcus (Lancefield group *D*) of the gut of man, and that this species is relatively rare in the gut of animals. Hence a specific means of demonstrating this organism would be of some value to sanitarians as a means of assessing human fæcal pollution, at least in certain climatic regions[5].

Slanetz and Bartley have recommended an agar medium which is capable of recovering larger numbers of types of enterococci than media formerly used, and the efficiency of this medium is under investigation. It is claimed[6] that strains of *Strep. faecalis* may be distinguished from other enterococci, when grown on Slanetz and Bartley's medium, by the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to the formazan, thus producing dark maroon colonies. Barnes[7] has shown that the reduction of TTC is reliable, however, only at pH 6·0–6·2 and not at pH 7·2 as in Slanetz and Bartley's medium.

In the present work, 201 strains of enterococci, isolated on Slanetz and Bartley's medium from sewage effluent and river water, were classified biochemically by conventional methods. More than a quarter of the 201 strains produced dark maroon colonies; but 41 per cent of this fraction were not 'typical' *Strep. faecalis*, as expected, but unidentifiable intermediate strains. Conversely, a number of colonies which were not maroon in colour were identified as *Strep. faecalis*. It appears, therefore, that a reliable quantitative determination of this organism cannot be obtained with this medium.

Central to the problem is the exclusion of the numerous intermediate types which apparently are not of critical significance in demonstrating human faecal pollution. An attempt has been made to formulate a medium which will considerably reduce the number of 'intermediates' and facilitate the recognition of *Strep. faecalis, sensu stricto*.

It was decided that the only satisfactory medium would be one that would select strains possessing several characteristics of the organism, and not merely the one factor of TTC reduction. An agar medium incorporating four characteristics was devised, as follows:

(1) *Ability to grow at 45° C following 4 h resuscitation at 37° C.* This technique has been successfully used by Burman[8] with Slanetz and Bartley's medium to eliminate many of the atypical strains encountered in water.

(2) *Ability to ferment sorbitol.* Kjellander[9] has shown that this characteristic is also apparent in a large number of human-derived enterococci, but is encountered more rarely in animal-derived strains.

(3) *Ability to decompose tyrosine.* This is another characteristic of the species, particularly of human strains, but in the medium described here the test is performed in a different manner from that originally devised by Sharpe[10].

(4) *Reduction of TTC at pH 6·2.* See above.

The medium has the following composition: peptone (Evans) 10 g; 'Yeastrel' 1 g; sorbitol 2 g; tyrosine 5 g; Oxoid agar 12 g. These constituents are dissolved in 1 litre of distilled water and autoclaved at 10 lb. for 10 min. The pH of the medium is then adjusted to 6·2 and 0·01 per cent TTC and 1 per cent thallous acetate added. An additional 4 g of tyrosine are also added while still hot to provide a suspension, so that decomposition of tyrosine by a colony will result in the formation of a clear zone surrounding it. It is important to note that autoclaving is required to dissolve some of the tyrosine completely, while the remainder forms the suspension. No other sterilization is necessary. Plates are poured when the temperature of the medium is near the setting point so that a uniform suspension of the undissolved tyrosine is obtained. A shallow layer of the medium without added tyrosine is poured first and allowed to set. This is followed by a layer of medium with the added tyrosine. The period of incubation is three days at 45° C after 4 h at 37° C. Only uniformly dark maroon colonies, encircled by clear zones, are regarded as 'typical' (Fig. 1). The medium as devised may be used in conjunction with membrane filters, though in this instance the membrane is placed face downwards on the surface of the agar after filtration.

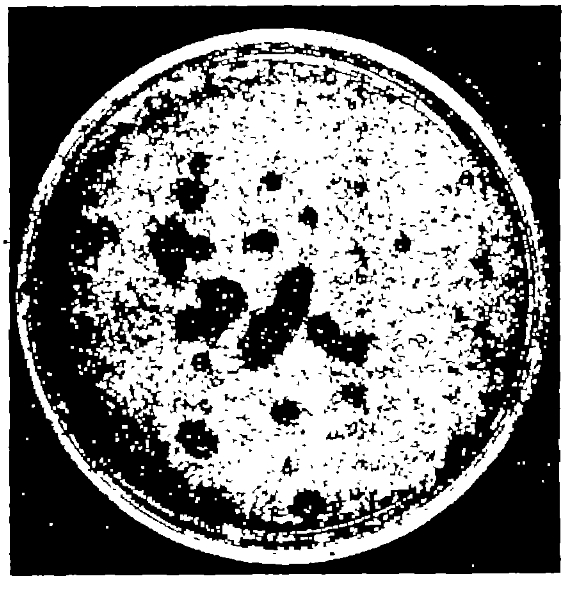

Fig. 1. A pure culture of *Strep. faecalis* on sorbitol/tyrosine/TTC medium. The zones of clearing appear as dark areas

The medium has been used successfully for the primary isolation of *Strep. faecalis* both from faeces and from water. Particular attention has been paid to strains of enterococci other than *Strep. faecalis* which may give a false 'positive' result when grown on this medium. Of the 201 strains of enterococci isolated on Slanetz and Bartley's medium, 26 strains which fermented sorbitol were not identifiable as *Strep. faecalis*. From these 26 strains, only three also produced dark maroon colonies, of which two did not attack tyrosine. Hence the proportion of false positives obtainable by the method described here, using the sorbitol/tyrosine/TTC medium, can be expected to be relatively low. An analysis of the faeces of eight animal species by direct plating on this medium revealed only two strains which gave false positive results.

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GENETICS

Multiple Allelic Control of Chlorophyll *b* Level in *Arabidopsis thaliana*

IN maize, the plant best known genetically, there are no viable chlorophyll *b* deficient mutants[1,2]. Röbbelen[3] described an *Arabidopsis* mutant devoid of this pigment which was, however, a lethal one. It has been reported that in barley only chlorophyll *a* is necessary for viability[4]. Among algae viable chlorophyll *b*-less mutants are not infrequent[5]. Since chlorophyll *b* is a normal constituent of the photosynthetic apparatus of all angiosperms and its precise role is little understood, viable mutants involved in the development of this pigment are of interest.

In our X-radiation experiments we obtained a few yellow-green mutants with reduced amount of chlorophyll *b*. Genetic tests have shown these to comprise a series of alleles. The locus (*ch*) has been assigned to our fourth linkage group with the following gene sequence: *gi, xv, P, ch, pa*. These *ch* mutants show fairly normal fertility and Mendelian inheritance. Detection of the chlorophyll *b* deficiency is easy. Good ascending paper-chromatograms can be developed (solvent: 10 vol.

petrol ether–1 vol. acetone) in 15–20 min at 5° C (Fig. 1) even from tissues weighing only a few milligrams.

Two of the alleles are of special interest: ch^1, which does not allow the production of any detectable amount (paper-chromatography, sugar-column chromatography, spectrophotometry) of chlorophyll *b*, and ch^2, which determines a reduced amount of this pigment. The phenotype of the mutants can be characterized by growth and pigment-content data (Tables 1 and 2). The size of the yellow-green plants is considerably reduced in comparison to that of the wild-type, but there is no difficulty in growing the mutants under the usual culture conditions. The appearance of these mutants (except for colour) is quite normal (Fig. 2).

When light intensity is reduced, the pigment content increases (on tissue weight basis), but still there is no chlorophyll *b* in ch^1. The percentage increase of the latter pigment in ch^2 is comparable with that in the wild-type. Apparently the reduction of the amount of chlorophyll *b* in *Arabidopsis* is not due to light destruction as observed in algal mutants by Allen[5].

Attempts to restore the normal chlorophyll *b* level by feeding the mutants on an aseptic agar medium supplemented with a variety of substances were unsuccessful. On irradiation or treatment with chemical mutagens of plants heterozygous for the locus, numerous *ch* sectors appear. These could be due to deletion of the critical segment of the wild chromosome, to mutation at the locus, or to non-disjunction. The margin of the genetically different tissues is clearly demarcated, showing that the immediate precursor cannot diffuse from the wild-type sector to the mutant.

Fig. 1 Contact print of a paper chromatogram of ch^1, ch^2 mutants and wild-type; *a*, chlorophyll *a*; *b*, chlorophyll *b*

Fig. 2. Phenotype of wild-type and mutants

Table 1. Some quantitative characteristics of wild-type and two *ch* mutants grown in the greenhouse, exposed to sunlight during the day and illuminated with 200-watt incandescent bulbs during the night. Temperature, 20°–25° C. Light intensity, 400–500 ft.-candles.

	Fresh weight (mg)	Dry weight (mg)	Length of stem (cm)	No. of buds, flowers and fruits
Wild	314·00	30·56	23·70	59·10
ch^2	60·13	7·13	10·19	18·80
ch^1	25·65	2·96	6·25	10·00

Table 2. Pigment content of wild-type and two *ch* mutants grown under two different light intensities (pigment γ/fresh weight g analysed by the technique of Röbbelen (ref. 3))

	Total pigment 1	Chlorophyll *a* 3	Chlorophyll *b* 4	Carotenoids 5	Ratios 3 : 4	Ratios (3 + 4) : 5
Strong light*						
Wild	1,184	736	255	193	2·9	5·1
ch^2	748	560	67	121	8·6	5·2
ch^1	508	416	0	92		4·5
Weak light†						
Wild	1,553	986	311	256	3·2	5·1
ch^2	1,058	815	93	150	8·8	6·1
ch^1	811	712	−13‡	99		7·1

* Culture conditions identical with that indicated in Table 1.
† Cultured in a cold room at 20° C and 400 ft.-candles illumination for 8 h/day.
‡ Negative value is due to technical error.

Chlorophyll *a* does not seem to be an immediate precursor of chlorophyll *b* as has been considered possible[6,7], since no accumulation of the former occurs in the mutant. Rather a decrease of chlorophyll *a* is found, which may be an indication of their common origin.

By means of lugol tests it was found that the absence of chlorophyll *b* has no important bearing on starch synthesis in these mutants. Thus Seybold's early suggestion and some other circumstantial evidence (cf. ref. 8) as to the role of this pigment are not applicable here in spite of the fact that some supporting data have been obtained with another *Arabidopsis* mutant[3].

It is possible that chlorophyll *b* has some stabilizing effect on the pigment system such as Allen[5] proposed, since all *ch* mutants are lower in the total amount of pigments.

Our preliminary experiments with intact plants appear to support the suggestion of Arnon *et al.*[9] that chlorophyll *b* is involved in photo-oxidation.

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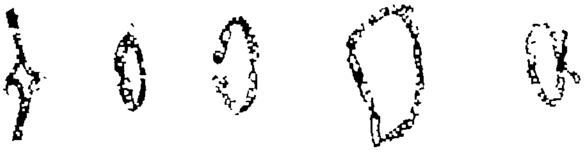
CYTOLOGY

Meiosis in the Male *Periplaneta americana*

CYTOLOGY of the common cockroach *Periplaneta americana* (Blattidae) dates back to 1909[1], and yet recent re-examination has not only brought to light interesting details, but also much controversy[2–4]. It was therefore felt necessary to examine the locally available species of cockroaches with the view of understanding the intricacies of the problem. Absence of diplotene-diakinesis and chiasmata in meiosis in the male has been claimed and various explanations have been marshalled[1,9,10]. But Lewis and John[7,8], taking into consideration all the aspects of indirect evidence, state that chiasmata might occur at the terminal ends of chromosomes and might terminalize rapidly.

Our observations on *Periplaneta americana* collected in and around Mysore City show that at the stage at which prometaphase stretch is operating, we come across open-cross bivalents (Figs. 1, 8, 10), ring-bivalents (Figs. 2, 3, 6, 7), rod-bivalents (Figs. 6, 7, 10), rod-bivalents with two chiasmata in the long arm of the chromosome (Fig. 9) bivalents with sub-terminal association (Fig. 5), tetravalents in the shape of rings (Figs. 4, 6) and open-chains (Fig. 7). These clearly indicate that chiasmata do occur and obviously for a very brief period. The point of special interest here is that the stages Lewis and John[7,8] have seen in the material treated with colchicine are seen in our untreated populations.


1 2 3 4 5

Figs. 1–5. Selected bivalents from different prometaphase figures (1) Open-cross bivalent with a single chiasma; (2) and (3) ring-bivalents; (4) tetravalent in the form of a ring; (5) bivalent with a sub-terminal association

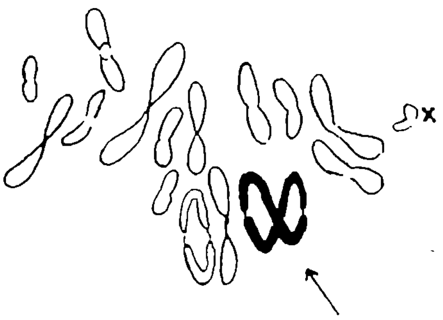


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Fig. 6. Late prometaphase showing a ring-tetravalent with a zigzag orientation (indicated by arrow). *X* is located to one side of the spindle. Except one ring-bivalent, the rest are rod bivalents

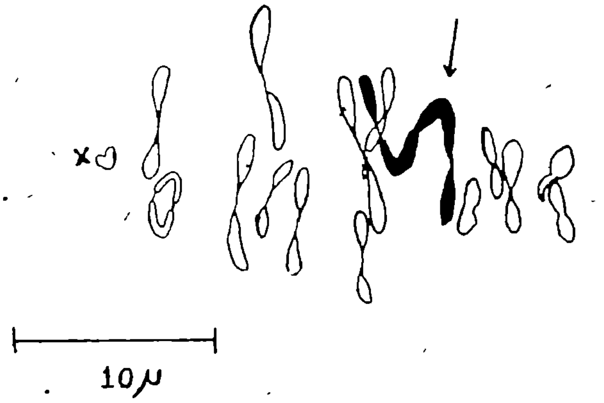

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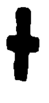
Fig. 7. Same as Fig. 6, except for a chain-tetravalent showing zigzag orientation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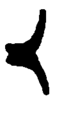
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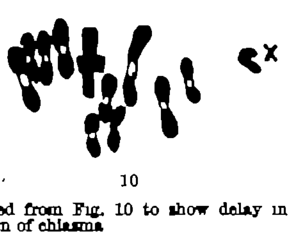

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Fig. 8. Open-cross bivalent selected from Fig. 10 to show delay in terminalization of chiasma

Fig. 9. A bivalent from metaphase I showing two chiasmata in the long arm of the chromosome

Fig. 10. Metaphase I showing an open-cross bivalent. Only 11 bivalents are shown

As to the nature of chromosomes, an examination of the gonial metaphase chromosomes reveals that most of the chromosomes are sub-metacentric but a few are acrocentric (contrary to the observations of Sharma *et al.*[3]). The gonial metaphases show 33 chromosomes ($2n = 32A + X$). Of the 85 spermatogonial plates examined, 3 of them distinctly showed 34, the extra chromosome being the smallest of the complement which perhaps represents a supernumerary chromosome. This becomes clear from an examination of early gonial prophases which show two condensed bodies differing in size: the larger is *X* and the smaller is supernumerary chromosome. There is an appreciable condensation of chromosomes by the end of pachytene. Sharma *et al.*[2,3] do not believe in the progressive condensation of the bivalents, but believe in the abrupt condensation of bivalents at prometaphase. However, in this observation we concur with John and Lewis[4–6]. In metaphase I, the *X* chromosome seems to orient itself on one side of the spindle and always shows 'precession'; but in 2 out of 93 anaphase I stages examined, *X* showed lagging.

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Negatively Stained Lipoprotein Membranes: the Validity of the Image

THE use of negative staining techniques in electron microscopy has recently been extended to the examination of lipoprotein membranes[1–3]. In view of present attempts to interpret data obtained by these techniques in terms of the molecular organization of the intact, hydrated membrane, attention must be directed to X-ray diffraction data which indicate that the dehydration of such lipoprotein systems generally leads to extensive reorganization of lipid and protein components. Freshly isolated preparations of red cell ghosts, mitochondria, and microsomes (unpublished results) do not give any low angle diffraction bands but the dried preparations all show a strong diffraction at about 60 Å, which is suggested to be characteristic of an independent lipid phase.

In the case of nerve myelin the detailed low-angle diffraction pattern from the fresh nerve is interpreted in terms of a single diffracting phase in which the fundamental repeating unit is the intact, hydrated lipoprotein layer, but studies of the effects of air drying[4–6] have revealed the formation of several independently diffracting phases in the dried specimen. These phases have been identified as a residual lipoprotein, cholesterol, and a residual mixed lipid. Although fixatives such as osmium tetroxide and potassium permanganate prevent the separation of independently diffracting units during drying, negative stains such as sodium phosphotungstate do not, and the unfixed, negatively stained lipoprotein preparation is undoubtedly a multi-phase system. This is confirmed by the low-angle diffraction pattern obtained from a saponin-treated preparation of myelin which has been mixed with 1 per cent sodium phosphotungstate at *p*H 7 and air dried. Electron micrographs of the same preparation revealed lamellar fragments which showed a hexagonal distribution of dense spots similar to that demonstrated in negatively stained preparations of saponin-treated red cell ghosts by Dourmashkin *et al.*[1].

Subsequent work[7,8] has shown that a very similar pattern can be obtained from a lecithin–cholesterol mixture or even from cholesterol alone when these have been allowed to interact with saponins. It therefore seems likely that the strikingly regular hexagonal pattern observed in negatively stained preparations of saponin-treated myelin or of saponin-treated red cells is due to a lipid phase, possibly pure cholesterol, which has separated from the lipoprotein structure during drying. This hexagonal phase may, in fact, be formed only during the final stages of dehydration, and the observed dimensions and form of the complexing between saponin and cholesterol may have little direct relevance to the structure of the intact membrane and its modification by saponin. A final assessment of the significance of these electron microscope observations will not be possible until the exact conditions necessary for the formation of this hexagonal phase in cholesterol-containing systems have been established. Meanwhile, it is essential that the electron microscopist who studies lipoprotein systems by negative staining techniques not involving the use of a fixative should be cautious in relating his observations to the intact lipoprotein membrane, for in all probability he is looking at several different 'breakdown products' of the original structure.

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VIROLOGY

Inhibition of *Enterovirus* Ribonucleic Acid Synthesis by 2-(α-Hydroxybenzyl)-benzimidazole

2-(α-HYDROXYBENZYL)-BENZIMIDAZOLE (HBB) is a specific chemical inhibitor of enterovirus reproduction[1,2]. Examination of its mechanism of action has shown that it prevents the production of the infective viral RNA of susceptible viruses[3]. These studies left open the question whether HBB in fact inhibited the synthesis of viral ribopolynucleotide strands, or merely prevented viral RNA from becoming infective. A simple experimental approach to this question was offered by the finding that actinomycin *D* is a specific inhibitor of cellular RNA synthesis, but does not affect viral RNA synthesis[4].

The virus-cell system used consisted of Coxsackie *A*9 virus and primary monolayer cultures of rhesus monkey kidney cells[3]. A coverslip technique[5] was used, and RNA synthesis was measured by incorporation of tritiated uridine, as follows: at intervals, cells were exposed to 1·0 μc. of tritiated uridine (specific activity 6·74 c./m.mole) in the presence of 20 μg of thymidine[6]. Incorporation was stopped after 15 min by fixation of cells in cold acetic alcohol (3 parts ethyl alcohol and 1 part glacial acetic acid). After 2 extractions with 5 per cent perchloric acid at 4° C the samples were washed 5 times with water, once with alcohol-ether (equal parts) and twice with ether. The coverslips were dried at 70° C for at least 2 h, placed on aluminium planchets, and counted in a windowless gas-flow counter.

It was determined in preliminary experiments with varying concentrations of actinomycin *D* that 1 μg/ml. reduced uridine uptake by uninfected cells by 90 per cent in 3–5 h and by 95 per cent in 7–9 h. In investigations with virus-infected cells, treatment with this concentration of actinomycin was begun 2 h before virus inoculation. In a single infectious cycle, with virus collected 8 h after inoculation, actinomycin caused no measurable decrease in virus yield. HBB was used at a concentration of 219 μM. At this concentration, HBB completely inhibits the production of the infective RNA of Coxsackie *A*9 virus and also of infective virus particles, but it has no effect on the uptake of uridine into cellular RNA[3].

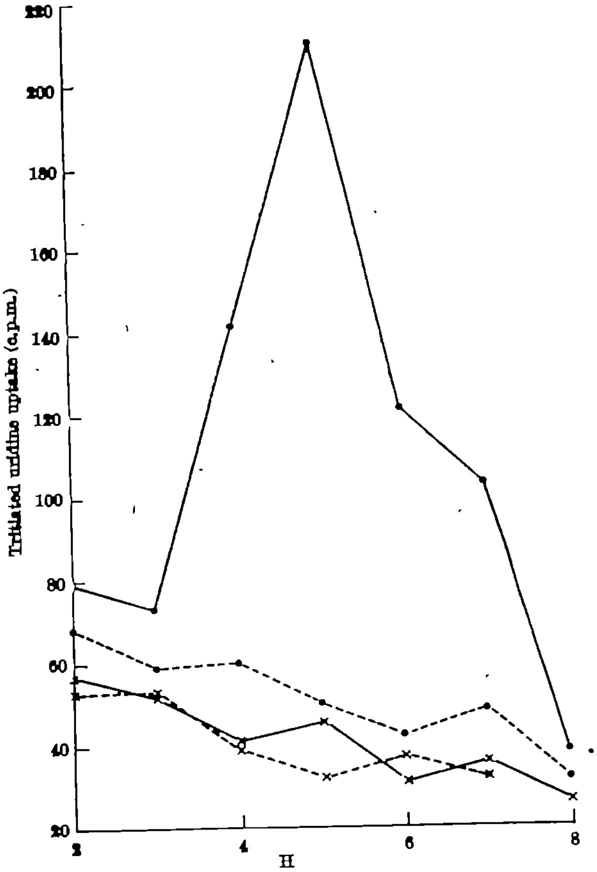

Fig. 1. Coxsackie *A*9 virus-induced stimulation of RNA synthesis in actinomycin-treated monkey kidney cells, and its inhibition by 219 μM 2-(α-hydroxybenzyl)-benzimidazole (HBB). ●—●, Infected; ●---●, uninfected; ×—×, infected + HBB; ×---×, uninfected + HBB

Fig. 1 shows the results of a typical experiment. In actinomycin-treated cells, infection with Coxsackie *A*9 virus induced a marked increase in the uptake of tritiated uridine into acid-insoluble form. The incorporated radioactivity could be removed by treatment of fixed cells with ribonuclease. The virus-induced RNA synthesis was first observed 4 h after inoculation, with a peak at 5 h; it then decreased, and was no longer detectable at 8 h. As can be seen in Fig. 1, 219 μM HBB completely prevented the virus-induced incorporation of tritiated uridine into RNA.

It is possible that not all the RNA synthesized under the control of the virus may have been incorporated into the virus[7]. Nevertheless, the time course of Coxsackie *A*9 virus-induced RNA synthesis, as determined in the present investigation, is similar to that of synthesis of the infective RNA of Coxsackie *A*9 virus[8]. Thus, the results reported here contribute additional support for the view that enterovirus RNA synthesis precedes virus maturation by only a short time[9].

The demonstration that HBB inhibits virus-induced ribopolynucleotide synthesis confirms and clarifies the previous finding of inhibition of infective viral RNA production by HBB.

This work was aided by a grant from The National Foundation.

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Specific Lipids produced by Sugar Beet infected with Yellowing Viruses

AFTER infection with beet yellows virus (SBYV) the older leaves of sugar beet (*Beta vulgaris* L.) become thickened, brittle and yellow, and develop red or brown necrotic spots. Infection with sugar beet mild yellowing virus (SBMYV), a virus unrelated to SBYV, produces an orange-coloured chlorosis of the older leaves with thickening and brittleness but without necrotic spots[1]. This communication is concerned with chromatographic differentiation of these viruses through characteristic lipids extracted from leaves.

Fully turgid mature leaves were collected from sugar beet plants grown in the field or in sterilized compost in a glasshouse. Material was taken from three fields near Cambridge, and in Bedfordshire from a trial consisting of commercial varieties and experimental breeding plants. Some plots had been inoculated with SBYV or SBMYV; control plots were uninoculated. Extracts were made from the yellowest parts of leaves showing SBYV or SBMYV symptoms, and were compared with similar extracts from leaves of apparently healthy plants. Acetone and light petroleum were used at room temperature to extract the lipids from about 500 mg of leaf. The extracts were applied to filter-paper treated with zinc carbonate: chromatograms were developed in two dimensions, then examined by daylight and under ultra-violet irradiation. Spots required for further examination were cut out and eluted into ethanol. The method has been briefly described[2] and is similar in extraction and chromatographic stages to that described in detail for the determination of α-tocopherol[3]. Some prominent lipids always found in healthy leaves, shown in Fig. 1, were used in locating lipids from leaves of virus-infected plants.

SBYV infection reduced the chlorophyll content and caused the appearance, on chromatograms prepared with extracts of leaves, of two prominent spots that fluoresced nearly white under each of two sources of ultra-violet irradiation having maximum emission at about 254 and 365 mμ respectively. The spectral absorption maxima in ethanol of the upper spot were at 243, 280 and 341 mμ and of the lower spot were at 243, 281 and 342 mμ. The lipids were produced in infected leaves of both field and glasshouse beet, but only in leaves that had turned yellow; they were not found in extracts from apparently healthy leaves of any age. These fluorescing lipids were not seen on chromatograms from green leaves of apparently healthy plants of 100 other species, nor on chromatograms from senescent leaves of several species. Two unrelated species, *Claytonia perfoliata* Donn., and *Chenopodium capitatum* Aschers, were grown in the glasshouse and inoculated with SBYV, but although the older infected leaves became prematurely yellow, the characteristic lipids found in SBYV-infected sugar beet leaves were not seen on chromatograms of their extracts.

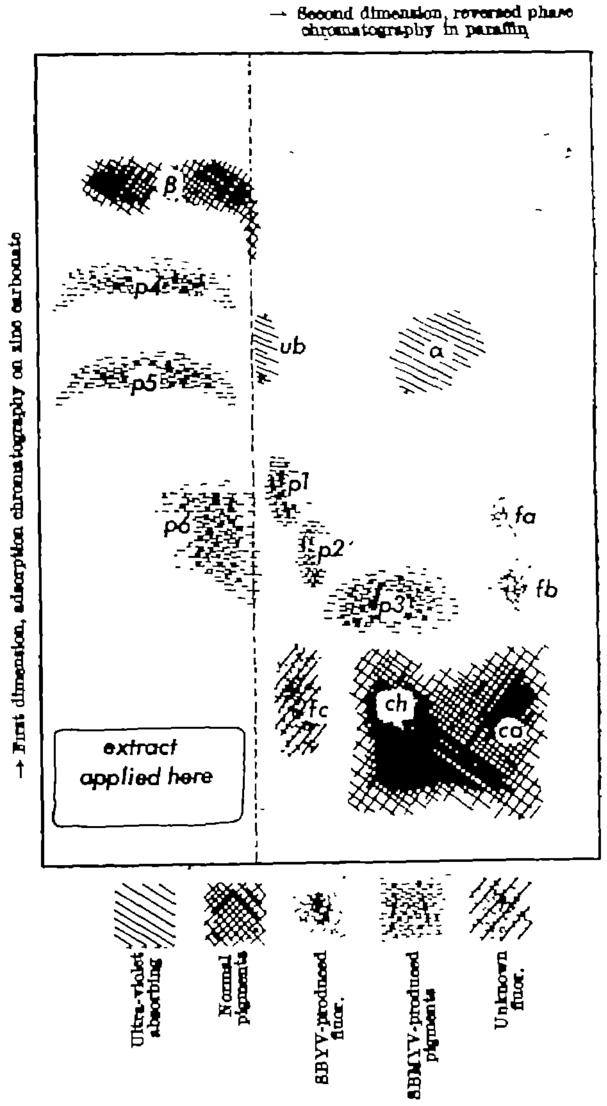

Fig. 1. Position on a two-dimensional chromatogram of phyllolipids extracted from leaves of sugar beet. Group I, lipids found in all leaves. *a*, α-tocopherol; β, β-carotene; *ch*, chlorophylls; *ca*, carotenols, *ub*, ubiquinone-50; group II, additional fluorescing lipids, *fa*, *fb*, from SBYV-infected leaves; group III, additional pigments *p*1–*p*6 from SBMYV-infected leaves; group IV, fluorescing lipid *fc* of unknown origin

SBMYV infection of field-grown beet also reduced the chlorophyll content, and caused the appearance of at least six yellow or orange pigments in addition to those found in healthy leaves. The positions of the most prominent pigments are shown on the chromatogram (Fig. 1). At least two other ill-defined yellow patches also appeared between the application area and pigment *p*6. Solubility and chromatographic behaviour of pigments *p*1–*p*6 were consistent with a carotenoid nature, and spectral absorption curves were characteristic of carotenoids. The pigments were tested for the presence of epoxide groups, by Curl and Bailey's method[4]: *p*6 gave a positive result in one min, *p*3 in 15 min, *p*5 in about an hour, whereas *p*1, *p*2 and *p*4 gave negative results. In some respects chromatograms of extracts from fully turgid orange leaves of SBMYV-infected beet resembled those of extracts from senescent leaves of uninfected plants. Leaf yellowing of SBMYV-infected sugar beet grown under glass was usually very slight, and the pigments were not always seen in this material.

On certain chromatograms, a strongly fluorescing spot was seen at *fc* (Fig. 1). This unknown fluorescing compound was found on about one-third of the chromatograms prepared from leaves of field-grown sugar beet, whether the plants were apparently healthy or were showing symptoms of SBYV, SBMYV or both. It was not seen in leaves of other species, nor was it obvious in preparations from leaves of beet grown under glass in sterilized compost. Its distribution was not correlated with any obvious symptoms, but it seems possible that those plants which produced this substance *fc* may have been infected with an unrecognized virus or other pathogen.

In the field, both yellowing viruses commonly occurred in the same plants, and leaf extracts from these showed the fluorescing spots characteristic of SBYV-infection, together with the pigments characteristic of SBMYV. From the experiments reported here it seems that SBYV produces specific fluorescing substances, and SBMYV produces pigments. This chromatographic method can therefore be used diagnostically. It also appears that the yellowing caused by SBYV resulted from the partial loss of chlorophyll without production of other pigments; whereas SBMYV caused some loss of chlorophyll but additional pigments were produced which gave the infected leaves their characteristic orange colour.

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SOIL SCIENCE

Identification of Soil-fertilizer Reaction Products in a Calcareous Saskatchewan Soil by Infra-red Absorption Analysis

Bouldin and Sample[1] and Beaton and Read[2] reported that uptake of phosphorus from calcareous soils was greater from monoammonium phosphate (MAP) than from diammonium phosphate (DAP) treatments. The formation of relatively unavailable reaction products was believed to be responsible for the differences observed in the latter work. Identification of the soil-fertilizer reaction products formed from MAP and DAP would assist in understanding the responses obtained with these materials since plants obtain most of their fertilizer phosphorus from such reaction products.

The soil used in this investigation and in the earlier investigation[2] was the Bradwell very fine sandy loam which had the following properties: $pH = 7 \cdot 7$, 6·6 per cent calcium carbonate, 6 p.p.m. of sodium bicarbonate extractable phosphorus and 1·4 per cent total carbon. Saturated solutions of MAP and DAP were prepared at 5° C and reacted with this soil according to the procedure of Lindsay and Stephenson[3]. 150 ml. of the fertilizer solutions were each shaken with 300 g of air-dried soil for 15 min at 5° C. Filtrates obtained from these suspensions were stored at 5° C and were examined periodically to follow the progress of precipitation of solids as was outlined by Lindsay *et al.*[4].

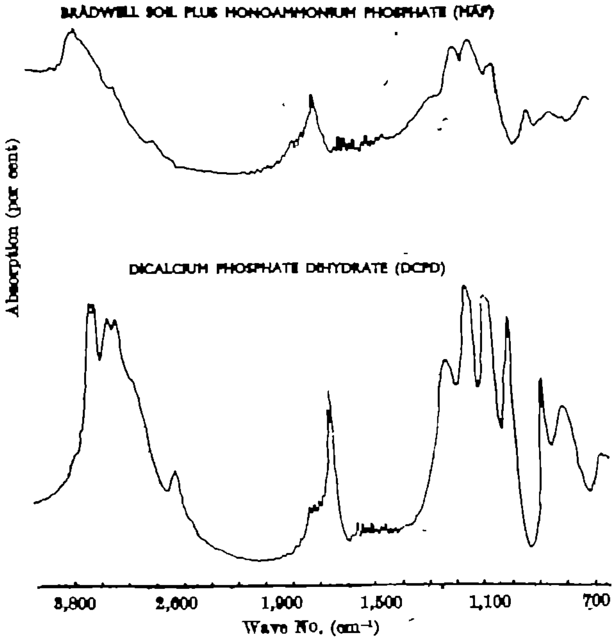

Fig. 1. Infra-red absorption spectra of monoammonium phosphate reaction product and dicalcium phosphate dihydrate

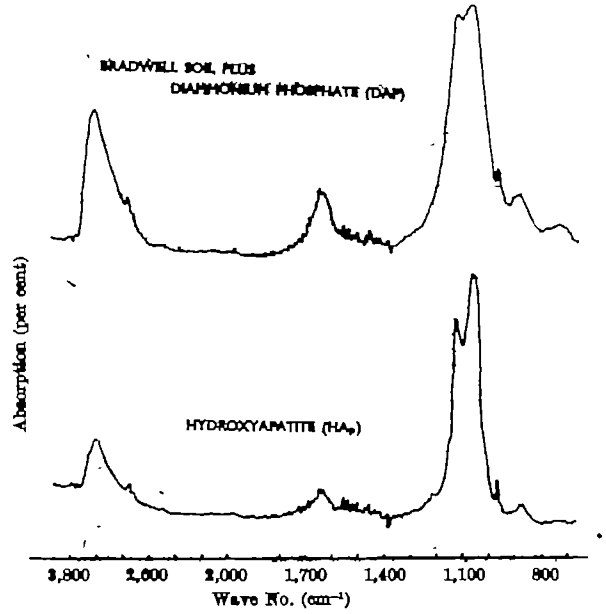

Fig. 2. Infra-red absorption spectra of diammonium phosphate reaction product and hydroxyapatite

At the end of 10 days the reaction products were collected, washed three times with distilled water and dried with acetone. Identification of the reaction products was attempted by X-ray diffraction; but this method failed apparently because the reaction products were too finely divided. However, infra-red absorption proved to be a satisfactory alternative method for identifying these materials.

Infra-red absorption spectra were obtained for a number of compounds that have been reported to be reaction products of MAP and DAP[4]. These spectra were compared with those of the soil-fertilizer reaction products. The spectra of MAP reaction product and dicalcium phosphate dihydrate (DCPD) are shown in Fig. 1. In Fig. 2 the spectra of DAP reaction product and hydroxyapatite are shown.

It is evident that the MAP reaction product and DCPD were similar while the DAP reaction product resembled hydroxyapatite. Our DCPD spectrum agreed closely with the brushite (DCPD) spectrum obtained by Jones and Cruickshank[5].

From these results it appears that the major initial reaction products of MAP and DAP in the Bradwell soil were DCPD and hydroxyapatite, respectively. Further investigations are being conducted to determine if infra-red absorption analysis can be used to identify reaction products from other fertilizers and soils.

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MISCELLANEOUS

Estimating the Thickness of Pulped Wood Fibres

IN examining the mechanical properties of dry, pulped wood fibres which comprise most papers, the dimensions of the fibres must be known. For quantitative purposes, their cross-sectional shape (Fig. 1) is best represented by the rectangle. The 'width' of the fibre is readily measured by the micrometer eyepiece of a microscope as is the length, the largest dimension by orders-of-magnitude. However, the thickness of the fibre is awkward to measure for a number of reasons:

(1) The thickness is in the μ range.

(2) The thickness is generally non-uniform at any one point along the length of the fibre because of the incompleteness of collapse; vestiges of the lumen are generally present along the edges. (Wood fibres are initially more or less circular, but the large pressures applied in the paper-making process cause their collapse.)

(3) Appreciable variations exist in the cross-sectional shape and thickness along the length of the fibre.

(4) Dimensional variations among fibres of any one pulp are extremely large.

(5) The top and bottom of fibres are difficult to define.

Clearly such conventional techniques for measuring the thickness of microscopic-sized particles as the vertical focusing of a microscope stage are unsatisfactory. The use of cross-sections, which require embedding, introduce an additional uncertainty in the possible changes in dimensions caused by swelling and microtoming. Thus a precise technique is not available.

We have applied two techniques, entirely different from each other, which give essentially the same results. These techniques have the further advantage of being quite rapid.

(1) *Retardation Method.* The cellulose chains in wood are aligned more or less parallel to each other and the central fibre axis: hence, fibres are birefringent. When examined between crossed Nicols and a first-order quartz retardation plate, fibres exhibit first-order retardation colours. The equivalent wave-length of the retardation colours, R, is related to the thickness of the cellulose of the fibre (double the wall-thickness), $\bar{\delta}$, by the relationship, $\bar{\delta} = R/(\eta_\gamma - \eta_\alpha)$, where η_γ and η_α are the indices of refraction parallel and perpendicular, respectively, to the principal axes of the fibre[1-3].

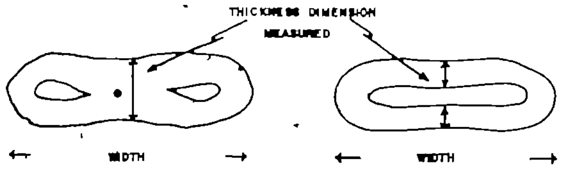

Fig. 1. Typical cross-sectional shapes of fibres

In the investigation recorded here we noted the colour of the central portion of the fibres where pits were absent. The selection of points of measurements is described later. Each value reported in Table 1 is the average of 200 measurements, requiring about 2 h.

(2) *Density Method.* Assuming fibres to have a rectangular cross-section, $\bar{\delta}$ can be calculated from the density equation, $\bar{\delta} = w/\gamma\bar{\omega}$, where w is the mean weight per unit length of fibre; γ, the density of cellulose; and $\bar{\omega}$, the mean fibre width. w, γ, and $\bar{\omega}$ are easily measured.

We measured γ of standard handsheets[4] with a Beckman air comparison pycnometer, model 930. In this instrument, the volume displaced by cellulose is measured.

Using 0·05 g of pulp instead of 1·2 g and large pressures in the standard sheet-forming process[4], ultra-thin (2–*D*) sheets were produced, the fibres of which are clearly visible. We scanned along a straight line of the 2–*D* sheets, noting the width and retardation colour of every fibre. w was calculated from the equation $w = 2W/(1{\cdot}1)\pi(N/L) = 0{\cdot}578W/(N/L)$, where W is the weight per unit area of the sheet; N, the number of fibres intersected in scanning along a line of length L; and 1·1, a factor taking into account the slight curvature of the fibres[5]. Three 15-cm scans, 60° apart, were made on each sample. A complete determination, excluding the retardation measurements, takes about 2 h. The results of the measurements on eight pulps are shown in Table 1. Agreement between the results of the two techniques is surprisingly good.

Table 1

Pulp	γ (g/cm³)	w (g/cm) × 10⁶	$\bar{\omega}$ cm × 10³	$\bar{\delta}$ cm × 10⁴ Retardation method	Density method
Spruce sulphite	1·63	1·3	3·7	2·7	2·2
Spruce kraft No. 1	1·60	1·5	3·5	2·6	2·7
Spruce kraft No. 2	1·60	1·4	3·5	2·6	2·5
Spruce magnifite	1·60	1·5	3·7	2·4	2·5
Southern pine kraft (U.S.)	1·59	2·2	4·5	3·5	3·1
Western mixed softwood kraft U.S.)	1·56	1·6	3·5	3·4	2·9
Jack pine	1·62	1·5	3·3	3·0	2·8
Mixed bleached hardwood	1·59	1·2	2·0	3·8	3·6

All samples mechanically untreated.

The retardation method requires a high-quality microscope and considerable training, but the density method involves only generally available equipment; where a pycnometer is not available, a value of = 1·6 g/cm³ can be used confidently. Thus the density technique is a simple and rapid means of estimating the wall thickness of pulped wood fibres.

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FORTHCOMING EVENTS

Monday, April 1

Royal Institution (at 21 Albemarle Street, London, W.1.), at 5.30 p.m.—Mr. Norris D. McWhirter: "Facts and How to Find Them".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Passenger Handling Within Large Buildings".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Tribes of the Xingu"—Films with commentary by Mr. Adrian Cowell.

Society of Chemical Industry, London Section (at 14, Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. Coates. "Modern Aspects of Phosphorus Compounds".

Tuesday, April 2

Polarographic Society (in the Optics Lecture Theatre of the New Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 2.30 p.m.—Meeting on "Polarography in the Teaching of Analytical Chemistry". 4 p.m.—Annual General Meeting.

Institution of Electrical Engineers, Science and General and Power Divisions (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. K. Wright and Mr. P. D. Dunn: "New Methods for the Generation of Electricity".

Plastics Institute (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Symposium on "The Mixing of Thermo-plastic Materials".

Tuesday, April 2—Wednesday, April 3

Faraday Society (at Queen's College (University of St. Andrews), Dundee), at 9.30 a.m. daily—General Discussion on "The Structure of Electronically-Excited Species in the Gas Phase". Chairman: Prof A. R. Ubbelohde, F.R.S.

Tuesday, April 2—Thursday, April 4

British Society for Parasitology (in the Zoology Department, University College of Wales, Aberystwyth)—Spring Meeting.

Wednesday, April 3

Colour Group (Great Britain) (in the Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 10 a.m.—Symposium in "The Colorimetry of Near Whites". 2 p.m. to 5 p.m.—Exhibition of Colorimeters, Spectrophotometers, Light Sources, Colour Atlases, Colour Filters, Colour Vision Tests and allied equipment. 5.30 p.m.—Prof. W. D. Wright: "The Rays are not Coloured" (First Newton Lecture).

Iron and Steel Institute, Iron and Steel Engineers Group (at 4 Grosvenor Gardens, London, S.W.1), at 10 a.m.—Discussion Meeting on "Universal Beams".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. D. J. Blundell, Dr. R. F. King and Dr. O D. V. Wilson: "Seismic Investigations of the Rocks Beneath the Northern Part of Cardigan Bay"; Dr. J. L. Daniels, Dr. W. J. Skiba and Dr. J. Sutton: "The Deformation of some Banded Gabbros in the Northern Somalia Fold-Belt".

Royal Society of Medicine, History of Medicine Section (at 1 Wimpole Street, London, W.1), at 5 15 p.m.—Dr. W. Hartston: "Medical Dispensaries in the 18th Century London".

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m —Mr. S. Elder: "The Umm Shaif Oilfield: The History of Its Exploration and Development".

Institution of Electrical Engineers, Power Division P G.P7 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. B. Madin and Mr. J. D. Whitaker. "The Dynamic Behaviour of a Transformer Winding under Axial Short-Circuit Forces—an Experimental and Theoretical Investigation".

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. G. E. Roberts, Mr. B. Parker and Mr. C. Powell: "The Display and Processing of Data from the Harco Navigation System"

Wednesday, April 3—Friday, April 5

Society of Chemical Industry, Agriculture Group (in Churchill Hall and the Engineering Laboratory Lecture Theatre, University of Bristol)—Symposium on "Soil Analysis and Its Relation to Plant Composition and Growth".

Thursday, April 4

Institution of Mechanical Engineers, Process Engineering Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10.30 a.m.—All-day Symposium on "Dust Control and Air Cleaning".

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. B. T. M. Willis: "Neutron Diffraction Studies of the Actinide Oxides".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. William S. Lacey· "A Possible Eleutherophyllum from Lower Carboniferous of North Wales"; Dr. John Attridge: "Triassic Vertebrates of Central Africa".

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 5.30 p.m.—Mr. K. Fowler "Air-Conditioning of Large Buildings".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2) at 5.30 p.m.—Mr. P L. Taylor: "Mathematics as an Educational Discipline for the Engineer".

Society of Chemical Industry, Microbiology Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting.

Society of Chemical Industry, London Section (joint meeting with the Mid-Southern Counties Section of the Royal Institute of Chemistry and the Plastics Institute at the University, Southampton), at 7.30 p.m.—Mr. K. C. Bryant. "Polymers—Old and New".

Institute of Metals (at 14 Belgrave Square, London, S.W.1), at 9 p.m.—Annual General Meeting, followed by The Viscount Boyle. "Architectural Metalwork".

Thursday, April 4—Friday, April 5

Mineralogical Society, Clay Minerals Group (at Plymouth Technical College, Tavistock Road, Plymouth)—Spring Meeting.

Friday, April 5

Royal Society of Medicine, Anaesthetics Section (at 1 Wimpole Street, London, W.1), at 6 p.m.—Dr. P. W. Nathan and Dr. T. A. Sears: "The Susceptibility of Nerve Fibres to Local Anaesthetics"; Dr. Ian Verner: "Some Problems of the Clinical Evaluation of Relaxant Drugs in Man, with special reference to a New Competitive Agent".

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting. Prof. R. A. Raphael, F.R.S.: "Acetylenic Chemistry".

Monday, April 8

Institution of Electrical Engineers, Science and General Division P.Gs. 31 and 32 (at Savoy Place, London, W.C.2)), at 5.30 p.m.—Discussion on "Applications of Superconducting Materials" opened by Dr W. F. Gauster, Dr. P. F. Chester, Dr. D. R. Chick and Dr. G. W. Wilson.

Royal Geographical Society (at 1 Kensington Gore, London, S W.7), at 8.30 p.m.—Mr. Charles A. Morris and Mr. A. C. Barrington-Brown. "Cambridge Expedition to South-East Asia".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer, Grade "B" (with a good honours degree in biological subjects, teaching experience, and preferably an interest in field work), to teach Botany and Zoology for General Certificate of Education Advanced Level and Zoology for the Ordinary Endorsed Certificate in Biology—The Principal, Acton Technical College, High Street, Acton, London, W.3 (April 6).

Lecturer or Assistant Lecturer in Numerical Analysis in the Department of Applied Mathematics—The Registrar, The University, Liverpool, quoting Ref. No. CV/231/N (April 6).

Research Assistant (with a good honours degree or diploma in technology and in mathematics, and preferably an interest in research in numerical analysis and statistics) in the Department of Mathematics—The Registrar, Department 27/E, Bradford Institute of Technology, Bradford 7 (April 6).

Scientific Assistant, to work on chemical and biochemical problems in the carbohydrate field—The Secretary, Royal Holloway College, Englefield Green, Surrey (April 6).

Lecturer (with a registrable medical qualification) in Physiology—The Registrar, Trinity College, Dublin 2, Republic of Ireland (April 8).

Programmer in the Department of Mathematics, to be concerned with the preparation of programmes for an English Electric KDF 9 Computer, which is to be installed in August 1963—The Secretary, Royal College of Advanced Technology, Salford 5, Lancs, quoting Ref. MA/6 (April 8).

Research Assistant (physicist or electrical engineer with a Ph.D. or equivalent research experience) in the Department of Electrical Engineering, to study processes associated with arc movement in a magnetic field—The Registrar, The University, Leeds, 2 (April 8).

Research Fellow in Pure Science—The Registrar and Secretary, The University, 38 North Bailey, Durham (April 8).

Assistant Lecturer in Social Anthropology and Sociology—The Registrar, The University, Manchester 13 (April 10).

Assistant Lecturers (3) (graduates in either metallurgy, physical chemistry, chemical engineering or physics) in the Department of Metallurgy—The Registrar, The University, Manchester 13 (April 10)

Lecturer or Assistant Lecturer (preferably with experience in communications, vacuum tubes, plasma physics or some other branch of electronics) in Electronics Engineering—The Registrar, University College of North Wales, Bangor, North Wales (April 10).

Research Assistant (with good qualifications in mathematics and preferably a knowledge of computing methods) to the Professor of Applied Mathematics, to assist with the analysis of magnetic observations made during the IGY—The Secretary, The University, Northcote House, The Queen's Drive, Exeter, Devonshire (April 11).

Biologist—Vermin Control (with a degree in science with a major in zoology or in agricultural science with postgraduate zoological experience, or equivalent)—in the Department of Agriculture, initially at Perth, to undertake biological work on the natural history, bionomics, ecology and physiology of pest or potential pest animals and birds—Public Service Commissioner, 184 St. George's Terrace, Perth, Western Australia (April 12).

Lecturer (preferably with an interest in insect physiology) in Zoology (Comparative Physiology) at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 12).

Research Fellow or Junior Research Fellow (with a sound statistical background, and capable of planning, executing and analysing mobility surveys) in Sociology at the Institute of Social and Economic Research, University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 12).

Assistant Lecturer in the Department of Philosophy—The Registrar, The University, Leicester (April 13).

Lecturer in the Department of Mathematics—The Secretary, The University, Aberdeen (April 13).

TEMPORARY ASSISTANT LECTURER or TEMPORARY LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Liverpool, quoting Ref. No. CV/287/N (April 18).

ASSISTANT LECTURER (preferably with some interest either in acoustics or in ionizing radiation) IN EXPERIMENTAL PHYSICS—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (April 17).

LECTURER IN THE DEPARTMENT OF ANIMAL GENETICS—The Secretary, The University, Edinburgh (April 17).

LECTURER IN APPLIED MATHEMATICS—The Secretary, The Queen's University, Belfast, N. Ireland (April 19).

LECTURER IN INORGANIC AND ANALYTICAL CHEMISTRY—The Secretary, The Queen's University, Belfast, Northern Ireland (April 19).

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Secretary, The Queen's University, Belfast, Northern Ireland (April 19).

LECTURER or ASSISTANT LECTURER IN SOCIAL ANTHROPOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (April 19).

LECTURER (preferably with postgraduate experience in any of the following fields: (*a*) physical organic chemistry; (*b*) spectrophotometry; (*c*) free radical chemistry; or (*d*) high molecular weight materials) IN ORGANIC CHEMISTRY—Head of the Department of Chemistry, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (April 19).

CHAIR OF GEOGRAPHY; SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN GEOGRAPHY AND GEOLOGY at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 20).

LECTURER (man or woman) IN PSYCHOLOGY AND MATHEMATICS—The Principal, Borough Road College, Isleworth, Middlesex (April 20).

POSTDOCTORAL RESEARCH FELLOW (preferably with a knowledge of many-electron atom theory and of computing) IN THEORETICAL CHEMISTRY, to work with Dr. H. O. Pritchard on exact calculations for lithium and beryllium using the Atlas Computer—The Registrar, The University, Manchester 13 (April 20).

PRINCIPAL PHYSICIST (with good academic qualifications (preferably including a higher degree) and several years experience in scientific research or development) IN THE REGIONAL PHYSICS DEPARTMENT, to be in charge of a section providing advice and scientific help on the application of radioactive isotopes in clinical research; most of this work is done in teaching hospitals and university departments—The Secretary, Western Regional Hospital Board, 351 Sauchiehall Street, Glasgow, C.2 (April 20).

SENIOR LECTURER (with a degree in either medicine, pharmacology or pharmacy, and previous experience in either teaching or industrial research) IN PHARMACOLOGY at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1 (April 23).

OFFICIAL FELLOW IN CHEMISTRY—The Principal, Hertford College, Oxford (April 24).

TECHNICIAN (male or female, F.I.M.L.T., with extensive experience in histological techniques) IN THE DIAGNOSTIC SECTION, Agriculture Department, Ruakura Animal Research Station, New Zealand, to take charge of the Histological Laboratory of the Diagnostic Section—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B 13/2/14/652 (April 24).

PROFESSOR OF AGRONOMY; and a SENIOR LECTURER/LECTURER or TEMPORARY LECTURER OF PARASITOLOGY in the Faculty of Rural Science, University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 26).

UNIVERSITY STUDENTS and FELLOWS for experimental or theoretical research in nuclear and elementary particle physics—The Professor of Natural Philosophy, The University, Glasgow, W.2 (April 27).

LECTURERS (2) (with qualifications in either pure mathematics or applied mathematics) IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Bristol 8 (April 29).

GORDON CRAIG FELLOW (graduate in medicine) IN UROLOGY—The Registrar, University of Sydney, Sydney, New South Wales, Australia (April 30).

LECTURER (well qualified in electrical engineering or science) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 30).

LECTURER (with experience in agricultural physics or soil physics) IN SOIL SCIENCE at the University of Sydney, Australia—The Association of Universities of the British Commonwealth (Branch Office), Pall Mall, London, S.W.1 (Australia and London, April 30)

READER IN ELECTRON MICROSCOPY IN THE DEPARTMENTS OF BOTANY, MICROBIOLOGY AND ZOOLOGY—The Registrar, University of Adelaide, Adelaide, South Australia (April 30).

RESEARCH ASSISTANT, Assistant Experimental Officer grade (with a chemical background, and a pass degree or equivalent qualifications) IN THE DEPARTMENT OF POTATO GENETICS, to help in biochemical investigations in potatoes—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts. (April 30).

RESEARCH WORKER, Scientific Officer grade (with biological, medical or veterinary training and preferably experience in virology or bacteriology), to join a team studying avian respiratory diseases—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (April 30).

SENIOR LECTURER IN MATHEMATICS; and a LECTURER IN MATHEMATICS—The Registrar, University of Adelaide, Adelaide, South Australia (April 30).

SENIOR LECTURER (preferably with experience in educational and vocational guidance and counselling, or in clinical psychology) IN PSYCHOLOGY at the University of Melbourne, Australia—The Secretary. Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 30).

SENIOR LIBRARIAN (with the A.L.A. qualification and preferably with experience in a special library concerned with biology or agriculture)—The Director, Commonwealth Institute of Entomology, 56 Queen's Gate, London, S.W.7 (April 30).

SENIOR RESEARCH ASSOCIATE (experienced solid state physicist), to carry out research on radiation damage and defects in ionic crystals—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (April 30).

UNIVERSITY DEMONSTRATOR (with interests in the economic aspects of geography) IN THE DEPARTMENT OF GEOGRAPHY—Dr. I. D. Muir, Department of Mineralogy and Petrology, The University, Downing Place, Cambridge (April 30).

OPPENHEIMER RESEARCH SCHOLARS (suitably qualified graduates in geology) IN THE RESEARCH INSTITUTE OF AFRICAN GEOLOGY, for work which will lead to a Ph.D., and will be done partly in Africa and partly in Leeds—Prof. W. Q. Kennedy, Department of Geology, The University, Leeds 2 (May 1).

PROFESSOR OF MATHEMATICAL PHYSICS; a LECTURER IN BOTANY; a LECTURER IN CHEMISTRY; a LECTURER IN MATHEMATICAL PHYSICS; and a LECTURER IN MATHEMATICS—The Secretary, University College, Galway, Republic of Ireland (May 8).

TUTORIAL RESEARCH FELLOW or STUDENT (man or woman graduate) IN CHEMISTRY, for duties which will include supervision of laboratory classes, time will be available for independent research—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (May 10).

CHAIR OF CHEMICAL ENGINEERING within the Faculty of Engineering at Monash University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, May 15).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Leeds 2 (May 25).

LECTURER (with an honours degree in physics or equivalent qualifications) IN PHYSICS at Wollongong University College—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (May 31).

ASSISTANT PHYSICIST (Senior or Basic grade) IN THE COUNTY MEDICAL PHYSICS DEPARTMENT—The Secretary, St. George's Hospital, Lincoln.

CHAIR OF THEORETICAL PHYSICS—The Registrar and Secretary, The University, Bristol.

EXPERIENCED MATHEMATICIAN capable of teaching up to "S" level—The High Master, Manchester Grammar School, Manchester 13.

GRADUATE TEACHING AND RESEARCH ASSISTANTSHIPS leading to M.Sc. and Ph.D. degrees—Head, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

LABORATORY TECHNICIAN, Grade II (aged 21 or over)—The Head Master, King Edward VI School, Southampton.

LECTURER or SENIOR LECTURER IN MATHEMATICS IN THE TRAINING DEPARTMENT—The Warden, University of London Goldsmiths' College, New Cross, London, S.E.14.

MASTER TO TEACH MATHEMATICS AND CHEMISTRY at Sixth Form level—The Headmaster, Greshams School, Holt, Norfolk.

POSTDOCTORAL RESEARCH FELLOW IN THEORETICAL NUCLEAR PHYSICS, for work involving the investigation of the structure of nuclei and hyperfragments—Head, Department of Physics, Battersea College of Technology, London, S.W.11.

READER (with considerable research experience) IN ELECTRICAL ENGINEERING—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

RESEARCH ASSISTANT IN MYCOLOGY—The Secretary, Wright-Fleming Institute, St. Mary's Hospital Medical School, Paddington, London, W.2.

RESEARCH ASSISTANT (graduate chemist or biochemist) IN THE ARTIFICIAL KIDNEY UNIT—The House Governor, The London Hospital, Whitechapel, London, E.1.

RESEARCH ASSISTANT (preferably with experience in autoradiography, embedding and section cutting for the electron microscope), for cytological work in algae in collaboration with Dr. M. B. E. Godward—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1.

RESEARCH DEMONSTRATOR (with a good degree in horticulture or botany) IN THE DEPARTMENT OF HORTICULTURE, to assist with practical classes in plant physiology—Prof. O. V. S. Heath, F.R.S., University of Reading. Horticultural Research Laboratories, Shinfield Grange, Shinfield, Berks.

SENIOR LECTURER IN THEORY OF MACHINES AND MACHINE DESIGN; LECTURER IN MATHEMATICS, an ASSISTANT LECTURER (Grade B) IN BOTANY, an ASSISTANT LECTURER (Grade B) IN PHYSICS; an ASSISTANT LECTURER (Grade B) IN ELECTRICAL ENGINEERING (heavy current); and an ASSISTANT LECTURER (Grade B) IN MARINE RADIO, at the College of Technology, Kingston upon Hull—The Chief Education Officer, Guildhall, Kingston upon Hull.

SENIOR LECTURER or LECTURER IN TEXTILE CHEMISTRY—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

SENIOR LECTURER (with a higher degree together with an effective knowledge of food processing methods) IN FOOD SCIENCE—The Secretary, Royal College of Science and Technology, Glasgow, C.1.

SENIOR II TECHNICIAN (with biochemical experience), for duties which will include the supervision of student technicians, supplies and storekeeping, and carrying out specialized biochemical investigations for diagnosis and research—Dr. G. H. Spray, Nuffield Department of Medicine. Radcliffe Infirmary, Oxford.

TECHNICIAN (preferably A.I.M.L.T., A.I.S.T., or equivalent status, and some experience in haematology) IN THE DEPARTMENT OF SURGICAL SCIENCE, to assist in research on tissue transplantation—The Secretary, Department of Surgical Science, University of Edinburgh Medical School, Teviot Place, Edinburgh 8, quoting Ref. No. T/88/3/63.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Ciba in Britain. (A brief historical survey of Ciba (A.R.L.), Ltd.). Pp 61. (Duxford: Ciba (A.R.L.), Ltd., 1963.) [122

Building Research Station Digest (Second Series). No. 31: Lightweight Aggregate Concretes—3: Structural Application. Pp. 6. (London: H.M. Stationery Office, 1963.) 4*d*. [122

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 73 Potatoes. Pp. 27. (Belfast. Ministry of Agriculture, 1962.) [122

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ANNOUNCEMENT

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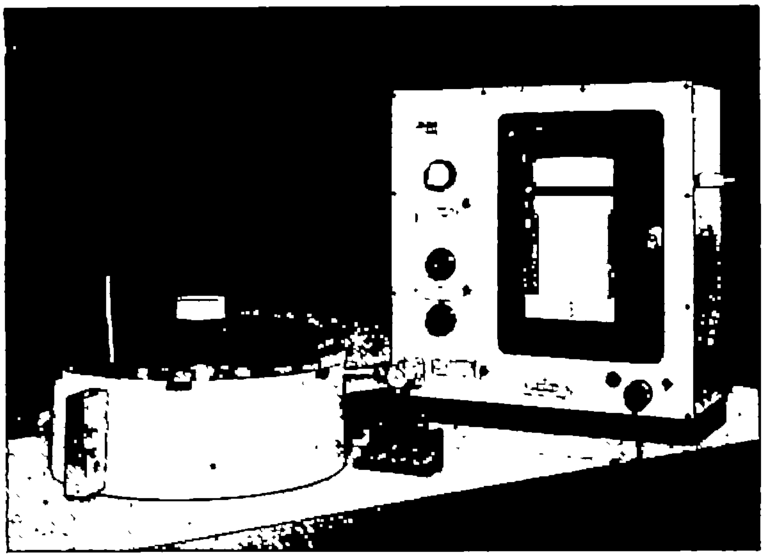

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'Metabolism of Resistant Mutants of Streptococcus faecalis—IV. Use of a Biophotometer in Growth-Curve Studies' by M. Katharine Coultas and Dorris J. Hutchison, Journal of Bacteriology (U.S.A.), Vol. 84, No. 3, pp. 393–401, Sept. 1962.

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APPOINTMENTS VACANT

UNIVERSITY OF BRISTOL

CHAIR OF THEORETICAL PHYSICS

The University is establishing a Chair of Theoretical Physics from August 1, 1963, and proposes to make an appointment thereto as soon as possible.

Suitably qualified candidates are invited to submit applications, including the names and addresses of referees, to the undersigned, from whom further particulars of the appointment may be obtained.

H. C. BUTTERFIELD,
Registrar and Secretary.

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI, GHANA

FACULTY OF PHARMACY

Applications are invited for the post of

SENIOR LECTURER

in Pharmacology.

Qualifications:

Applicants should hold a degree in either Medicine, Pharmacology or Pharmacy and should have previous experience in either teaching or industrial research.

Duties:

Successful candidates will be expected to teach up to the level of the general Degree in Pharmacy of the University. In addition to teaching duties, candidates will be expected to participate in a research programme of investigation of the Medicinal and toxic plants of Ghana. This programme is being carried out in conjunction with the Chelsea College of Science and Technology, London.

Salary Scale:

Ghanaian Senior Lecturer, £G.1,750 by £G.75 to £G.2,200 per annum.

Non-Ghanaian Senior Lecturer, £G.2,100 by £G.90 to £G.2,640 per annum.

Entry at appropriate point.

Appointments are normally for five years in the first instance and may be renewed; one term's notice of resignation or termination of appointment is required on either side. Outfit allowance £G 60 (where applicable). Car maintenance allowance £G.150 per annum. Children's allowances are paid in respect of expatriates at the rate of £G.50 per annum per each child resident in Ghana and £G.100 per annum per each child in full-time education overseas up to a maximum of 21 years of age (maximum: five children). Accommodation with basic furniture. Rent not exceeding £G.150 per annum. F.S.S.U. policies maintained if already held or superannuation on similar basis.

Annual leave with three free return passages in every four years for non-Ghanaians and families and one in every three years for Ghanaians.

Application forms and further information may be obtained from the Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1. Completed application forms should reach the above-mentioned address not later than April 23, 1963

UNIVERSITY OF CAMBRIDGE

DEMONSTRATOR IN GEOLOGY

Applications are invited for the post of University Demonstrator in the Department of Geology, tenable from October 1, 1963, for three years in the first instance. Applicants should have interests in palaeontology, stratigraphy, sedimentary petrology, or structural geology. Salary scale (now under review): £1,000 by £50 to £1,250. Membership F.S.S.U. and Children's Allowance Scheme.

Further details may be obtained from Dr. I. D. Muir, Department of Mineralogy and Petrology, Downing Place, Cambridge, to whom applications (including the names of two referees), should be sent by May 18, 1963.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF CHEMICAL ENGINEERING

Applications are invited for the undermentioned positions with the Organization's Division of Chemical Engineering at Fishermen's Bend, Melbourne, Victoria.

The Division of Chemical Engineering undertakes fundamental and applied investigations into fields of process and equipment development and unit operations research. Subjects covered include: water desalination, gasification of coal, comminution, fluidization, mixing and mass transfer operations.

CHEMIST OR CHEMICAL ENGINEER

(No. 608/27)

The appointee will be required to undertake fundamental and applied research in various fields of physical chemistry and chemical engineering. Initially work would be undertaken on a promising new method for the prevention of scale formation on the heating surfaces of evaporators (e.g., in water desalination equipment), by the introduction of selected polymeric compounds which form regenerating protective films. This would involve the building up of a small team to prepare and characterize suitable polymers, and to examine their behaviour under both practical and idealized laboratory conditions.

Applicants should possess at least a University honours degree in Science with Chemistry as a major subject, or in Chemical Engineering, together with some years of postgraduate research experience. An interest, or experience in physical or surface chemistry would be of particular value.

CHEMICAL ENGINEER

(No. 608/34)

The appointee will be required:

(a) To undertake work on plant design, and evaluation and costing of proposed new processes.

(b) To carry out investigations into applications of computer techniques to process design.

The work will be mainly of a theoretical nature, but the opportunity will arise from time to time to take part in experimental investigations.

Applicants should possess at least a Degree in Chemical Engineering with first- or second-class honours, together with some years of postgraduate research experience. Additional experience in process design and/or the commissioning of plant would be desirable.

SALARY: Dependent on qualifications and experience, salary for both positions will be determined within the ranges

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Promotion within C.S.I.R.O. is by merit and may go beyond the upper limit of the scale within which the original appointment is made.

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Fares paid for the appointees and their dependent family and return fares to point of origin will be paid for an appointee who accepts appointment for a fixed term. Further particulars supplied on application to:

Mr. P. F. Butler, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting the relevant appointment number), should be addressed by May 25, 1963

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Apply Head, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

JOHN INNES INSTITUTE REQUIRE A RESEARCH Assistant with chemical background to help in biochemical investigations in potatoes in the Department of Potato Genetics. The appointment will be in the Assistant Experimental Officer grade, and a Pass Degree or equivalent qualifications is required. Salary will be in the range £622 to £1,022 per annum, according to age and experience. Superannuation.—Applications, with the names of two referees, should be addressed to the Secretary, Bayfordbury, Hertford, Herts, not later than April 30, 1963.

HOUGHTON POULTRY RESEARCH STATION

Applications are invited for a research worker with biological, medical or veterinary training preferably with experience in virology or bacteriology to join a team studying avian respiratory diseases. Grading Scientific Officer (£768 to £1,721 per annum) or Senior Scientific Officer (£1,396 to £1,720 per annum), according to qualifications and experience. Superannuation under F.S.S.U.

Applications, with names of three referees, to the Station Secretary, Houghton, Huntingdon, before April 30.

ELECTRON MICROSCOPE UNIT, DEPARTMENT of Zoology, require person who should preferably be able to assist both with the research and the general running of an electron microscope. Salary in the range £785 to £1,335, plus London weighting.—Applications, in writing, stating qualifications, age and experience, should be sent to the Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, E.C.1.

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UNIVERSITY COLLEGE OF SOUTH WALES AND MONMOUTHSHIRE

LECTURESHIP IN PSYCHOLOGY

Applications are invited for appointment to a Lectureship in Psychology. A good honours degree in psychology is required and applicants should preferably have university teaching experience and a research degree or research experience. The post will involve responsibility for laboratory practical courses.

Applications (six copies), containing the names of three referees, should be received not later than May 4, 1963, by the Registrar, University College, Cardiff, from whom further particulars may be obtained.

E. R. EVANS,
Registrar.

Cathays Park,
Cardiff.

THE ROYAL COLLEGE

NAIROBI

Applications are invited for newly created Chair of Pathology in Faculty of Veterinary Science. Appointee expected to develop Department in all aspects, but with special reference to gross pathology, histopathology, and food hygiene. Salary: £2,600 per annum. (Revision of salary scales under consideration.) Expatriate allowance £400 per annum. Permanent or contract terms (contract terms carry 25 per cent gratuity). F.S.S.U. Education allowance. Housing at subsidized rental. Passages for appointee and family (up to five adult passages) on appointment, termination and leave (three months every 21 months).

Detailed applications (eight copies), naming three referees, by May 31, 1963, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF ZOOLOGY

VANCOUVER 8, B.C., CANADA

Applications are being sought for a post as Physiologist effective September 1, 1963. The appointment will be made at the Assistant Professor rank (salary $7,000 to $8,400 per annum, plus superannuation, insurance and fully comprehensive medical benefits). Teaching duties will include a course in elementary vertebrate physiology at the undergraduate level. The pursuit of an active programme of research and graduate student training are also essential. Persons will be considered irrespective of the field (vertebrate or invertebrate) but candidates with interests in nerve, muscle, cellular and general physiology will be particularly welcome. Curriculum vitae, publication list, a brief outline of current activities and a list of three referees should be submitted before April 30, 1963. References will only be sought in the cases of applicants who are short-listed for the appointment.

Applications should be addressed to: The Chairman, Department of Zoology, University of British Columbia, Vancouver 8, B.C., Canada.

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ANIMAL GENETICS

LECTURESHIP

Applications are invited for the post of Lecturer in the Department of Animal Genetics. Salary scale (under review): £1,150 by £50 to £1,350; bar: £1,400 by £50 to £1,500 by £75 to £1,950 per annum, with placement according to qualifications and experience, and with superannuation benefit and family allowance where applicable. Some research experience in genetics is essential, and preference will be given to candidates prepared to teach either cytology or classical segregational genetics. It is hoped the successful candidate could take up his appointment on October 1, 1963.

Further particulars may be obtained from the undersigned, with whom applications (six copies), giving the names of two referees, should be lodged not later than April 17, 1963.

CHARLES H. STEWART,
Secretary to the University.

ST. GEORGE'S HOSPITAL

LINCOLN

COUNTY MEDICAL PHYSICS DEPARTMENT

Applications are invited for a post of Assistant Physicist in the County Medical Physics Department. The successful applicant may be appointed either in the Senior or Basic grade, depending on qualifications and experience. For the Senior grade appointment, experience in Radiotherapy Physics or Isotope Technique will be necessary. There are good facilities for work with modern Radiotherapy equipment, Radioactive Isotopes and in general medical physics. The salary is according to the Whitley Council scale.

Applications, giving details of qualifications and experience, together with the names of two referees, should be addressed to the Secretary, St. George's Hospital, Lincoln, from whom further particulars can be obtained.